STRUCTURED MONITORING OF GAS EXCHANGE

~~IN AEROBIC FERMENTATIONS~~

IN FERMENTERS FOR CONTROL.

A thesis submitted for the degree

of

Doctor of Philosophy

by

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August 1992

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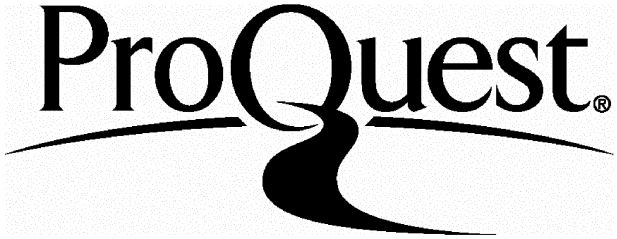

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...to my parents, and Patricia

ACKNOWLEDGEMENTS

I gratefully acknowledge the help of my supervisor, Nina Thornhill, and the electronics workshop manager, Martin Vale. I would also like to thank the staff of the pilot plant and the workshop for their assistance at various times in the course of this project, and the academic staff and students of the Department for making my stay at UCL enjoyable and profitable. I gratefully acknowledge the financial support of the SERC.

ABSTRACT

Information from measurements made by on-line sensors are, directly or indirectly, critical to strategies for improved monitoring and control of industrial fermentations. Over the past 20 years, a large body of research has, with little success, attempted to expand the library of on-line measurements routinely used in industrial fermentation. Partly as a result, research efforts have increasingly been targeted at the development of models incorporating on-line data, that describe the time-profiles of unmeasurable variables of importance to fermentation monitoring. Due to the complexity of most of these models, industrial applications have largely been limited to the simplest of empirical models, based around "derived variables" (that derive directly from one or more on-line measurements), most of them associated with gas exchange, examples being the carbon dioxide evolution rate (CER) and respiratory quotient (RQ). Improvements in the conditioning, analysis and application of gas exchange data would, therefore, be of considerable benefit in improved monitoring, modelling and control of fermentation. This project examines opportunities for such improvements.

It was shown that the oxygen transfer rate (OTR) data contain a significant component of uncorrelated Gaussian noise arising from their calculation as a small difference between two large numbers. A chi-square filter was used to fit a linear model to a reduced data set containing only the most recent OTR data, in order to remove this noise. The benefits of applying such a filter were illustrated by the improvement in the quality of OTR data, and related derived variables (the mass transfer coefficient, $K_L{}^{O2}a$, and the respiratory quotient, RQ), during a *Streptomyces clavuligerus* fermentation.

Theoretical work supported the view that carbon dioxide transfer can be treated as a purely liquid-film limited physical process, as for oxygen. Concerning the error involved in the (widely-used) assumption that the dissolved CO_2 partial pressure is equal to the CO_2 partial pressure in the exit gas, practical factors were shown to limit the maximum error possible. This error varies with $K_L{}^{O2}a$, and the aeration rate, being 20-30% in small fermentors, and less in large fermentors. The theoretical results were supported with experimental data from *Escherichia coli* fermentations. For fermentations run above pH 6.5, the high effective solubility of dissolved carbon dioxide can cause changes in the pH and CER to make unsteady-state terms in the CO_2 mass balance important. An effect is to cause the "measured respiratory quotient" as apparent from gas analyses (called here the transfer quotient, or TQ) to differ from the real underlying respiratory quotient (RQ). A model to predict such effects agreed well with experimental results from fermentations of *E. coli* and *S. clavuligerus*. The control of pH by on-off additions of acid or base introduces regular fluctuations into the TQ that are not present in the underlying RQ. During exponential growth, the TQ is smaller than the RQ. The RQ can be estimated on-line from the TQ using the model developed.

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1. BACKGROUND

This thesis describes a project to examine opportunities for improving the monitoring and control of gas exchange in aerobic fermentations. The author was employed on the Fermentation Monitoring and Control Project, and the project was defined following an extensive review of the literature that sought to identify areas of fermentation monitoring and control research that could have the most direct impact in improving industrial fermentation operation. The project reported here examined 'derived fermentation variables' (ie. those that can be evaluated directly from on-line sensor measurements) associated with gas exchange, which form the basis of applications of models to industrial fermentation control. In particular, conditioning of these data, and factors that affect their accuracy, were examined. The utility of the results is illustrated by application to a fermentation control problem.

The first chapter "BACKGROUND", explains the title of the project and the background work that led to the identification of the project as an important and, until now, neglected area of research. The second chapter, "LITERATURE SURVEY", is a concise examination of the current state of research relating to gas exchange in fermentation, providing a point from which the work reported in the fourth chapter, "RESULTS AND DISCUSSION" leads on.

1.1 PROJECT DEFINITION

The objective in this section is two-fold. Firstly, to summarize the conclusions arising from a review entitled "Recent Trends and Developments in Fermentation Operation", which is presented in full in Section 1.2. Secondly, to describe how the conclusions of this review were used to define the project.

Prior to discussing the conclusions reached in the review in Section 1.2, some initial decisions were made concerning the fermentation subject group of interest. It was decided to consider only aerobic fermentations in this project. Aerobic fermentations were chosen for two reasons: firstly, that they represent the most important industrial fermentations, and secondly, that the presence of an oxygen demand increases the number of fermentation variables that can be monitored routinely. The work in the project was also intended to be most relevant to production-scale fermentations. It was felt that problems whose solutions would be applicable at production scale would be more interesting and important from an engineering point of view that those associated with research and scale-up stages of fermentation operation.

The principal conclusions arising from the review of fermentation operation presented in Section 1.2 are summarized in Table 1, which targets areas which most require research in order to advance industrial fermentation operation. The areas identified in Table 1 are divided by product sector (primary/secondary metabolite) and stage of fermentation (research/scale-up/production stage). It was found that cost/benefit and other practical considerations meant that the importance of some avenues of research was affected by whether the product was a primary or secondary metabolite. As it had been decided to concentrate on problems most relevant to production-scale fermentation, the areas identified in Table 1 concerning research and scale-up stages of fermentation were not considered. One of the conclusions of the review, summarized in Table 1, concerns the relatively greater incentive in the case of production-scale secondary metabolite fermentation for efficient data analysis

	PRIMARY METABOLITE	SECONDARY METABOLITE
RESEARCH		- further development of automated biochemical analyses - dynamic optimization of fed-batch fermentation conditions?
SCALE-UP		- scale-up of batch sterilization - scale-up of fermentation control - effect of scale on mixing especially of non-Newtonian fermentation broths
PRODUCTION	- improved fixed-parameter models - optimal temperature and pH profiles - feedforward control of environmental variables - automated dissolved oxygen control - better understanding of the merits of agitator retrofitting - effect of heterogeneous conditions on productivity	- effect of heterogeneities in fermentor on micro-organisms and fermentation productivity - data reduction - efficient data archiving - better (automated) use of historical data - automated model synthesis and identification - improve quality of derived variables - better use of off-line data in identifying events in on-line data

Table 1: Developments required for improved fermentor operation

and application of data, by comparison with primary metabolite manufacture. This incentive arises because of the higher sensitivity of secondary metabolite fermentation productivity to operating conditions, and also because regular changes of production strain in secondary metabolite manufacture means that there is less trial and error experience for a given production strain on which to base production fermentor control. It was decided to focus the project on areas of data analysis and application identified in Table 1 concerning production-scale secondary metabolite fermentations. Clearly, however, advances in data analysis most relevant to secondary metabolite manufacture, will also be of benefit to the operation of primary metabolite fermentations.

In secondary metabolite manufacture, the constantly moving 'goalposts' arising from development of new production strains means that modelling work for application in the monitoring and control of production-scale fermentation must, from cost-benefit considerations, be directed towards simple models and automated data analysis for identification of model parameters. The benefits of modelling and control of secondary metabolite fermentations at production scale have as much to do with achieving reproducibility (for the benefit both of downstream processing and product safety in the case of therapeutic products) as with maintaining high productivity. The industry has been relatively reluctant to implement novel measurement strategies in a production setting due to the increase risk of contaminating the fermentation, and this situation will continue. Hence this project worked within the constraints imposed by the most commonly-used on-line instrumentation, and novel sensor measurements were not considered.

It was decided to focus on the problem of improving the quality of 'derived fermentation variables', identified in Table 1 as an area requiring additional research. By 'derived variables' are meant those variables that can be evaluated more or less directly from one or more raw on-line fermentation measurements, and include the oxygen uptake rate (OUR), carbon dioxide evolution rate (CER), respiratory quotient (RQ), and the mass transfer coefficient ($K_L{}^{O2}a$). In addition to these well known derived variables, it was concluded in Section 1.2 that the concentration of dissolved

carbon dioxide could also be considered to be a derived variable. These variables represent the most important information 'handle' available during fermentations. Used in physiological models, these variables have facilitated most of the work reported in the literature concerning the use of models in monitoring and controlling industrial fermentation. However, there has been no systematic study of their evaluation, or of factors that can affect the quality of derived variable data. This is in spite of the fact that several authors have noted that derived variables have differing qualities, and can be affected by operating conditions. For example, the amplitude of random noise in the OUR as apparent from gas analyses is higher than that apparent in the CER. Also the CER as apparent from gas analyses is known to be affected by changes in the pH of the fermentation broth.

The object of this project was therefore to improve understanding of the quality of derived variables, and use this information to improve the control of fermentations. This examination was viewed as consisting of two parts. Firstly, an examination of opportunities for signal conditioning of derived variables. Signal conditioning involved in particular examination of noise, and to a lesser extent, bias in derived variables. Secondly, the methodology in the calculation of derived fermentation variables has to some degree been taken for granted, even though several authors have alluded to difficulties inherent in their calculation. Such derived variables form the building blocks for the synthesis of models and their identification. Further, such models form the key to more advanced control of fermentation. Hence, there is a clear dividend to structuring the monitoring of derived variables so that more robust, accurate and reliable values for these variables are incorporated into modelling and control strategies. The structured monitoring of derived fermentation variables forms the basis of this project. As most derived variables relate directly or indirectly to gas exchange (CER, OUR, RQ, K_La), the project was entitled "Structured Monitoring of Gas Exchange in Aerobic Fermentation".

1.2 RECENT TRENDS AND DEVELOPMENTS IN FERMENTATION OPERATION

This section presents a review of the interaction between academic research and production-scale fermentation in industry, in the area of fermentation operation. Section 1.2.1 presents an overview of the fermentation industry and fermentation operation in order define more clearly the objectives of the review. Fermentation operation is viewed as consisting of ten areas, which can broadly be divided into two groups, under the headings "Monitoring" and "Control". "Monitoring" is viewed as encompassing information acquisition and analysis, while "Control" encompasses the application of information with the ultimate objective of improving the profitability of the fermentation process. Sections 1.2.2 and 1.2.3 make a critical assessment of the relationship between academic research and industrial practice in the ten areas under these headings "Monitoring" and "Control", respectively.

1.2.1 OVERVIEW

The section examines the fermentation industry in broad economic terms, and shows that achieving high product yield is a dominant objective in fermentation operation. The areas encompassed by the term "fermentation operation" are described. It is argued that while improvements in all these areas lead to improved fermentation operation, the nature of the product (whether a primary or secondary metabolite) has a large impact on the relative importance of particular areas.

Fermentation is used to produce products with a very wide range of value, as indicated in Figure 1 (based on Dunnill, 1987). At either extreme of the product value range, rather special solutions as regards fermentor design are encountered. At the highest value end of the fermentation product market, typified by the products of mammalian cell culture, product purity is a dominant need, and the product cost reflects the costs of research and achievement of regulatory approval far more than

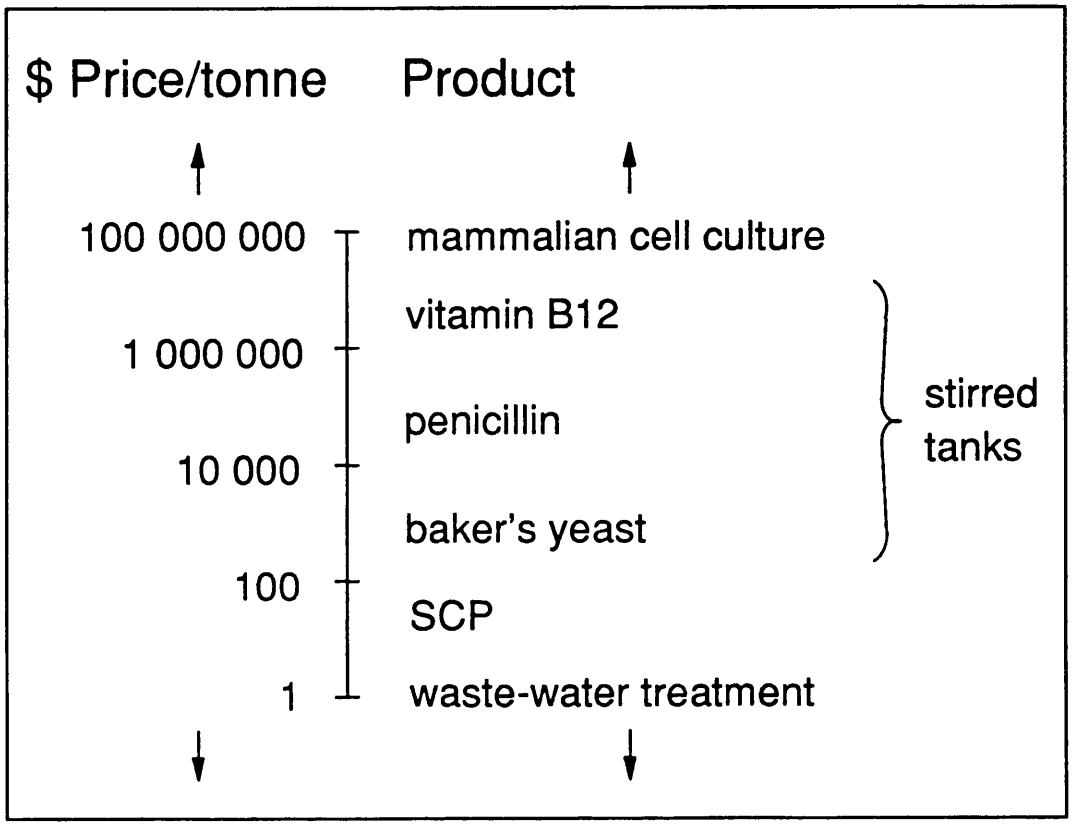

Figure 1: Range of product value in the fermentation industry (1992 prices)

the cost of operating the fermentation. As a result, rather unusual fermentation systems have been developed, and of the five commercial biotechnology products produced by cell culture in 1992, only one is produced in stirred vessels (D.R. Omstead*). Further, for such products, the focus is on speed to market, while production efficiency and scalability are less important. Additionally, regulatory restrictions make production in stirred tanks difficult. At the opposite end of the scale, in anaerobic digestion of sewage and industrial waste, the dominating objective is to achieve a required purification at minimum cost. This single dominating objective has given rise to the evolution of rather novel fermentor designs, a recent development being the upflow anaerobic sludge blanket reactor (Kosaric, 1991). In between these two extremes of product value, however, a large proportion of fermentation products are produced in agitated stirred tanks (Sittig, 1982) typically with a working volume of $100m^3$ (Dunnill, 1987), in particular under the fed-batch feeding strategy (Johnson, 1987), with the fermentation normally lasting a few days. Although there are a few important fermentation processes, such as alcohol production, which are anaerobic and non-sterile operations, most industrial fermentations require sterile operation and the provision of adequate amounts of oxygen to the culture (Lilly, 1987) (anaerobes being less widely used perhaps because of their relative metabolic inefficiency (Curran and Smith, 1989)). For these products, operating costs are an important cost term. This uniformity of fermentation system is useful in providing a basis on which to examine developments in fermentor operation from a generic standpoint. This review concerns in particular this latter group of fermentation products, and the problems tackled in the project are directed towards this group.

These fermentation products broadly fall into two categories, dependent on whether the product is associated mainly with a microorganism's primary or secondary metabolism. Products associated with primary metabolism tend to be of relatively low value, because they involve production of simple biochemicals in relatively large volumes. Ethanol, citric acid, biomass and lactic acid fall into this category. The products of secondary metabolism have to be relatively high value products at their introduction, as the yield of secondary metabolites is so low as to require this for an

* refer to p69

economic process. Examples are antibiotics (the most important sector of the fermentation industry), and vitamins. Although enzymes are generally considered as intermediate metabolites, they are included with this latter group as the economics of enzyme fermentations share important features with secondary metabolite production.

With this division of fermentation products are associated two important aspects which have a great impact on fermentation economics and therefore on optimal fermentation operation.

The first of these concerns the gross economics of fermentation. Total energy consumption for a fermentation in a stirred tank is on average 8.2kW.m^{-3}, for agitation, air compression (in aerobic systems) and the provision of chilled water (Curran and Smith, 1989). Electricity costs in the UK for a large industrial user are US $0.07 per kWh. Hence a 6-day antibiotic fermentation in a 100m^3 fermentor with a one-day turnaround requires around $8000 of power. For high value antibiotics, the media costs for a fermentation, even at pilot scale, can be $100 000 (P.M. Salmon*), and hence energy costs are much less important than raw material costs for high value products. Even for the case of relatively low value products such as ethanol produced from sugar cane, the raw materials represent 70% of the operating costs of the process (Maiorella et al, 1984). Hence, the dominating objective in the operation of production fermentors is to achieve high yield of product, energy economy being in general a secondary, although not negligible, objective.

The economic importance of high yield, while implying the need for tight and reproducible control of production fermentation, also implies the need for strain development programmes. The maximum theoretical yield for a fermentation product is ultimately limited by thermodynamical considerations of the biochemical reactions constituting a cells metabolism. Not surprisingly, therefore, there is considerably more scope for improving the relatively low yields of secondary metabolites, than there is for improving the yields of the products of primary metabolism. Up to the

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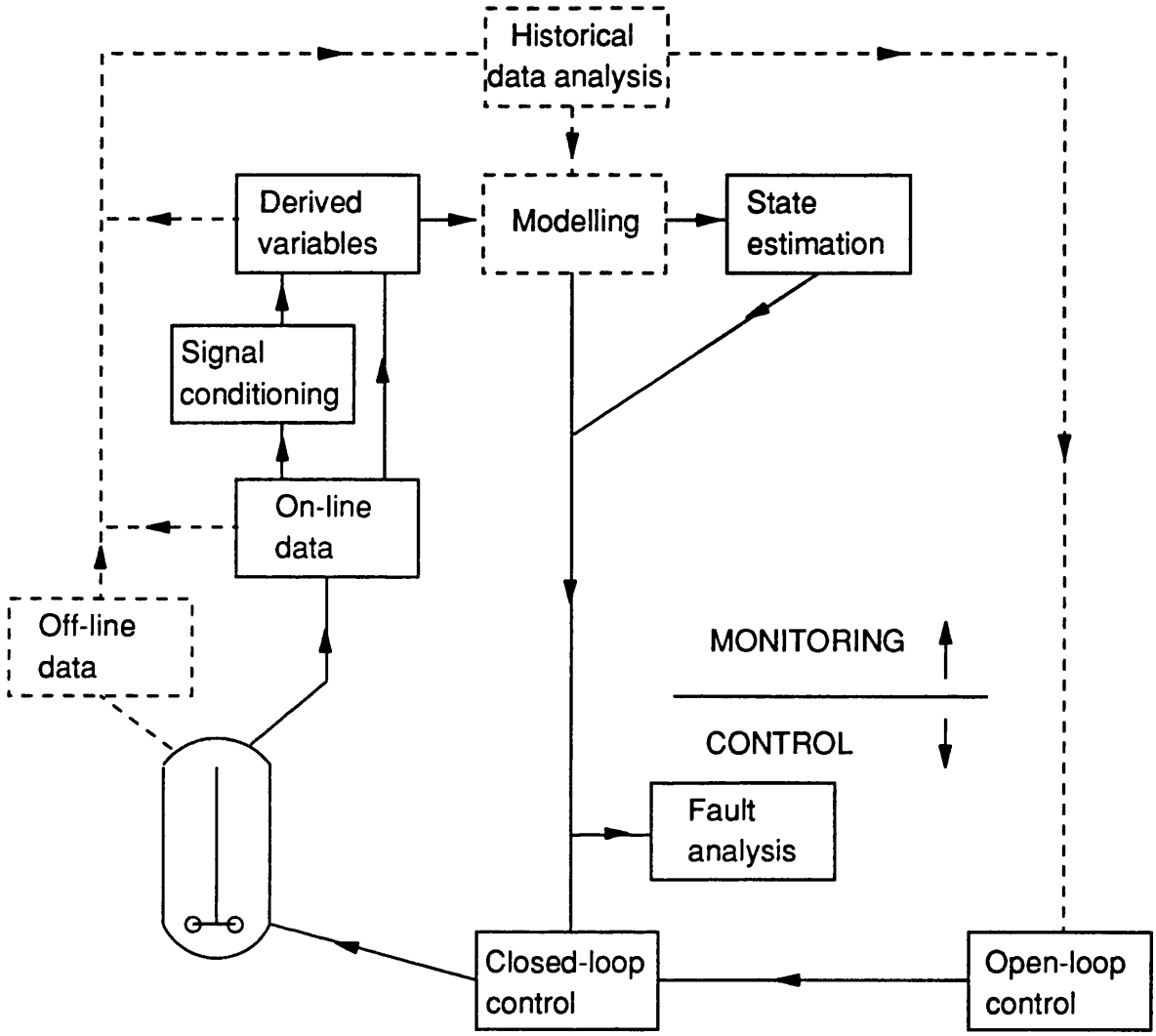
present time, strain improvement was mainly based on induced mutagenesis and only during the last few years has genetic recombination based on protoplast fusion become an additional practicable strategy (Schwab, 1988). The productivity of the penicillin process between 1950 and the present has been increased by more than two orders of magnitude. The fermentation industry has continued to achieve significant improvements in penicillin titre more recently, with a 4-fold increase in production titres between 1970 and 1985 at Gist Brocades being reported (van der Meer and Valkema, 1985). DNA technology may facilitate 30-60 fold increases in secondary metabolite yields (Earl, 1991), although the yield of penicillin, at least, must ultimately be limited by the achievement of almost 100% of conversion of carbon in the substrate to the product, current levels of conversion being only around 10% (Moller and Schugerl, 1987). In work describing the history of yield improvements of asperlicin, production of asperlicin by the original soil isolate of *Aspergillus alliaceus* of 15-30mg/L was increased to greater than 900mg/L in nine months, by isolation of high yield mutants and media optimization (Monaghan et al, 1989). It is not unusual that mutant strains can produce many times the yield of enzyme than obtainable from the wild parent culture in industrial enzyme production (Volesky and Luong, 1984). By contrast, application of process engineering techniques to biotechnology could result in biological reactors which are up to 50% more efficient (Morgan, 1992). This clearly indicates that in secondary metabolite fermentation industry, the identification of higher yielding strains and their rapid introduction into the production process is of greater importance in achieving competitive advantage, than improved process engineering. In the primary metabolite industry, the rather less spectacular increases in product titre and yield mean that improvements in process engineering can yield significant competitive advantage. One of the effects of this situation is that manufacturers of secondary metabolites can justify far greater investment in industrial research and pilot plant facilities than can generally be justified in the primary metabolite industry (G. Austin, personal communication).

The two aspects of industrial fermentation outlined above, namely gross economics and strain development, have had far reaching consequences for the sorts of

fermentation operating strategies employed in industry. Fermentor operation can broadly be divided into the areas of monitoring and control. While these terms have been widely used in the biochemical engineering literature to convey a range of concepts, they are nevertheless useful in allowing a broad division of ideas in fermentor operation into those that involve "information acquisition and analysis" with those that involve "information application". This division of areas is illustrated in Figure 2. Within monitoring is included any activity that is concerned with improving the ability to track the critical variables that affect the fermentation. A control activity entails the use of monitored information in order to take decisions that affect the profitability of the process in some desirable way.

Monitoring of a process is not limited to its measurement. This is especially the case for fermentation processes, where industrially reliable instruments and analyzers capable of directly measuring metabolic variables are lacking. In addition, production fermentors typically only have a few measuring points as there is an incentive to minimize the number of ports to facilitate cleaning and so reduce the risk of contamination (Chisti, 1992). The incorporation of measurements into models and state estimators that provide information that cannot be measured directly is another aspect of process monitoring.

The ability to accurately and reliably monitor a fermentation state is normally the greatest hurdle to be overcome in automatic control of that state. Two factors have, however, tended to limit such applications. Firstly, the dynamics of fermentation processes are very slow, events frequently occurring over periods of hours rather than seconds. Indeed, it is quite feasible to effect control action manually, and such manual control is commonly used in industry for the control of dissolved oxygen concentrations. Further, for products with therapeutic application such as antibiotics, the entire production process has to be validated and in process controls have to be established, to guarantee consistent product quality and safety of the product (Gerson et al, 1988; Werner and Langlouis-Gau, 1989). Once a company has overcome the



MONITORING - information acquisition and analysis

CONTROL - information application

——— normally on-line

- - - - - - - normally off-line

Figure 2: Components in fermentation operation

hurdle of getting a process licensed, there may be little incentive to apply sophisticated control algorithms to improve the process productivity.

One of the most striking developments in operation of industrial fermentation over the last 20 years has been the almost universal acceptance of the role of computers. It was rightly pointed out some years ago (Dobry and Jost, 1977) that fermentation computer systems were justified not only in their potential to allow modelling and optimization techniques to be applied to industrial fermentation, but for achieving more uniform and reliable fermentation operation. Computing allows, for example, that data can be logged at variable frequencies to correspond to critical periods of the fermentation, and that data can be stored for later processing and modelling. Computers are used to improve operations within the process plant for metering of nutrients, sterilization cycles and batch sequencing (Hatch, 1982). Surprisingly, the potential for computer application of modelling and optimization techniques remains to be fulfilled.

Fermentation operation can therefore be viewed as consisting of ten areas, seven of which are monitoring activities, and three being control activities. Throughout the fermentation industry, yield improvements are the most important factor in improved profitability. In secondary metabolite manufacture, the profitability of production depends critically on continual process development, and so improvements in fermentation operation applicable to the research and scale-up stages of operation can be of significant benefit to the overall profitability of a fermentation facility. In primary metabolite manufacture, energy economy at the production scale is a more important objective than improving fermentation operation in process development.

The review presents a examination of progress towards improving the monitoring and control of fermentation. A parallel examination is made of trends in industry and in academia relating to improving fermentation monitoring and control. In particular, an attempt is made on the basis of practical considerations, to identify area of research that could have the most direct impact on industrial fermentation *monitoring and control, an assessment that is* potentially important as an essential criterion of value for technological research and

development is that it must be useful to society (Gaden, 1982). Section 1.2.2 discusses the areas encompassed by "Monitoring", as indicated in Figure 2, while Section 1.2.3 considers the areas of fermentation operation covered by the term "Control". The Review Conclusions (Section 1.2.4) discusses how progress or lack of progress in areas of fermentor operation can be related back to the two aspects of gross economics and strain development already noted.

1.2.2 MONITORING

This section considers the interaction between academic research and industrial practice under seven subheadings, which, as can be seen in Figure 2, are the areas making up fermentation monitoring. It is assumed that academic research in fermentation operation can only be justified if it leads to advances in industrial practice. It is concluded that such advances in industrial practice are relatively few in comparison with the volume of academic research, and are unevenly divided amongst the seven areas constituting "Monitoring". It is also concluded that a structured examination of academic and industrial work in fermentation monitoring allows the targeting of areas that have been inadequately researched, and in which improvements could relatively easily see application in industry.

On-line measurement

Without any practical exceptions, commonly-used industrial on-line measurements are limited to the measurement of physico-chemical states by physical means. Examples are measurements of flowrate, temperature, pH, dissolved oxygen and gas analyses. While sensors for the measurement of dissolved carbon dioxide have been commercially available for some time (Puhar et al, 1980), they are not widely used in the fermentation industry, as this concentration can be estimated from the exit gas composition, an approach that has been widely used in the fermentation industry for

some time (Carleysmith and Fox, 1984). Surprisingly, little work has reported the on-line measurement of broth level using ultrasonic level detectors, in spite of their widespread use in the chemical industry (Davies, 1986). Such a measurement, in combination with differential pressure cells or load cells for broth weight, allow the determination of broth void fraction, an important economic and process term. On-line instruments currently used in production fermentors share certain features detailed in Table 2, these features including cost, reliability and accuracy. It is not unreasonable to expect that if novel on-line measurements developed in academia are to be implemented in an industrial setting, they will share most if not all of the features that can be seen in Table 2. It is interesting to note that the desire to avoid increased risk of contamination of production fermentors often leads to well-instrumented research and development fermentors, while production fermentors are the least well instrumented (Montague et al, 1989). Hence, researchers may be overestimating the interest the fermentation industry has in implementing new on-line measurements in a production environment.

A great deal of work has gone into the development of new on-line measurements of chemical and biochemical variables. Many of these developments do not meet any of the features suggested in Table 2 as being generic to currently-used on-line industrial instruments, and it is worth bearing in mind the distinction between novel ideas and practical solutions. These efforts have been focused mainly in two areas:

a) the development of new *in situ* sensors, most noticeably enzyme-based biosensors for some substrates and products, and sensors for the measurement of biomass concentration

b) the utilization of on-line sampling devices (Kroner and Papamichael, 1988) that can be linked to analytical techniques, such as flow injection analysis (FIA) (Nalbach et al, 1988) and high performance liquid chromatography (HPLC)

PERFORMANCE INDICATORS	- work reliably for >80% of fermentations - accuracy 1-2% of full scale - low maintenance - calibration simple and fast - steam sterilizable (in-situ probes) - cross-sensitive only to other on-line measurements - drift < 1-2% of full scale over period of fermentation
COST-BENEFIT CONSIDERATIONS	- information provided is of use in controlling fermentation to increase profitability - instruments cost less than $5 000 per fermentor

Table 2: Features generic to the most commonly-used on-line fermentor instrumentation

The routine *in situ* on-line determination of biomass concentration in industrial fermentation, the most universally important fermentation state, cannot be achieved with existing measurement technology, in spite of a large variety of techniques proposed by researchers (Clarke et al, 1986). The wide use of complex insoluble medium ingredients in industry make it impossible to meaningfully measure dry weights. Optical density measurements are not possible due to interference from medium ingredients (Gbewonyo et al, 1989).

Biosensors have not fulfilled their initial promise (Cook and Livingstone, 1991), due to problems of robustness and long-term stability (Montague et al, 1988). The enzymes and cells involved in such sensors cannot be steam sterilized, and have a limited lifetime (Hatch, 1982). Chemical sterilization poses an unacceptable risk in production fermentors (Carleysmith, 1985). The problem of steam-sterilizability can be overcome by sterilizing the probe empty, and then inserting the enzyme solution or immobilized enzyme slurry behind the semipermeable membrane (Enfors and Nilsson, 1979). While this circumvents the problem, it is not convenient for industrial application. Some research is focusing on designing synthetic enzymes with structural attributes that would enable them to remain stable at high temperatures. The realization of this goal seems unlikely in this decade, however (Shamel and Brown, 1992). This lack of biosensor reliability and more importantly the financial consequences of sensor failure in its widest sense has served to maintain the prevalence of off-line sample analysis for the biochemical monitoring of fermentation (Carleysmith, 1987; Di Massimo et al, 1991).

There has been much effort put in to devising what is basically an automated version of off-line analyses, with automatic sampling of the fermentor. Yet, as with the off-line procedure, the cost and complexity of such a system makes it uneconomic in all but the most intensive single-vessel installations. In a production plant with many fermentors, the effort is not justified (Fox, 1984). Further, the sampling system must be sterilized by pressure steam and then maintain a barrier against contamination for the length of the fermentation. Many novel on-line analytical techniques fail this test.

While for the fermentation industry as a whole, the picture in terms of implementation of novel on-line sensors in production-scale fermentation is discouraging, there have been certain localized developments. Some progress has been made with the measurement of broth dielectric permittivity at radio frequency to measure biomass concentration (Harris et al, 1987) in yeast fermentations in industry. The measurement of the concentrations of volatiles in the gas phase, to indicate their concentration in the broth, is employed in the vinegar and bakers yeast industries using metal-oxide sensors as well as various analytical techniques (Kempe and Schallenberger, 1983). On-line viscometry for mycelial fermentations has been described (Endo et al, 1990; Kemblowski et al, 1985) and on-line viscometers are commercially available (Tily, 1983). Off-line viscometry is more normally applied during industrial mycelial fermentations, as it is often the broth rheology rather than simply its viscosity that is of interest (Carr-Brion, 1991).

Off-line measurement

On-line monitoring of all variables would be the best solution, as off-line methods mean loss of information density, delay in getting the results and normally require a great deal of manpower (Meiners and Rapmundt, 1983). Two barriers to the availability of on-line sensors for all process variables include absence of suitable detection methods, and the uneconomically high cost of some of the well known methods arising from the comparatively small market in biotechnological application. Hence, as already noted, biochemical characterization of the fermentation broth is dominated by off-line analysis of broth samples taken manually. The off-line analytical techniques commonly available in industrial fermentation facilities, as well as some recently-developed techniques are shown in Table 3, with an indication of the typical analysis time required. Common biochemical analyses in industrial fermentation are for carbohydrates, proteins, phosphate, lipid, enzyme activities, and precursors and intermediates related to the product, and are taken every 4-8h, being

Method	State measured	Analysis time (h)
COMMONLY-AVAILABLE OFF-LINE ANALYSES		
Spectrophotometry	various	< 0.5
Atomic absorption	metals	< 0.5
GC	volatile components	< 0.5
HPLC	substrates, products, proteins	0.5-2
enzymatic assay	common substrates and products	0.5-1
Chitin assay	biomass	8
DNA	biomass	6
RNA	biomass	6
ATP	biomass	0.5
Dry weight	biomass	min. 1-3
Chemical analysis	substrates and products	1
Rheology/viscosity	related to biomass concentration and morphology of filamentous organisms	< 0.5
DEVELOPMENTS IN OFF-LINE MONITORING		
P^{31} NMR	intracellular sugar phosphate concentration (Seo and Bailey, 1987)	< 0.5
Laser-flow cytometry	biomass (Seo and Bailey, 1987) and cell size distribution	< 0.5
Image analysis	biomass concentration and morphology of filamentous organisms (Packer and Thomas, 1990)	1
HPTLC*	same as HPLC (Kreuzig, 1983)	< 0.5
HPLAC†	proteins, monoclonal antibodies (Compton et al, 1989)	< 0.5
Enzyme-FIA	substrates and products (Ruzicka and Hansen, 1988)	< 0.5
Immuno-FIA	peptide hormones (Bradley et al, 1991)	< 0.5
FIA-AC‡	monoclonal antibodies (Stocklein et al, 1991)	< 0.5

* high performance thin-layer chromatography
† high performance liquid-affinity chromatography
‡ flow-injection analysis-affinity chromatography

Table 3: Off-line techniques for fermentation monitoring.

logged onto the process computer between 2-24h after manual sampling. The large number of off-line analyses involved in a large industrial fermentation facility has served to make laboratory automation a cost-effective approach (Carleysmith, 1987), to the point that the use of robots in the laboratory for sample preparation has been reported (P.M.Salmon*).

Off-line techniques can give quite rapid results if samples are taken frequently under shift operation, but this is not normally the case. In practise, such information is more commonly used for post-experimental evaluation of the process (Tannen and Nyiri, 1979). Chemical analysis of cell components such as protein, DNA and RNA to indicate biomass concentration require sophisticated procedures and are too labour intensive, and cannot be generated on a real-time basis for control or fault detection in industry (Gbewonyo et al, 1989). Even off-line analyses that can be available faster, such as dry weight measurements, cannot be used during the seed stage, where active growth requires an analytical technique taking less than 30 minutes, and sedimentation or oxygen uptake rate data are used in preference (Cook and Livingstone, 1991). The increasing number of flow-injection analysis (FIA) publications and of commercially available FIA and sampling systems reflects the high interest and acceptance of this approach at both research and industrial levels (Bradley et al, 1991). While any manner of biochemical analysis can be made, the choice is determined by cost-benefit considerations.

Data Acquisition and signal conditioning

Data acquisition and signal conditioning concern the manner in which (especially on-line) measurements are collected, and the way in which individual raw measurements are treated prior to storage for use in numerical analysis of the fermentation. Referring to the map of fermentation operation presented in Figure 2, this section can be seen to be the third area coming under the heading "Monitoring".

With the wide application of computerized data acquisition in industry, the rate of

* refer to p69

data acquisition from analog *in-situ* on-line instruments such as pH and temperature measurements is effectively unlimited. However, as important process changes occur over relatively long time frames in fermentation, the rate of data acquisition does not need to be particularly fast. The sampling period of analogue signals for DDC is commonly 1-5 s. Short-term data storage is with a frequency of 1-2 min^{-1}. The frequency of long-term data storage (archiving) is normally 1-4 h^{-1} (Spark, 1987). Expensive instruments such as gas analyzers are multiplexed between several fermentors. This typically means that each fermentor exit gas is analyzed at least every 15 minutes.

The widespread application of computers to industrial fermentation systems in recent years has produced an explosion in the amount of data that can be acquired during the fermentation, and management of the gigabytes of data involved is an increasing problem in industry (P.M. Salmon*). Surprisingly little research has considered methodology relating to data acquisition, conditioning and storage, although such issues have a strong impact on opportunities for later data analysis. Noise in pH and dissolved oxygen measurements can seriously affect the accuracy of the readings (Gomes and Menawat, 1992). Events requiring control action during a fermentation are often difficult to detect amongst the noise of the process (O'Connor et al, 1992). Although noise in a measurement can reflect heterogeneities in large fermentors, the response time of the pH and dissolved oxygen probes is usually sufficiently slow to smooth such variations. The membrane probe measurement noise is due to mains noise and Johnson' noise (thermal fluctuations) (N. Thornhill, personal communication) in the high impedance amplifiers used, amplification gains used being of order 10^{12} (Clarke et al, 1982). The period of mains noise is so much smaller than the time period over which process changes in fermentation occur that the noise in such measurements can be treated as pseudo-random. As these measurements are analog, this pseudo-random noise can be reduced by filtering of the signal in the amplifier, or by averaging over several measurements after digitization of the signal (Meiners and Rapmundt, 1983). Frequently, both methods are available, with the result that random noise in pH and dissolved oxygen measurements can be reduced to a very low level, where desired, prior to its use in

* refer to p69

modelling and estimation.

Somewhat less flexibility is available in the case of gas analysis data, which cannot generally be sampled at a high frequency. Gas analyzers are, however, high performance instruments, and an accuracy of 1% and noise of ±0.1% for a mass spectrometer with fixed magnetic field has been reported (Buckland et al, 1985). There is relatively little opportunity for signal conditioning of off-line data because, as has already been noted, these measurements are taken relatively infrequently in industry. There has been little structured examination of noise in such measurements, although off-line biomass measurements can be in error by 15% (Bush, 1989). The effect of off-line measurement technique on variability in dry weight measurement has been examined (Stone et al, 1992).

To summarize, the primary sampling rate of on-line sensors in fermentation is of order 0.2-1 Hz for analog sensors, and 0.1 min^{-1} for gas analyses. Of the analog sensors, noise in pH and dissolved oxygen are the most important, but this noise can be digitally filtered. Digital filtering of gas analysis data is not practical because of their relatively low frequency. Little work has examined noise in off-line measurements.

Derived fermentation variables

The most important fermentation variables cannot be measured directly, but can be calculated directly from measurements. The most commonly encountered of these are the oxygen uptake rate (OUR), carbon dioxide evolution rate (CER), respiratory quotient(RQ) and mass transfer coefficient (K_La). In view of the importance of these derived variables, surprisingly little research has examined the factors that corrupt the derivation of such variables, and how their quality can be improved.

Chattaway and Stephanopoulos (1989) noted that their oxygen uptake rate (OUR) data were much noisier than their carbon dioxide evolution rate (CER) data, but did

not explain their finding. This same observation had been made by two workers at UCL (C. Marshall and M. Suphantharika, personal communications). Some derived fermentation variables are relatively easier to monitor at large scale than at small scale, and viceversa. Measurement of the heat of fermentation during full-scale production of lager beer has been reported (Ruocco et al, 1980), a measurement that is relatively difficult at small scale due to the importance of ambient heat exchange (Figure 3). The use of oxygen balancing for the calculation of mass transfer coefficients is well documented, particularly for small-scale fermentations. Less well documented are the difficulties entailed in obtaining a meaningful value for this coefficient in large-scale fermentation. For this type of equipment, models more sophisticated than perfect mixing and plug flow are necessary in order to accurately describe the fluid-dynamic behaviour of both the gas and the liquid phase (Nocentini, 1990). The calculated mass transfer coefficient in large-scale fermentation is quite sensitive to the assumed extent of gas backmixing owing to the low aeration rates commonly employed at larger scales. For fermentations operated at neutral or alkaline pH, the apparent values of the CER and RQ can deviate significantly from their real underlying values, as a result of changes in the concentration of dissolved carbon dioxide in the broth, which has a high solubility in fermentation media (Esener et al, 1980; Meiners and Rapmundt, 1983). Several authors have noted the effect of pH control action on the CER (Buckland et al, 1985; Stephanopoulos and San, 1984). It has been pointed out (Fox, 1984) that as the concentration of dissolved carbon dioxide is dominated by its solubility, this concentration can be deduced approximately from the exit gas analysis, and hence the concentration of dissolved carbon dioxide may be considered to be a derived variable. The concentration has been variously suggested as being 10% (Carleysmith, 1985) and 10-30% (Fox, 1984) more than the concentration implied by assuming equilibrium between the fermentation broth and exit gas.

Clearly, any attempt to model fermentation systems using data that are inaccurate and noisy simply makes the task of accurate modelling more difficult. Noise in input data necessarily limits the accuracy of model predictions, while inaccuracies in input data can lead to errors in model parameters and even in the model structure. Almost

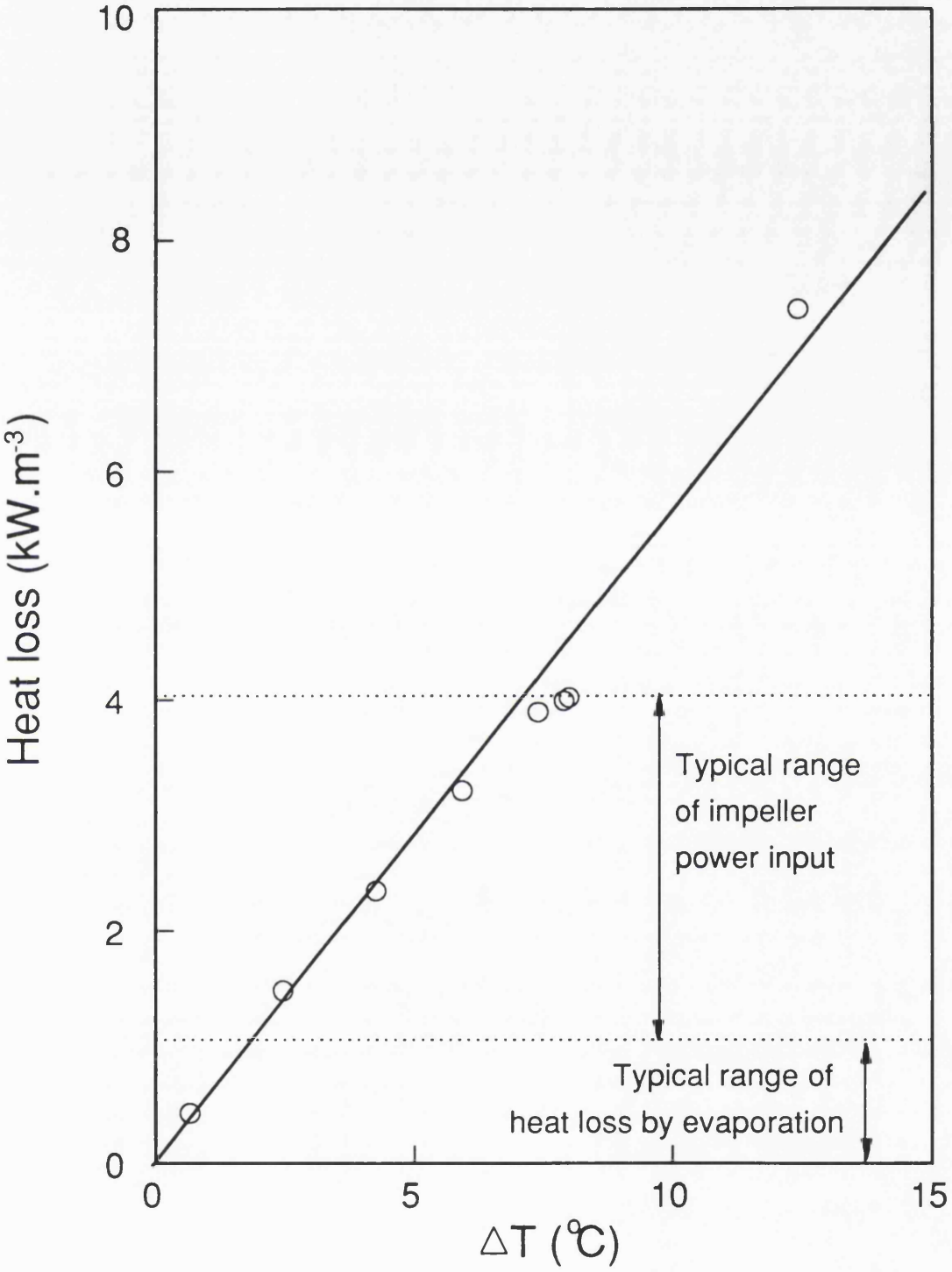


Figure 3: Rate of heat loss to the environment from the UCL 42L unlagged fermentor, as a function of the difference between the broth temperature and ambient

no work in the literature has made a structured examination of the way in which derived fermentation variables are monitored.

Modelling and model identification

"Modelling" (incorporating model identification) can be seen, by referring to the map of fermentation operation presented in Figure 2, to be the fifth area falling under the domain of "Monitoring". Prior to discussing modelling and identification of fermentation, the meanings intended by some of the terms used in this section are briefly described. By "variable" is meant a time-varying quantity that defines an important condition of the fermentation, and includes the temperature, biomass concentration, concentrations of substrates and products, and gas exchange rates, amongst others. "Model synthesis" concerns finding a way to express the relationships between variables, in a mathematical model. Models usually involve "parameters", which are (ideally time-invariant) quantities, sometimes representing unit conversions and physical properties/constants, required to make the model fit the data. "Identification" concerns finding the values of these parameters. Model "identifiability" concerns the ease with which it is possible to identify the model parameters. Model "robustness" describes how stable a model output is in the face of large errors in one or two input data points. Model "stability" concerns the extent to which small errors in input data are magnified in the model output.

There has been some criticism of mathematical modelling of fermentation in recent years. It was commented that an important factor contributing to the disproportionate increase in the volume of publication relative to real research activity in biotechnology was the ease with which masses of numerical "results" from mathematical modelling can be published, without the burden of experiment (Gaden, 1982). In spite of the large volume of work relating to modelling and identification of fermentation processes reported in the literature, the lack of industrial application of the increasingly overwhelming number of models has been pointed out (Fox, 1984). Modelling is not an important part of current industrial

practice (Beck and Young, 1989), and the application of process models to industrial fermentation has been achieved in only few cases (Halme, 1979; Hopkins, 1981). The reason for this is in essence that model based methods used in the academic community have been rendered uneconomic by the too complicated modelling procedure (Bellgardt et al, 1989). One of the central causes of this situation is that mathematical modelling is rarely viewed in academia as being subject to the same cost-benefit considerations that have limited, for example, industrial fermentation measurement to its current makeup. Several reviews of mathematical modelling in biotechnology have been compiled (Fish, 1987; Kleinstreuer, 1987; Thornhill and Royce, 1991) and the object here is not to add to these. Instead, some of the reasons for the limited uptake of research in fermentation modelling by the industry are examined.

A great deal has been made of the distinction between "structured" and "black box" models. Basically, the former involves development of the model around physically meaningful state variables, while the latter involve empirical identification of input-output relationships. This distinction is often nominal rather than real, as even complex structured models do not fully to fully describe microbial dynamics. It is more useful to view these two terms as representing different levels of complexity within a continuum. With the increase in complexity in the transition from black box to structured models is normally associated a concomitant decrease in robustness and identifiability. Comprehensive models demand a great investment in coming to grips with the fundamentals of the particular process, then in evaluating all the constants that go into the equations, and finally in validating the finished model. Many industrial concerns have evidently been unwilling to commit funds to the complete undertaking when partial and empirical models give almost the same benefit with a fraction of the effort (Carleysmith and Fox, 1984). Interestingly, however, in many areas of importance to improved fermentor operation, even empirical understanding of the processes involved is poor.

There are, for example, large spatial variations in the shear to which organisms are exposed in stirred tanks, which can have a significant impact on shear-sensitive

fermentations, such as mycelial and polysaccharide fermentations. Little is known either of shear distribution in the heterogeneous environment of stirred tanks, or of its effect on micro-organisms. In most fungal fermentations, some fragmentation due to agitation is beneficial, but excessive liquid shear leads to damage and reduced yield. Industry tends to use a rule of thumb that the maximum impeller tip speed should be 5-6m.s^{-1} (Einsele, 1978), although the shear experienced by the organisms must depend on the type of impeller used (Buckland et al, 1988). There is evidence that the gas-liquid interface, and bubble breakage in the foam layer can cause shear-damage to cells in some systems (Tramper et al, 1991). The higher the frequency of circulation of mycelia though the dispersion zone in the vicinity of the agitator, the more damaged the hyphae and the lower the rate of penicillin synthesis by *P.chrysogenum* (Smith et al, 1990). Interestingly, these results are the opposite than was predicted by a model reported in the literature for *Rhizopus nigricans* (Reuss, 1988). Accurate modelling of, for example, shear distribution in fermentors will greatly assist understanding of the effect of agitation rate on microbial growth and productivity (Papoutsakis, 1991) in stirred tank fermentors.

Mixing times of tens of seconds on larger fermentors (Nienow, 1990) lead to spatial variations in the dissolved oxygen concentration (caused by axial variations in hydrostatic pressure and gas-phase composition), pH, and in fed-batch fermentations, of nutrients. Little is known about the effect of these temporal and spatial variations on the performance of fermentation processes (Lilly, 1987). In the case of dissolved oxygen in large fermentors, the variation is both axial, as can be seen in Figure 4 (Manfredini et al, 1983) and radial (Oosterhuis and Kossen, 1984), and the organism is exposed to a cycling concentration, which been shown to reduce penicillin production in fermentations of *P. chrysogenum* (Vardar and Lilly, 1982). In yeast fermentation, production fermentors have been found to be 15% less productive than expected from a well-mixed reactor, an effect caused by the yeast producing ethanol in regions of higher than average glucose concentration (Bajpai and Reuss, 1982). Better models describing both the hydrodynamic performance of fermentors, and the behaviour of microbial systems would be of great utility, particularly in the case of secondary metabolite fermentations, where the need for rapid and reliable scale-up

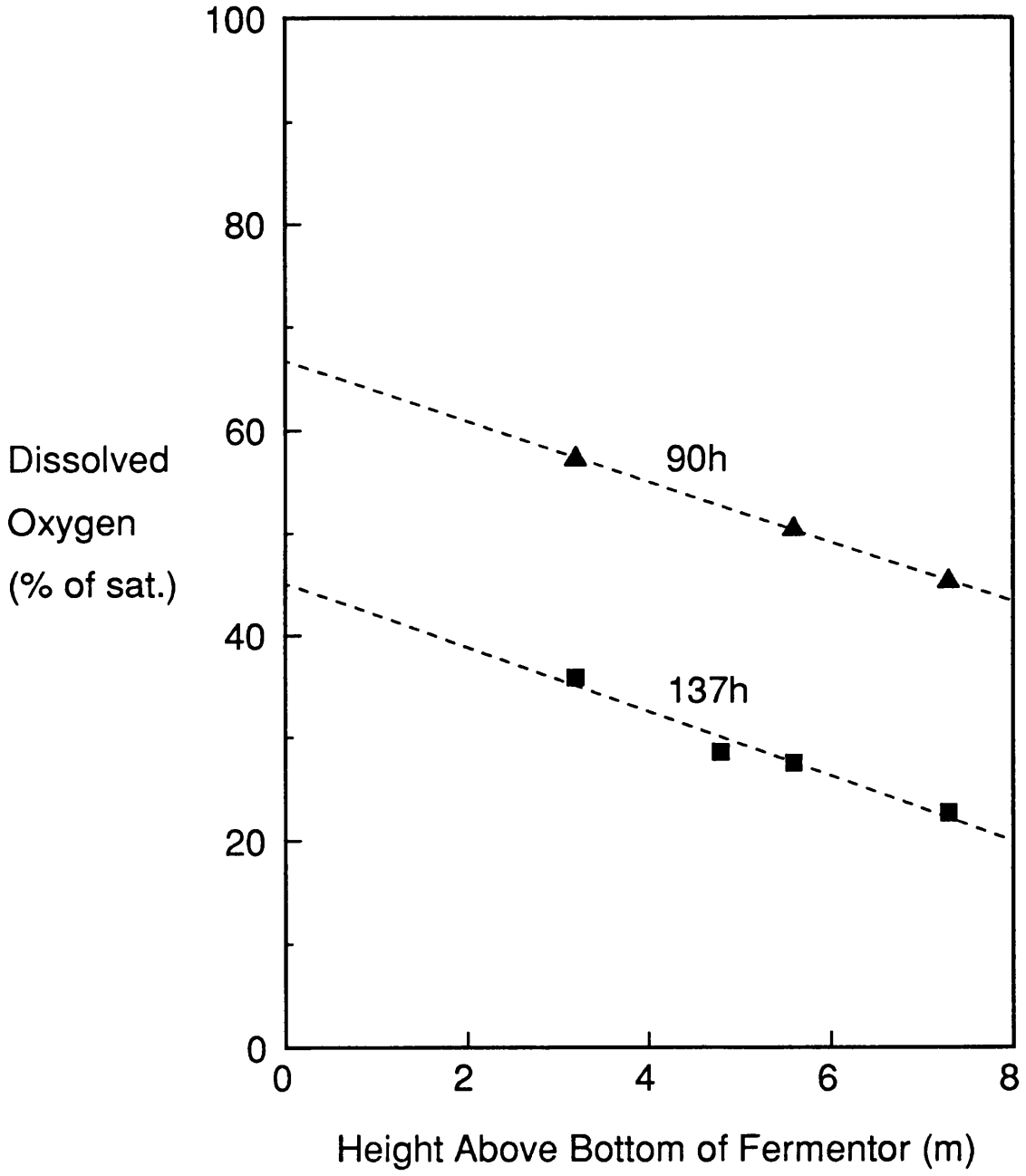


Figure 4: Axial variation of dissolved oxygen with height at different times during a 112m^3 chlortetracycline fermentation (Manfredini et al, 1983)

of newly-identified high yield strains is paramount.

Even such apparently simple problems such as the scale-up of batch sterilization can lead to problems at industrial scale and have a very great impact on the fermentation productivity (Jain and Buckland, 1988). While on newer facilities, design features such as continuous sterilization can circumvent this problem, it serves to illustrate that there are problems that are unique to large-scale fermentation that aren't generally encountered in university research, and as a result have not been widely researched.

Mass balance models have been widely applied to estimate biomass concentrations from secondary variables, as the elements carbon, hydrogen, oxygen and nitrogen represent more than 95% of biomass (Fish and Thornhill, 1988), an approach first proposed in 1969 (Yamashito et al, 1969). This method has not been directly applicable to industrial fermentations with complex media, where it is difficult to define the elemental composition of the substrate (Montague et al, 1988). This problem can be avoided by balancing energy using degrees of reductance (Erickson et al, 1978). However, solution of such balances generally requires measurement of substrate or product concentrations for robust estimation, without which extreme sensitivity problems arise (Chattaway et al, 1992). In industry, such measurements, in common with biomass measurements, are generally made off-line, and so little advantage is gained by balancing.

Modelling has an important role to play in improving fermentor operation, and for example, Gerson et al(1988) noted that while it is relatively expensive to investigate a series of possible useful control schemes experimentally, it is relatively inexpensive to develop a simulation model of a process, however unknown, and use it to guide experimental programmes. However, some caution is required when modelling fermentation, regarding the level of complexity that is practical and necessary. The more complex models may, for example, not be identifiable from input-output behaviour alone. This problem is exacerbated by the fact that large and abrupt changes in process variables, such as the test signals used for model identification

are usually unacceptable in fermentation (Johnson, 1987). It is therefore important to develop a model the complexity of which balances identification difficulties with its ability to represent the dynamic behaviour (Montague et al, 1988). Some authors (Bastin, 1992; Montague et al, 1986) have alluded to the lack of identifiability of the more complex structured models, and it is a common misconception in the academic community that fermentation model synthesis is more important than identification (Beck and Young, 1989). At the same time, while the classical methods of identification (Scheiding et al, 1986) might allow the model to be fitted to the data, this does not imply that a correct structure for the model has been elucidated. On this theme, it is pertinent to note that of four models to describe a cell culture, all were overparametrized, all were plausible, all fitted the data and all were contradictory! (G. Bastin*). The practical problem of having several model structures that can all be made to fit the data equally well caused Fogler and Brown(1986) to suggest that a model should contain at most two parameters, a constraint that is far exceeded in most of the models reported describing fermentation.

That the problem of model identification is receiving greater recognition is indicated by recent work that attempts to reduce or avoid the problem of identifying multi-parameter models. Some practical issues specific to fermentation have also caused researchers to question the appropriateness of classical modelling methodology. An example of such an issue concerns the significant batch to batch variations which have been reported in what were initially expected to be nominally identical fermentations (Leigh and Ng, 1984). In addition, it is difficult to keep all of the characteristics of a micro-organism constant for a long period, and the improvement of strains by genetic engineering and/or screening techniques is often tried in order to improve industrial fermentation processes. As a result, a model of a real fermentation process usually applies only for a limited period, and it is virtually impossible to construct a comprehensive and robust model fixed parameter model (Kishimoto et al, 1991). Modelling of a higher yielding strain inevitably requires reidentification of the model parameters relating to product synthesis, and sometimes affects other parameters such as those relating to substrate inhibition (Gbewonyo et al, 1991). This suggests that fixed-parameter multi-parameter modelling may

* refer to p69

not be the most cost-effective way of describing, in particular, secondary metabolite fermentations. Alternative approaches may broadly be divided into two groups, below:

In the first of these initiatives, classical modelling and identification are modified, but remain purely quantitative in nature. Examples are the decoupling of the identification of bioprocess stoichiometry and kinetics (Bastin, 1992), and the use of adaptive parameter approaches, in which time-varying model parameters are identified on-line (Dochain and Bastin, 1990). In adaptive estimation, further simpification can be achieved if the time variation of all parameters are lumped into one global adjustment parameter (Flaus et al, 1991).

In the second initiative, qualitative as well as quantitative information is used in modelling (Cheung and Stephanopoulos, 1990; Marshall et al, 1992). For example, several archetypal fermentation patterns could be allowed for, a concept familiar to managers of production plants (Fox, 1984). Another example is to use simpler models to describe individual growth phases, rather than the entire fermentation, and use knowledge-based reasoning to detect growth-phase transitions on-line (Halme, 1989; Kishimoto et al, 1991).

The above two initiatives may be further aided by concerted attempts to find invariant parameters describing microbial metabolism that will apply to all future production strains, such as the Gibbs energy dissipation per mole of carbon in biomass, suggested as fundamental by Heijnen and van Dijken(1992).

Probably the most "user-friendly" improvement in fermentation modelling would be the automation of model synthesis and identification. While a fundanmetal obstacle to automation of the identification of models is the need to decide, part by part, whether the model is credible (Norton, 1986), this is likely to be less critical where the model is for a new strain rather than a new organism or media. A recent development in this area concerns the ability of artificial neural networks (ANNs) to automatically generalize from previous experience and abstract essential characteristics from data, which may allow process models to be identified in a more

cost-effective way than with conventional model-based techniques (Di Massimo et al, 1991). This ability has been used to show (Montague et al, 1989) that a backpropagation network could satifactorily describe the relationship between carbon dioxide evolution and biomass for an industrial mycelial fermentation. In one work (Lubbert et al, 1992), the performance of ANNs was comparable with that of a fuzzy-supported extended Kalman filter, but required one tenth the development time. However, the new technology is not without its problems, and a feed-forward three-layer neural network did not perform well in data extrapolation outside of the learning set (Thibault et al, 1990). A more complex network did not give improved performance. G. Stephanopoulos* pointed out that, for the error to be bounded when data outside the domain of the learning set are encountered requires the use of wavelet functions in place of sigmoidal functions normally used as the neurons activation function. There is, however, little indication that ANNs can be used to yield physically meaningful fermentation parameters, and it is therefore pertinent to note that automation of model synthesis and identification can be achieved within the framework of classical modelling techniques. Further, while some general rules assist in the choice of ANN architecture, such as that recurrent networks are best suited to the representation of time-varying process states (Rivera and Karim, 1992), there is as yet no clear guidance on identifying the simplest ANN for a given application. While ANNs will undoubtedly become an additional important tool for fermentation monitoring and control, until some of these problems are resolved, ANNs will be prone to overparametrization and hence poor robustness, as commonly encountered with the more complex structured models.

To summarize, the classical approach of using fixed-parameter multi-parameter models to describe industrial fermentation is rendered impractical by the complexity of identifying such models, and the fact that such models have a limited lifetime, especially in secondary metabolite manufacture, where new production strains are regularly introduced. There is a need for automation of modelling, as can be achieved, albeit imperfectly, by the use of artificial neural networks. In the authors view, the best approach is to use qualitative and quantitative information to detect phase changes, so yielding simple models for each individual growth phase. It would be possible to reliably automate the identification of such simple models using

* refer to p69

classical methodology.

State estimation

The quality of output from a model can sometimes be improved by incorporation into an estimation scheme. Such estimators often implicitly incorporate additional information about the process that cannot easily be incorporated directly into the model, such as stochastic information and that of times characterizing process dynamics.

Incorporation of fermentation models into estimation schemes has been widely reported. However, it is increasingly apparent that the success of the approach depends on the accuracy and robustness of the process model, the state estimator chosen being of secondary concern. While industrial application of process observers has been reported for the estimation of biomass in fed-batch and continuous fermentations (Montague et al, 1988), it cannot be described as a widely-used technique in industry. The process observer approach is not without problems because fixed-parameter model-based observers suffer from errors due to the gross simplifications made in fermentation system models (Montague et al, 1988). The problem with estimators is that quite an accurate model is usually needed which in turn requires much *a priori* knowledge about the process (Halme, 1989). The performance of the Kalman filter (a stochastic estimator), for example, is seriously degraded by inaccuracies in the process model (Jefferis, 1979), and good models for fermentation processes are rarely available.

The need for stochastic estimators in fermentation, such as the extended Kalman filter, is claimed to have arisen because of the large amount of random noise in the process and secondary variable measurements (Bull, 1983). It has already been pointed out that much of this noise can be removed with suitable hardware and signal conditioning. It has in any case been pointed out that the treatment of disturbances to a fermentation as being stochastic in nature is not a relevant approach

because a bioreactor in practice is usually a very stable and undisturbed environment (Bellgardt et al, 1989).

Stochastic estimators are one of several groups of estimators that have been applied to fermentation. Such applications have been reviewed (Reinier et al, 1989), and have included fuzzy state estimators (Postethwaite, 1989), and minimum variance approaches (Solomon and Erickson, 1982). In spite of the many academic applications of estimators to the monitoring of biomass, their implementation to industrial fermentations has been rather slow (Gbewonyo et al, 1989). The possibility of improving estimates by the incorporation of delayed off-line data into the estimation algorithm (Halme et al, 1986; Stone et al, 1992) is an interesting approach. However, it seems clear that progress in the estimation of unmeasurable states will depend in large part on improved process measurement and modelling, and only to a small extent on improved estimation methodology.

Historical data analysis

This section examines activities relating to improving the use of stored information collected from previous fermentation runs. Referring to the map of fermentation operation presented in Figure 2, this is the last area associated with process monitoring.

Efficient and competitive manufacturing is becoming more and more dependent on information management/processing activities (Saraiva and Stephanopoulos, 1992). It is not unusual to find plants where the values of as many as twenty thousand variables are being continuously monitored and accumulated (Taylor, 1989). Data from previous fermentation batches includes not only the on-line and off-line data for a particular run, but also setup data, like inoculation and harvest times, feedrates, and operator comments. The nature of the problem of analysis of previous batch data depends on whether the analysis is concerned with industrial research and development laboratories or with the production setting.

In industrial research and process development, analysis of previous batch data is intimately linked with the statistical design of experiments, whose analysis is used to direct improvements in strain, media and operating conditions. Inoculum variability often requires batch fermentations to be run in parallel, commonly six fermentors from the same inoculum (Bush, 1989). It is common to be evaluating several process changes simultaneously. A major reason for this is that the baseline is constantly moving as new culture strains and treatments are incorporated as soon as possible for economic reasons (Fox, 1984). Statistical experimental design such as multifactorial methods (Plackett and Burman, 1946), and response surface analysis (Box and Behnken, 1960; Dempsey, 1991) are widely used in the fermentation industry for the design of experiments and analysis of data (Bull, 1983; Monaghan et al, 1989; Moresi et al, 1991) so that the individual effects of simultaneous process changes can be assessed.

The nature of the problem in a production setting is quite different, where previous batch data are analyzed principally for the benefit of improved monitoring and control. The ability to efficiently analyze previous batch data is clearly closely linked with the choice of database structure for long term data storage, and one of the reasons that relatively little has been reported on the methodology of fermentation data storage is that there is very little automated analysis of such information in industry at present. It has been pointed out (P.M. Salmon*; U. Saner*; M.N. Liebman*) that while ever increasing quantities of data can be collected using the increasing power of computers in fermentation, work in automated fermentation data management, data reduction and data analysis is not keeping pace. The long-term database of fermentation data often involves simply selecting data points at a fixed frequency of once per hour or so, although a data storage algorithm that responds to rates of change in measured variables has been reported (Buckland, 1989), as have efficient data storage algorithms, particularly for variables that may remain at constant levels for long periods (Hale and Sellars, 1981). A database for an industrial fermentation plant was described and its application in advising process operators on solving emergencies suggested (Endo and Nagamune, 1987).

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Artificial intelligence has been applied by several authors for the abstraction of information in previous batch data, although most this work has been with the objective of testing new technology rather than for solving practical problems. An expert systems approach was used to determine harvest time based on data from previous runs (Halme, 1989). A shortage of experienced *toji* (sake-brewing experts) has become a serious problem in sake brewing processes in Japan recently, and is providing motivation for the extraction of fuzzy rules from previous batches for fermentation control (Oishi et al, 1991). A knowledge and model based system which can extract information from a database of thousands of historical files, to provide information for the operation and control of industrial fermentations has recently been reported (Fowler et al, 1992). The application of expert systems to data analysis has, however, been interpreted as a reaction to the deluge of available process information (Benson, 1987), and this emphasizes a simultaneous need for work to focus on automated quantitative historical data analysis and data reduction, as well as qualitative approaches. Once data can be provided in an optimal form to expert systems, it is possible that their true potential power to extract qualitative information from the process data that the numan operator is not aware of, may be realized.

Summary of monitoring

A full discussion of the conclusions from the above section on Monitoring is made in the Review Conclusions (Section 1.2.4). Some of the main points already made are outlined here. Little progress has been made regarding information available from on-line instrumentation for production-scale fermentation in the last twenty years. Almost any variable can be measured using off-line techniques, and these analytical techniques are becoming faster and increasingly automated. However, cost/benefit considerations and concerns over the risk of contaminating production fermentors will continue to limit their application to the analysis of broth samples taken manually and therefore infrequently. These points suggest that little progress will be made in industrial fermentation measurement, and therefore that research in monitoring should make more effective use of existing information. Activity should focus in particular on improved data acquisition, signal conditioning, calculation of

derived variables and historical data analysis. Modelling still has the (as yet, largely unrealized) potential to become an important part of industrial fermentation monitoring and control, but cost/benefit considerations, which include issues such as identifiability and the extent of automation of modelling, must be taken into account in designing models.

1.2.3 CONTROL

The widespread use of computers in the fermentation industry in recent years has provided the power with which sophisticated control methods could be applied to improve fermentation productivity. It is particularly in the areas of optimization and control, in which the capabilities of the computer can truly be realized (Rolf and Lim, 1982). The success of computer application depends on the development of better on-line instrumentation and reliable mathematical models (Dobry and Jost, 1977). Computer applications in industrial fermentation facilities is still largely limited to data acquisition and information management. In order to improve fermentor performance, control should move from the off-line to the on-line domain. Due to the problems of obtaining process variable measurements, little has been achieved to date on the industrial scale (Montague et al, 1988). However, the financial benefits from process control improvement can be very significant, particularly in primary metabolite fermentations as the present level of control is low and the running costs in terms of percentage of final product are very high in most cases (Montague et al, 1988).

This section discusses the three areas identified in the map of fermentation operation (Figure 2) as constituting fermentation control, namely fault analysis, open-loop control, and closed-loop control.

Fault analysis

Early detection of faults during fermentations has been an important area for research in recent years. Prior to examining the nature of these faults, it is interesting to note the comments of one author (Benson, 1987) who expressed the view that sometimes academic research describes at great length techniques for solving problems, but does not adequately address the issue of why a problem exists, nor of who the process could be changed to remove the problem.

Data for a fermentation pilot plant containing 20 fermentors (Meiners and Rapmundt, 1983) suggests that failures during fermentations from all causes occur in 15-20%

of fermentations. Such failures don't necessarily result in loss of the batch. Typically, around 7% of batches in an industrial pilot plant are actually lost, about half of which are due to contamination as a result of failure of the basic sterilization, and the other half due to services failures. The rates and patterns of failure inevitably vary considerably according to the type of fermentation. A rate of contamination of 17% for the industrial-scale production of β-interferon from human fibroplasts in stirred tanks has been reported (Morandi and Valeri, 1988), a fermentation which lasts several weeks. Up to 98% of process disturbances and economic losses in the dairy industry are due to phage development (Wunsche, 1989). It has been estimated that only 6-20% of production problems are due to special causes, while the remaining bulk of 80-94% are due to common and sustained causes (Hart and Hart, 1989). The widespread application of computers to fermentation operation in recent years has not changed the nature of fermentation faults, but has instead provided new and easier opportunities for making old errors, particularly where the computer is treated as a black box (Kletz, 1991).

Fermentation faults can be divided into two categories, these being equipment-type faults (failure of sensors and services, pipe blockages) and faults with the culture itself (contamination by foreign organisms or by a mutant).

With regard to equipment faults, some rates and modes of failure of equipment are presented in Table 4, with methods for their detection. Equipment faults are most simply detected at source. For example, a failure in a sensor will often lead to an inadmissible change/rate of change in its output, and/or a change in the noise characteristics of its output. There is a trend in the chemical industry towards 'intelligent' sensors that are able to analyze their own performance (Hilton and Oughton, 1991). Faults such as pipe blockages can be detected if flow is sensed. As this is not normal industrial practice, it suggests that such problems are not a common occurrence in industry. The reliability of pH probes is excellent (Fox, 1984) at 98%, although the basis for this reliability measure is not reported. The high value

EQUIPMENT	MEASURE OF RELIABILITY	DETECTION	
		FAILURE	DRIFT
pH probe	1) 98% (Fox, 1984) 2) MTBF† 9 - 12 weeks (Lees, 1976; Carleysmith, 1985)	1) Multiple probes 2) No response to pH control action 3) pH probe output suddenly changes to electrical zero	Compare against pH of manual samples every few hours (Carleysmith and Fox, 1984)
Dissolved O_2 probe	1) 50-80% (Fox, 1984) 2) MTBF† 9 - 20 weeks (Carleysmith, 1985)	Probe output suddenly changes to zero or full-scale	1) Anomalous mass transfer coefficient 2) Change in noise characteristics (Spark, 1987)
Temperature probe	MTBF† 150 - 200 weeks (Lees, 1976; Carleysmith, 1985)	Multiple probes	
Mass spectrometer	MTBF† 1) 10 weeks (Carleysmith, 1985) 2) 50 weeks (Heinzle et al, 1990)		1) Compare inert gas balances for Ar and N_2 (Heinzle et al, 1990) 2) Compare air analysis with historical data
Computer systems	MTBF† 4 weeks (Meiners and Rapmundt, 1983)	Self-diagnostic	Not applicable

† MTBF = mean time between failures

Table 4: Rates of failure of fermentation equipment and methods for detecting such failure

reflects in part the fact that pH probes are only used for four or five production scale fermentations before being replaced. The productivity of the highly selected organisms used by industry can be very sensitive to pH drift. The need to rapidly detect errors in pH measurements means that the broth pH in industry may intentionally be only loosely controlled so that the response of the probe to acid and base additions associated with pH control action, can be observed (P.M. Salmon*). Excluding pH measurement, gas analysis is the most widespread and most reliable on-line analytical method in industrial fermentation processes (Heinzle et al, 1990), and five years of trouble free operation has been reported (Buckland et al, 1985). There are several approaches to diagnosing faults at source, particularly in the case of gas analysis by mass spectrometry (Heinzle et al, 1990) which allows consistency checks to be made on measurements. Dissolved oxygen probes are the least reliable of on-line instruments. Because oxygen probes are kinetic rather than equilibrium devices like pH probes, deposits affect their accuracy, and microbial fouling of the membrane can be a problem (Clarke et al, 1986). Only severe drift in probe output is of concern, as on larger fermentors, loose manual control of dissolved oxygen is generally practiced. Further, fouling may not be a problem for at least 1-2 weeks if the dissolved oxygen probe is not sited in a stagnant region (Carleysmith, 1987).

There are therefore several simple ways to detect and counteract sensor failure, such as signal analysis, procedural techniques, and hardware redundancy. Process control hardware are more reliable than sensors, and again hardware redundancy can be put in place to combat loss arising from failure of computer systems and power supplies. In one fermentation facility, for example, computer failures did not affect the process if the system had been set up correctly (Meiners and Rapmundt, 1983). However, while such methods exist to reduce the impact of failures on production facilities, the consequences of failure in the case of fermentations for therapeutic products can be severe even if the batch is not actually lost, as the batch may not meet the FDA requirement for being "in control" at each step.

With regard to faults with the culture itself, in economic terms, contamination faults are an important cause of financial loss in the fermentation industry. Such contamination can be either by a foreign micro-organism, by genetic drift, or by

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"revertants" (Imanaka and Aiba, 1981) in the case of cultures of recombinant organisms. Genetic drift in the highly selected strains used in secondary metabolite production is a common problem (Reusser, 1963). The risk of contamination by foreign organisms can be reduced by suitable fermentor design and maintenance (Chisti, 1992). Genetic drift and contamination by revertants can be countered by giving priority to the selection of genetically stable organisms (Schwab, 1988). Despite the economic importance of contamination, the identification theory has not often been applied to this problem (Chattaway and Stephanopoulos, 1989). However, it is questionable whether there is much economic benefit in early detection systems for contamination, and whether the advice of such systems would be taken before microbiological confirmation in the laboratory, although an early indication of contamination could bring forward a laboratory check by a few hours. The approaches adopted frequently require assumptions to be made that aren't relevant to industrial systems, such as, for example, the assumption of approximately constant yield (Chattaway and Stephanopoulos, 1989). Early detection of contamination in industry is frequently by rules-of-thumb distilled from experience, and is likely to remain so, particularly as recurrent contamination by a known contaminant is a frequent problem. An example of such a rule-of-thumb is the knowledge that viral infection of cell culture is rapidly betrayed by a change in the population distribution of cell volume, which can be detected using a flow cytometer (D.R.Omstead*)

In summary, faults in on-line instrumentation are best detected at source, using noise analysis, or combated by means of hardware redundancy. Other equipment-associated faults, such as pipe blockages and pump failures can be detected if suitable instrumentation and alarms are put in place when designing the process, the implementation of such measures being determined by cost-benefit considerations. In terms of software fault detection using analytical redundancy, tracking of the oxygen volumetric mass transfer coefficient could be useful in detecting drift in dissolved oxygen measurements, or air leaks in gas analysis systems. Soft detection of microbial contamination in the authors view might assist, but will not replace laboratory analyses for contamination.

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Optimal open-loop control

The earliest attempts at fermentation control used the tracking of time profiles for important variables taken from successful previous batch fermentations (Yamashita et al, 1969). Interestingly, many industrial fermentations are still operated using this method (Johnson, 1987), and feed-forward strategies using pre-programmed feed profiles are widely employed in industry (Minihane and Brown, 1986). Environmental variables like the pH are commonly controlled to optimal constant setpoint although constant control is generally suboptimal(Biryukov, 1982; Dekkers, 1983; Wang and Stephanopoulos, 1985). The greatest hurdle in the optimal open-loop control of fermentation is the identification of optimal operating conditions from information from previous batch data, which has already been considered. However, what has not been considered are the practical benefits that are likely to arise from such an exercise, and in this section, an attempt is made to put into perspective what are realistic objectives and expectations in such work.

In terms of optimizing energy input to fermentation, it is interesting to note that the most important energy term in the running of a fermentation is not agitation, but the provision of chilled water (Curran and Smith, 1989), and so costs of refrigeration present interesting problems in the optimization of refrigerant flowrate and broth temperature. Some studies have considered the problem of optimizing the profile of environmental variables like pH and temperature. In most cases this has involved mathematical optimization of a model of the process, and relatively fewer studies have tested their conclusions experimentally. Of the studies that compared optimal pH and temperature control relative to constant control of these variables, one found no benefit for a gluconic acid fermentation (Rai and Constantinides, 1973), a second found a 15% improvement in the productivity of a penicillin fermentation (Constantinides et al, 1970), and a third found no improvement in yield but a 33% decrease in fermentation time, in an erythromycin fermentation (Cheruy and Durand, 1979). The opportunity for productivity improvements in which an optimal pH profile, rather than constant pH, is needed arises when the optimum pH for specific growth rate for cells and metabolite are different (Andreyeva and Biryukov, 1973).

There are wide variations with regard to the perceived importance of feed strategy in increasing fermentation productivity, which tends to suggest that the opportunities for productivity improvement are very much system dependent. It was found (Heijnen et al, 1979), in simulations, that the feed rate profile during a penicillin fermentation showed the final product concentration varying by a factor of three, according to whether the feedrate was increasing, decreasing or constant. By contrast, no difference in productivity between four different feeding strategies (constant feed, constant specific feed per gram of cells, a complex pattern to achieve a predetermined growth pattern, and simple control of glucose concentration) was found in computer simulations, again of a secondary metabolite fermentation (Bajpai and Reuss, 1981). While this latter finding was implicit in the model used, and the theory was not tested in practise, it is, however, broadly in agreement with industrial findings (Carleysmith and Fox, 1984). A recent observation reported is that nutrient uptake and antibody production in cells are not continuous processes, but tend to occur in fits and starts (Kirkby and Faraday, 1992). It was further reported that while all cells in a fermentation do the same things in the same order, they do not do them at the same time. Bringing the cells in step with each other by oscillating the feed rate may allow for improvements in productivity of up to 50% (Morgan, 1992). Enhanced yields of tylosin by *Streptomyces fradiae* over those previously obtained using a constant feed rate were found (Vu-Trong and Gray, 1984) by feeding both glucose and monosodium glutamate in a cyclic fashion.

While yield improvements are possible by the rigorous application of optimization methods to fermentation analysis, the improvements reported have been relatively modest by comparison with yield improvements obtained from strain development programs in secondary metabolite manufacture. Hence, rigorous optimization of a fermentation process is most applicable in the case of primary metabolite manufacture, where yield improvements arising from strain development are more modest. In secondary metabolite manufacture, only simple (suboptimal) optimization strategies are likely to be cost-effective.

Closed-loop control

Closed-loop control is the third (and last) area identified in the map of fermentation operation (Figure 2) as falling under the title Control. By closed-loop control is meant process control where measurements available from the process are used in real-time to determine control action.

Effective closed-loop control is intimately linked with effective, usually on-line, monitoring. For automatic control, the monitored information used must be highly reliable, since, if a sensor forms part of a computer control system, then the consequences of its failure may be loss of the batch due to uncontrolled physical conditions or inappropriate feedrates, the cost of which could be $100 000. The feedback control based using on-line measurements is often the only form of closed-loop control in industrial fermentation plants. As a control problem, it is normally straightforward, although the problem of accurate temperature control in large-scale fermentation should not be underestimated (Strandberg et al, 1991). Automatic feedback control of dissolved oxygen concentration is not normally used in industry, as dissolved oxygen probes are considered too unreliable. A difficulty in applying automatic closed-loop control strategies concerns the accommodation of spurious or transient results in data. For example, transient peaks in the OUR and CER covering a period of 1/2 hour have been reported (Strandberg et al, 1991), arising as a result of changes in the aeration rate. Such effects can be accommodated if the feedback control algorithm includes process information about mass transfer rates. Tight closed-loop control of a variable does not necessarily lead to improvements in the fermentation productivity. The pH control of an Efrotomycin fermentation resulted in reduced lysis of cells (previously caused by large pH changes associated with a runaway metabolic reaction), but did not improve product titres (Jain and Buckland, 1988). There are, however, some less obvious but important advantages to control based on information fed back from the process. For example, fed-batch control strategies which depend on measurements of substrate or product concentrations will be much less dependent on scale and should provide acceptable product formation rates with less scale-up research (Gerson et al, 1988). This is an important advantage

in secondary metabolite fermentation and the biotechnology industry, where reducing the time required to go from the pilot plant to production is of significant economic benefit. Further, feedback control can have advantages over preset profiles, such as making easier the accommodation of, for example, an unusually long lag phase, or seasonal variations caused by changes in complex feeds such as those derived from agricultural sources.

The performance and robustness of single input-single output control feedback control can be considerably improved by the addition of a feed-forward control action component that uses process information. There are several opportunities for this in fermentation. Information about the rate of heat production during the course of a fermentation (Ruocco et al, 1980) obtainable from the temperature rise of cooling water can be used for feedforward temperature control. Although oxygen uptake is proportional to microbial heat production (Cooney et al, 1968), microbial heat production forms a small part of total heat production in an agitated fermentor. Another example of the opportunity for feed-forward control concerns dissolved oxygen control in the face of sudden changes in the mass transfer coefficient associated with addition of antifoam. To avoid the risk of dissolved oxygen limitation (since the mass transfer coefficient usually falls with increasing antifoam concentration), dissolved oxygen is sometimes controlled at a higher than optimum level (Schugerl, 1988). Process information about the effect of the antifoam on mass transfer could be included into a feed-forward control scheme, to allow this controlled level to be reduced, hence reducing energy consumption. A mass transfer correlation incorporating process information about the effect of agitation, aeration and pressure on the average mass transfer coefficient (Moresi and Patete, 1988) could be used to feedforward control dissolved oxygen where such control is achieved automatically.

As it is possible with off-line analysis to measure virtually any state of interest, it is in principal possible to control almost any state to a desired value. Control theory would suggest, however, that in order to control the process correctly, samples should be taken at a rate that is somewhere between one-fifth and one-tenth of the

major system time constant. In continuous fermentation, for example, time constants for bacterial systems are of order one hour, while for mycelial fermentations they can be several hours (Montague et al, 1988). It is therefore usually the case that off-line measurements either cannot be, or are not taken frequently enough to maintain "tight" process regulation (Di Massimo et al, 1991), due to the financial constraints of staffing and maintaining laboratories. Effective control based on information available from analytical techniques normally performed off-line either must be by automated sampling of the fermentor to increase sampling rate, or by their use in conjunction with estimation methods and process models. The reluctance of industry to use automated broth sampling in production-scale fermentation due to the risk of contamination, has already been noted, and so approaches using process models are the most practical way forward.

The application of process models to closed-loop control of industrial fermentation has been achieved in only a few cases, although industry has adopted control using partial and empirical models ("physiological control") rather more extensively (Carleysmith and Fox, 1984). Closed-loop control of the respiratory quotient of bakers yeast fermentations is used in industry to minimize the Crabtree effect, and several alternative schemes to achieve this objective have been developed in the academic community. Some examples of these and other physiological approaches to closed-loop control of fermentation are given in Table 5. Quite clearly, successful control can be exercised in the absence of complete understanding, by application of input-output models. And the success in practise of such approaches provides little immediate incentive to industry to improve that understanding (Beck and Young, 1989).

There appears to be relatively little need for the application of advanced control theory to fermentation systems because, as has already been noted, the principal hurdle in closed-loop control of fermentation concerns reliable monitoring of the process. Benson(1987) commented (although not in reference to a bioprocess, but of relevance) that it is disappointing to some to find that the optimal control of

MONITORED VARIABLE	MANIPULATED VARIABLE	OBJECTIVE
Exit gas analysis	Glucose feed rate	Control of specific growth rate in *S. cerevisiae* (Takamatsu et al, 1985)
Acid production rate	Chemically-defined nutrient media	Control of specific growth rate of *Methylomonas methylotroph L3* (Agrawal, 1989)
pH	Glucose feedrate	pH during *E. coli* fermentation for optimal growth (Robbins and Taylor, 1989)
pH	Glucose feedrate	Maximal production of bacitracin by *Bacillus licheniformis* (Suzuki et al, 1988)
Total oxygen consumed	Glucose feedrate	Growth rate control for minimal byproduct formation for an *E. coli* fermentation (Paalme et al, 1990)
Oxygen transfer coefficient	Glucose feedrate	Growth rate control of *Penicillium chrysogenum* (Nelligan and Calam, 1983)

Table 5: Some recent applications of physiological control

a simple process (achieved with much labour) produces results which are about the same as with conventional proportional integral control action which is properly tuned and has an adequate sampling frequency.

1.2.4 REVIEW CONCLUSIONS

Some years ago the view was expressed (Bull, 1983) that operating aids of a more mundane, but extremely useful and important nature, such as sequencing control, would be areas for future progress in computer applications to fermentation, and might turn out to be more useful than much of the sophisticated control and optimization already at hand. The evidence is that this view has been disconcertingly accurate up to the present. This section summarizes the most important points arising from the preceding review, with a view to identifying some of the stumbling blocks to improved fermentation operation.

In secondary metabolite manufacture, competitive advantages arises primarily from the identification of higher yield strains and their rapid deployment at the production scale. On-line biochemical measurement may facilitate the dynamic optimization of fed-batch fermentations, a technique which has thus far largely been limited to continuous processes (Ko et al, 1982). This could speed the introduction of new production strains in secondary metabolite manufacture, and facilitate the derivation of complex optimal open loop control strategies. This provides additional justification for the drive to automate off-line analyses for research and pilot plant fermentors, over and above the benefits of reduced manpower costs. Automated fermentor sampling is, however, unlikely to be accepted in a production setting, as it offers too much opportunity for contamination of the fermentor, opportunity that could be particularly acute during failures of computer control systems and steam supply. Manual sampling allows, for example, blockages to be detected, while automatic sampling may be less than adequately reliable (Carr-Brion, 1991). The importance of measurement in research facilities for secondary metabolites is indicated by the level of investment in such facilities. In the late 70s, average

investments in automation equipment were 4-7% of all plant, while in the late 80s this had risen to 7-10%. In biotechnology, the trend has been the same, investments in automation and computer systems being even higher (up to 15%) especially in new pilot plants where products and processes are developed. Hence, steady development of measurement techniques in research facilities is likely to continue.

The regular introduction of new production strains in secondary metabolite manufacture necessitated by the yield improvements achievable makes rapid scale-up of crucial importance in this industry. Most academic studies of scale-up have been limited to aspects of fermentor design, such as of the agitation and heat transfer systems. Many factors concerning the scale-up of microbial processes are by no means fully understood (Hamer, 1985). These factors include the scale-up of batch sterilization, and the impact of imperfect mixing at large scales on the fermentation.

The benefits of modelling and control of secondary metabolite fermentations at production scale have as much to do with achieving reproducibility (for the benefit both of downstream processing and product safety in the case of therapeutic products) as with maintaining high productivity. The industry has been relatively reluctant to implement novel measurement strategies in a production setting due to the increase risk of contaminating the fermentation, and this situation will continue. Hence modelling and control of the biochemical state of the fermentation is of particular importance in a production setting. However, there is a need to recognise the importance of strain development programmes in these industries, and so modelling strategy must be so packaged that it can adapt rapidly to new production strains. It is questionable whether, in the case of secondary metabolite fermentation, complex multiparameter models can fill this role. The key need is flexibility in modelling and control, and this need is additionally true of the fermentation products of the biotechnology industry (Zanetti, 1992). The most important component in achieving this objective is the better use of variables that can currently be monitored on-line, rather than application of models to estimate unmeasurable variables. This requires improvement in the quality of measurements, by suitable noise filtering, and improving the quality of derived fermentation variables, which are the foundation to

any attempt at fermentation modelling.

Very little has been written of efficient schemes for fermentation data archiving, that anticipate the needs of historical data analysis. Once such improvements are made, there may well be considerable increase in the scope for recognising qualitative or quantitative trends in on-line data that correspond to important events in off-line data, a concept ("physiological control") that has thus far seen little industrial application beyond the RQ control of yeast fermentations. Once a simple model structure for the fermentation has been identified, it might be possible to automate further development and identification of the model, particularly if the fermentation data base has been constructed for this purpose. There is a need to strike a balance between automated data collection and automatic data interpretation. Currently, the balance is well to the former (Saner*).

Energy costs are a much more important cost term in the case of primary metabolites, than is the case for the secondary metabolite industry. Tight control of dissolved oxygen would be beneficial not so much because of improved productivity (as for many organisms, productivity is a fairly weak function of dissolved oxygen concentration within a wide range) but rather in allowing reductions in energy consumption. As the production strain in primary metabolite production may be used for a considerable period of time, it is more practicable to spend resources on identifying accurate multi-parameter models than is the case for secondary metabolites. The relatively small improvements in productivity achievable by optimal control of pH and environmental conditions can yield disproportionately large benefits in an industry that works with tighter profit margins. An important component of optimal temperature control is the optimal use of expensive refrigeration. Feedforward control of environmental variables may allow reductions in running costs and tighter control. Few novel on-line measurements are likely to become more routinely applied, with the possible exception of biomass concentration (by radio frequency dielectric permittivity for example) which is the only fermentation state of universal interest in the industry. An important point concerns the extent to which process design can be used to influence fermentation

* refer to p69

profitability. For example, it was found (Buckland et al, 1988) that agitation power could be reduced 34% by retrofitting of Rushton impellers with Prochem agitators. However, without adequate understanding of the effect of shear and inhomogeneities on microorganisms, and the effect of broth rheology on mixing, it cannot by assumed that such a successful outcome will always be the result of an impeller retrofit.

Concerning the fermentation industry as a whole, development of more robust dissolved oxygen sensors would ultimately allow automatic control of dissolved oxygen, although the opportunity for this may be limited as most fermentors have fixed-speed agitators (Bryant, 1977; Lilly, personal communication) and compressors are often limited in flexibility. There is scope for the more structured automatic control of dissolved carbon dioxide during fermentation. Dissolved carbon dioxide can be derived directly from the exit gas analysis in large fermentors, although in small fermentors, information about the mass transfer coefficient is also required (Royce and Thornhill, 1991). Wider application of RQ control seems natural, as this variable, in addition to being one of the few metabolic indicators that can be monitored on-line routinely, is also "normalized" by being a quotient and thus has a well defined range. As the level of understanding of fermentation processes increases, real-time information on cell growth kinetics could become useful as a key control parameter in directing the fermentation along its optimal production pathway (Gbewonyo et al, 1989).

Substrate concentration control by manipulation of feedrates provides a realistic and practical route to increased productivities in fermentation. The effectiveness and efficiency of fed-batch fermentation systems either with or without feedback control of substrate concentration has been repeatedly demonstrated. With improved techniques for planning experiments through process simulation and optimization, computer controlled fermentation will be developed more easily and will become the norm in industrial fermentation processes (Gerson et al, 1988). Computer applications are already of crucial importance in the rapid scale-up of new processes from laboratory to production scale (Jain and Buckland, 1988). It should not, however, be expected that all of the most advanced concepts of point and path

optimization will be utilized to solve many of the problems of industrial complexity. As in many areas of the chemical industry, there is a very large gap between advanced optimization and control research from academia, and actual practice in the chemical process industry.

The project background has been described above. The conclusions reached were used to define the project in Section 1.2.1. The next chapter examines the published literature relating to the projects' defined area of interest.

* *comments made at ICCAFT5 (5th International Congress on Computer Applications to Fermentation Technology) together with IFAC-BIO2 (2nd International Federation of Automatic Control Symposium on Modelling and Control of Biotechnical Processes), held in Keystone, Colorado, USA, 29 March - 2 April, 1992*

2. LITERATURE SURVEY: GAS EXCHANGE IN AEROBIC FERMENTATION

This chapter presents a literature survey of gas exchange in aerobic fermentation. Such gas exchange consists in the transfer of oxygen from the gas phase to the fermentation broth, and the transfer of carbon dioxide produced by microbial respiration from the broth to the gas phase. This chapter is divided into two sections, Section 2.1 dealing with oxygen transfer, while Section 2.2 deals with carbon dioxide transfer.

2.1 OXYGEN TRANSFER

The volumetric mass transfer coefficient for oxygen

Mass transfer equations assume proportionality between a molecular flux, and a chemical potential gradient across a mass transfer resistance. The proportionality constant that relates the molar flux with this potential gradient is termed the mass transfer coefficient, and consists of the product of the film mass transfer coefficient, with an interfacial area. In the case of aerobic fermentations, the transfer of oxygen is the process most commonly associated with the evaluation of mass transfer coefficients in fermenters. There are conceptually several resistances to the transfer of oxygen from the gaseous phase into the cell (Bailey and Ollis, 1986), these being the gas film, the gas-liquid interface, the liquid films at the gas-liquid and cell interfaces, and the cell membrane. It is generally considered that transfer of oxygen from the gas to liquid phase represents the rate-limiting step (Bartholomew et al, 1950, Steel and Maxon, 1966), although in certain viscous fungal and streptomycete fermentations or where cells are immobilized, transfer from the liquid into the cell may be rate-limiting (Wang et al, 1977). Of the resistances across the gas-liquid interface, only the liquid film resistance is important, since at the interface itself the gas and liquid are normally considered to be in equilibrium, while the gas-phase resistance is negligible (Fair et al, 1973; van 't Riet, 1983; Calderbank, 1959). Although Dunn and Einsele(1975) concluded that the gas film resistance could not be ignored, Calderbank(1958) has shown that even for the extreme case of a rigid spherical bubble with no internal circulation, the ratio of the concentration gradients in the liquid and gas films is 44. Hence mass transfer of oxygen in fermentation is commonly described in terms of an "overall" liquid film mass transfer coefficient, K_L^{O2}.

As fermentation broths are relatively dilute, they are commonly treated as thermodynamically ideal, so that the chemical potential gradient can be expressed as a concentration gradient. This concentration gradient can be converted to a partial pressure gradient by using the Henrys constant for oxygen, and expressed as such,

is more in keeping with the measurements that can be made during fermentations. In describing mass transfer across the liquid film, the relevant interfacial area is the surface area of gas bubbles. For gas-liquid contactors, this is most conveniently normalized as the product of the local gas-liquid interfacial area per unit dispersion volume with the dispersion volume, V_d. In such a representation, surface aeration is ignored. Although it has been shown that surface aeration is significant in small contactors at low aeration rates, it is a small component of total aeration for aeration rates commonly employed in fermentation systems (Nienow and Chapman, 1979; Fuchs, 1971). While the film mass transfer coefficient, K_L^{O2} and the local interfacial area, $(a)_l$, are conceptually different quantities, they cannot be evaluated separately without special experimental apparatus, such as photography (Calderbank, 1958), light transmittance (Lopes de Figuerdo and Calderbank, 1979), X-ray transmittance (Sridhar and Potter, 1980) and ultrasound (Bugmann and von Stockar, 1989; Stravs et al, 1986). Hence it is usually their product, the local volumetric mass transfer coefficient for oxygen transfer, $(K_L^{O2}a)_l$ that is of interest in fermentation.

Hence the differential equation for oxygen transfer can be written:

$$dN^{O_2} = (K_L^{O_2}a)_l \cdot ([O_2]_g - [O_2]_L) \cdot dV_d$$

$$= \frac{(K_L^{O_2}a)_l}{H^{O_2}} \cdot \left(p_g^{O_2} - p_L^{O_2}\right) \cdot dV_d \qquad (1)$$

where N^{O2} = molar flux of oxygen across interface ($mol.s^{-1}$)

$(K_L^{O2}a)_l$ = local volumetric mass transfer coefficient for oxygen (s^{-1})

H^{O2} = Henry's law constant for oxygen ($Pa.(mol.m^{-3})^{-1}$)

p_g^{O2} = partial pressure of oxygen in the gas phase (Pa)

p_L^{O2} = partial pressure of oxygen in the liquid phase (Pa)

$[O2]_g$ = concentration of oxygen in the gas phase ($mol.m^{-3}$)

$[O_2]_L$ = concentration of oxygen in the liquid phase ($mol.m^{-3}$)

V_d = dispersion volume or aerated broth volume (m^3)

For Equation (1) to be useful in fermentation monitoring requires its integration over the entire fermentor. The integral of the left hand side in Equation (1) over the entire fermenter is equal to the total oxygen transfer rate (OTR). Integration of the right hand side of Equation (1) requires several assumptions to be made, as the spatial variations of the terms on the right hand side with position in the fermentor are rarely known. Normally, integration of the right hand side is achieved by assuming all variables with the exception of the gas-phase oxygen partial pressure, p_g^{O2}, to be constant with position. The Henry's law constant for oxygen is a physical property that is independent of position in the fermenter, though it may vary slightly with time during the fermentation as a result of changes in the broth composition (Schumpe and Quicker, 1982). $(K_L^{O2}a)_l$ is normally assumed not to vary with position, and hence the subscript l (to indicate "local") can be dropped, so yielding the fermentor volumetric mass transfer coefficient, $K_L^{O2}a$. Further, the fermentation broth is assumed well mixed, so rendering p_L^{O2} constant with position. This latter assumption has been found to be good for a pilot-scale fermentor (Steel and Maxon, 1966), but not to be exact for the very largest fermentors (Manfredini et al, 1983).

The variation of the gas-phase oxygen partial pressure with position is generally assumed to be described by one of two idealised models of gas mixing, the 'plug-flow' model (Yoshida et al, 1960; Cooper et al, 1944) and the 'well-mixed' model (Westerterp et al, 1963; Calderbank, 1958). The former has been qualitatively associated with large fermenters (Shioya and Dunn, 1979) and low specific power inputs, the latter with small fermenters and high specific power inputs (Westerterp et al, 1963). In practise for many systems, the gas flow will be intermediate between these two idealised states (eg. Gal-Or and Resnick, 1966). As the oxygen content of gas in the fermentor often only falls from 21% at the inlet to 18% at the outlet, the difference between the well-mixed and plug-flow assumptions, while significant in physical terms, may have a relatively small impact on the calculated volumetric mass transfer coefficient (Buckland, 1985). For some systems, however, the difference can be important (Chang et al, 1989; Nocentini, 1990). Integration of Equation (1) for these two models of gas backmixing yields, for a system in which the hydrostatic pressure effects are negligible yields:

$$OTR = G.\frac{\left(\%O_2^{in} - \%O_2^{out}.\frac{\%N_2^{in}}{\%N_2^{out}}\right)}{100}$$

$$= \frac{K_L^{O_2}a.V_d}{H^{O_2}}.\left(p_g^{O_2,out} - p_L^{O_2}\right). \qquad (well\ mixed) \qquad (2)$$

$$= \frac{K_L^{O_2}a.V_d}{H^{O_2}}.\frac{\left(p_g^{O_2,in} - p_g^{O_2,out}\right)}{\ln\left(\frac{p_g^{O_2,in} - p_L^{O_2}}{p_g^{O_2,out} - p_L^{O_2}}\right)} \qquad (plug\ flow) \qquad (3)$$

where OTR = oxygen transfer rate ($mol.s^{-1}$)

G = inlet air flowrate (dry basis) ($mol.s^{-1}$)

$\%O_2^{in}$, $\%O_2^{out}$ = mole percentages of oxygen in the inlet (in) and exit (out) gas streams respectively (dry basis)

$\%N_2^{in}$, $\%N_2^{out}$ = mole percentages of nitrogen in the inlet (in) and exit (out) gas streams respectively (dry basis)

$p_g^{O2,in}$ = partial pressure of oxygen in the inlet gas (Pa)

$p_g^{O2,out}$ = partial pressure of oxygen in exit gas (Pa)

$K_L{}^{O2}a$ = average volumetric mass transfer coefficient for oxygen in the fermentor (s^{-1})

Gas dispersion in aerobic fermentors

Air is introduced into agitated fermentors via a perforated ring sparger, placed just below the bottom impeller. Mechanical agitation of the broth has two principal functions: firstly, to produce a small bubble size (and hence large surface area for mass transfer) by generating an environment in which high liquid shear rates, particularly in the immediate vicinity of the impellers, break up large bubbles; secondly, to ensure the bulk mixing of the broth. On a large scale, especially in high viscosity broths, it is probable that bulk blending is the most demanding of these two functions (Nienow, 1990). Good bulk mixing of the broth ensures adequate

dispersion of the gas. The agitation rate used must be above that which just prevents flooding, so that gas passes through the gas-filled cavities behind the impeller blades (Nienow and Chapman, 1979). A significant proportion of the gas held-up in the dispersion may be recirculated gas, rather than newly sparged, and for small gas-liquid contactors, this recirculated gas flowrate may be an order of magnitude greater than the sparge rate (Westerterp et al, 1963).

It is known that addition to water of small amounts of organic or inorganic substances sensibly decreases the average bubble size, although in many cases, viscosity, density and even surface tension are left practically unchanged (Marrucci et al,1967). These authors showed this observation to be related to the electrical effects at the gas-liquid interface which hinder coalescence, in solutions of acids, bases and salts, although some authors have claimed hindered coalescence to be the result of the Marangoni effect (Middleton, 1985; Zuiderweg and Harmens, 1958). Marrucci et al(1967) also found that the average bubble diameter decreased with electrolyte concentrations, with an asymptote to 0.41mm diameter. The bubble size distribution became sharper as the electrolyte concentration increased. Most fermentation media contain significant quantities of salts, both to buffer the media against pH changes, and also to supply nutrients (such as nitrogen in the form of NH_4^+, and sulphur in the form of SO_4^{2-}). Fermentation broths are usually non-coalescing due to the presence of certain types of surface-active materials which tend to stabilize foams when they are formed (Kawase and Moo-Young, 1990). The range of bubble size in fermentors is, not surprisingly therefore, rather narrow (Nienow, 1990). It is claimed that as the interfacial tension has only a slight influence on the bubble coalescence, the bubble size distribution changes only a little during cultivation (Frolich et al, 1991).

Bubble size affects the interfacial area for mass transfer in a gas-liquid contactor in two ways. For a given overall gas holdup, the interfacial area per unit volume is inversely proportional to the bubble diameter. The bubble diameter also affects the gas holdup, however, as the bubble rise velocity normally increases with bubble diameter. This latter dependency is complex, however, and Peebles and Garber

(1953) identified five different regimes of bubble rise, dependent mainly on bubble diameter. Gas recirculation within the dispersion is important and depends in a complex way on fluid dynamics. Computational fluid dynamics is only now becoming available on workstations (Butcher, 1991). It is questionable how useful this will be to the fermentation industry as most fermentation broths display a variable plastic rheology (Blanch and Bhavaratu, 1976). Correlations for the *a priori* prediction of gas holdup not surprisingly display a large uncertainty.

Methods for monitoring mass transfer coefficients

Chemical systems, such as the sodium sulphite system (eg. Moresi and Patete, 1988) have been widely employed for the measurements of mass transfer coefficients in gas-liquid contactors in the chemical industry. The purpose of such chemical systems is to provide a molar flux of a component to generate a concentration gradient which can be measured. In the case of fermentation, such chemical systems are unnecessary as the aerobic organisms used themselves generate fluxes of oxygen and carbon dioxide across the gas-liquid interface in the course of respiring.

Referring back to Equations (2) and (3) it can be seen that if a dissolved oxygen sensor and gas analyzer are available, so allowing the monitoring of gas and liquid phase oxygen partial pressures, $K_L{}^{O2}a$ can be evaluated directly from these equations. Such measurements, as has already been noted, are routinely available during fermentations, and so such an approach to monitoring of the volumetric mass transfer coefficient, called static oxygen balancing, is widely employed to routinely monitor the volumetric mass transfer coefficient for oxygen transfer during fermentations (Carleysmith and Fox, 1984).

Where only one of these measurements (ie. gas analyzer or dissolved oxygen sensor) are available, it is still possible to evaluate the volumetric mass transfer coefficient by a dynamic method, involving a differential equation. Dynamic methods have been widely applied in the chemical industry for some time. Many works in the

biochemical engineering literature have appeared more recently, discussing the application of these same methods to fermentation monitoring, using dynamic changes such as steps in head-space pressure (Linek et al, 1989), inlet gas composition (Chandrasekharan and Calderbank, 1981; Chang et al, 1989), agitation (Mignone, 1990) and aeration (Taguchi and Humphrey, 1966). The resulting dynamic methods for monitoring mass transfer in aerobic fermentation are inappropriate for two reasons. Firstly, they are not required because, as has already been noted, gas analyzers and dissolved oxygen sensors are routinely available in fermentation applications, and so static oxygen balancing can be used. Secondly, large and abrupt changes in process variables, such as test signals used for model identification are usually unacceptable in fermentation (Johnson, 1987). Montague et al(1986) have observed that the application of steps, pulses or pseudo-random binary sequence signals to the fermenter feed addition to a fed-batch penicillin fermentation, subjects the mould to conditions which are unfavourable to growth, and the same is known to apply in the case of some environmental variables such as dissolved oxygen. For example, organisms in larger-scale fermentors are likely to be subjected to rapid and significant perturbations in dissolved oxygen as a consequence of imperfect mixing (eg. Bryant, 1977; Jansen et al, 1978), and such fluctuations in dissolved oxygen have been observed to lead to reduced product yield in penicillin fermentations (Vardar and Lilly, 1982). A further issue countering the application of dynamic methods to mass transfer monitoring in fermentation is that the equations defining the relationship between $K_L{}^{O2}a$ and the dynamic changes become far more complex in non-ideal situations, than the static case. For example, if the dynamic response of the dissolved oxygen sensor output to a change in a process variable is being monitored, this either requires a non-standard fast-response oxygen probe (eg. Gibilaro and Davies, 1985), or requires the dynamic response of the probe itself to be well characterized (eg. Linek et al, 1981). Lastly, dynamic methods do not work for viscous mycelial fermentations, as these broths degas very slowly (Wax et al, 1976).

The most appropriate method for monitoring the mass transfer coefficient during fermentations is therefore the static oxygen balance. The evaluation of $K_L{}^{O2}a$ by this

method is relatively simple, although in the case of some gas analyzers, correction factors may be required relating to the instrument calibration (Spriet et al, 1982). Further, dissolved oxygen measurements may be rendered inaccurate as a result of the liquid film effect at the membrane surface of dissolved oxygen sensors. This effect tends to reflect as a relatively small error in the monitored value of the volumetric mass transfer coefficient as long as the dissolved oxygen concentration is well below its saturation value. This is usually the case in industrial fermentation, where there is a strong economic incentive to operate at low dissolved oxygen concentrations (Fox, 1984). Some references have indicated the range of mass transfer coefficient that is practicable in industrial fermentors. Atkinson and Mavituna(1983) place values for $K_L{}^{O2}a$ for large fermentors in the range 0.01-0.04s^{-1}. Buckland et al(1988) indicate that a value of 0.04s^{-1} is well within the capabilities of a 19m^3 fermentor at a pharmaceutical company (Merck, Sharp and Dohme, Rahway, NJ). In another work, van 't Riet (1983) say that OTR = 0.028mol.m^{-3}.s^{-1} must be regarded as a rather high value for many types of fermentor. This OTR implied a maximum value for $K_L{}^{O2}a$ of about 0.175s^{-1} for operation at atmospheric pressure. This seems optimistic for mycelial fermentations, as Buckland et al(1985) report that for a viscous avermectin fermentation, the maximum oxygen transfer rate achievable with existing production fermentors is 0.01mol.$m^{-3}s^{-1}$, which implies a maximum value for $K_L{}^{O2}a$ of about 0.06s^{-1}.

Mass transfer correlations for fermentors

Most studies of mass transfer in gas-liquid contactors have been undertaken with the aim of providing generalized power-law correlations of the volumetric mass transfer coefficient, in terms of easily measurable quantities, such as the agitation and aeration rates, for the design and scale-up of processes. The data used to obtain most correlations has involved the study of chemical systems. In principle, these correlations are equally applicable to fermentation systems. Mass transfer in fermentation is a physical process as in the case of gas-liquid contactors used in the chemical industry, and Yagi and Yoshida (1975) have, for example, shown that mass

transfer enhancement in the liquid film due to the accumulation of cells at the gas-liquid interface is negligible. However, a difficulty that limits the direct applicability of mass transfer correlations to fermentation systems concerns the complexity of rheology normally displayed by fermentation broths. Most fermentation broths do not display simple Newtonian rheology, but are plastic (Blanch and Bhavaratu, 1976), although pseudo-plastic behaviour has also been described (Jurecic et al, 1984; Paca et al, 1976). Such complexities may in part explain the wide range of exponents proposed for power law correlations. However, attempts to reduce such variability by expressing mass transfer correlations in terms of variables that cannot be designed for, such as the gas hold-up (Mooyman, 1987), seem of limited value.

Such variability is inevitably exacerbated by problems in integrating Equation (1), presented earlier. Middleton (1985) pointed out that as the assumed gas flow pattern has a dramatic influence on the value of the calculated volumetric mass transfer coefficient, and this flow pattern is neither approximately plug flow nor backmixed, this means that most of the published K_La data are suspect since an ideal gas flow pattern is implicitly assumed. He was, however, referring to chemical systems, and it has already been noted that the sensitivity of the calculated $K_L^{O2}a$ to the assumed extent of gas backmixing is relatively weak for many fermentation systems. The question of the appropriateness of assumptions made does, however, become acute when dealing with large fermentors. At large scale, aeration rates tend to be smaller than those employed at small scale (Sittig, 1982; Yagi and Yoshida, 1977), in order that the superficial gas velocity remains in a range that gives an economically appropriate gas void fraction in the fermentor. Perhaps partly as a result, authors who have generated mass transfer correlations for large fermenters (Fukuda et al, 1968a,b) have obtained somewhat anomalous exponents for the correlation. Some examples of the exponents in power law correlations involving the gassed power per unit volume and the gas superficial velocity are tabulated in Table 6.

α	β	Reference
0.58	0.75	Lopes de Figueredo and Calderbank (1979)
0.55	0.55	Chandrashekharan and Calderbank (1981)
0.95	0.67	Cooper et al (1944)
0.62	0.63	Moo-Young and Blanch (1981)
	0.40	Linek et al (1988)
0.49	0.2-0.7	Jurecic et al (1984)

Table 6: Exponents in power law correlations for the mass transfer coefficient reported in the literature ($K_La \propto (P_g/V_d)^{\alpha}(v_s)^{\beta}$ where P_g is gassed power, V_d is dispersion volume and v_s is superficial gas velocity)

2.2 CARBON DIOXIDE TRANSFER

Work reported in the literature concerning carbon dioxide transfer is examined here under the three subheadings. Under the first heading "Effect of dissolved carbon dioxide on microorganisms", the importance of dissolved carbon dioxide as a fermentation variable is established. The second heading, "Chemistry of carbon dioxide in aqueous solution" notes some of the complexities of carbon dioxide chemistry, by contrast with oxygen in solution. Under the third heading, "Dissolved carbon dioxide as a derived variable", justification for considering dissolved carbon dioxide to be a derived variable (i.e. obtainable directly and easily from other on-line measurements) is provided.

Effect of dissolved carbon dioxide on microorganisms

The regulatory effect of dissolved carbon dioxide on microorganisms has been considered to be as important a variable as the oxygen concentration (Tannen and Nyiri, 1979). Its measurement or estimation is important because high dissolved carbon dioxide levels generally inhibit growth and often reduce production of secondary metabolites (Carleysmith, 1987). The concentration of dissolved carbon dioxide that microorganisms are exposed to can affect the rate of spore germination, the development of hyphae of filamentous organisms, the rate of carbohydrate uptake and the rate of product synthesis (Nyiri and Lengyel, 1968). Ho and Smith (1986a,b) found growth of *P. chrysogenum* reduced by 25% for an influent gas composing 12.6% carbon dioxide. Pirt and Mancini (1975) found that the penicillin synthesis rate of *P. chrysogenum* was decreased 33% at a carbon dioxide partial pressure of 0.05atm, and 50% at one of 0.08atm compared to the control case. Belmar-Campero (1988) found that 8% carbon dioxide in the influent air had no effect on morphology and biomass production of an *S. clavuligerus* fermentation, though the length of the lag phase was increased, and clavulanic acid productivity reduced by 35% . At 18% carbon dioxide, growth was strongly inhibited. Bylinkina et al(1973) found dissolved carbon dioxide inhibition to be reversible for *S. aureofaciens* and *S.antibioticus*, but

irreversible for *S. griseus*. Inosine production by *B. subtilis* was found to be inhibited above 0.03atm (Ishizaki et al, 1973). Han and Mudgett(1992) found improvements in pigment production by *Monascus purpureus* with decreasing partial pressure of dissolved carbon dioxide. The maximum productivity corresponded to the minimum carbon dioxide partial pressure investigated, of 0.01 atm. There is evidence that for some fermentation systems, increases in dissolved carbon dioxide tension may, within limits, stimulate growth (Gandhi and Kjaergaard, 1975; Gill and Tan, 1979; Repaske et al, 1974). It is known that bicarbonate ions formed from the hydration of carbon dioxide in solution can inhibit or promote growth of *H. eutropha* independently of dissolved carbon dioxide (eg.Repaske et al, 1974), although these effects were not observed in *S.sanguis*. Carbon dioxide is known to be generally required as a substrate for growth, and its optimal concentration may be defined within narrow limits, although for most organisms, this requirement for CO_2 can be satisfied by their net production of metabolic CO_2.

Jones and Greenfield (1982), Molin(1987) and Onken and Liefke(1989) have reviewed the inhibitory effect of carbon dioxide on different microbial species. From their results it is clear that microbial carbon dioxide resistance differs widely between species. For many organisms, there is negligible limitation below a carbon dioxide partial pressure of 0.1atm. No work has, to the authors knowledge, been published that specifically examines the sensitivity to dissolved carbon dioxide of highly-selected industrial strains for secondary metabolite production. Nevertheless, it is clear that in some instances, fermentation productivity is highly sensitive to the dissolved carbon dioxide concentration, and therefore that the ability to monitor and hence control this variable could be of significant value in many fermentation processes.

Chemistry of carbon dioxide in aqueous solution

As the cell membrane is relatively impermeable to ionic species (Jones and Greenfield, 1982), the carbon dioxide produced by microbial metabolism enters the broth as dissolved carbon dioxide, $CO_2(l)$. It can then be involved in any of the reversible processes below:

$$CO_2(g) \underset{k_L^{CO2}a}{\overset{k_L^{CO2}a}{\rightleftharpoons}} CO_2(l) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H_2CO_3 \overset{K_{acid}}{\rightleftharpoons} H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

where k_1, k_2 = rate constants for the indicated reactions (s^{-1})

K_{acid} = dissociation constant for carbonic acid ($mol.m^{-3}$)

The range of pH values normally encountered in fermentation systems is around 4-8. At such pH values the concentration of carbonate ions, CO_3^{2-}, is negligibly small (van Beusekom et al, 1981). In addition, while $CO_2(l)$ can react directly with hydroxyl ions, this reaction is of no importance in this pH range (Sherwood et al, 1976). The rate constants governing the dissociation of carbonic acid (H_2CO_3) are very large (Ishizaki et al, 1971), so that this reaction is effectively always at equilibrium, determined by the acid equilibrium constant, K_{acid}, at the applicable temperature. The concentration of bicarbonate ions increases with pH, being approximately equal to the dissolved carbon dioxide concentration at pH 6.3, and five times at pH 7. Data for this equilibrium were presented in Arrua et al(1990). Pinsent et al(1956) and Sirs(1958) presented data for the rate constants k_1 and k_2 in water, respectively, and their data were used in Chapter 4.

Some authors have noted ways in which the above reactions involving carbon dioxide can be modified when solutes are present in the water. The hydration of carbon dioxide can be catalysed by the enzyme carbonic anhydrase which is found in red blood cells, and has been found in a certain species of bacteria (Veitch and Blankenship, 1963). This effect is not normally significant (Yagi and Yoshida, 1977),

as most microbes do not contain this enzyme (Yoshida, 1982). Nyiri and Lengyel(1968) claimed that the hydration reaction could be catalysed by HPO_4^{2-} which is present in some fermentation media to buffer against pH changes. The reaction stoichiometry they suggested, however, appeared to imply catalysis of an already instantaneous reaction. Additionally, the effect they detect is small, and measured under unsteady state conditions at high pH, although they did not take the unsteady state conditions into account. They may instead have been observing mass transfer enhancement due to the effect of the buffer in reducing local pH variations.

It is, however, known, that carbon dioxide can bind to some complex organic molecules, to form carbamates and colloids. Carbon dioxide may be chemically bound to the various proteins present in a complex medium. For example, carbamate (a cation combining ammonia and carbon dioxide) can arise by the interaction of dissolved CO_2 and the free amino groups of the proteins. This reaction is favoured at pH values above the isoelectric point of a protein and competes successfully with the CO_2 hydration reaction (Jones and Greenfield, 1982). The reaction of carbon dioxide with ammonia to form carbamate consumes only a minor amount of the carbon below pH 9 (Noorman, 1992). Very weakly acidic organic hydroxides such as glucose, can combine with CO_2 (Faurholt, 1927). It is usually not a realistic expectation to hope for complete understanding of every reaction that occurs in the complex system. However, consideration must be given to the dominating reactions in a system if proper monitoring of a particular component must be performed (Ho et al, 1987). The dominant reaction in the case of fermentation media is that forming bicarbonate ions, and is discussed further below.

The dependence of the bicarbonate concentration on pH is responsible for the observed dependence of the CTR on the pH. Buckland et al(1985) said that it might not be possible to use RQ control in combination with acid pH control, because of the large quantities of CO_2 liberated each time the acid mixes with the fermentation broth. In fermentations for the production of efrotomycin, the application of RQ control was made difficult by the large amount of CO_2 liberated each time the acid was added to the broth to control the pH. For fermentations producing avermectin

where the pH was not controlled and hence varied between pH 5 and 7, the OUR was used in preference to the CER for biomass estimation, because of the latters distortion by the changing pH (Buckland et al, 1985). These effects similarly resulted in the calculation of yield based on O_2 consumption being more accurate than that based on CO_2 consumption (Gbewonyo et al, 1989). Changes in the pH can cause changes in the amount of CO_2 evolved which temporarily aren't related to the culture's production of CO_2 (O'Connor et al, 1992), and which may be misinterpreted as changes in the rate of growth of the organism (Stephanopoulos and San, 1984). A 10% variation in the exit gas carbon dioxide content is typical of pH variations within 0.1 of a pH unit. Meiners and Rapmundt(1983) noted that a plot of carbon dioxide evolution rate versus oxygen uptake rate yields a straight line that doesn't pass through the origin. This can probably be explained by the different solubilities of oxygen and carbon dioxide, which always lead to smaller RQ values at the beginning of the process. This effect can be observed in almost all fermentation processes (Meiners and Rapmundt, 1983). Similar observations were made by Esener et al(1980) who performed a carbon dioxide balance to evaluate the effect of the discrepancy on the calculated RQ.

In summary, the most important liquid-phase reactions involving carbon dioxide in fermentation broths result in the formation of bicarbonate ions. As the concentration of bicarbonate ions is affected by the pH, pH changes cause changes in the concentration of bicarbonate in solution, which can affect the exit gas carbon dioxide content. Low values of the respiratory quotient tend to be observed at the beginning of fermentations, and this effect may arise as a result of the differing solubilities of oxygen and carbon dioxide.

Dissolved carbon dioxide as a derived variable

Techniques for the measurement of dissolved carbon dioxide partial pressures have been available for thirty years, and sterilizable sensors for the measurement of the concentration of dissolved carbon dioxide in fermentation broths have been

commercially available for more than ten years (Puhar et al, 1980). In spite of this, it cannot be described as an established technique (Fox, 1984). The output is logarithmic, so that two calibration points are necessary. Their reliability is lower than that for oxygen probes, which have an estimated reliability of 50% - 80% (Fox, 1984). The probes can be calibrated against a calibration gas (giving an accuracy of ±2%) or against bicarbonate buffers (giving an accuracy of ±10%) (Ingold literature). The latter technique is more practical and hence more likely to be applied in industry. However, as carbon dioxide desorption is dominated by its solubility, with kinetic effects usually contributing only 10-30% to the dissolved carbon dioxide concentration (Fox, 1984), measurement using a probe calibrated in this way may appear to offer little information beyond that provided by the assumption of gas-liquid equilibrium. This latter approach has been used in industry for some time (Carleysmith, 1985). Smith and Ho(1985) however, demonstrated that no equilibrium exists when the culture reaches its maximum growth phase. Accordingly, any efforts to utilize an equilibrium expression given effluent gaseous CO_2 partial pressure to obtain the dissolved carbon dioxide concentration are not advisable. Instead, they recommended the use of a steam-sterilizable dissolved CO_2 electrode. There is increasing evidence that the quantity of CO_2 in fermentation media is much higher than that predicted by Henry's law (Bardford and Hall, 1979; Esener et al, 1980; van Beusekom et al, 1981). Smith and Ho(1985) found that equilibrium between the fermentation broth and the exit gas was not a valid assumption for a *P. chrysogenum* fermentation in a 14L fermentor. They found that the partial pressure of dissolved carbon dioxide was up to four times that in the exit gas.

It has been shown in chemical systems that the mass transfer of carbon dioxide can be enhanced or 'facilitated' by ions and groups in the liquid phase (Otto and Quinn, 1971). The mass transfer enhancement is particularly associated with local pH variations. Buffers in the media can suppress the pH profile that would otherwise exist in the liquid film, so enhancing mass transfer (Lander et al, 1979). Yagi and Yoshida(1977) found no facilitation of CO_2 transfer during a fermentation, because no pH dependence of the mass transfer coefficient for carbon dioxide was measured. They concluded that CO2 transfer in fermenters takes place as a purely physical

process.

Ho et al(1987) point out that carbon dioxide, as a polar molecule, can quickly be transported across cell membranes to the cell-liquid interface. As the cell-liquid interfacial area per unit volume for micro-organisms is very large by comparison with the gas-liquid interfacial area, it is the latter that presents the principal resistance to mass transfer of carbon dioxide, as for oxygen. Some authors have noted that as the same gas-liquid interfacial area per unit volume, **a**, is involved in the transfer of both oxygen and carbon dioxide, a calculated value for the oxygen mass transfer coefficient could be used to estimate the concentration of dissolved carbon dioxide using a mass transfer equation analogous to that for oxygen transfer. Schneider and Frischknecht (1977) reported that, within the measurement error of their technique, the volumetric mass transfer coefficient for carbon dioxide transfer, $K_L^{CO2}a$, was the same as that for oxygen transfer, $K_L^{O2}a$. However, in view of the complexity of their fast transient technique, their idealised gas backmixing assumption, and their ignoring the response time of the sensors for dissolved gases, their claimed measurement accuracy of ± 5-7% is questionable. Yagi and Yoshida (1977) noted that from both the theory and experiment of mass transfer, the mass transfer coefficient depends on the component diffusivity. Hence the ratio of $K_L^{CO2}a$ to $K_L^{O2}a$ should depend on the ratio of the liquid diffusivities of carbon dioxide and oxygen. They suggested that the relevant quantity is the square root of the diffusivity ratio, which yielded a mass transfer coefficient ratio of 0.93. Fox(1984) reported that his experimental results suggested a ratio of 0.8. Carleysmith(1987) said that the concentration of dissolved carbon dioxide in aerobic fermentations is, as a rule of thumb, only 10% greater than the equilibrium concentration, which suggests that the chosen ratio of mass transfer coefficients has little quantitative impact on the calculated concentration, but is principally of interest from an academic point of view.

Some of the work reported above suggests that the concentration of dissolved carbon dioxide can be deduced directly from gas analyses and measurements relating to oxygen transfer. The extent to which dissolved carbon dioxide may be treated as a

"derived variable" (like the OUR, CER and RQ) will be examined in this project.

2.3 SUMMARY OF RESEARCH STRATEGY

While a large amount of work in the literature has examined aspects of oxygen transfer in fermentation, relatively much less has considered carbon dioxide transfer. Carbon dioxide transfer is of interest for two reasons. Firstly, monitoring and, ultimately, control of the concentration of dissolved carbon dioxide may facilitate productivity improvements in industrial fermentation. Secondly, it has been noted that carbon dioxide in solution can affect the evaluation of derived variables that are likely to become increasingly important in fermentation control, such as the RQ and CER. In formulating a research strategy, it was decided, therefore, to concentrate on the monitoring of dissolved carbon dioxide, its control, and the way it affects the evaluation of derived variables.

There were, however, two aspects of oxygen transfer that were first considered prior to examining carbon dioxide transfer. The first of these concerns the validity of describing oxygen transfer in a fermentor in terms of a single value for the oxygen volumetric mass transfer coefficient, $K_L{}^{O2}a$, examined in Section 4.1. This question is important for later work, as it has already been noted that carbon dioxide transfer can be described in terms of $K_L{}^{O2}a$. Rigorous analysis of this question is complex and outside the scope of this project, so instead some practical arguments are presented for the usefulness of $K_L{}^{O2}a$ as a variable describing fermentation. The second aspect concerns the quality of the OUR, an important derived variable. It was noted above that one author had found the noise in the OUR to be much larger than that in the CER, but did not explain this observation. This question is examined in Section 4.2, both within the project scope of improving the quality of derived variables, and also because of the effect the quality of the OUR has on other derived variables, such as $K_L{}^{O2}a$ and RQ.

To study the monitoring and control of dissolved carbon dioxide requires a clarification of the nature of carbon dioxide transfer in fermentation, regarding both the extent of any enhancement of mass transfer of carbon dioxide by liquid-phase reactions, and the relation of the mass transfer coefficient for carbon dioxide to that

of oxygen, and these issues are discussed in Section 4.3. The theoretical reasons for the influence of dissolved carbon dioxide on the CER and RQ are examined in Section 4.4, while experimental validation of these theoretical arguments is presented in Section 4.5. Having established the basis for monitoring dissolved carbon dioxide, Section 4.6 presents and discusses a simple algorithm for the routine control of dissolved carbon dioxide.

3. EQUIPMENT DEVELOPMENT AND FERMENTATION MATERIALS AND METHODS

In December 1988, a new 42L fermentor was supplied to the (then) SERC Centre for Biochemical Engineering by LH Fermentation (Maidenhead, UK). The fermentor specification included automatic sterilization, a fermentor load cell, and pressure control. The fermentor was earmarked for use by the Control Group, and the author was given responsibility for commissioning this fermentor, which was then used in all fermentation work reported in this thesis. Commissioning of this fermentor is described in Section 3.1 of this chapter, as are details concerning calibration and system characterization of equipment and sensors associated with the fermentor.

For reasons discussed in this chapter, it was felt necessary to design and develop a standalone PC data acquisition and control system for this fermentor, rather than to base data acquisition on the BIO-i software used by other fermentation workers. The background to this decision, and the design and development of this system are described in Section 3.2 of this chapter.

The micro-organisms used in fermentation work were *S. clavuligerus* and *E. coli*. The original intention had been to use *S. clavuligerus* in all fermentation work, but problems encountered in sterilizing media in the new fermentor necessitated the use of a fast-growing organism that did not require strict sterility, for most fermentation work, and *E. coli* was chosen on this basis. Section 3.3 of this chapter describes materials and methods associated with these two fermentation systems.

3.1 FERMENTOR

3.1.1 Modifications resulting from commissioning of the LH 42L 3000 Series fermentor

This section describes issues relating to the commissioning of a 42L fermentor newly supplied to the SERC Centre for Biochemical Engineering in December 1988 by LH Fermentation (Maidenhead, UK), and on which all fermentation runs analyzed in this thesis were conducted. Figure 5 presents a P & I diagram for the fermentor, as modified by the author during the commissioning period.

Mechanical agitation is by bottom entry impeller. This necessitates lubrication of the mechanical seal by a lubricant circuit, in this case using 30%v/v glycerol as lubricant.

Although the fermentor was intended to be sterilizable automatically, the long lines leading to steam traps and the abscence of automatic steam flow control resulted in flash boiling of the broth during sterilization. Hence sterilization required a component of manual control, in regulating steam flow during heating up and cooling down.

The fermentor had originally been designed for broth temperature control whereby the temperature of water supplied to the fermentor jacket was controlled by the mixing of water and steam in a mixing vessel upstream of the jacket. However, as steam is only available to the UCL pilot plant from 7.30am to 5.30pm, temperature control of higher-temperature fermentations was not possible outside these hours with this design of temperature control system. Temperature regulation also oscillated slowly because the long delay time inherent in the temperature control system necessitated a small temperature difference across the jacket for stable control. As a result of these problems, the temperature control system was respecified by the author so that heating was from an 1kW electrical heating element inserted into the

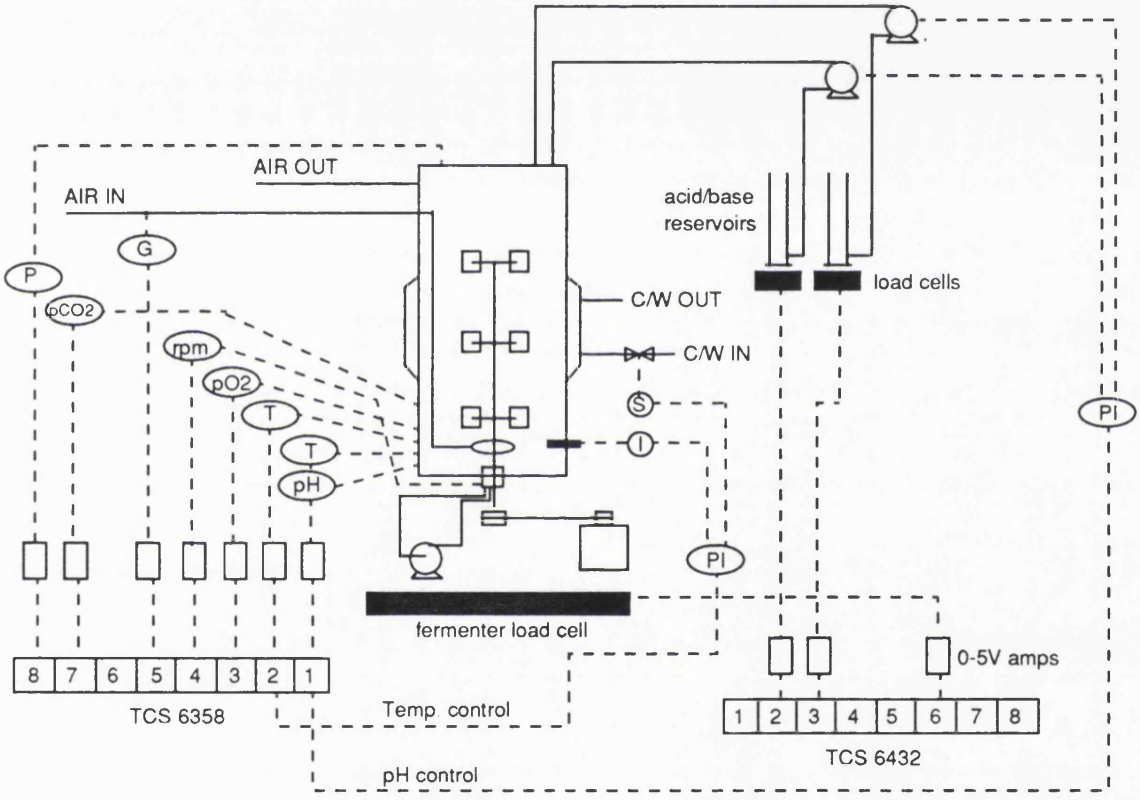


Figure 5: P&I diagram for the 42L fermentor

broth through one of the instrument ports, instead of by steam. Cooling continued to be provided by tap water to the cooling jacket. The electical workshops assembled a switch box that allows for the selection between electrical or steam heating of the broth. The mechanical workshop manufactured a port for the heating element. The author tested the resulting system and found that the larger temperature difference across the jacket enabled much tighter and responsive temperature control.

The fermentor was supplied with a pressure controller. This was removed as it was not required, and imposed a significant pressure drop during atmospheric operation, as can be seen from Table 7. The position of impellers on the impeller shaft was adjusted to meet the standard dimensions suggested by Middleton (1985). The geometry of the vessel is indicated in Figure 6. The maximum working volume of the fermentor is approximately 30L.

Although the fermentor was supplied with a load cell for the vessel contents, this load cell also weighs the fermentor platform and impeller drive motor and fan. The total dead weight is about 300kg. The resolution of the fermentor load cell is nominally 0.1kg, although in practice the measurement is only reproducible to ±0.3kg. This is of little use except in fed-batch fermentations where there are large volume changes. For the fermentations reported here, the output of the fermentor load cell was not used.

All other instrumentation on the vessel is standard to UCL fermentors with the exception of the dissolved carbon dioxide probe (Ingold, Urdorf, Switzerland).

3.1.2 Sterilization

This section describes the fermentor sterilization protocol developed by the author, which was used in all fermentations analyzed in this thesis. It is reported here as there was no documentary information provided in this regard by the manufacturers of the fermentor.

Aeration rate (L/min STP)	Pressure drop (kPa)		
	Air filter	Sparger + inlet + outlet fittings	Pressure controller
5	0.13	0.07	0.56
10	0.26	0.17	1.74
15	0.39	0.32	3.41
20	0.52	0.49	5.4
30	0.74	1.04	10.3

Table 7: Pressure drops in the 42L Fermentor System

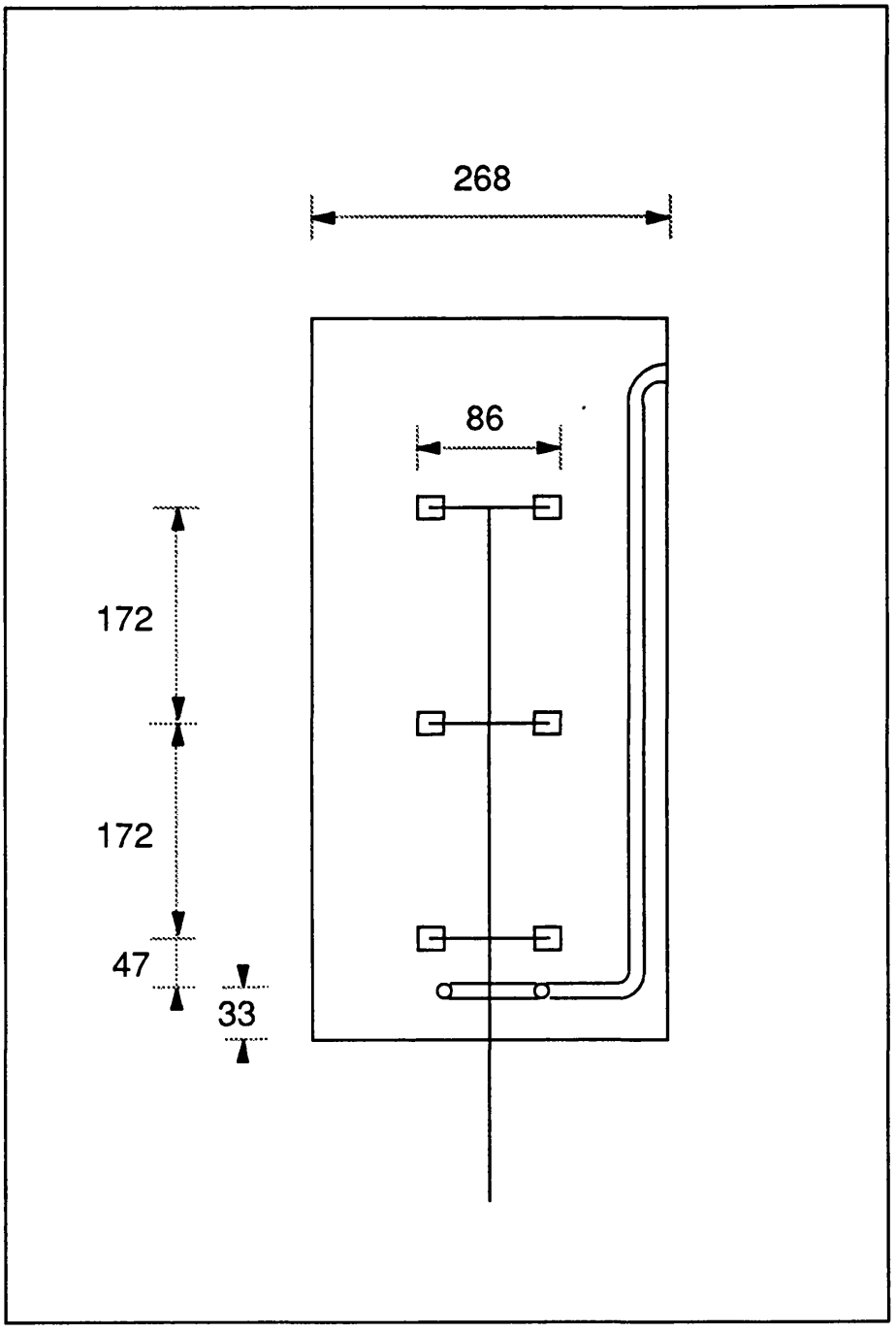


Figure 6: Geometry of impeller arrangement in the 42L fermentor (all dimensions in mm)

The method used to sterilize the fermentor involved sterilizing it in three stages. Firstly, the pressurized lubricant system for the mechanical seal was sterilized, and then maintained at elevated pressure (1.5-2 bar). This was followed by sterilization of the vessel contents, and finally sterilization of the sample port and harvest valve.

The lubricant system was sterilized by opening the steam valve to the lubricant reservoir steam coil, with the air valve closed and lubricant circulating. Once the lubricant has reached 120°C, the valve linking the reservoir head space to the condensate system was opened so as to purge air from the system, and sterilize the lubricant reservoir air filter. The lubricant drain line was sterilized by switching off the lubricant pump, and opening the valve linking the lubricant and condensate systems. Once sterilized, the valves to the condensate system were closed. The air valve was then opened slowly so as to maintain a positive pressure in the condensate circuit relative to that in the fermentor.

The fermentor contents were sterilized by steam to the fermentor jacket. A steam pressure of200kPag. was found to be most suitable. The sterilization cycle was commenced by pressing the sterilization switch on the main unit. During the initial stages of steam flowing to the jacket, the jacket drain valve was be left open, so as to drain the cold water in the jacket. Once the broth temperature had reached 100°C, the steam flowrate was manually reduced by closing the isolation valve on the steam line. This was to allow time for the long lines to the steam traps to get hot. Without this action, vigorous boiling of the broth contents results would occur, which could block the filters if the broth had a tendency to foam. Once the steam traps were hot, the steam flow could be raised.

The automatic sterilization cycle ended once the temperature had been above 120°C for 45 minutes. If the broth temperature didn't reach 120°C (because of too low a steam pressure, say), then the sterilization would not be terminated automatically. Sterilization could be terminated manually by pressing the "Standby" switch on the Control unit. Sterlization for more than 45 minutes was difficult. No information on programming the sterilization cycle was available from LH Fermentation. Simply

running the cycle twice was complicated because the sterilization cycle overrided all other switches, and the cycle couldn't be restarted until the temperature had fallen below 42°C. The system could be "fooled" by manually ending the cycle, switching the temperature amplifier to "calibration" (which caused the sterilization controller to read a temperature of 5°C), restarting sterilization, and then switching the temperature amplifier back to its measurement position. This series of operations needed to be done quickly, as flash boiling ensued when the sterilization cycle was interupted.

In spite of the rigorous sterilization regime, repeated changes of fermentor seals and pressure testing, several replacements of air filters, long sterilization times (75 minutes), and various other measures undertaken with the assistance and advice of pilot plant staff, only a 25% success rate in sterilizing fermentation media was achieved. In a series of 12 sterilizations of *S. clavuligerus* media, over a period of 3 months, on only 3 occasions were the media successfully sterilized. It is understood that other LH Fermentation customers using this design of fermentor had encountered similar problems (C. Turner, personal communication). This low success rate in sterilizing fermentation media made it impractical to run long fermentations, and hence the bulk of fermentations used in the project were short, as these did not require strictly sterile media.

3.1.3 Analog instrument calibration

Analog signal amplification is by 0-5V amplifiers (LH Fermentation, Slough, UK). All analog measurements, with the exception of the pH were 12-bit digitized. The temperature, dissolved oxygen, agitation, aeration, pressure and dissolved CO_2 appeared on an 8-loop controller (Model 6358, TCS Ltd., Worthing, UK), which provided PID control of the temperature, agitation and aeration rates. Load cell outputs from the acid and base reservoir load cells appeared on a signal processor (Model 6432, TCS Ltd., Worthing, UK) (Figure 5).

The pH measurement (Combination pH electrode Model 465, Ingold, Ufdorf, Switzerland) was 16-bit digitized by a signal processor (Model 6B, Analog Devices, Norwood, MA). The control of pH was by PI control through the PC used for data acquisition.

The pH was calibrated prior to sterilization of the fermentor contents, against buffers produced using buffer tablets. The tablets were preferred over commercial buffer solutions, because the latter can age quickly by picking up carbon dioxide from the atmosphere. Three commercial buffer solutions covering a range of pH were also found to give poor linearity.

Temperature measurement by platinum resistance thermometers (RTDs) does not in principle require calibration. The indicated temperature was compared with the average from three mercury thermometers, the spread of measurements being 0.5°C, which was considered satisfactory.

The dissolved oxygen sensor used was a polarographic probe (Ingold, Urdorf, Switzerland). The full scale was calibrated against air, at the agitation rate to be used during the fermentation. The zero point was calibrated against nitrogen from a cylinder.

The agitation rate was calibrated using a tachometer. Although such a calibration is often not regarded as necessary, there was found to be a considerable discrepancy between the agitation rate displayed, and that measured, in the agitator calibration as supplied by LH Fermentation, as can be seen in Figure 7.

The air mass flowmeter was calibrated against a 14G rotameter with aluminium float, whose calibration was checked against standard rotameters in the Chemical Engineering Department. There was found to be a considerable error in the mass flowmeter output as calibrated by LH Fermentation, as can be seen in Figure 8. The rotameter readings were used to correct this calibration by adjusting the TCS 6358

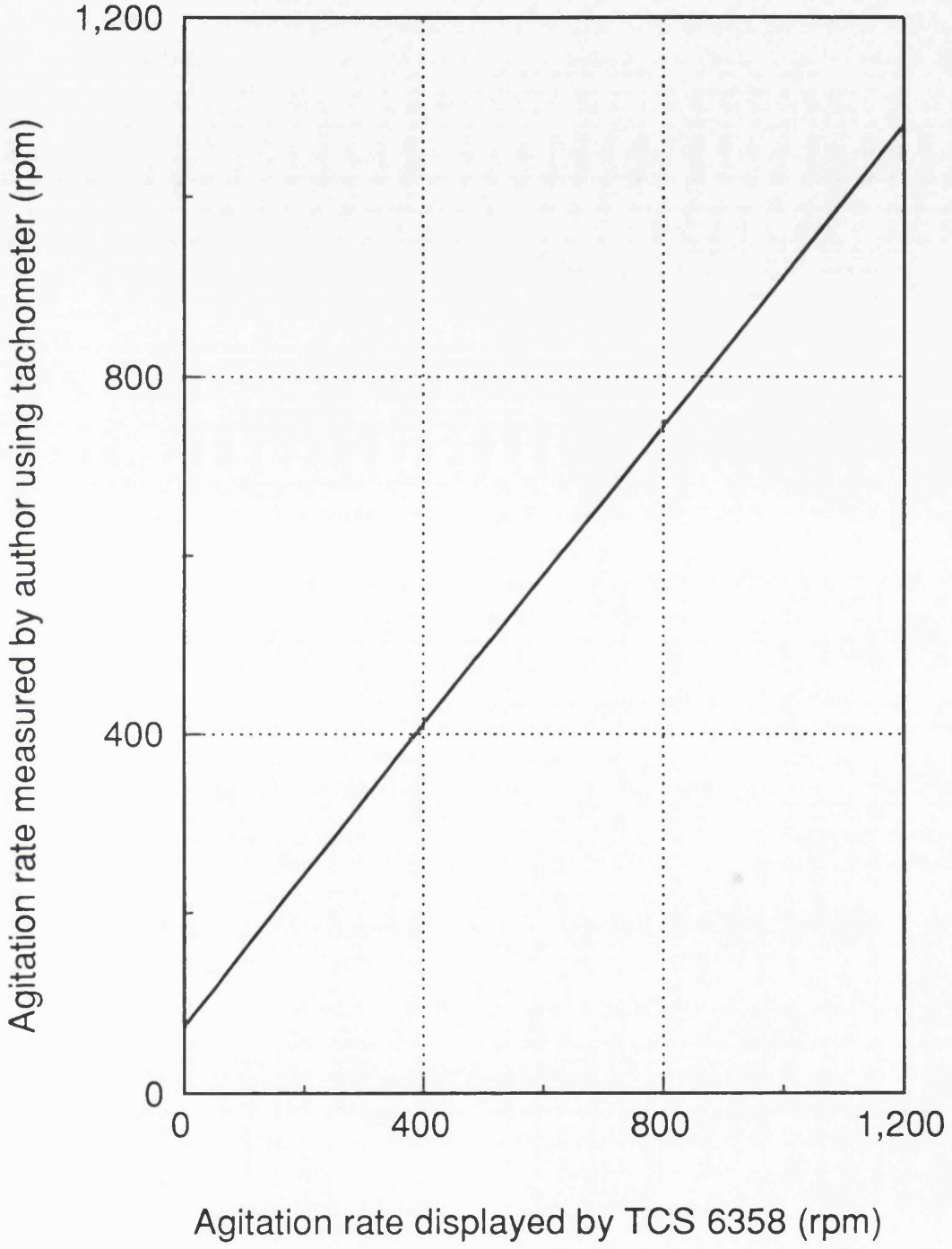


Figure 7: Error apparent in the calibration of the 42L fermentor agitator as supplied by the manufacturer. Manual measurements were made using a tachometer

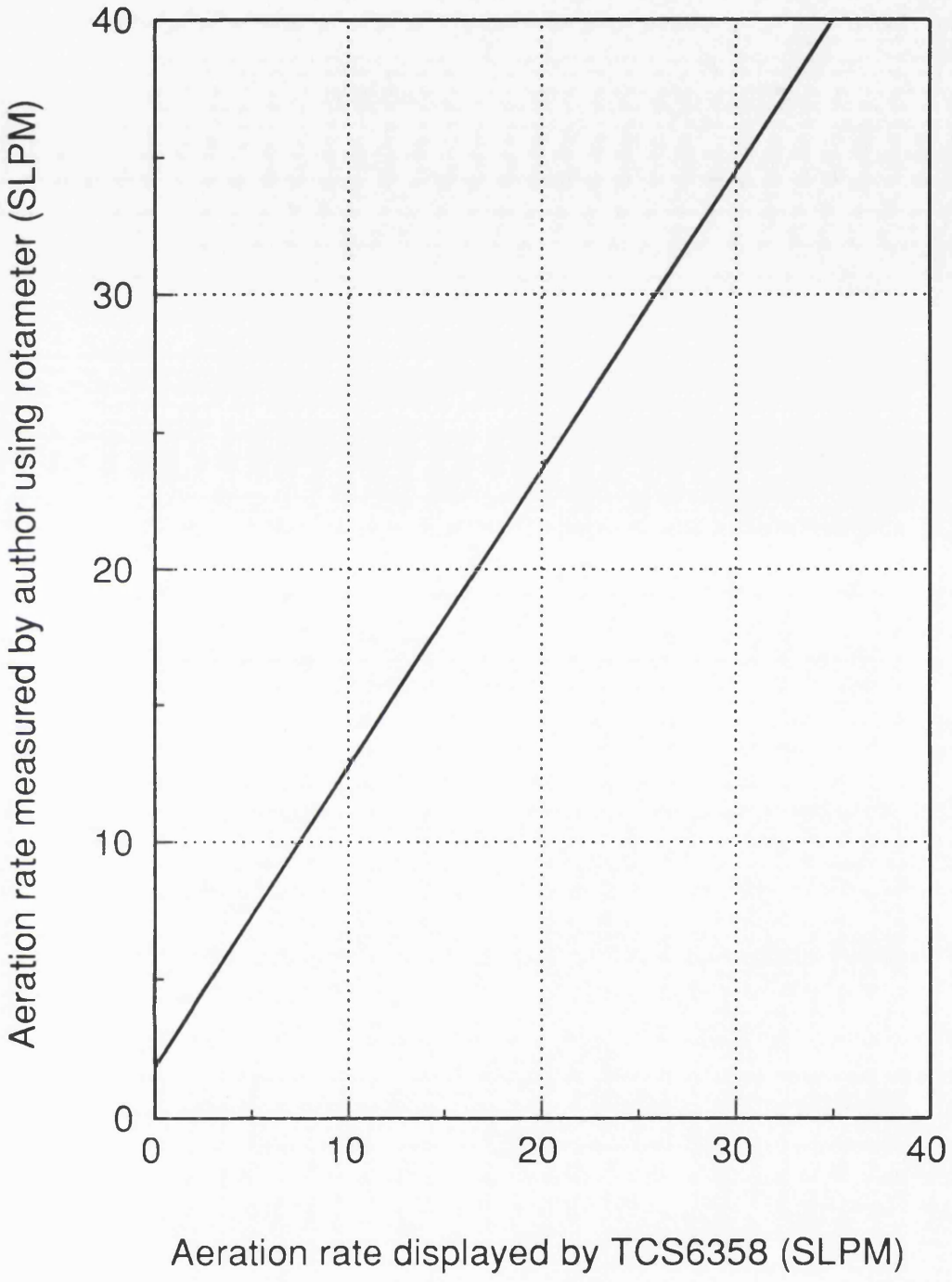


Figure 8: Error apparent in the calibration of the 42L fermentor mass flowmeter as supplied by the manufacturer. Manual measurements were made using a calibrated rotameter

range values, rather than by making adjustments to the mass flowmeter itself. Linearity was good above a flowrate of 5L/min.

Two methods for calibrating the dissolved carbon dioxide probe (Sterilizable dissolved CO_2 probe, Ingold, Urdorf, Switzerland) are to use the buffers supplied by the manufacturer, or to use a standard gas. The manufacturers' literature says that calibration against buffers is accurate to ±10%, while that against a standard gas is accurate to ±2%. For measurements in aerobic fermentations, calibration against buffers was insufficiently accurate, as the partial pressure of dissolved carbon dioxide is in any case only 10-30% above the equilibrium concentration. Calibration against a standard gas can be directly against the standard gas before sterilization, or against standard gas blown through the fermentation broth after sterilization. The disadvantage of the former method is that drift of the probe as a result of sterilization is unknown. As accurate measurements were required in this work, this approach could not be used. Therefore, the calibration was after sterilization, by blowing standard gas through the broth before inoculation. A standard gas of 3% CO_2 was used, which could be diluted with air to give a second point for calibration. The mass spectrometer was used to indicate accurately the dilution effected. As CO_2 has a high solubility in fermentation broths, long periods (½-1h) were required for approach to equilibrium. Rather than attempt to adjust the amplifier slope and zero point, it was simply ensured that the amplifier was within the linear range, and the probe output at two points was used to generate a calibration curve which was used off-line to calculate the dissolved CO_2 pressure. As the amplifier for the dissolved CO_2 probe in any case outputs a pH value rather than a partial pressure of dissolved CO_2 (p_L^{CO2}), this method was most practicable. Figure 9 shows a typical calibration curve for the dissolved CO_2 probe. It can be seen that the probe output is non-linear below a p_L^{CO2} of 400Pa. Above this level, the probe response is still very slow until the p_L^{CO2} reaches about 1000Pa. When the p_L^{CO2} is small and changing rapidly, such as during the early stages of exponential growth, the p_L^{CO2} indicated by the probe output is therefore likely to be unreliable.

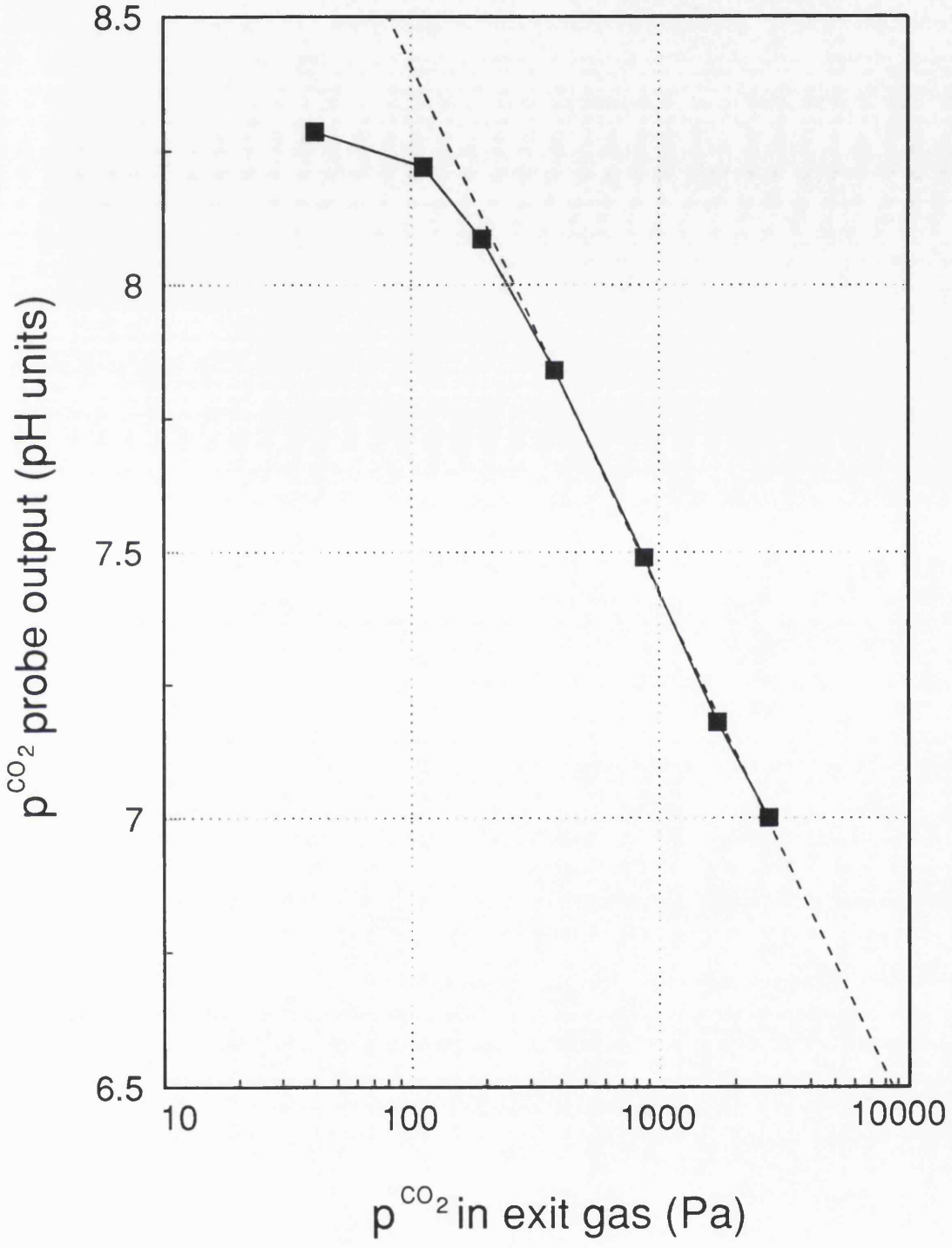


Figure 9: Typical calibration curve for the dissolved carbon dioxide probe. Only the range above 400 Pa was used for calibration and quantitative measurement

As the dissolved CO_2 probe is an equilibrium device rather than a kinetic device, deposits do not affect its accuracy. However, over a series of fermentations, while the dissolved CO_2 probe was found to have a stable sensitivity (ie. slope), it was found to have a highly variable zero point. Changes in zero point from one fermentation to the next, and even over the duration of a single fermentation, could amount to 30% of full scale. These large zero point changes persisted even though the probe membrane and electrode were changed three times during the course of the experimental program. Where very accurate dissolved CO_2 data were required, the probe was recalibrated at the end of the fermentation in order to quantify the extent of zero point drift.

3.1.4 System characterization

Film effects in the measurement of dissolved oxygen

By contrast with the pH and dissolved carbon dioxide sensors, which are equilibrium devices, the dissolved oxygen probe is a kinetic device (Fox, 1984). At low agitation rates, a film can develop at the membrane surface which becomes depleted of dissolved oxygen relative to the surrounding broth, so giving erroneously low dissolved oxygen measurements. To quantify this effect the fermentor was filled with water and aerated, and the probe full scale calibrated at the maximum agitation rate. The effect of reductions in the agitation rate on the probe output is indicated in Figure 10. It can be seen that the film effect is small for agitation rates above 400rpm. The error introduced by the film effect into calculation of mass transfer coefficients can be reduced by calibrating the probe at the same agitation rate as used during the fermentation, and by operating at a dissolved oxygen concentration well below the saturation level.

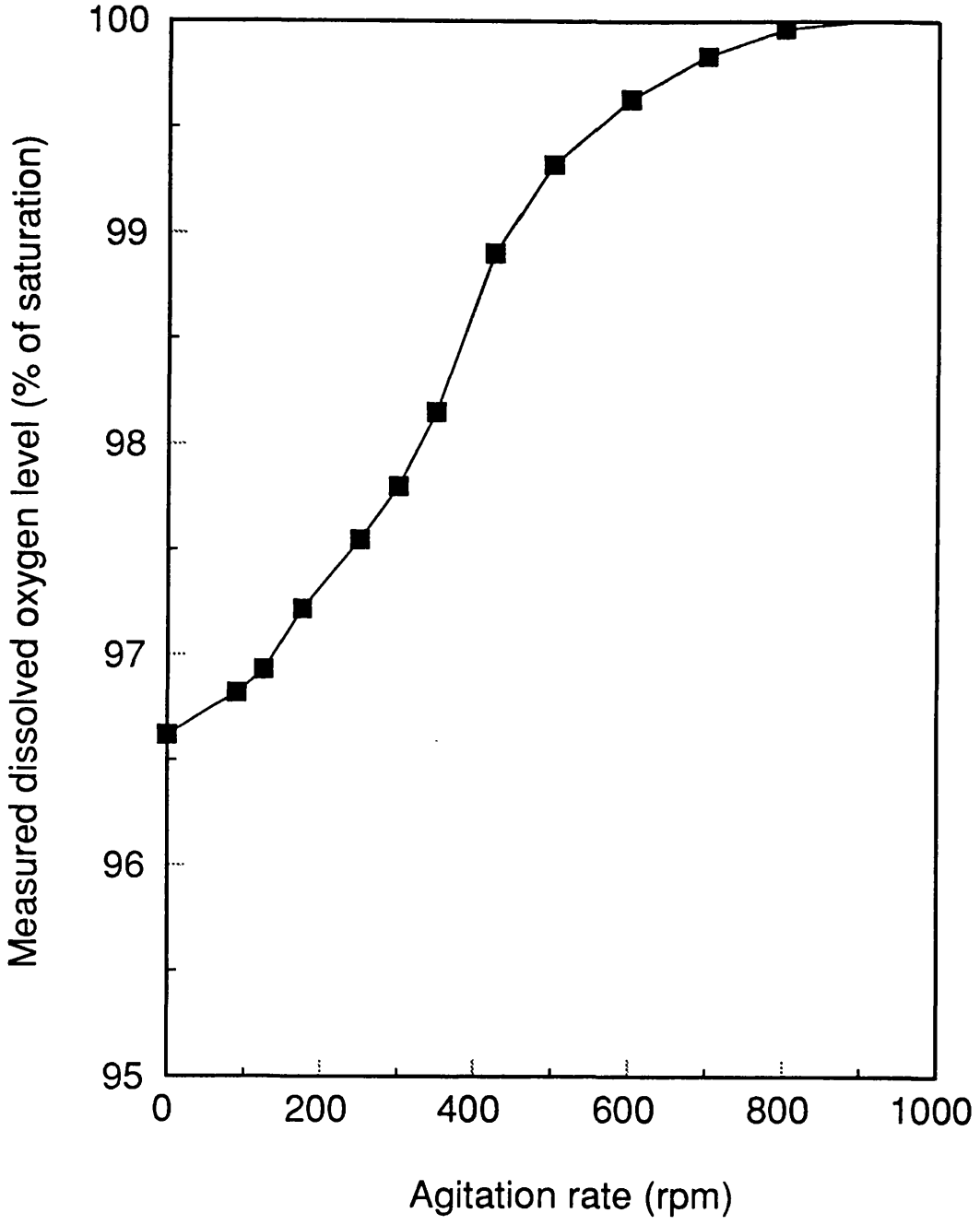


Figure 10: Change in the measured dissolved oxygen level with change in agitation rate, due to the film effect, for zero oyxgen demand.

Peristaltic Pump Calibration

In all fermentation work, the peristaltic pumps used were Model 101U/R (Watson Marlow, Falmouth, UK). The pump speed could be controlled either manually (by a switch setting from 01 to 99 on the pumps) or automatically by a voltage input of 0-10V. Within a measurement accuracy of ±1%, there was no detectable effect of ambient temperature (over 20°C range), or temperature of fluid being pumped (over 20°C range), on the flowrate generated. There was negligible drift in flowrate over periods of several days. The technique of clamping tubing into the pump had no measureable effect on flowrate. The difference in pumping rate between two 101U/R pumps was, however, found to be as much as 10% at the same speed setting. A calibration curve for a 101U/R pump, for 1.6mm diameter silicone tubing (Type TU092, Watson Marlow, Falmouth, UK) used in all fermentations reported here (for supplying both acid/base and substrate), is presented in Figure 11.

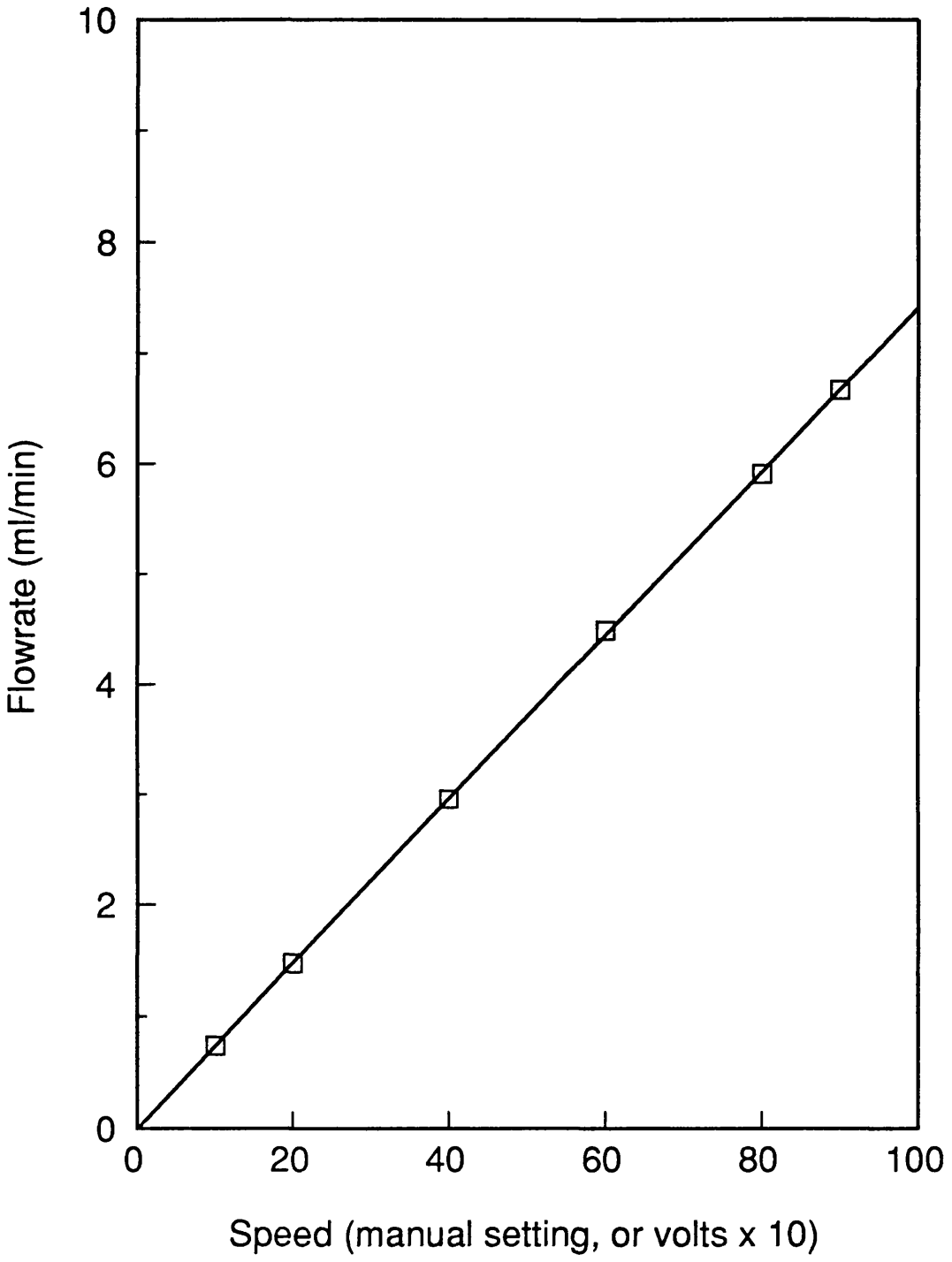


Figure 11: Calibration curve for a 101U/R pump, for 1.6mm diameter silicone tubing

3.2 DESIGN AND DEVELOPMENT OF A PC-BASED FERMENTOR DATA ACQUISITION AND CONTROL SYSTEM

3.2.1 PC-based data acquisition

This section describes the justification for a standalone PC-based data acquisition and control system for the fermentor described in Section 3.1, and its design and development.

Data from fermentations needs to be acquired from two sources, the gas analyzer, and the analog sensors. Gas analyses are made by a mass spectrometer (VG model MM80, VG Gas Analysis Ltd., Cheshire, UK). The results of analyses are stored on a dedicated IBM-compatible PC. The data can be picked up asynchronously via an RS-232 serial communications port. Analog fermentation measurements are digitised and stored on an 8-loop controller (Model 6358, TCS Ltd., Worthing, UK) and 32-channel signal processor (Model 6432, TCS Ltd., Worthing, UK). These units are on a multi-drop bus (RS422) and hence can both be addressed via the same serial transmissions cable.

At the start of this project, data acquisition from the TCS units attached to all UCL fermentors was managed by a multi-user software package (BIO-i, BCS Ltd., Maidenhead, UK) running on a micro-computer (PDP 11, Digital Equipment Corp., USA). A single monitoring rate could be specified, which then applied to all fermentors equally. Clearly, it would be desirable to vary the monitoring rate according to the duration of the fermentation, and the type of data being acquired. The software had a low reliability, and frequently 'crashed', causing data losses during all of the first six fermentations run relating to this project. In addition, there was no facility for closed-loop control of fermentation variables, nor for the on-line calculation of derived variables. The setting of open-loop control strategies was laborious, and could only be effected via expensive TCS hardware. The set-up could not be modified without the assitance of the system manager, who often required the assistance of BCS Ltd. Data acquired from fermentations was not stored as ASCII

characters, but in a relational database format. The information was difficult to retrieve, and sometimes required screen dumping and capture by a spreadsheet package. There were typically many errors in retrieved data (Figure 12), as well as lines of text that had to be edited out. This software was therefore deemed not suitable for a project dedicated to improved fermentation monitoring and control.

In specifying an alternative data acquisition system, it was felt that the most important requirement was for a system independent of the existing software and hardware (Thornhill and Royce, 1989). This would allow the monitoring and control team to test strategies without adversely affecting the rest of the Department's research effort. The most cost effective way of achieving such independence was considered to be by PC-based data acquisition. The PC chosen was an IBM compatible PC with an AT-bus, already existing in the Department (Model Vaxmate, Digital Equipment Corp., California, USA), as most of the Department's PC-based data analysis is on IBM compatibles. It was also felt that, as IBM compatibles represent 80% of the market in PCs, the choice of data acquisition software and hardware would be wider, its quality greater, and its price less than for other PCs. The choice of an IBM-compatible machine with an AT rather than Microchannel bus halves the cost of add-on boards.

In terms of software for data acquisition, the requirements were for a system capable of data storage in ASCII or Lotus-123 format, and for closed-loop control capability. The available systems fell into two groups, namely those designed for laboratory environments, and those intended for industrial production. The industrial systems were much more expensive than the laboratory systems, as can be seen in Table 8, and so were not considered. Data acquisition toolkits were not considered, as quick implementation that avoided any "custom" software elements was desired. Any of the remaining systems (eg. Notebook, Measure, LabWindows) would have been suitable. Notebook was chosen as it is produced by a manufacturer specializing in software, rather than as an off-shoot to a hardware companys' activity, which would

0.0506	7.010	86.700	500.000	0.000	7.730
0.0639	7.020	86.700	500.000	-9999.000	7.730
0.0803	7.010	86.600	-9999.000	0.000	7.730
0.1136	7.010	86.500	500.000	0.000	7.740
0.1300	-9999.000	86.500	500.000	0.000	7.730
0.1469	7.010	86.400	500.000	0.000	7.730
0.1633	7.010	86.400	500.000	0.000	-9999.000
0.1800	7.010	100.800	500.000	10.200	7.740
0.1967	7.010	108.300	500.000	-9999.000	7.720
0.2133	7.020	101.400	500.000	5.200	7.730
0.2300	7.020	100.300	-9999.000	5.000	7.870
0.2467	7.030	100.100	500.000	5.000	7.880
0.2800	7.020	100.000	500.000	5.000	6.940
0.2967	-9999.000	100.000	500.000	5.000	6.900
0.3133	7.030	100.000	500.000	5.000	-9999.000
0.3300	7.030	100.100	500.000	5.000	8.020
0.3467	7.030	100.100	500.000	5.000	8.130
0.3633	7.030	100.100	500.000	-9999.000	8.160
0.3800	7.030	100.100	500.000	5.000	8.220
0.3967	7.020	100.100	-9999.000	5.000	7.030
0.4300	7.030	100.100	486.000	5.000	6.990
0.4467	-9999.000	99.100	125.000	5.000	7.010
0.4633	7.030	98.500	125.000	4.900	7.010
0.4803	7.030	97.900	125.000	5.000	-9999.000
0.4967	7.030	97.500	124.000	5.000	7.010

Figure 12: A sample of data retrieved from Bio-i in 1989 by screen dumping, after removal of lines of text. The -9999.000 entries indicate failed data acquisition.

PACKAGE NAME AND SUPPLIER	COMMENTS	COST (1989 prices)
LT/Control, Laboratory Technologies, Wilmington, USA	Industrial data acquisition;	£2750
CAMM Technology, Worthing, UK	Industrial data acquisition;	£3250
Paragon 500, Intec Controls Corp., Chichester, UK	Industrial data acquisition;	£3500
Tactician, TCS Ltd, Worthing, UK	Industrial data acquisition;	
LT/Notebook, Laboratory Technologies, Wilmington, USA	Supports many different suppliers hardware platforms;	£ 895
Asyst, Keithley Instruments, Reading, UK	Drives Metrabyte hardware only;	£1670
LabWINDOWS, National Instruments, Austin, USA	Emphasizes interfacing over data analysis;	£ 349
Measure, National Instruments, Austin, USA	Program calls not supported; recent change of ownership;	£ 395

Table 8: Commercial software packages considered for use for the monitoring and control of the 42L fermentor

increase the availability of supported hardware. It was also well reviewed in the literature (eg. Tinham, 1989).

A block diagram of the data acquisition system that was set up is shown in Figure 13. Labtech Notebook is able to acquire data serially via an RS-232 port. To communicate with the TCS units, it was necessary to install a 422/232 converter, already existing in the Department. Notebook does not support process control via a serial port, but only by analog output. For control of flowrates (of acid/base and substrate) this was not a disadvantage, as automatic control of the 101U/R pumps is in any case by an analog signal. A 6-channel analog output board was specified (Model DDA06, Metrabyte Corp, USA, supplied by Keithley Instruments, Reading, UK). A four serial-port board (Model Async 4-II, Accent Computers Ltd., Burgess Hill, UK) was also specified, to add to the single RS-232 port that is standard on the Vaxmate PC.

3.2.2 Data Analysis

In this work, Notebook was directed to store data in files in ASCII format. The files were copied onto 5¼" floppy disks. Off-line data analysis was performed on a PC (Model 310, Dell Ltd., USA). The data analysis packages used were Lotus 123 (Version 3.0, Lotus Development Corp., Cambridge, USA) and Freelance Plus (Release 3.0, Lotus Development Corp., Cambridge, USA). Where sophisticated mathematical analyses were required, a library of mathematical routines (Numerical Recipes C library, Numerical Recipes Software, Cambridge, USA) was used. These routines were applied using the Quick C and C5.1 compilers (Microsoft Corp., California, USA).

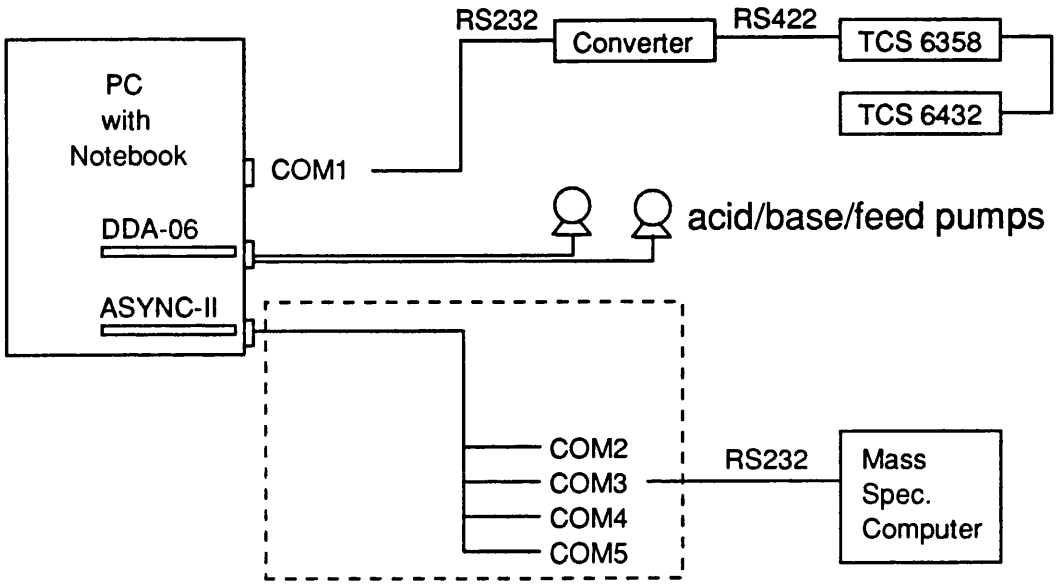


Figure 13: Block diagram of the data acquisition system

3.2.3 Control of fermentations

As Notebook supports analog control, open-loop feed profiling was easily implemented, by manipulating the voltage to the feed pump. As pH control is effected by manipulating the feedrates of acid and base to the fermentation, which again requires analog signals, for the acid and base pumps, profiling of pH was also easily achieved using the data acquisition system specified. Most of the control strategies of interest in the project involved profiling of feedrates and pH, and therefore simply involved control of the voltage to the peristaltic pumps.

Open-loop setpoint profiling of other environmental variables, such as the agitation and aeration rates, could also be easily implemented by manipulating the voltage to the analog inputs on the TCS units. Such control was not required in this project.

Control strategies that involve gas analysis data could not be easily implemented using Notebook. Inclusion of gas analysis data introduces two problems. Firstly, the frequency of gas analyses is outside the control of the PC-based data acquisition system, but depends instead on the number of fermentations logged onto the mass spectrometer software system. As Notebook acquires data at a rate that is fixed at the beginning of a run, too high a rate leaves holes in the gas analysis data file, while too low a rate causes loss of some gas analysis data. Secondly, the string produced by the mass spectrometer each time a gas analysis is performed must be parsed on-line, and while Notebooks' parsing facility was adequate for data from the TCS units, it was not powerful enough for data from the mass spectrometer. As only one fermentation required control involving gas analysis data, an elegant long-term solution was not sought to these problems. Instead, the fact that data acquisition was PC-based meant that a C program could be written to perform the necessary fermentation control. This program is listed in Appendix A2 (undocumented), and was used to control the aeration rate to a fermentation, based on gas analyses. The part of the program that handles communication with the TCS units was written by the author, while that involving communication with the mass spectrometer was an

edited version of the program presented in Mavrikis(1992).

3.3 FERMENTATION SYSTEMS

As noted in the introduction, the project was directed towards the monitoring of aerobic fermentations. As the focus was on generic approaches to fermentation monitoring, there were no constraints on the fermentation systems chosen to test monitoring methodology. It seemed appropriate, therefore, to use fermentation systems of which there was experience in the Department. It was also desired to choose two contrasting fermentation systems, one involving a simple organism grown on defined media, the other a mycelial organism of industrial importance grown on undefined media. The organisms chosen were *E. coli* and *S. clavuligerus*. Unfortunately, due to the difficulties experienced in sterilizing fermentation media in the 42L fermentor as already noted, the program of *S. clavuligerus* had to be curtailed. On only three occasions out of twelve were the media for this fermentation successfully sterilized, and hence only three *S. clavuligerus* fermentations were run. As *E. coli* is a fast growing organism, strictly sterile media weren't required for this fermentation. The data from a total of nine *E. coli* fermentations, and three *S. clavuligerus* fermentations are analyzed in this thesis.

3.3.1 *S. clavuligerus*

An isolate of *S. clavuligerus* ATCC 27064 was used for this work.

Storage and preparation of inocula

The methods of preparation are those of Belmar-Campero (1989), but were adapted to be suitable for 42L spore-inoculated fermentations. Spore suspensions of the organism, used by previous workers, were available in the Department. The spores were suspended in Tween saline sucrose solution (Table 9) and stored at -70°C. The spore concentration was approximately 10^{10} spores.ml^{-1}. A volume of 0.5ml spore

TWEEN SALINE SUCROSE

COMPONENT	CONCENTRATION	SUPPLIER AND CODE
Tween 80	$0.8ml.L^{-1}$	Sigma, St. Louis USA
NaCl	$5.7g.L^{-1}$	
Sucrose	$102g.L^{-1}$	

GROWTH MEDIA (MYEA)

COMPONENT	CONCENTRATION	SUPPLIER AND CODE
Malt extract	5 g/L	Oxoid, UK, L39
Yeast extract	5 g/L	Oxoid, UK, L21
Agar technical No.3	20 g/L	Oxoid, UK, L13

SPORULATION MEDIA

COMPONENT	CONCENTRATION	SUPPLIER AND CODE
Tomato puree double concentrate	10 g/L	Sainsbury
Milupa Oatmeal with apple	10 g/L	Boots
Agar technical No. 3	20 g/L	Oxoid, UK, L13

GLYCEROL MEDIA

COMPONENT	CONCENTRATION	SUPPLIER AND CODE
Bacteriological peptone	20 g/L	Oxoid, UK, L37
Malt extract broth	10 g/L	Oxoid, UK, CM57
Glycerol	20 g/L	FSA, UK

Table 9: Recipes for *S. clavuligerus* fermentations (from Belmar-Campero, 1989)

suspension was used to inoculate a light-walled Thompson bottle (Hampshire Glassware, Southhampton, UK) containing the sporulation media (Table 9). Each Thompson bottle contained 500ml of sporulation media, whose pH was adjusted to 7 prior to sterilization for 20 minutes at 120°C.

The inoculated Thompson bottles were placed in an incubator at 26°C. Sixteen days were required for sporulation. In practise, the bottles were left in the incubator for considerably longer, until they were required for a fermentation. The data of Belmar-Campero (1989) suggested that reproducible fermentations could be obtained as long as the same generation of spores was used in each fermentation.

Fermentation media and operation

The media used for *S. clavuligerus* fermentations are reported in Table 9. These media were reported in Belmar-Campero(1989). All media components could be sterilized together in the fermentor, in the manner described in Section 3.1.2. The media components were weighed to give the indicated concentrations for a 30L working volume. The media were made up to 32L prior to sterilization, to allow for the loss of approximately 2L of water during sterilization. The media have a strong tendency to foam during sterilization and during the early stages of the fermentation. Hence, 500ppm of polypropylene glycol antifoam (PPG) was added to suppress foaming.

The fermentor was inoculated directly from the light-walled Thompson bottles, using the apparatus indicated in Figure 14. The spores were removed from the surface of the sporulation media using 200g of 4mm glass beads (Ballotini, Jencon, Leighton Buzzard, Beds, UK). Using less than this quantity of beads led to only partial removal of spores. Once removed, 50ml of Tween saline sucrose was introduced into the bottle, and the bottle was agitated vigorously. The spore suspension was introduced directly into the fermentor.

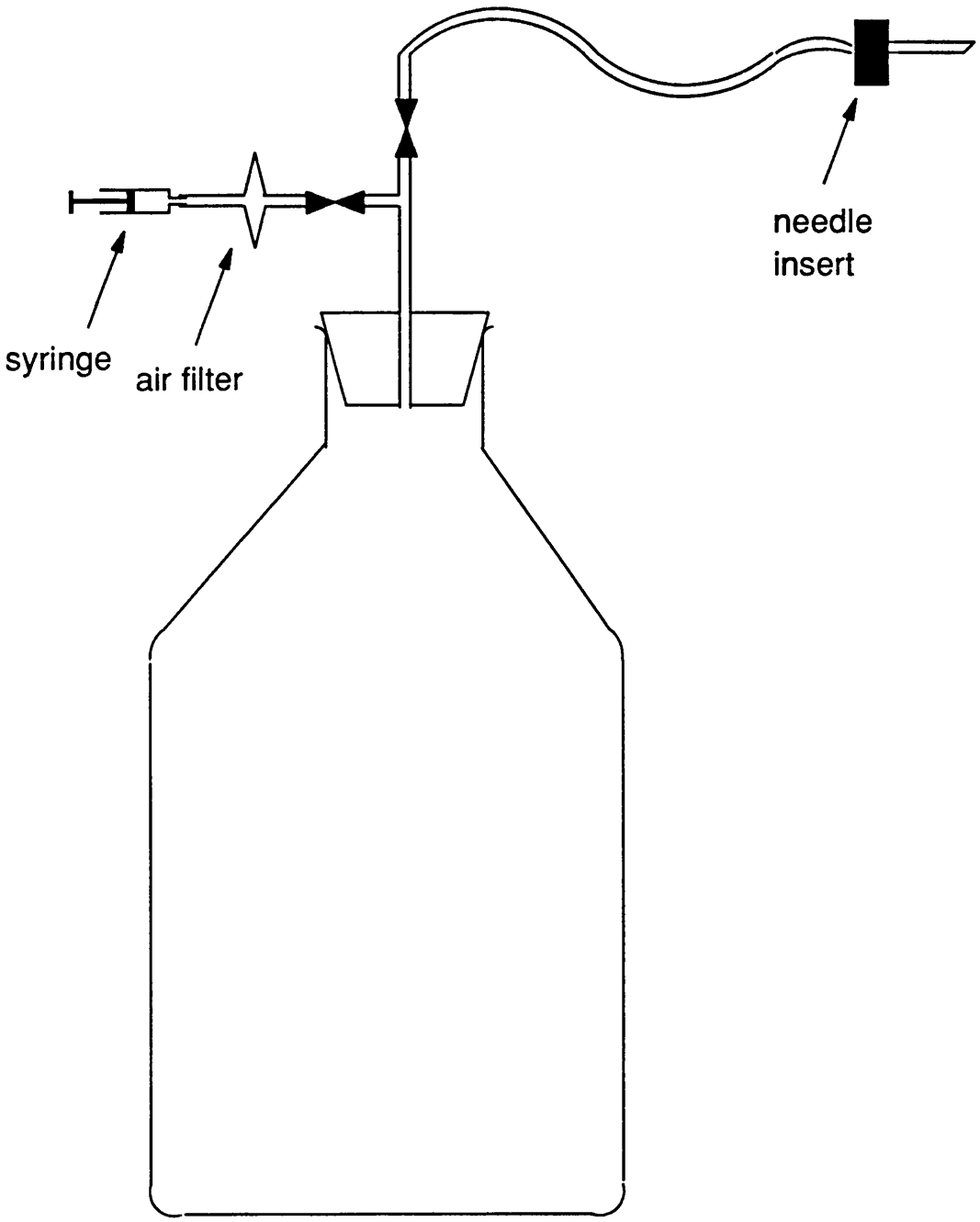


Figure 14: Attachment to Thompson bottles for inoculating the *S. clavuligerus* fermentations

The fermentation environmental conditions were: agitation 400-650rpm, aeration 10 SLPM (≡⅓vvm), pH 7, temperature 26°C, pressure 1kPag (ie. atmospheric operation). The dissolved oxygen level was maintained above 30% of saturation. The control of pH was achieved by the addition of 2.5M NaOH and 1.25M H_2SO_4.

The fermentation media recipe reported by Belmar-Campero(1989) differed from those of Reading and Cole (1977), to which Belmar-Campero(1989) had referred. The difference was that the concentration of bacteriological peptone reported in Belmar-Campero(1989) was twice that of Reading and Cole(1977), which was initially thought by the author to represent an improved formulation of the media, developed by Belmar-Campero(1989) for the isolate used. However, the effect of the difference was to cause the initial biomass concentration achieved after exponential growth to be 7-8g.L^{-1}, rather than the 5g.L^{-1} obtained by Belmar-Campero (1989). This resulted in exhaustion of glycerol at 60h, followed by a second peak in the CER, and then a rapid fall in the CER to very low levels, as can be seen in Figure 15. Hence the difference between the reported media of Belmar-Campero(1989) and Reading and Cole(1977) was due to a typographical error in Belmar-Campero(1989). The *S. clavuligerus* fermentations resulting from the reported media of Belmar-Campero(1989) would not have been satisfactory had the purpose in the project reported here been to examine the productivity of the *S. clavuligerus* fermentation, as glycerol is required for clavulanic acid synthesis (Romero et al, 1986). However, as the objective in this project was improving the monitoring of gas exchange rather than studying clavulanic acid synthesis, the work reported in this thesis was not adversely affected by the unusual growth curve.

Off-line analyses

Dry weight measurement

For each dry weight measurement, two 20ml samples of fermentation broth were removed from the sample port. They were separately vacuum filtered

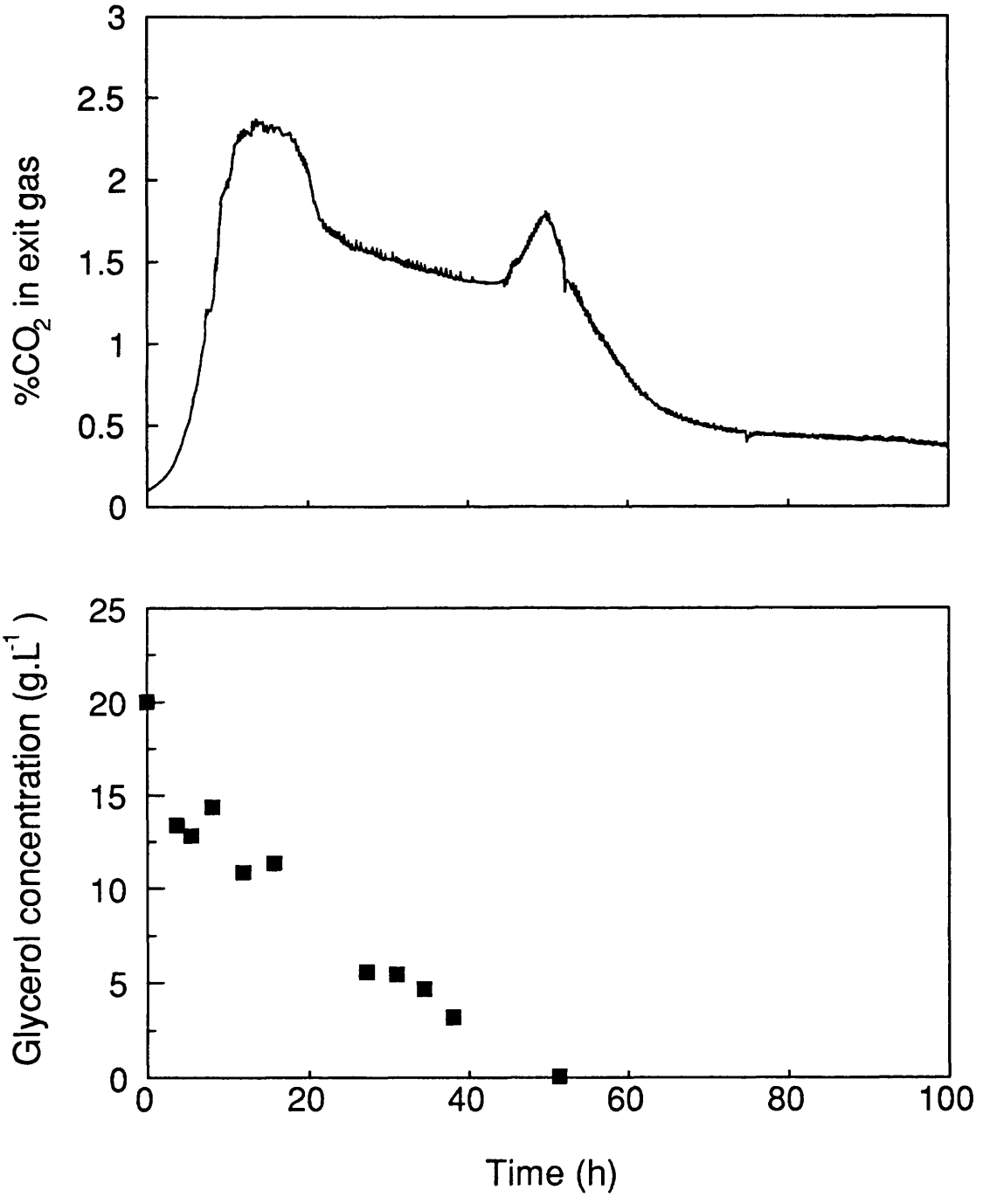


Figure 15: Data from a *S. clavuligerus* fermentation. The second peak in the exit gas carbon dioxide content starting around 45h (top graph) may have been caused by the exhaustion of glycerol around this time (bottom graph).

(Model SM16510/11, Sartorius Instruments Ltd., Sutton, UK) on 47mm diameter filter papers (GF/C grade, Whatman, Mainstone, UK), each rinsed twice with 10ml distilled water, and left overnight in an oven at 105°C.

Manual pH measurement

A pH meter (Model 7020, Electronic Instruments Ltd., Chertsey, UK) was used to manually measure the pH of broth samples in order to establish whether the output of the *in situ* fermentor pH probe had drifted.

Glycerol Assay

The assay for glycerol during the *S. clavuligerus* fermentations was done enzymatically (No.148 270, Boehringer Mannheim UK, Lewes, UK).

Spore counts

Spore counts were made by serially diluting the spore suspension (1:500; 1:200: 1:100 in 150ml bottles) to a final dilution of $1:10^7$. The resulting suspension was inoculated onto Petri dishes containing growth medium (MYEA) (Table 9). The spore suspension used to inoculate the fermentor had a spore concentration of $2x10^9$ ml^{-1}, giving a spore concentration in the fermentor upon inoculation of $3x10^6ml^{-1}$.

Sterility Control

At the end of each fermentation, five 0.2ml samples of fermentation broth were inoculated onto Petri dishes of 13 g/L nutrient agar (Oxoid, UK) and incubated at 37°C for 48 hours.

Viscosity

The viscosity of the broth was measured periodically over a range of shear rates by a rheometer (Bohlin VOR Rheometer, Bohlin Rheologi, Lund, Sweden), using the 3.84gcm torque bar and the C25 measuring system (a 25mm diameter cup and bob system). At the lowest broth viscosities (1-2cP) the shear rate had to be fairly high ($>50s^{-1}$) for reasonable accuracy to be achieved (±10%).

It has been found that thinning at the measuring system walls can cause erroneously low apparent viscosities for filamentous organisms (Bongenaar et al, 1973; Charles, 1978) in some measuring systems. Allen and Robinson (1990) used eight different methods for viscosity measurement on broths of *A. niger*, *S. levoris* and *P. chrysogenum*, including three cup and bob systems of similar dimensions to the one used in this work. They found reasonable agreement between the different methods. In this work, reproducible measurements that drifted only slowly with time suggested this method to be adequate for *S. clavuligerus*, in line with the findings of Allen and Robinson(1990).

Surface Tension

Measurements were made using ground coverslips suspended from a microbalance (Mettler ME30, Mettler Toledo, Leicester, UK). The methodology was developed at UCL (D. Allen, personal communication).

3.3.2 *E. coli*

The strain of *E. coli* used in this work was HB101.

Storage and preparation of inocula

The organism was available in the Department, and was stored on nutrient agar slopes at 4°C. The composition of the media excluding carbon source (D-glucose) are presented in Table 10. A wire loop was used to inoculate 2x750ml of the media in 3L conical flasks. Although it is usual for such flasks to contain only 250ml of inoculum, it was found experimentally (Figure 16) that there was little adverse impact on the dry weight achieved or the length of the lag phase, as a result of using a volume of 750ml. The glucose was present in the flasks at a concentration of 5g.L^{-1}. The inocula were incubated for 12h at 37°C, by which time the optical density at 670nm was 2 (dry weight ≈ 1 g.L^{-1}).

Fermentation media and operation

Under this heading are described the fermentation operating conditions that were used in eight out of nine *E. coli* fermentations that were run to provide data for this project, the ninth fermentation being described in Section 4.5.4.

The fermentor (42L 3000 Series, LH Fermentation, Maidenhead, UK) contained a working volume of approximately 30L of media, so that the inoculum represented 5% of the working volume. The initial glucose concentration in the fermentor was 2g.L^{-1}. Polypropylene glycol (PPG) antifoam was introduced at the beginning of the fermentation at a concentration of 50ppm. The operating conditions were: aeration 10 SLPM (≡⅓vvm), agitation 500 rpm, temperature 37°C, pressure 1 kPag (ie. approximately atmospheric). Glucose was fed to the fermentation (150 g.L^{-1} solution)

TRACE ELEMENTS STOCK SOLUTION (1L)			
$FeCl_3.6H_2O$	5.40 g	$MnCl_2.4H_2O$	2.00 g
$CoCl_2.6H_2O$	0.48 g	ZnO	0.41 g
H_3BO_4	0.06 g	$CuCl_2.2H_2O$	0.17 g
HCl	10 ml 36% solution		
SALTS STOCK SOLUTION (1L)			
KCl	74.5 g	$NaSO_4$	57.3 g
Citric acid	42.0 g	$MgCl_2.6H_2O$	25.4 g
$CaCl_2.2H_2O$	0.29 g	Na_2MoO_4	0.002 g

1L MEDIA EXCLUDING CARBON SOURCE

- 5 ml trace elements stock solution
- 10 ml salts stock solution
- 5 $g.L^{-1}$ NH_4Cl
- 1.5 $g.L^{-1}$ $NaH_2PO_4.2H_2O$

Table 10: Defined media used for *E. coli* fermentations

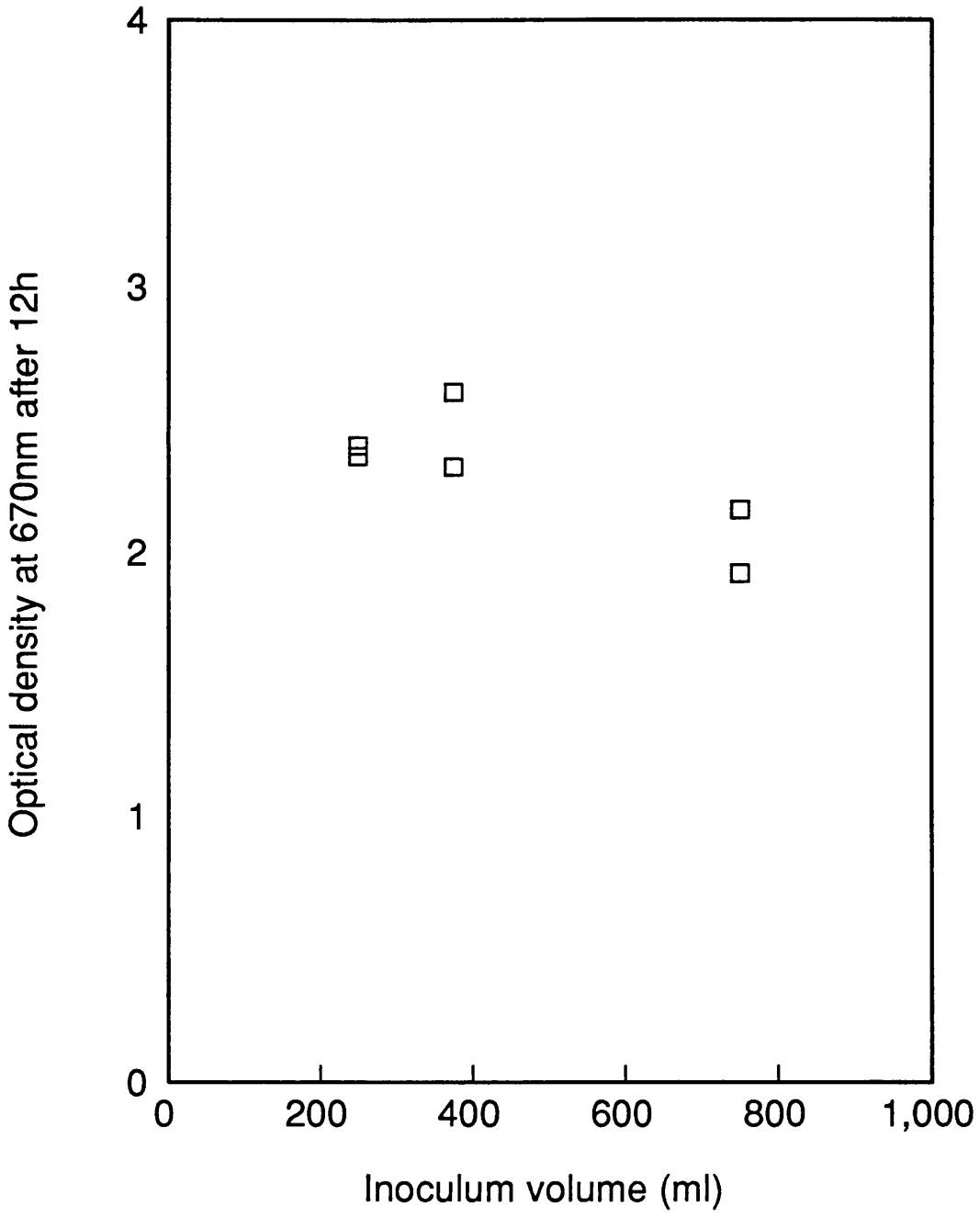


Figure 16: Impact of inoculum volume in a 3L conical flask on the optical density of *E. coli* inocula after 12h incubation. The graph basically shows that there is a relatively small price to pay in terms of reduced biomass concentration if a high inoculum volume is used. Hence a 5% inoculum for the 42L fermentor can be generated in just two flasks each containing 750ml inoculum.

when the difference between the percentage of oxygen in the inlet and exit gas first exceeded 2.0%. In all fermentations reported here, the initial rate of glucose feeding was set at 3.3 $ml.min^{-1}$ so that the mole percentage of carbon dioxide in the exit gas was about 2% once the fermentation had adjusted to fed-batch conditions. The flowrate corresponded to a pump speed setting of 40-45 (manual setting) or 4-4.5V (automatic control) for a tubing diameter of 1.6mm, depending on which 101U/R pump was used. In all fermentations, only base additions (of 2.5M NaOH) were required in the (on-off) control of the pH.

Off-line analyses

Optical Densities

The optical density of the broth was measured at 670nm every 15 minutes using a spectrophotometer (Model SP6-250 Visible Spectrophotmeter, PYE UNICAM Ltd., Cambridge, UK). As the linear range for optical density measurements is below 0.5, dilution of the broth samples with distilled water was necessary once the biomass concentration in the broth was substantial, to keep the measurement in the linear range.

4. RESULTS AND DISCUSSION

Earlier, Section 2.3 summarized the conclusions from a literature survey of work on gas exchange in fermentation, and, from these, proposed a research strategy. It was pointed out that carbon dioxide transfer is of interest not only from the point of view of monitoring and controlling dissolved carbon dioxide (an important fermentation variable) but also because of the effect dissolved carbon dioxide can have on the apparent values of important derived variables like the CER and RQ. The body of this chapter therefore concerns carbon dioxide transfer. Prior to examining carbon dioxide transfer, however, two aspects of oxygen transfer were also considered, and which have an impact on later work on CO_2 transfer. The first concerns the utility of a single value of the oxygen mass transfer coefficient in characterizing mass transfer in fermentation. The second, while addressing signal conditioning of all fermentation measurements, finds that the oxygen transfer rate or OTR (which is approximately equal to the OUR) has some unique features that necessitate the signal conditioning of OTR data.

The order of presentation of work in this chapter is not chronological, but hierarchical, later work, either explicitly or implicitly using the results of earlier work. Table 11 lists the sections in this chapter and summarizes the objectives of the work presented. The mathematical symbols used are explained in the text, and are also listed under 'NOMENCLATURE' on Page 262. The first section (Section 4.1) examines the evaluation of the oxygen mass transfer coefficient for a fermentor, $K_L{}^{O2}a$, with a view to establishing if a single variable such as this, evaluated from a simple expression in terms of fermentation measurements, can provide a useful description of mass transfer in production-scale fermentors. Much of the argument provided is theoretical chemical engineering, and the reader may prefer simply to read to conclusions of this section which are summarized in Table 12 on Page 148.

CHAPTER SECTION	OBJECTIVE
4.1 $K_L{}^{O2}a$ as a characteristic variable in fermentation	1. To show that a value for $K_L{}^{O2}a$ for a fermentor, defined only in terms of the local mass transfer coefficient, can be easily calculated, and which retains an element of fundamental physical information 2. To show that the result obtained is characteristic of a given fermentation, and reproducible from one batch to the next
4.2 Random noise in fermentation data, and its removal from OTR data	1. To qualitatively examine the magnitude of noise in fermentation measurements 2. To show that oxygen transfer rate (OTR) data are unique amongst fermentation data in requiring special conditioning *3. To show that conditioning of OTR data improves their quality, and that of related derived variables*
4.3 Monitoring dissolved carbon dioxide under steady-state conditions	*1. To show theoretically that mass transfer of CO_2 is a purely liquid-film limited physical process* 2. To examine the factors that affect the accuracy of the assumption of equilibrium between dissolved carbon dioxide and that in the fermentor exit gas 3. To examine if direct measurement of dissolved CO_2 yields results compatible with theoretical work
4.4 The development and examination of the behaviour of a model of unsteady-state CO_2 transfer	1. To develop a model describing CO_2 transfer under conditions where the concentration of CO_2 in the liquid or gas phase is changing with time *2. To use the model developed to show that the respiratory quotient, as apparent from gas analyses, can be an inaccurate indicator of the real underlying RQ, when the fermentation pH or CER are changing*
4.5 Experimental observations of unsteady-state CO_2 transfer in fermentations of *E.coli* and *S.clavuligerus*	1. To experimentally validate the model developed in Section 4.4 *2. To use the model of Section 4.4 to recursively estimate the "real" RQ from the "apparent" RQ during a fermentation* 3. To apply the model of Section 4.4 to detect a simulated failure of a pH sensor
4.6 Automatic control of dissolved carbon dioxide	1. To show independent control of dissolved O_2 and dissolved CO_2 is feasible *2. To show that a simple ratio controller can control dissolved CO_2 to a constant level*

Table 11: Summary of chapter sections and objectives in Chapter 4. The most important objectives are shown in *italics*

4.1 THE OXYGEN MASS TRANSFER COEFFICIENT, $K_L^{O2}a$ AS A CHARACTERISTIC VARIABLE IN FERMENTATION

The fermentor volumetric mass transfer coefficient, $K_L^{O2}a$ was noted in Section 2.1 to be a variable that is commonly monitored in industrial fermentation. Also noted were the assumptions that are usually made to allow the translation of the differential equation describing local mass transfer (Equation 1) into an equation involving the average volumetric mass transfer coefficient over the entire fermentor (Equations 2 & 3). In this section, points are made relating to the validity of these assumptions and the utility of the resulting variable. The points made are necessarily intuitive, as an exact examination would be a lengthy undertaking. The points are intended to provide a perspective on later theoretical and experimental work that uses $K_L^{O2}a$.

It is commonly assumed that mass transfer in a fermentor can be described in terms of a single value of the mass transfer coefficient, $K_L^{O2}a$. In Section 4.1.1 it is noted that the local mass transfer coefficient (i.e. that at a point in the fermentor) varies strongly with position. Hence the most appropriate definition for a single value of $K_L^{O2}a$ is as the volume integral of the local mass transfer coefficient. It is shown in this Section 4.1.1 that this (average) $K_L^{O2}a$ retains at least some of the fundametal properties of the local mass transfer coefficient. It is also shown that only an approximate value for this coefficient can ever be obtained from fermentation measurements normally available, the size of this error being dependent on the number of impellers in the fermentor. The sensitivity of the calculated $K_L^{O2}a$ to the assumed extent of gas backmixing is examined in Section 4.2.2, and shown to be weak in many instances. It is shown in Section 4.2.3 that the use of the average hydrostatic pressure in large fermentors in the equations for the evaluation of $K_L^{O2}a$ will lead to negligible error by comparison with the rigorous results. The overall conclusion in Section 4.1 is that simple equations for the evaluation of $K_L^{O2}a$ can be used to yield a result that contains an element of fundamental information about the process (albeit imperfect) that is useful in characterizing fermentations, and this conclusion is supported by examining the evaluated mass transfer coefficient over several fermentation batches of *E. coli* and *S. clavuligerus* in Section 4.2.4.

4.1.1 The average fermentor $K_L{}^{O2}a$ as a fundamental variable

The differential equation describing local mass transfer presented in Section 2.1 was:

$$dN^{O_2} = (K_L^{O_2}a)_l.([O_2]_g - [O_2]_L).dV_d$$

$$= \frac{(K_L^{O_2}a)_l}{H^{O_2}}.\left(p_g^{O_2} - p_L^{O_2}\right).dV_d \qquad (4)$$

where N^{O2} = molar flux of oxygen across interface ($mol.s^{-1}$)

$(K_L{}^{O2}a)_l$ = local volumetric mass transfer coefficient for oxygen (s^{-1})

H^{O2} = Henry's law constant for oxygen ($Pa.(mol.m^{-3})^{-1}$)

$p_g{}^{O2}$ = partial pressure of oxygen in the gas phase (Pa)

$p_L{}^{O2}$ = partial pressure of oxygen in the liquid phase (Pa)

$[O2]_g$ = concentration of oxygen in the gas phase ($mol.m^{-3}$)

$[O_2]_L$ = concentration of oxygen in the liquid phase ($mol.m^{-3}$)

V_d = dispersion volume or aerated broth volume (m^3)

It was noted in Section 2.1 that integration of this differential equation is usually achieved in industry by assuming the local volumetric mass transfer coefficient to be independent of position. In reality, the local gas-liquid interfacial area per unit volume is a strong function of position (Calderbank, 1967), while the local film mass transfer coefficient for oxygen is also a function of position (Lopes de Figuerdo and Calderbank, 1979), albeit a weak function. For both these variables, the spatial variations are due to the spatial variation of liquid turbulence which arises as a result of power input to the broth being highly localized in mechanically agitated multi-impeller fermentors. The most direct definition of the average volumetric mass transfer coefficient for a fermentor, $K_L{}^{O2}a$, in terms of the local volumetric mass transfer coefficient, $(K_L{}^{O2}a)_l$ is:

$$K_L^{O_2}a = \left(\frac{\int (K_L^{O_2}a)_l.dV_d}{V_d}\right) \qquad (5)$$

As K_L^{O2} is a relatively weak function of position, it can be brought outside the integral sign.

$$K_L^{O_2}a = K_L^{O_2} \cdot \frac{\int (a)_l \cdot dV_d}{V_d} \qquad (6)$$

In the above equation, the local interfacial area, $(a)_l$, has a clear physical meaning at a given point in a fermentor. By contrast, the average interfacial area for the fermentor, **a**, has only mathematical meaning, without necessarily retaining any of the physical properties of $(a)_l$, except in hypothetical systems where the local coefficient is independent of position.

The question arises as to whether **a** retains any of the information contained in $(a)_l$. With a view to establishing whether this is the case, Figure 17 presents the data of Calderbank(1959) for the axial variation in the local interfacial area per unit dispersion volume, $(a)_l$ at several agitation rates. In the lower graph, for each agitation rate, the ratio of the local interfacial area to the average interfacial area along the fermentor is shown. It can be seen that, with the exception of the result at 200rpm, the curves are all approximately identical. This indicates that the sensitivity of the average interfacial area to the agitation rate is more or less the same as that of the local interfacial area. This result, in showing that the average interfacial area retains some of the same information as contained in the local interfacial area, is significant in providing at least some evidence to support the use of the average volumetric mass transfer coefficient as a "fundamental" variable in fermentation.

Having established that $K_L^{O2}a$, as defined in Equation (6) contains physical information, the next question concerns whether it is possible to evaluate this quantity from what can be measured routinely during fermentations. Clearly, values for the local interfacial area, $(a)_l$, aren't normally available during fermentations. Hence, evaluation of $K_L^{O2}a$ during a fermentation, in such a way that it will have the same numerical value as that defined in Equation (6) requires that the integration of $(a)_l$ be separable from the integration of the partial pressure gradient driving mass

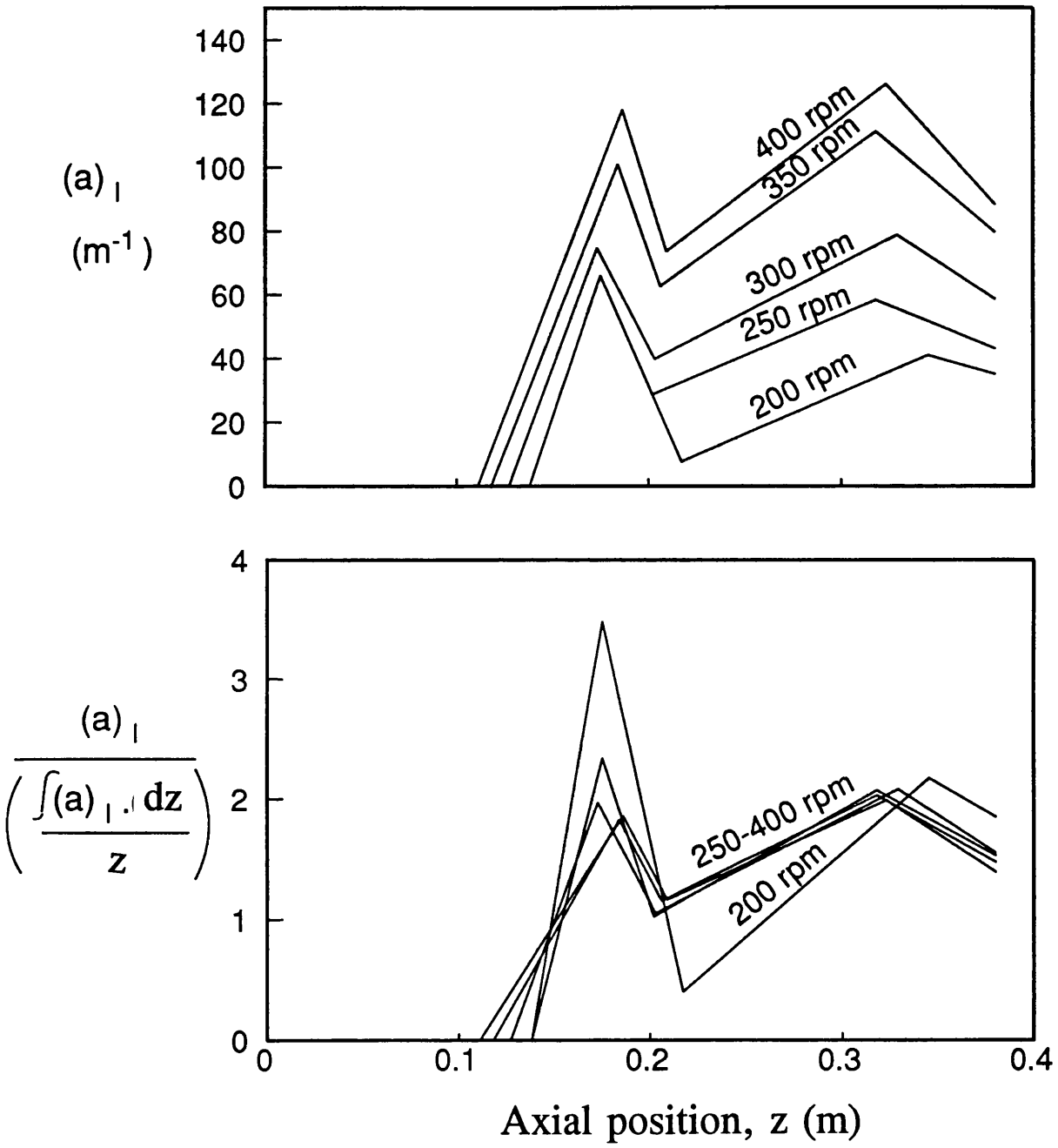


Figure 17: Relationship between the local and average interfacial area as a function of the agitation rate (local interfacial area data taken from Calderbank, 1959)

transfer of oxygen, as can be seen by referring to Equation (4). Although integration of these functions cannot be exactly separable, they may be approximately so. Integration along the angular and radial axes of a fermentor can be considered separable, as the partial pressure gradient for oxygen transfer is relatively constant in these directions. Integration in the axial direction may be approximately separable, as $(a)_l$ may be expected to be a periodic function in multi-impeller fermentors, that reflects the periodicity of the intensity of liquid turbulence in the fermentor (Figure 18). Integration of the product of a smooth function with a periodic function is approximately the product of their integrals, an approximation that improves as the number of periods increases. As fermentors have 3-5 impeller, hence providing 3-5 wavelengths of the periodic $(a)_l$, and there are end effects associated with the first and last impellers in particular, the assumption of separability can only be approximate. However, as the partial pressure gradient doesn't vary greatly in the axial direction (up to a factor of 2 in the very largest fermentors), it is reasonable to expect that an approximate numerical value for $K_L{}^{O2}a$ as defined in Equation (6) can be obtained from the integrated form of Equation (4). For all further discussion of mass transfer, the mass transfer coefficient at any point in the fermentor is considered to be pseudo-constant, and therefore can be represented by the average volumetric mass transfer coefficient for the fermentor, $K_L{}^{O2}a$.

4.1.2 Idealized states of gas and liquid backmixing

Most studies of on-line monitoring of the mass transfer coefficient in fermenters assume the gas flow to be described by one of two idealised states, namely well-mixed, or plug flow. These models represent states of infinite and zero backmixing of the gas. Extremes of axial flow, such as produced in airlift fermentors, can produce results outside the range of these idealized states, but for mechanically-agitated fermentors, these two states define the range of physical realizability for the gas phase.

These same two states of zero and infinite backmixing define the range of physical

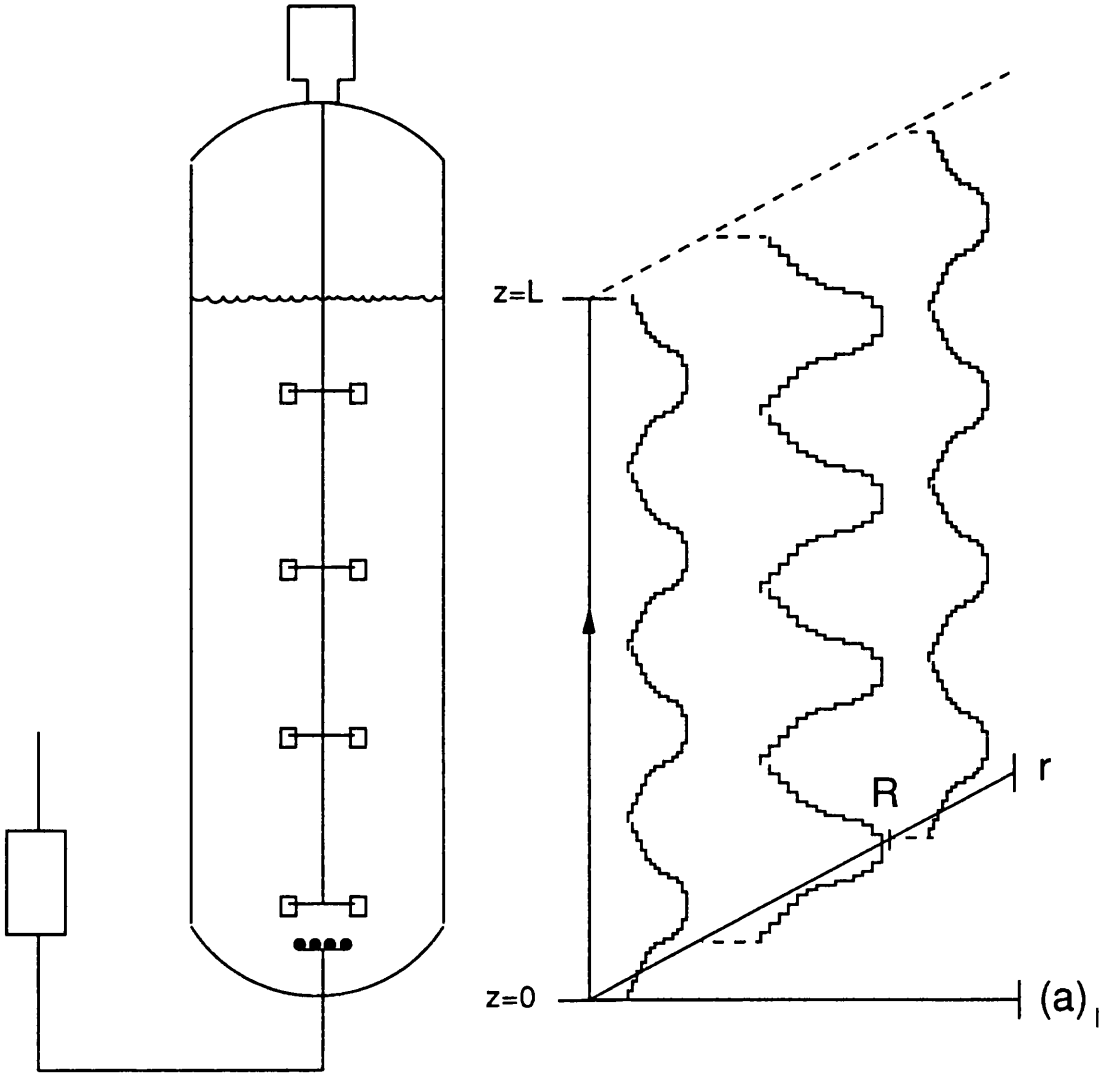


Figure 18: Qualitative representation of the variation of the local interfacial area per unit volume, $(a)_l$, with position, in a multi-impeller fermentor, where z is the height above the fermentor bottom (L being the height of the broth surface) and r the radial distance from the impeller shaft (R being the fermentor radius)

realizability for the liquid phase as well. Hypothetically, if there is no backmixing in the liquid phase (ie. plug-flow liquid), then the variation in the dissolved oxygen partial pressure in the gas phase will be mirrored in the liquid phase (Figure 19). In practice, there is always a substantial degree of liquid backmixing, and so the variation in the liquid phase partial pressure with axial position is much less than that in the gas phase (Fox, 1984). In the evaluation of $K_L{}^{O2}a$ in fermentors, the liquid phase is commonly assumed to be infinitely backmixed (ie. well-mixed). This is known to be a good assumption even at pilot scale (Steel and Maxon, 1966). However, in an extreme situation, such as that reported in Manfredini et al(1983), in which the dissolved oxygen partial pressure was measured in a 112m^3 fermentor, for a highly viscous mycelial broth, the assumption of a well-mixed liquid phase is inaccurate. Interestingly, however, the inaccuracy of this assumption does not necessarily result in very great errors in the calculated $K_L{}^{O2}a$, as the variation of dissolved oxygen in the liquid phase with axial position remains small in comparison with that in the gas phase (Figure 20). Hence, the assumption of a well-mixed liquid phase in respect of oxygen for the evaluation of $K_L{}^{O2}a$ will be good in the large majority of fermentors. Hence the two idealized cases of gas and liquid backmixing of greatest interest are the well-mixed liquid/ well-mixed gas and the well-mixed liquid/ plug-flow gas (Figure 19). Calculation of the mass transfer coefficient for these idealized states (for systems in which hydrostatic pressure effects are negligible) is indicated by Equations (2) and (3) in Section 2.1, which are repeated below:

$$OTR = G.\frac{\left(\%O_2^{\,in} - \%O_2^{\,out}.\dfrac{\%N_2^{\,in}}{\%N_2^{\,out}}\right)}{100}$$

$$= \frac{K_L^{O_2}a.V_d}{H^{O_2}}.\left(p_g^{O_2,\,out} - p_L^{O_2}\right). \qquad \textit{(well-mixed gas)} \quad (7)$$

$$= \frac{K_L^{O_2}a.V_d}{H^{O_2}}.\frac{\left(p_g^{O_2,\,in} - p_g^{O_2,\,out}\right)}{\ln\left(\dfrac{p_g^{O_2,\,in} - p_L^{O_2}}{p_g^{O_2,\,out} - p_L^{O_2}}\right)} \qquad \textit{(plug-flow gas)} \quad (8)$$

where OTR = oxygen transfer rate ($mol.s^{-1}$)

G = inlet air flowrate (dry basis) ($mol.s^{-1}$)

$\%O_2^{in}$, $\%O_2^{out}$ = mole percentages of oxygen in the inlet (in) and exit (out) gas streams respectively (dry basis)

$\%N_2^{in}$, $\%N_2^{out}$ = mole percentage of nitrogen in the inlet (in) and exit (out) gas streams respectively (dry basis)

$p_g^{O2,in}$ = partial pressure of oxygen in the inlet gas (Pa)

$p_g^{O2,out}$ = partial pressure of oxygen in exit gas (Pa)

p_L^{O2} = partial pressure of dissolved oxygen (Pa)

In Figure 21, the sensitivity of the calculated $K_L^{O2}a$ to the assumed extent of gas backmixing is examined. Using Equations (7) and (8) to calculate $K_L^{O2}a$, it can be seen in Figure 21 that if the change in oxygen mole fraction between the inlet and exit gas streams (indicated on the X-axis) is small, the ratio of $K_L^{O2}a$ obtained assuming a well-mixed gas phase to that obtained assuming a plug-flow gas phase is close to one, indicating that the assumed gas flow model has little impact on the calculated mass transfer coefficient. As the change in the gas-phase oxygen mole fraction across the fermentor becomes larger, the gas flow model has an increasing impact on the calculated mass tranfer coefficient. It can be seen in Figure 21 that the mass transfer coefficient calculated from an inappropriate gas flow model will vary with %DO, even if the integral of the (unknown) local mass transfer coefficient, as defined in Equation (6) hasn't changed.

For many industrial fermentations, the change in the gas-phase oxygen mole fraction is sufficiently small that the assumed extent of gas backmixing has little effect on the calculated value of $K_L^{O2}a$ (Buckland et al, 1985). This is fortunate for, in practice, the extent of gas backmixing is often neither approximately well-mixed or plug-flow. Further, this extent may vary as a result of changes in broth physical properties, in particular of viscosity during mycelial fermentations (Figure 22), as well as as a result of changes in agitation and aeration rates. Where the extent of gas backmixing has an important impact on the calculated value of $K_L^{O2}a$, a model to

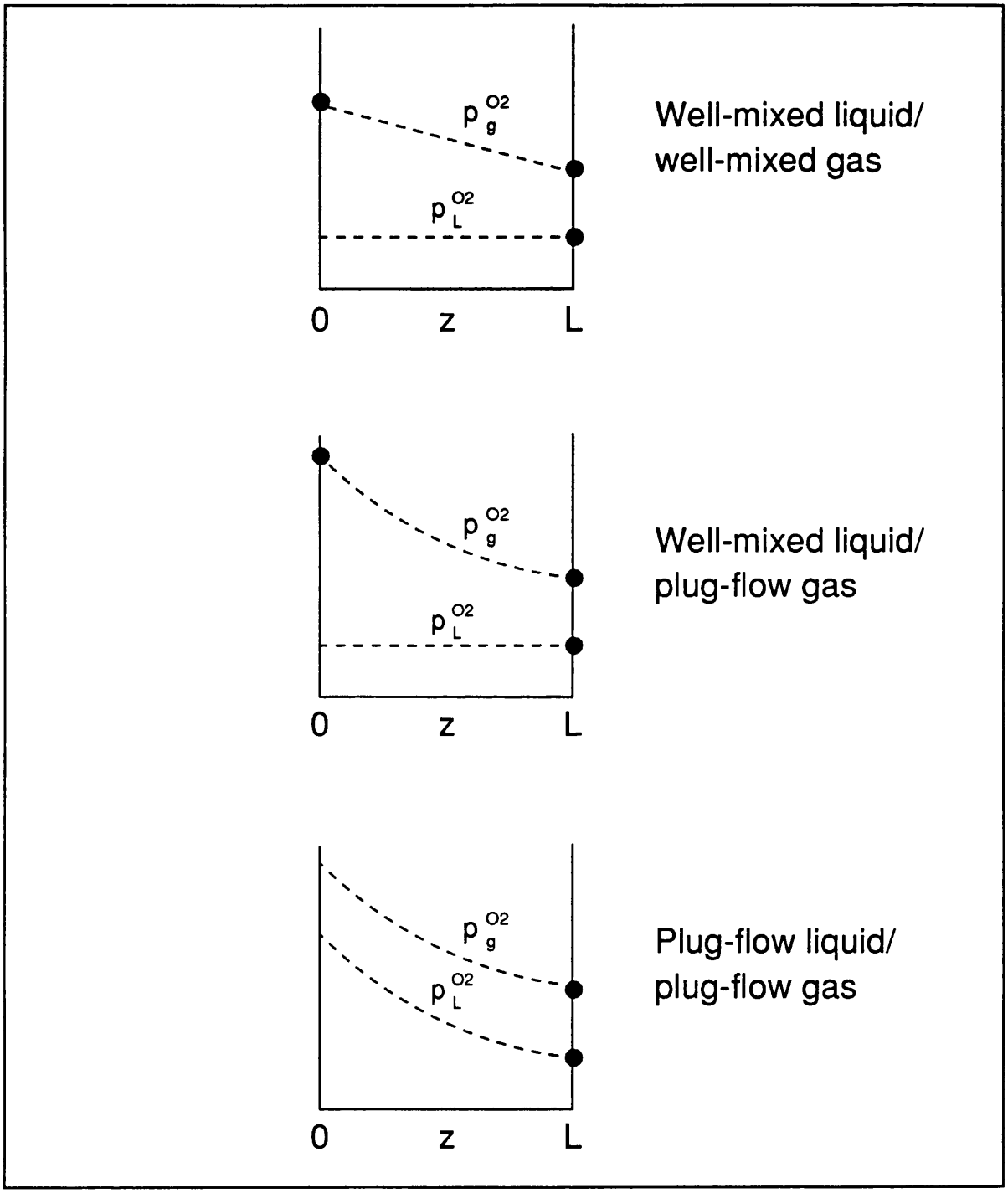


Figure 19: Graphical representation of extreme cases of liquid and gas backmixing, where z = height above fermentor bottom, L = height of broth surface above bottom, and p_L^{O2} and p_g^{O2} are the partial pressures of oxygen in the liquid and gas phases respectively

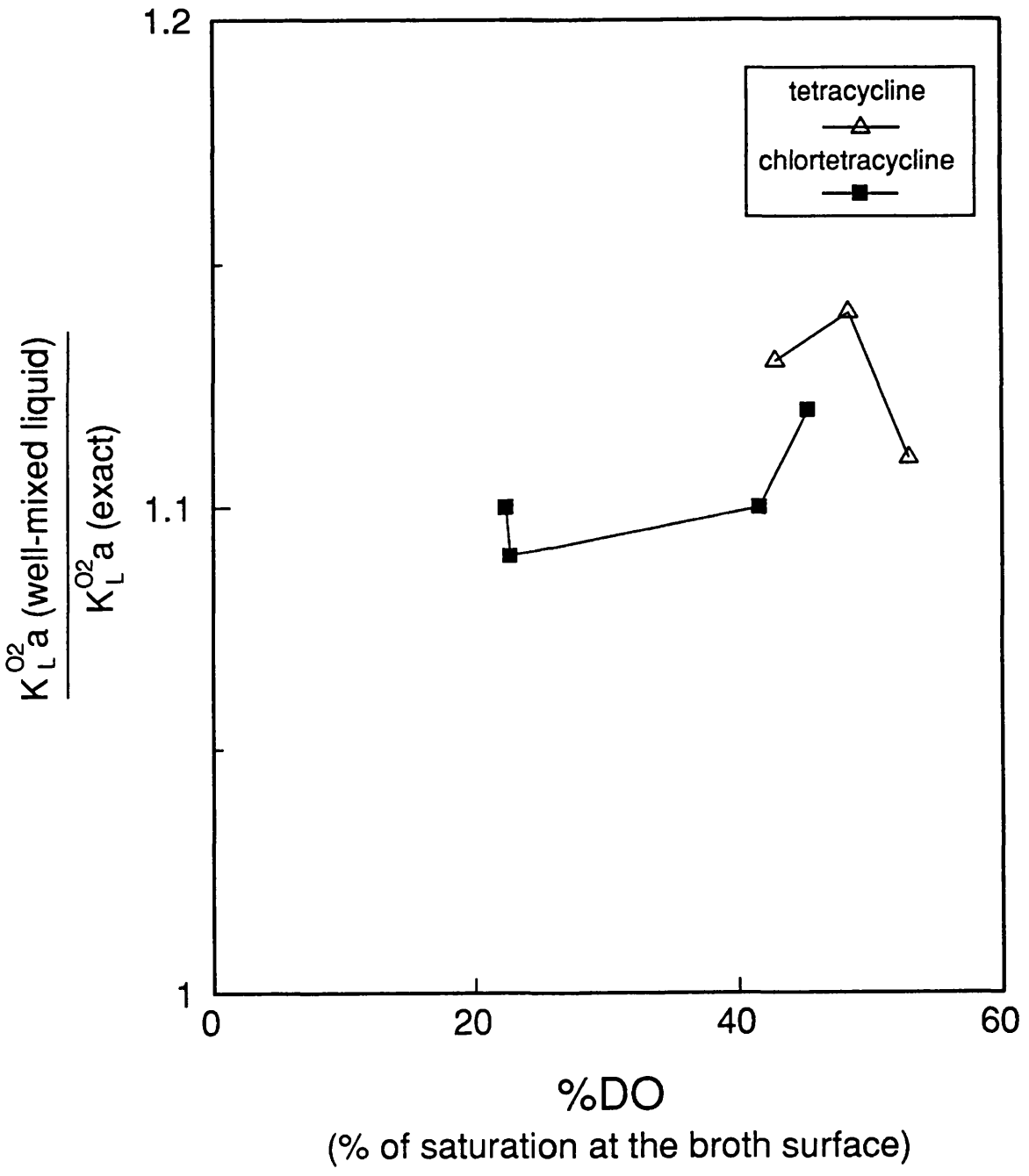


Figure 20: Error incurred in the calculation of the mass transfer coefficient, $K_L{}^{O2}a$, in a 112m^3 fermentor, by assuming a well-mixed liquid phase (deduced from the data of Manfredini et al, 1983). The X-axis indicates the dissolved oxygen level at the broth surface since, when dissolved oxygen is only measured at a single point in the fermentor, the measurement is made near the broth surface, where the dissolved oxygen is at a minimum.

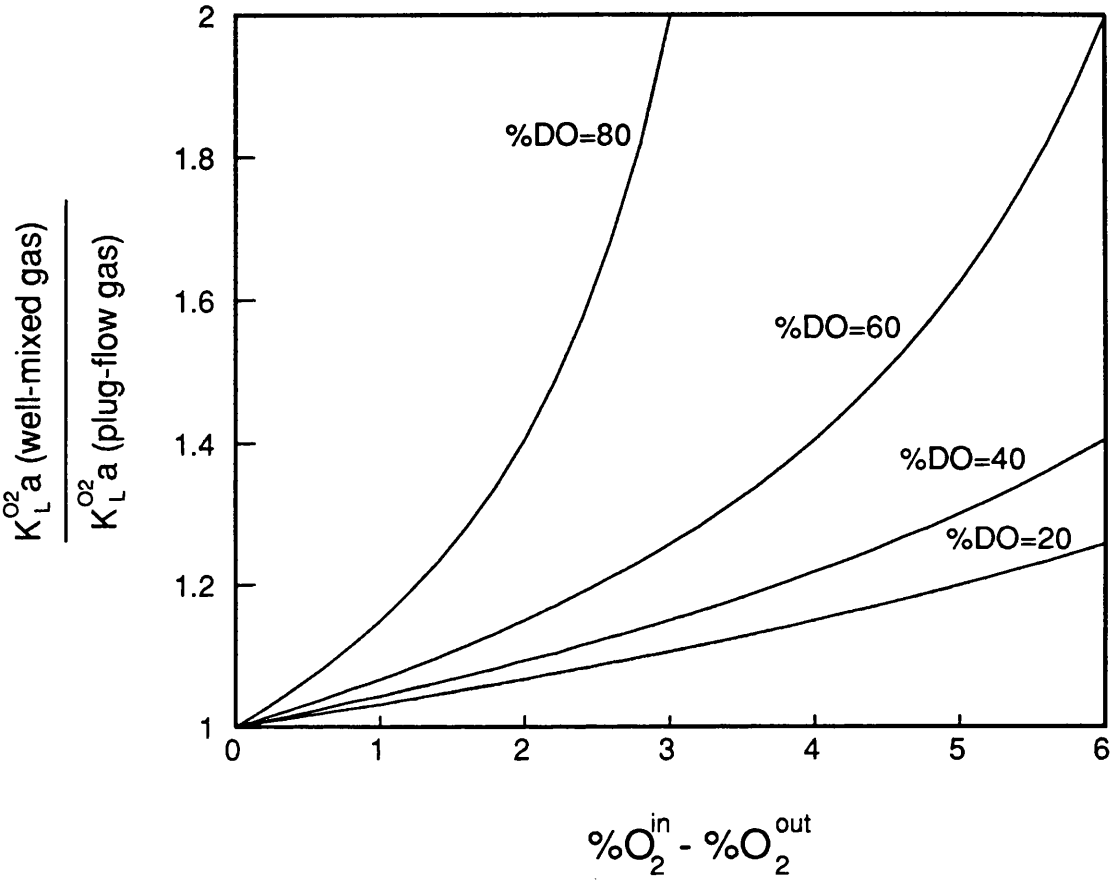


Figure 21: Sensitivity of the calculated mass transfer coefficient, $K_L{}^{O2}a$, to the assumed extent of gas backmixing, as a function of the difference between the inlet and exit gas mole percentages of oxygen, and of dissolved oxygen (as a percentage of saturation). Assumed temperature 25°C and pressure 101.3kPa. Inlet air assumed to contain 21% oxygen on a molar basis

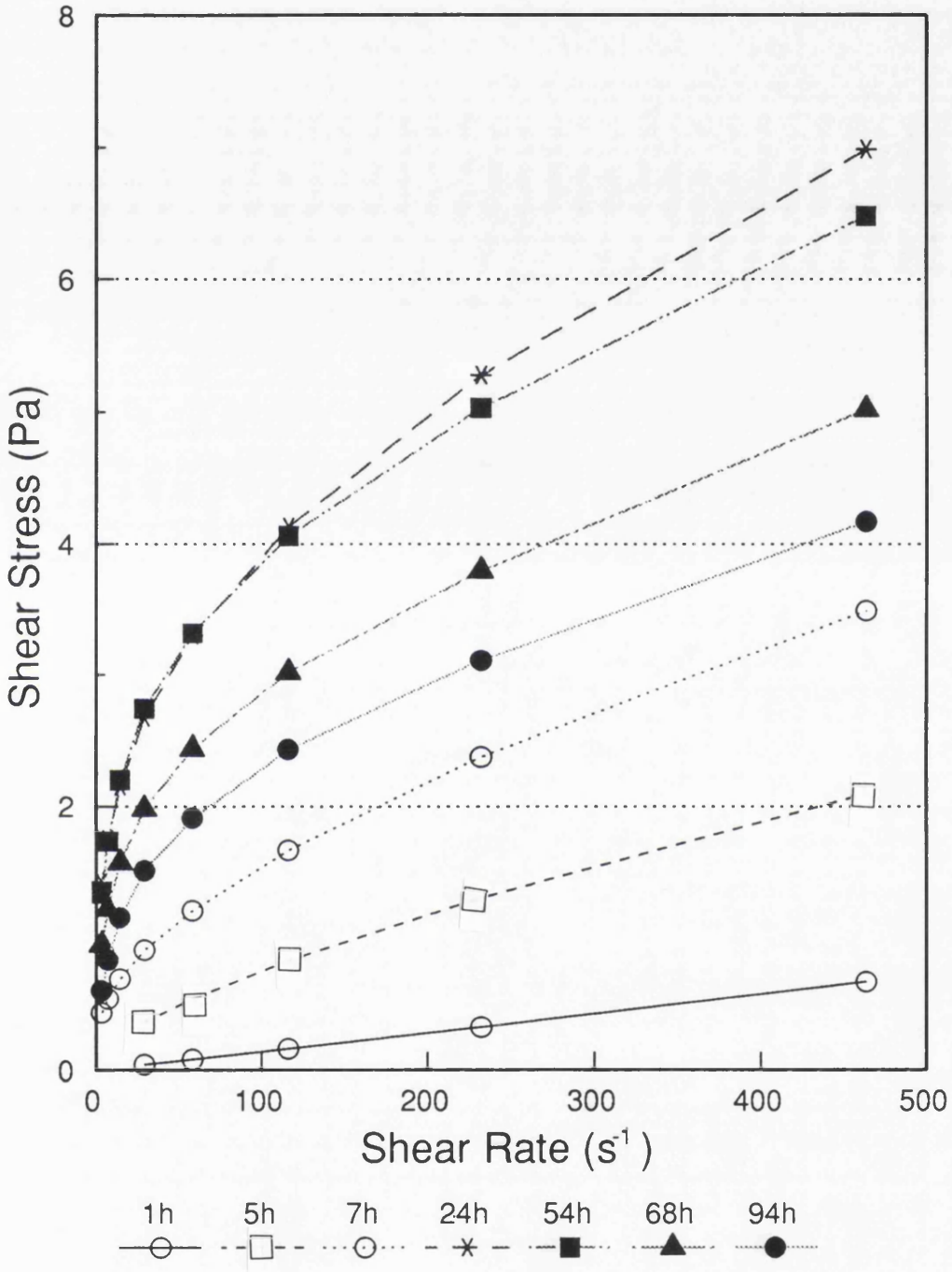


Figure 22: Changes in broth rheology during the course of a *S. clavuligerus* fermentation. The measurements were for increasing shear rates, but there did not appear to be any hysteresis, although wall-thinning caused a slow drifting of measurements with time.

describe an intermediate state of gas-backmixing may be necessary. Figure 23 presents common examples of models to describe such intermediate states of backmixing.

4.1.3 Hydrostatic pressure in large fermentors

The two most important features of large fermentors that impact on the evaluation of $K_L^{O2}a$ concern the extent of liquid backmixing, and the effect of hydrostatic pressure on the calculation. Liquid-backmixing in the largest fermentors has already been considered, and it was noted in Figure 20 that an error of perhaps 10% would be incurred in the calculated $K_L^{O2}a$ as a result of assuming a well-mixed liquid phase. Hence, in the large majority of fermentors, relatively little error will be incurred in the calculated $K_L^{O2}a$ as a result of this assumption. This is again fortunate, as there is rarely any quantitative information about the extent of liquid backmixing.

The second question posed concerns the effect of the presence of large hydrostatic pressures on the calculation of $K_L^{O2}a$. For the well-mixed liquid/well-mixed gas case (Figure 19), it can be seen graphically that integration of the partial pressure gradient for oxygen transfer, required for the calculation of $K_L^{O2}a$, simply requires that the average hydrostatic pressure (ie. half of the maximum hydrostatic pressure) be added to the head-space pressure, because p_g^{O2} varies linearly with height. The same is not true exactly of the well-mixed liquid/plug-flow gas case, as integration of the partial pressure gradient for this case results in an expression involving the error function, erf(z), which cannot be evaluated analytically. However, if instead Equation (8) is used, but with the operating pressure defined to include the average hydrostatic pressure, then the numerical result for $K_L^{O2}a$ is very close to the exact result for this case, as can be seen in Figure 24.

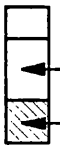
MODEL	DIAGRAM / PARAMETER	BEST DESCRIBES...
Well-mixed		Small highly-agitated fermenters
Plug flow		Large fermenters with low agitation and high aeration rates
Series of well-mixed cells	 N = number of cells	Well-agitated multi-impeller fermenters with radial-flow impellers
Plug flow with axial dispersion	 D = axial dispersion coefficient	Fermenters where broth turbulence is not a strong function of axial position
Well-mixed/Plug flow in series	α = fraction of volume well-mixed  $(1-\alpha)V_d$ αV_d	Single-impeller fermenters
Well-mixed/plug flow in parallel	α = fraction of volume well-mixed  $(1-\alpha)V_d$ αV_d	Fermenters with axial-flow impellers

Figure 23: Models commonly used to describe intermediate states of gas backmixing. Hatched areas indicate zones of well-mixedness

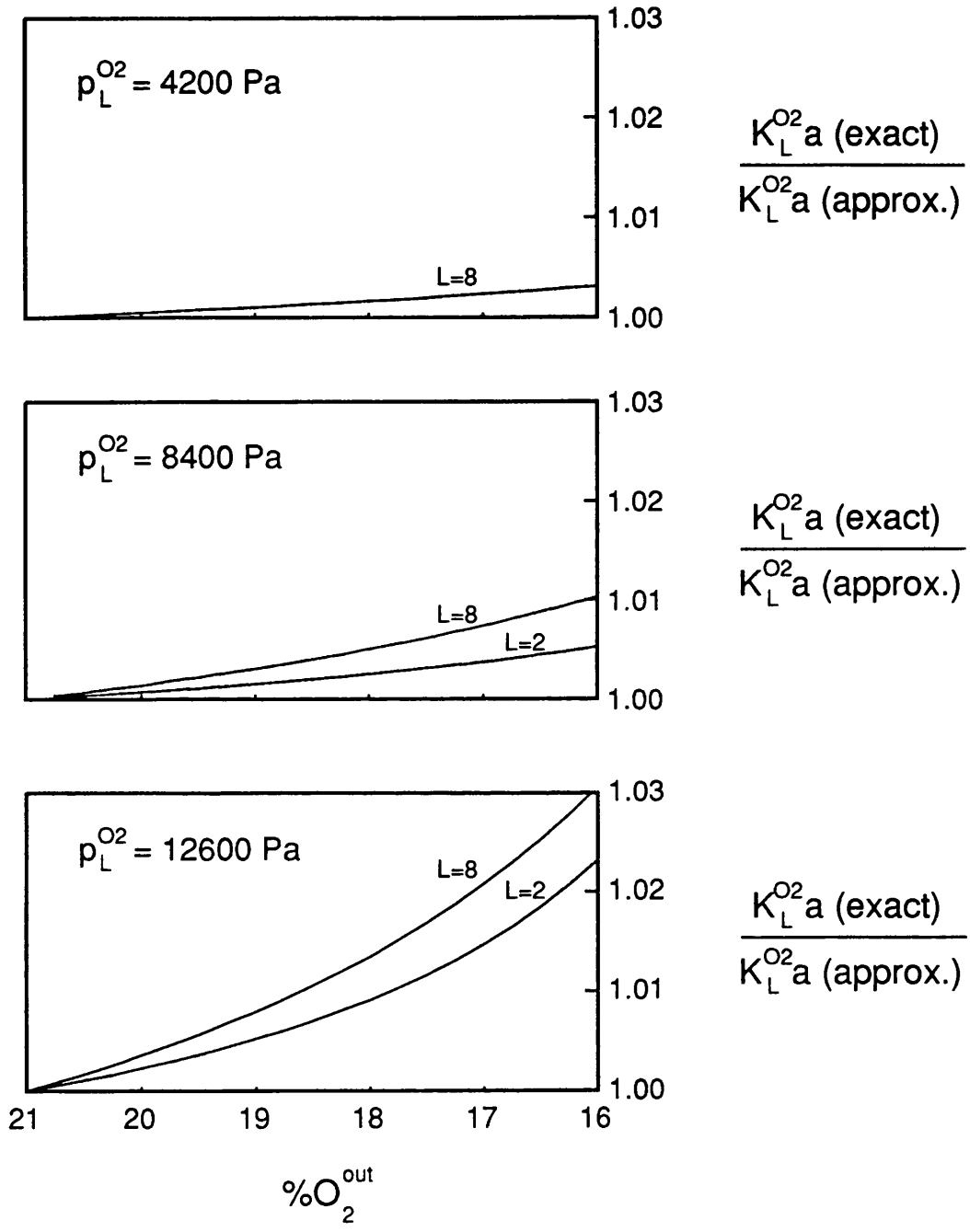


Figure 24: Accuracy of the use of the average hydrostatic pressure for the well-mixed liquid/ plug-flow gas case (pressure 1kPag., temperature 30°C, aspect ratio of working volume = 2, liquid density 1000 $kg.m^{-3}$, $\%O_2^{in}$ = 21, L = height of broth surface above fermentor bottom in m)

4.1.4 $K_L^{O2}a$ as a variable characterizing a given fermentation

The practical test of $K_L^{O2}a$ as a variable characterizing a fermentation concerns the utility of the result obtained. Mass transfer studies have long been used as a basis for scaling up fermentation agitation and aeration, by making use of mass transfer correlations. In terms of monitoring an existing fermentor rather than designing a new fermentor, the usefulness of $K_L^{O2}a$ depends to a large degree on its reproducibility from one fermentation to the next, for fermentations that are intended to be identical. A high reproducibility implies that $K_L^{O2}a$ is highly characteristic of certain conditions inherent in a given fermentation, and as such would be useful in data analysis, control and fault detection.

An indication of the reproducibility of $K_L^{O2}a$ for the *E. coli* and *S. clavuligerus* fermentations run for this project is given in Figures 25 and 26. The profiles for $K_L^{O2}a$ can be seen to be highly reproducible from one batch to the next, for both fermentation systems, which implicitly supports the conclusions already reached regarding the utility of a single value of $K_L^{O2}a$ to characterize mass transfer in fermentation.

This concludes the examination of $K_L^{O2}a$ as a variable characterizing fermentations. While a single value of $K_L^{O2}a$ is inevitably an imperfect description of mass transfer in fermentation, such a quantity retains an element of physical meaning that makes it of utility. Table 12 lists the principal points made and results obtained in this section.

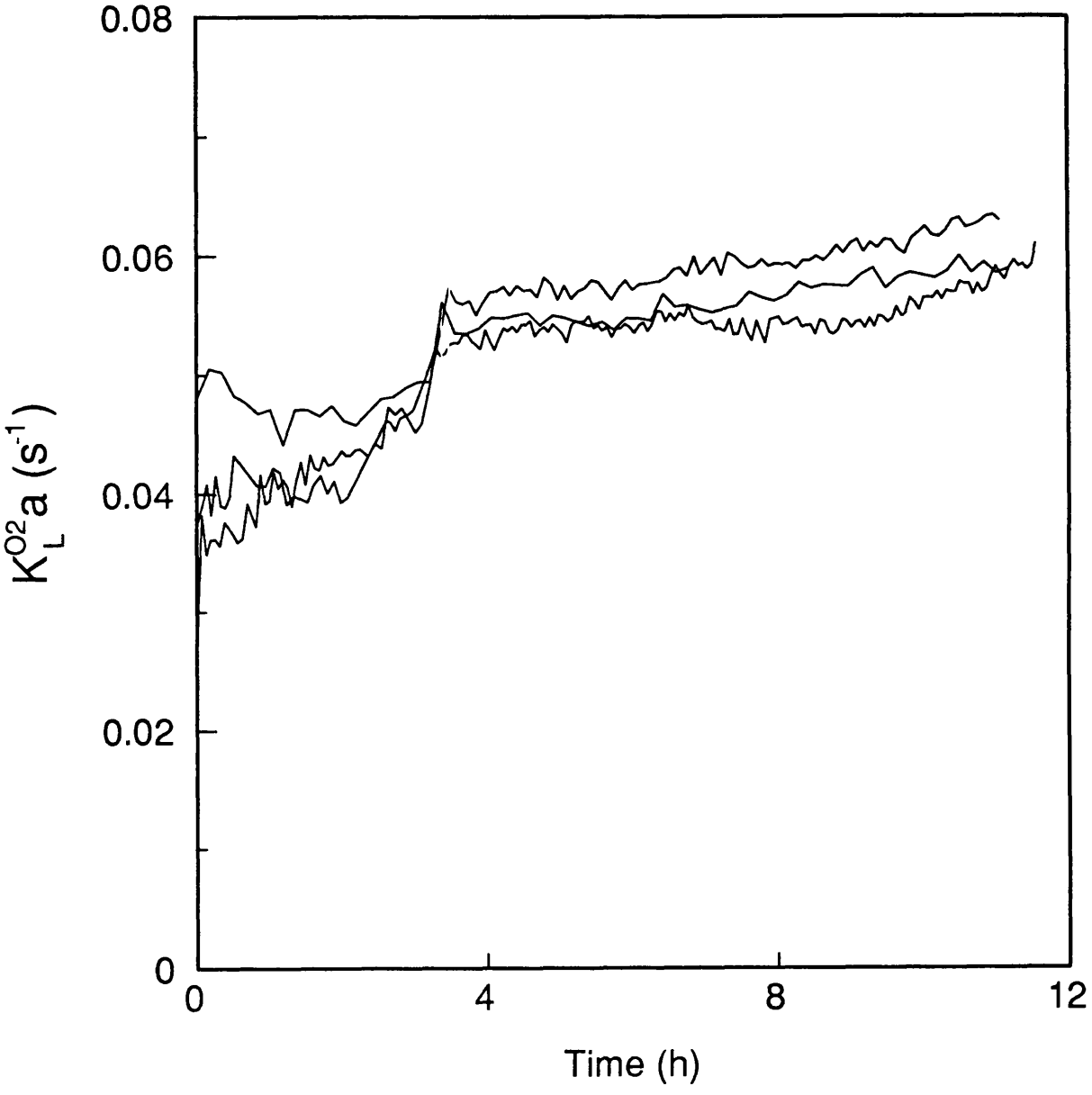


Figure 25: Comparison of the mass transfer coefficient for three *E. coli* fermentations. The values were calculated assuming well-mixed liquid and gas phases, and negligible hydrostatic pressure (Equation 7).

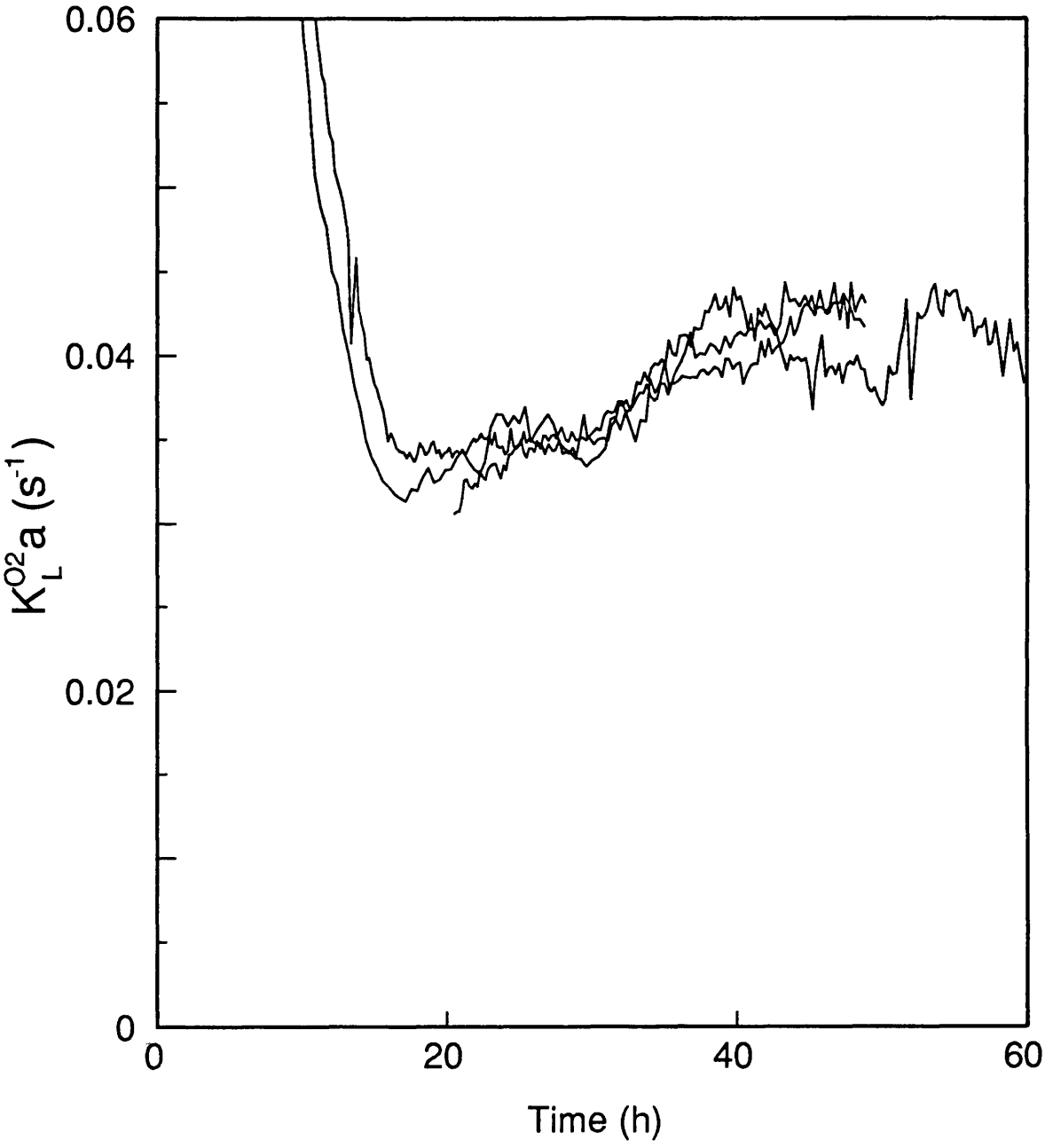


Figure 26: Comparison of the mass transfer coefficient for three *S. clavuligerus* fermentations. The values were calculated assuming well-mixed liquid and gas phases, and negligible hydrostatic pressure (Equation 7).

1. If $K_L{}^{O2}a$ for a fermentor is defined as the volume integral of the local oxygen mass transfer coefficient, it still retains some of the fundamental physical properties of the local mass transfer coefficient
2. Having defined $K_L{}^{O2}a$ *only* in terms of the local oxygen mass transer coefficient, it is possible to obtain an expression for this quantitiy that does not involve the local mass transfer coefficient. This is important because the local mass transfer coeffient is not known in pratical situations
3. The assumption of a well-mixed liquid phase will lead to, at most, an error of 10% in the calculated $K_L{}^{O2}a$, even in the very largest fermentors
4. The assumed extent of gas backmixing (from well-mixed to plug-flow) can have a significant impact on the calculated $K_L{}^{O2}a$ if the difference between the percentages of oxygen in the inlet and exit gas streams, and the level of dissolved oxygen are both large
5. Use of the average hydrostatic pressure in large fermentors, for calculating $K_L{}^{O2}a$ leads to negligible error, irrespective of the assumed extent of gas backmixing
6. The calculated $K_L{}^{O2}a$ for a given fermentation system is reproducible from one fermentation batch to the next, and therefore a useful and meaningful parameter for characterizing fermentations

Table 12: Principal results and conclusions arising from Section 4.1 concerning the justification for treating $K_L{}^{O2}a$ as a physically meaningful parameter characterizing a given fermentation

4.2 RANDOM NOISE IN FERMENTATION DATA, AND ITS REMOVAL FROM OTR DATA

This section examines opportunities for signal conditioning of fermentation measurements, in particular concerning the magnitude of noise in fermentation data, and ways to reduce any noise present. It is concluded that noise in outputs from analog sensors (like temperature, pH and dissolved oxygen) is not a problem because the noise is of sufficiently high frequency, and these measurements available at a sufficiently high sampling rate, that noise can be reduced to very low levels by digital filtering. Gas analyses, by contrast, are only available relatively infrequently (say with a frequency of 0.1 min^{-1}). This does not necessarily pose a problem, as gas analyzers are high performance instruments, and their measurements contain little noise and bias. However, the oxygen transfer rate, or OTR, (which indicates the OUR, an important derived variable) is calculated as a small difference between two large numbers (the inlet and exit gas oxygen analyses). As a result, small amounts of noise and bias in raw oxygen analyses which appear in the calculated OTR, can be a much more significant proportion of the OTR. The problem is quantified, and a way to remove noise in OTR data that can be applied on-line during fermentations, is proposed.

The work on OTR data conditioning reported in this section was published in Royce and Thornhill(1992), which is presented in Appendix A1.

4.2.1 High-frequency noise in analog sensor outputs

The most commonly encoutered *in situ* analog sensors measurements in fermentation are the pH, temperature, dissolved oxygen, agitation, aeration and pressure. All these measurements, with the exception of the pH and dissolved oxygen sensors, are highly reliable and robust, and noise in these measurements is not a problem. However, the pH and dissolved oxygen probes produce very small signals, which necessitates the use of high amplification gains. As a result, the amplified signals from these sensors contain a component of mains noise and Johnsons' noise.[†] The noise is of relatively high frequency by comparison with the time scale over which changes in fermentation variables take place. Hence such noise can be digitally filtered at a rate that is only limited by the capabilities of the hardware. The analog signal in addition is often itself considerably filtered within the amplifier.

Problems could arise, however, if a significant amount of noise is present in pH and dissolved oxygen measurements with a much lower frequency than that associated with mains noise. Figure 27 shows the output from the pH probe (immersed in pH 7 buffer) and the dissolved oxygen probe (immersed in saturated water), after being 16-bit digitized[‡] and sampled at 1Hz. The noise with frequency of order 1 Hz in this figure is the Johnson' and mains noise which can easily be digitally filtered. There is no evidence in this figure of significant amounts of noise with a much lower frequency than 1 Hz. Hence, noise in analog measurements was not considered further in this project.

4.2.2 Analysis of noise and bias in gas analysis data from an *S. clavuligerus* fermentation

Instruments available for the analysis of fermentation gases are both highly accurate and reliable. For example, an accuracy of gas analyses of ±0.1%, and calibration drift of less than 1% per month have been reported (Buckland et al, 1985) for a mass

[†] due to thermal fluctuations

[‡] 16-bit digitization implies higher resolution than available from 12-bit digitizers that are more normally used for signal digitization

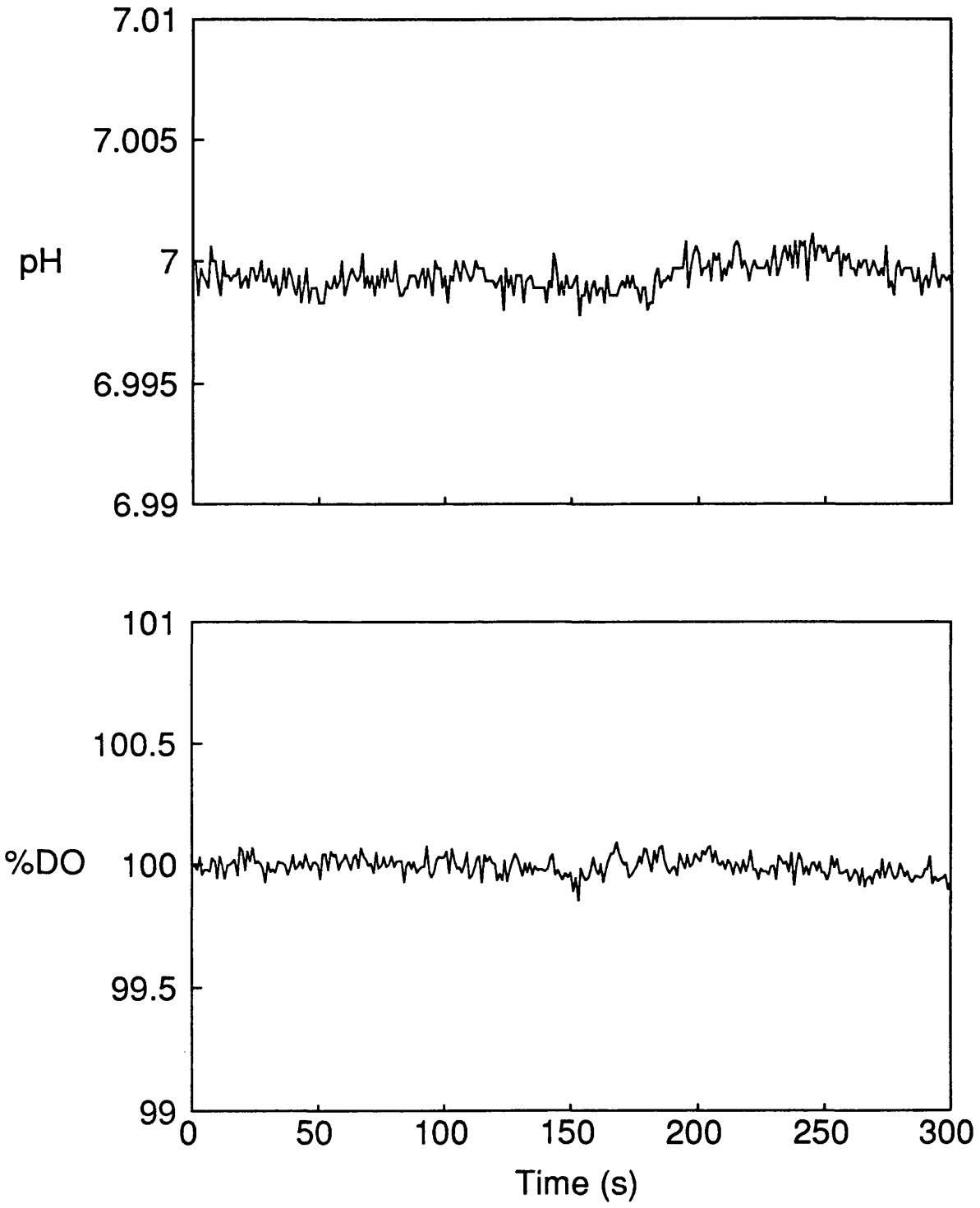


Figure 27: Noise in the output from the pH probe (immersed in pH 7 buffer) and the dissolved oxygen probe (immersed in saturated water), sampled at 1Hz and 16-bit digitized

spectrometer with a magnetic sector of fixed field strength, used in a fermentation facility. The fermentation variables of greatest interest depend on the difference between the molar percentages of oxygen and carbon dioxide in the inlet and exit gas streams, which, being indicative of the molar oxygen and carbon dioxide transfer rates, are here called the %OTR and %CTR respectively.

$$\%OTR = \%O_2^{in} - \%O_2^{out} \cdot \left(\frac{\%N_2^{in}}{\%N_2^{out}} \right)$$

$$\%CTR = \%CO_2^{out} \cdot \left(\frac{\%N_2^{in}}{\%N_2^{out}} \right) - \%CO_2^{in} \qquad (9)$$

The superscripts indicate the inlet (in) and exit (out) gas stream analyses.

Fermentation variables such as the carbon dioxide evolution rate (CER), oxygen uptake rate (OUR), mass transfer coefficient ($K_L{}^{O2}a$), and respiratory quotient (RQ) require the %CTR and %OTR for their calculation, and hence improving the quality of the %CTR and %OTR improves the quality of derived variables. Under steady-state condtions, the %CTR and %OTR can be used directly to yield these fermentation variables:

$$CER = \frac{\%CTR}{100} \cdot G \qquad OUR = \frac{\%OTR}{100} \cdot G$$

$$RQ = \frac{CER}{OUR} = \frac{\%CTR}{\%OTR} \qquad (10)$$

$$K_L^{O_2}a \propto \%OTR$$

where G = molar aeration rate

Air fed to a fermentor contains about 0.04% CO_2 and 21% O_2. At most one quarter of the oxygen fed is consumed (Fox, 1984). As the RQ is generally around 1, the %CTR is approximately equal to $\%CO_2^{out}$, while the %OTR is calculated as a small difference between two large numbers. Gas analyses contain an element of noise and fixed bias, which is inevitably reflected to some extent in the calculated %CTR and %OTR. As $\%CO_2^{in}$ is close to zero, the noise and bias in the %CTR will be of order

that in $\%CO_2^{out}$ analyses, and should therefore be very small. The purpose in this section is to examine the extent of noise and bias reflected in the %OTR, on which the most important on-line derived fermentation variables depend.

Bias in the %OTR

If the cause of bias affects the instrument slope (ie. sensitivity) or zero point, then the proportion of bias in the %OTR will be no greater than that in the raw oxygen analyses, and may even be less, as the individual bias' cancel out to some extent. If, however, a source of bias affects the inlet or exit gas stream analyses independently of one another, then this fixed error will become an increasing proportion of the %OTR as the %OTR falls. Truncation error (arising because single-precision calculations on computer are truncated to eight figures) may also become an increasing proportion of the %OTR as the %OTR falls.

Circumstantial evidence for this effect has been found by examining gas analysis data during the early part of a fermentation. Figure 28 presents RQ and %OTR data at the beginning of a fermentation of *S.clavuligerus*. Initially the calculated RQ is considerably above 1, settling to a value of 0.9 at 2h, at which time the %OTR is around 0.2. The high values of the RQ during the earliest stages of the fermentation are due to bias in the %OTR because the direction of settling to RQ=0.9 was found not to be reproducible from one fermentation to the next, sometimes being from above the 0.9 level, and sometimes from below. Such variability is unlikely to reflect real variations in the RQ, as the variability is large, when the "real" RQ is normally highly reproducible from one fermentation to the next. As the %OTR is much greater than 0.2 during most fermentations, bias in the %OTR will not normally be a problem for the gas analysis system used here. For other systems this threshold may be higher.

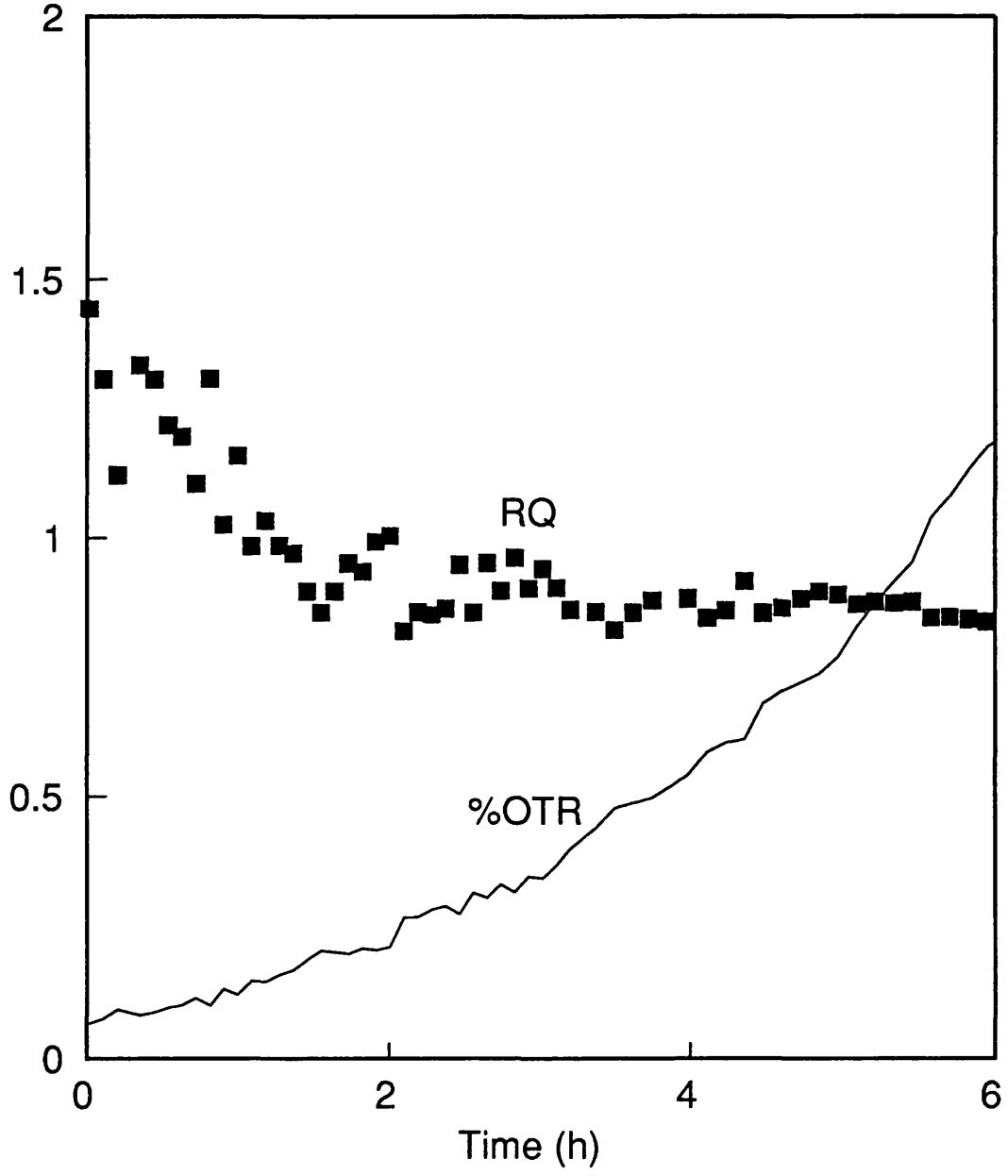


Figure 28: The RQ and %OTR during the early stages of a *S. clavuligerus* fermentation

Noise in the %OTR

Noise in the $\%O_2^{in}$ analysis, for the mass spectrometer used here, has a standard deviation of 0.04% of the signal, the effects of which can be seen in Figure 29, which presents the reference (ie. inlet) gas analyses over a 12h period. The exit gas oxygen content from the fermentor is only slightly lower than the inlet content. The absolute standard deviation of noise in the %OTR will therefore be independent of the %OTR. This noise will become a larger proportion of the %OTR as the %OTR falls. This can be seen in Table 13, which tabulates the standard deviation of noise in samples of 10 %OTR data points during a fermentation of *S.clavuligerus*. For %OTR < 0.2, the %OTR data are dominated by noise, and, for example, a noise standard deviation of 22% was found for a %OTR=0.1. At higher values of the %OTR, the noise will still significantly corrupt derived variables.

Characterization of noise in a signal requires that the noise amplitude be measured relative to some smoothed signal baseline. Where the signal is known to be constant, this baseline is easily generated. Where the signal is varying, the method used to generate a baseline may influence the resulting analysis of noise. In this work, the noise data set were generated from a 500 point set of %OTR data shown in Figure 30, which were available at the relatively high (and constant) frequency of $0.67min^{-1}$ and which, while not constant, only decreased slowly and approximately linearly with time, being from the glycerol-based growth phase of a fermentation of *S. clavuligerus*. This meant that the method used to generate the baseline should have little impact on the conclusions of the noise analysis, and this was confirmed by using both chi-square and moving average filters to generate the baseline, and comparing the results.

The noise data set is plotted in Figure 31, and in Figure 32 the autocorrelation for this 500 point noise data set is presented. A criterion for white noise (Norton, 1986) is that the autocorrelation at non-zero lags as a fraction of that at zero lag should be

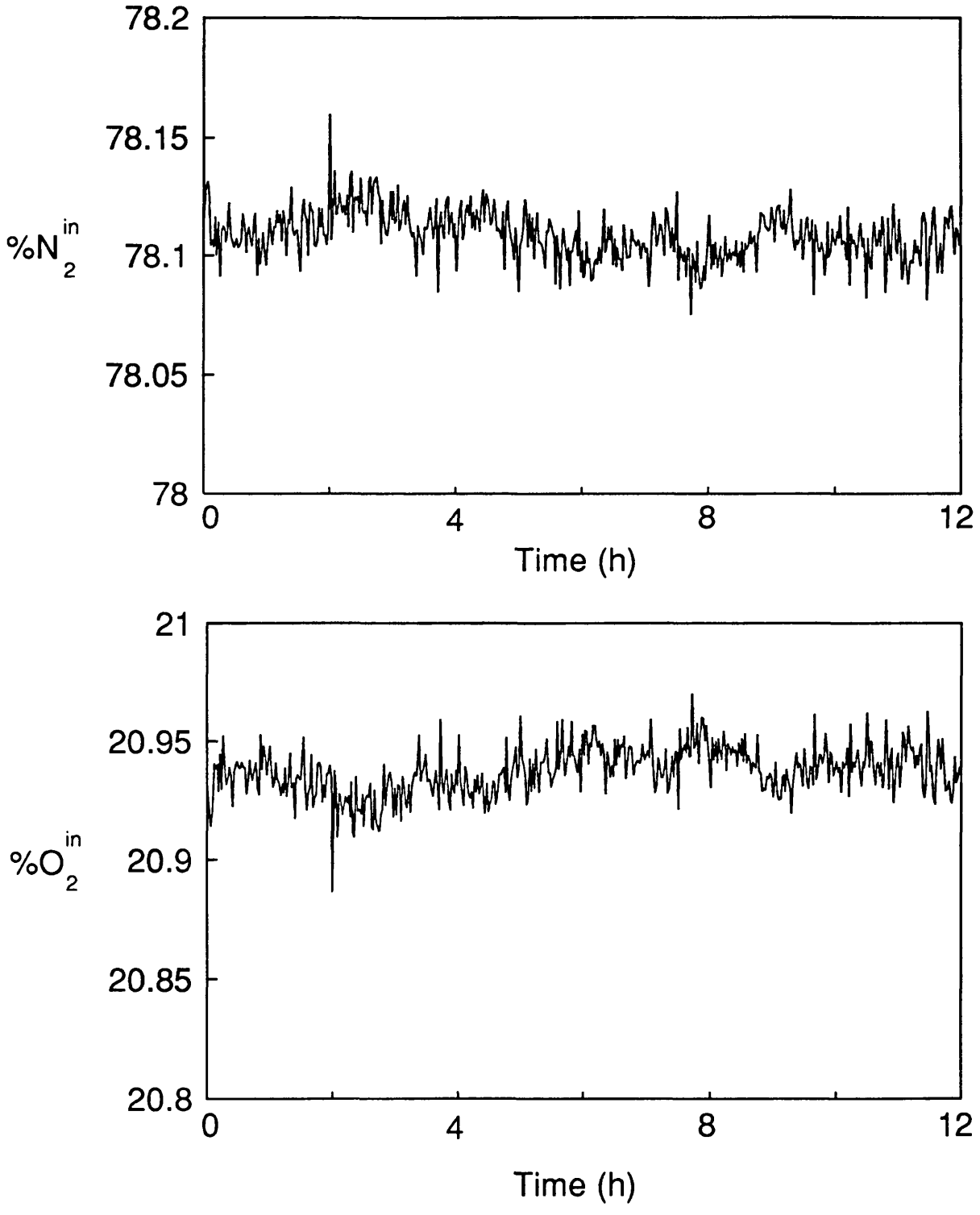


Figure 29: A 12h sample of reference gas analyses

%OTR	Standard Deviation of Noise as a percentage of the %OTR
2.0	0.8%
1.0	1.5%
0.5	2.1%
0.2	8.5%
0.1	22.0%

Table 13: The standard deviation of noise in 10-point sets of %OTR data as a percentage of the signal, during a fermentation of *S. clavuligerus*

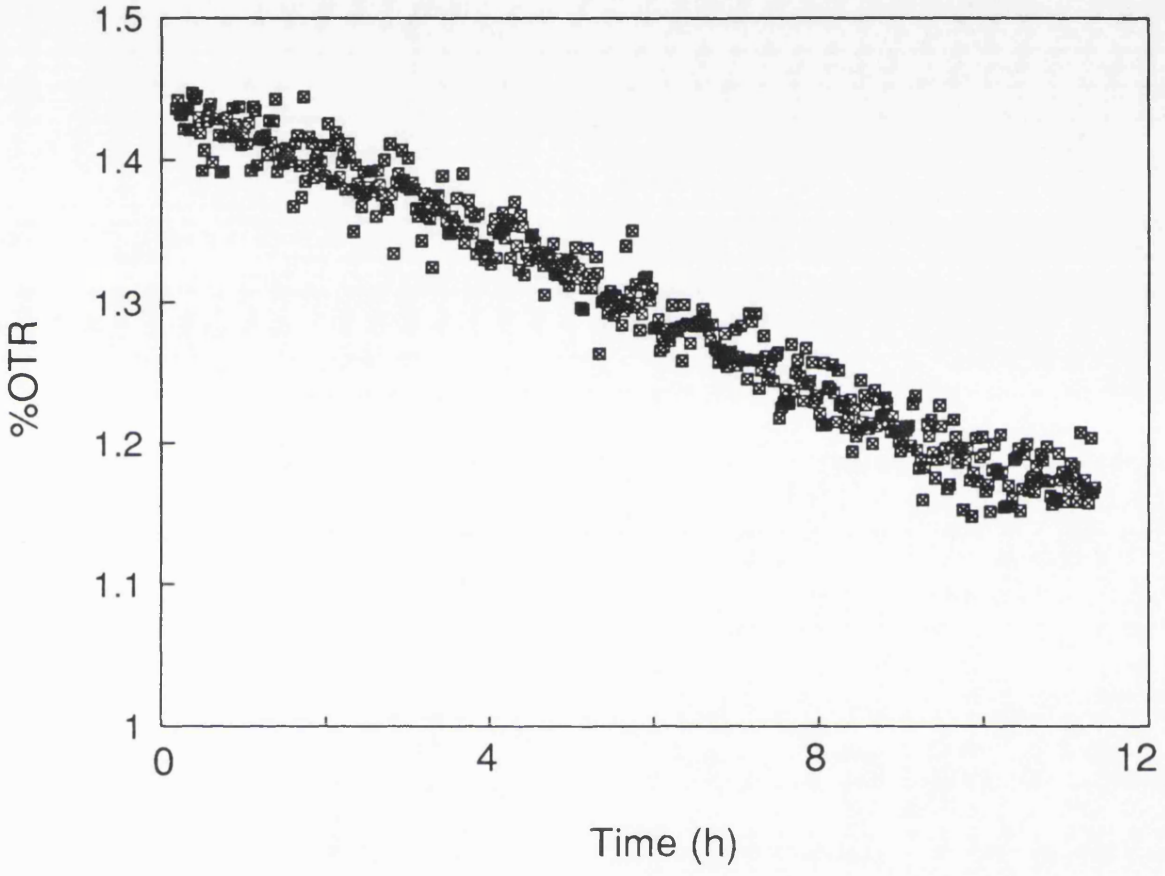


Figure 30: Section of 500 %OTR data points from the glycerol-based growth phase of a *S.clavuligerus* fermentation, which were used for the analysis of noise in OTR data

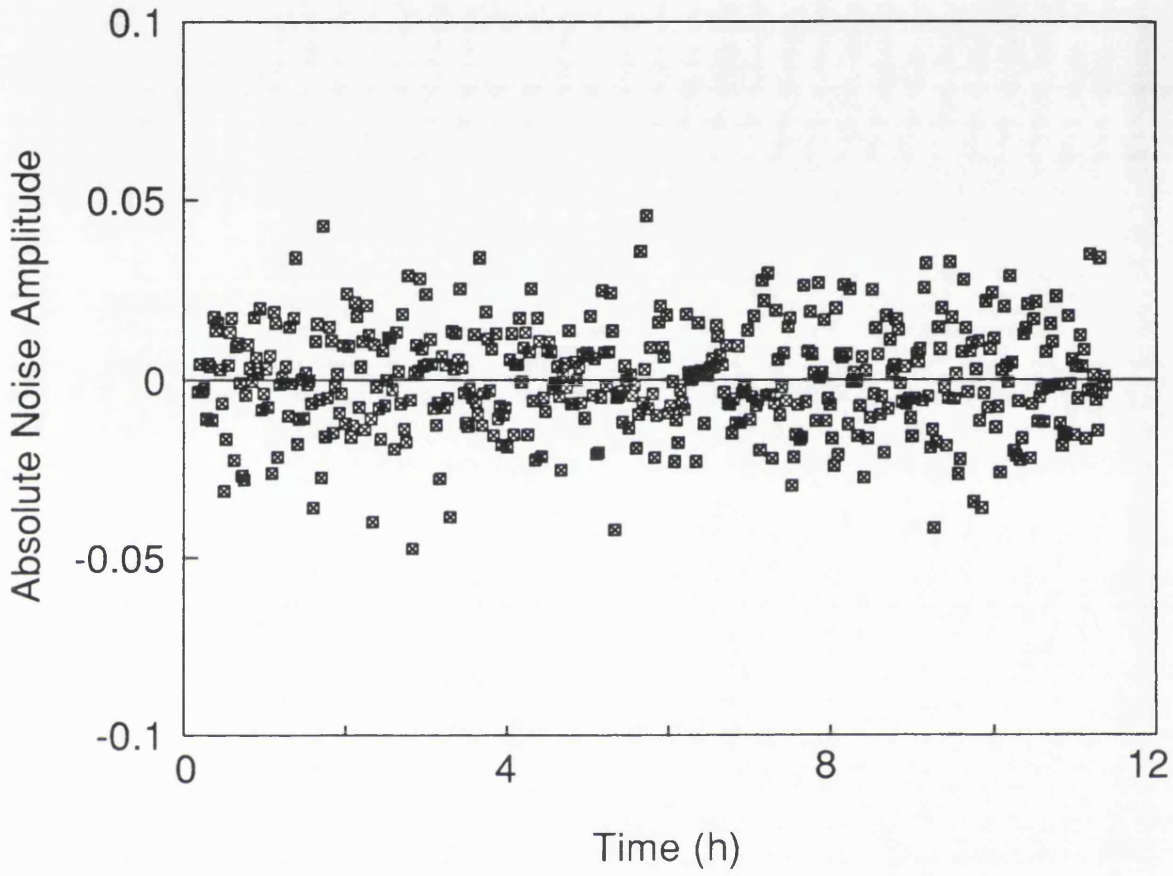


Figure 31: Noise in the %OTR data displayed in Figure 30, from a *S. clavuligerus* fermentation

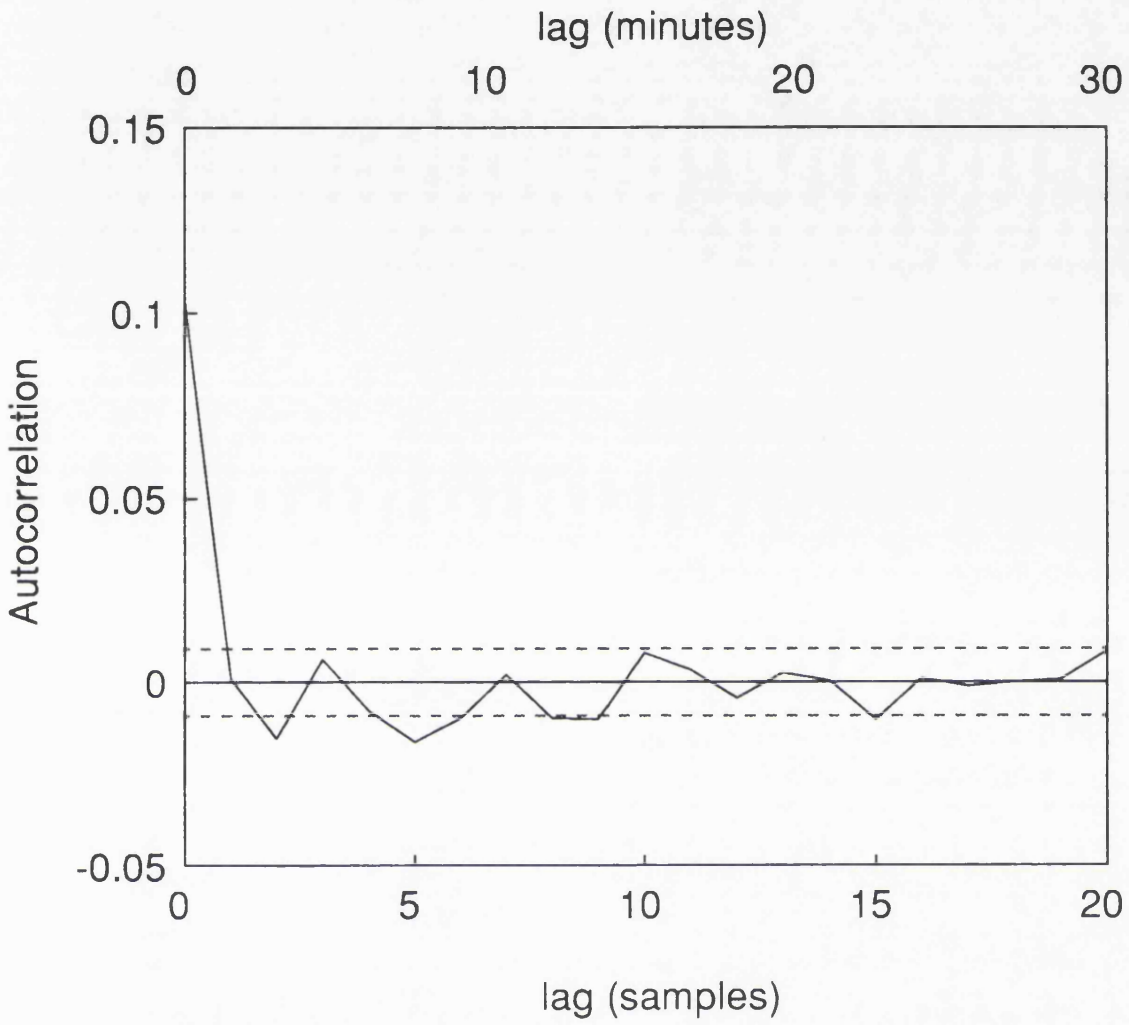


Figure 32: Autocorrelation of the noise data set presented in Figure 31. According to the criterion of Norton(1986), the noise data are essentially uncorrelated (ie. random)

less than $\pm 2/\sqrt{N}$, where N is the number of samples of noise. For a 500 point data set, this requires the autocorrelation at non-zero lags to be less than 9% of that at zero lag. This criterion is also indicated in Figure 32. It is slightly exceeded at lags of 2 and 5 samples, but the data are not strongly correlated. Analysis of other %OTR noise data sets showed similar results, although the lags at which the criterion was exceeded were different, again indicating the noise in the %OTR data to be very weakly autocorrelated, and hence approximately white.

The probability distribution of noise amplitude for the %OTR data set can be seen in Figure 33 to be approximately Gaussian, with an amplitude standard deviation of 0.0145. This reflects the individual contributions of noise in the inlet and exit gas analyses. The standard deviation of noise in the $\%O_2^{in}$ and $\%O_2^{out}$ analyses is 0.008 (0.04% of the signal), as already noted. When $\%O_2^{out}$ is based on the inlet aeration rate, its noise standard deviation increases to 0.012, due to noise in the nitrogen analyses. The standard deviation of noise in the %OTR is the square root of the sum of the inlet and exit gas oxygen analysis noise variances, as is to be expected for normally-distributed noise data sets.

4.2.3 Removal of noise in OTR data from an *S. clavuligerus* fermentation

Since the noise in the %OTR is approximately white, a model-based method for noise removal rather than a spectral method is required. As the noise amplitude probability distribution is Gaussian, a least squares method such as a chi-square filter will give a maximum likelihood estimate of the %OTR and model parameters. The %OTR profile for an entire fermentation is commonly made up of two or more growth phases, and it is rare for a good model to be available to describe this profile. It is more practical to use a convenient class of function, such as polynomials, applied to a reduced data set. The simplest polynomial is a constant function, which could be used to produce a moving average of the most recent %OTR data points. This is not a suitable filter for real-time application to %OTR data, as the estimate

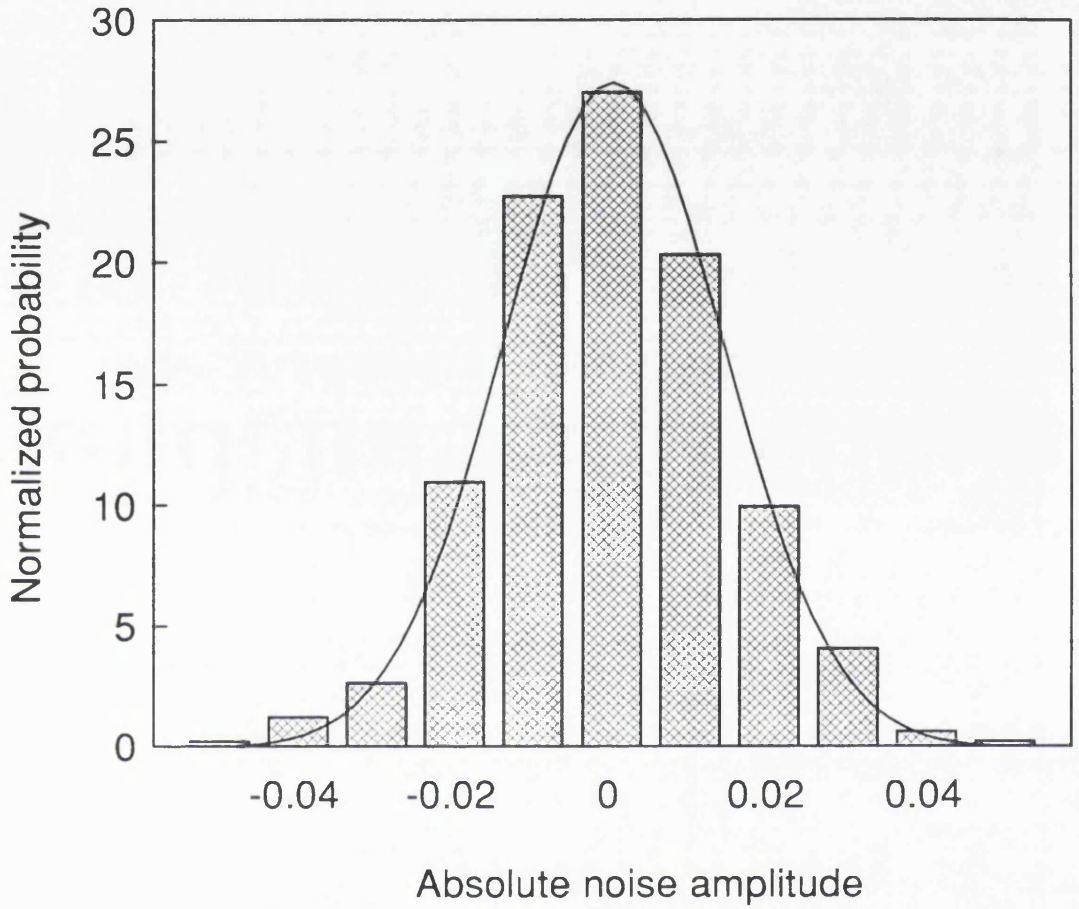


Figure 33: Noise amplitude probability distribution for the noise data set presented in Figure 31. The continuous line is the Gaussian distribution with standard deviation 0.0145, indicating the noise to be normally distributed

applies to the midpoint of the time range covered by the reduced data set to which the moving average is applied. Gas analyzers for fermentation applications, being expensive instruments, are multiplexed between several fermentors, which means that gas analyses for a given fermentor are typically only available at a relatively low frequency of around 0.1-0.2min^{-1}. Hence a 10-point moving average, for example, would produce %OTR estimates around 40 minutes old. The next simplest polynomial is a linear model, which was used in this work. To obtain a real-time estimate of the %OTR, a chi-square filter with the noise standard deviation specified as 0.0145, is used to fit a straight line to a time window of %OTR data, as can be seen in Figure 34. Clearly, the time window has to be narrow enough so that the data within the window are at least reasonably linear, while at the same time, containing a sufficient number of data points for the parameter estimation. In Figures 35, 36, 37, 38 and 39, the effect of increasing the window width from 0h to 3h can be seen, for gas analysis data that were available at a rate of one data point every 6 minutes (frequency 0.17min^{-1}), a figure that is compatible with data frequencies commonly achieved in industry. Increasing the window width has the effect of reducing high frequency noise in the %OTR data, but also causes variations in the %OTR that are not random, to be increasingly smoothed. An example of such a non-random variation is the transient variation in the %OTR at 7h, that was caused by a change in the agitation rate. An intermediate window width, of say 1 h is best, in removing a large proportion of the noise, while not excessively smoothing real changes in the %OTR, and was used in the examination of the effect of %OTR filtering on the calculated $K_L^{O2}a$ and RQ.

In Figure 40, the RQ and $K_{LO2}a$ data for a fermentation of *S. clavuligerus*, calculated from unfiltered %OTR data, are presented. The $K_L^{O2}a$ data assumed well-mixed liquid and well-mixed gas phases. During the first 10h of the fermentation, the broth displayed Newtonian rheology, giving very high mass transfer coefficients (off-scale) at the chosen agitation rate. Once the concentration of mycelial biomass had risen sufficiently, broth rheology became plastic as is the case for most mycelial

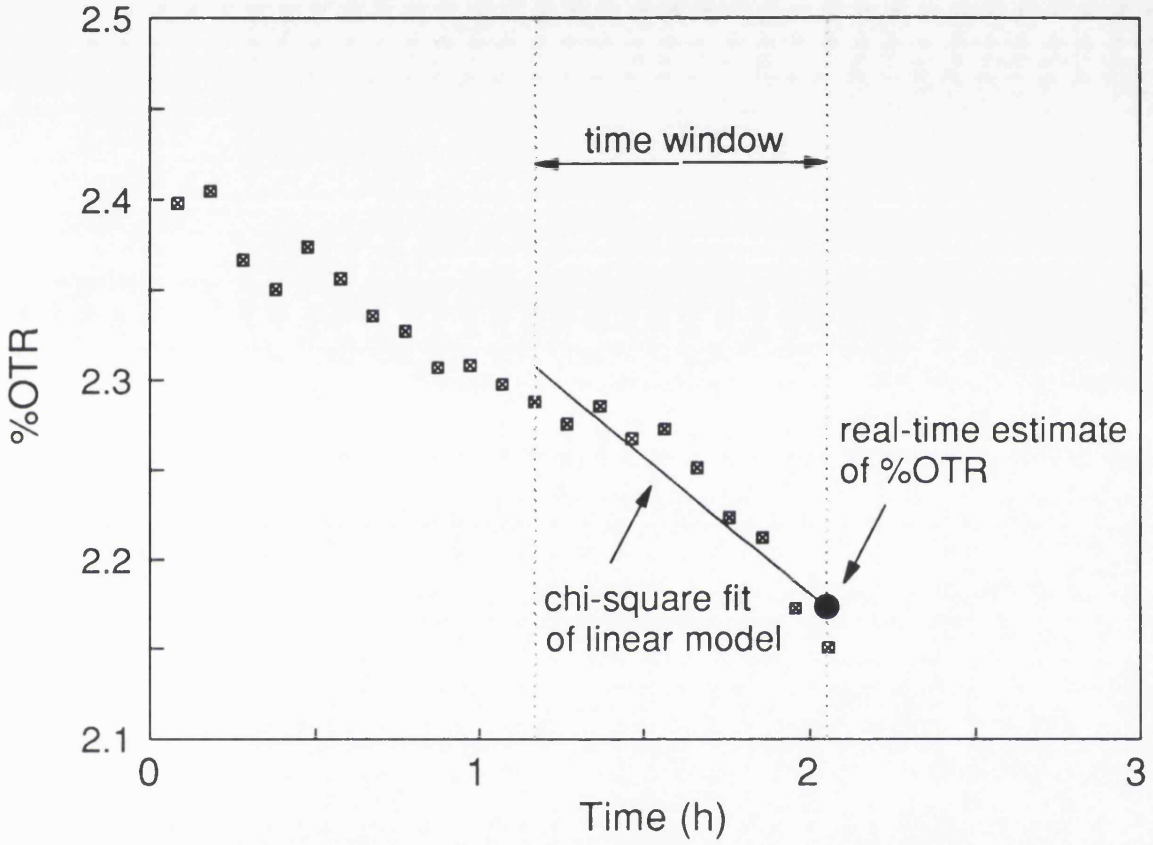


Figure 34: Methodology for removing noise from %OTR data using a chi-square fit of a linear model to a moving window of %OTR data

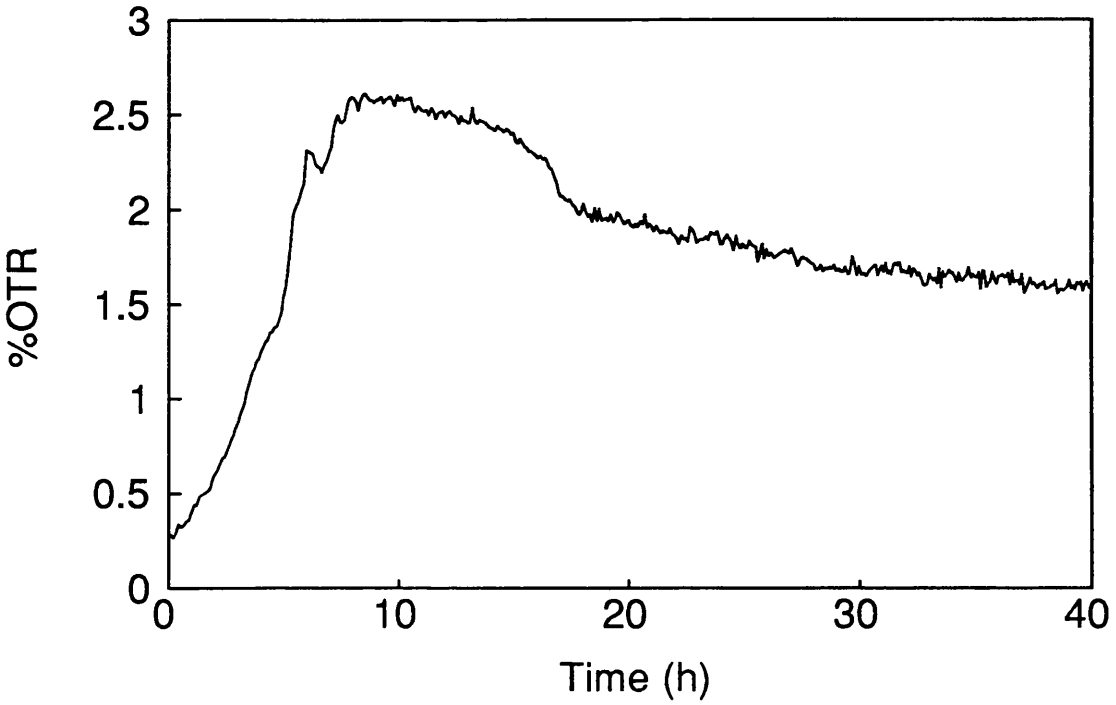


Figure 35: Unfiltered %OTR data during a *S. clavuligerus* fermentation. The noise visible is random, with the exception of the disturbance at 7h, which is due to an increase in the agitation rate

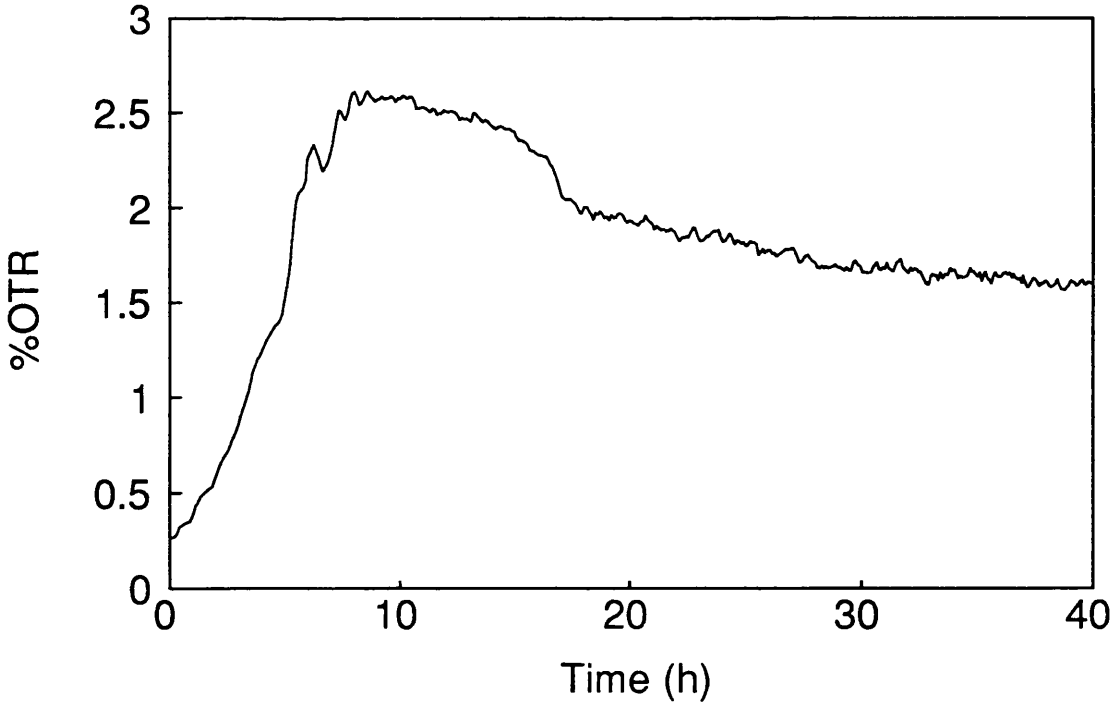


Figure 36: The %OTR after filtering with a time window of ½h (5 points)

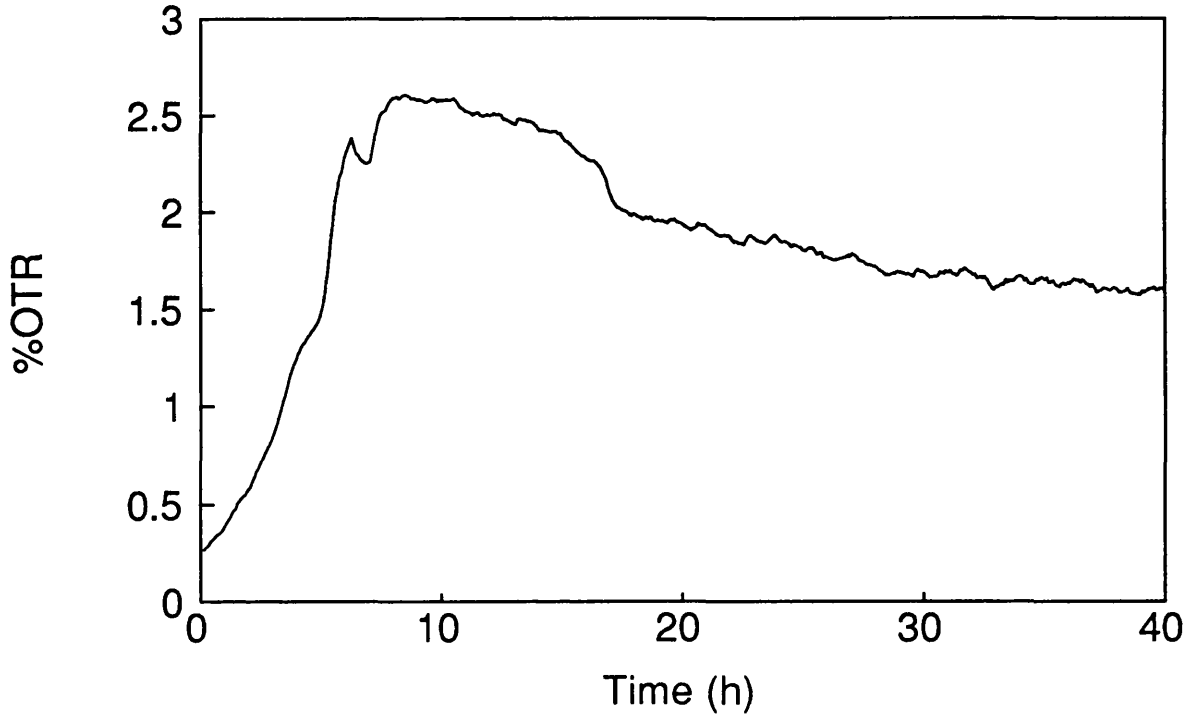


Figure 37: The %OTR after filtering with a time window of 1h (10 points)

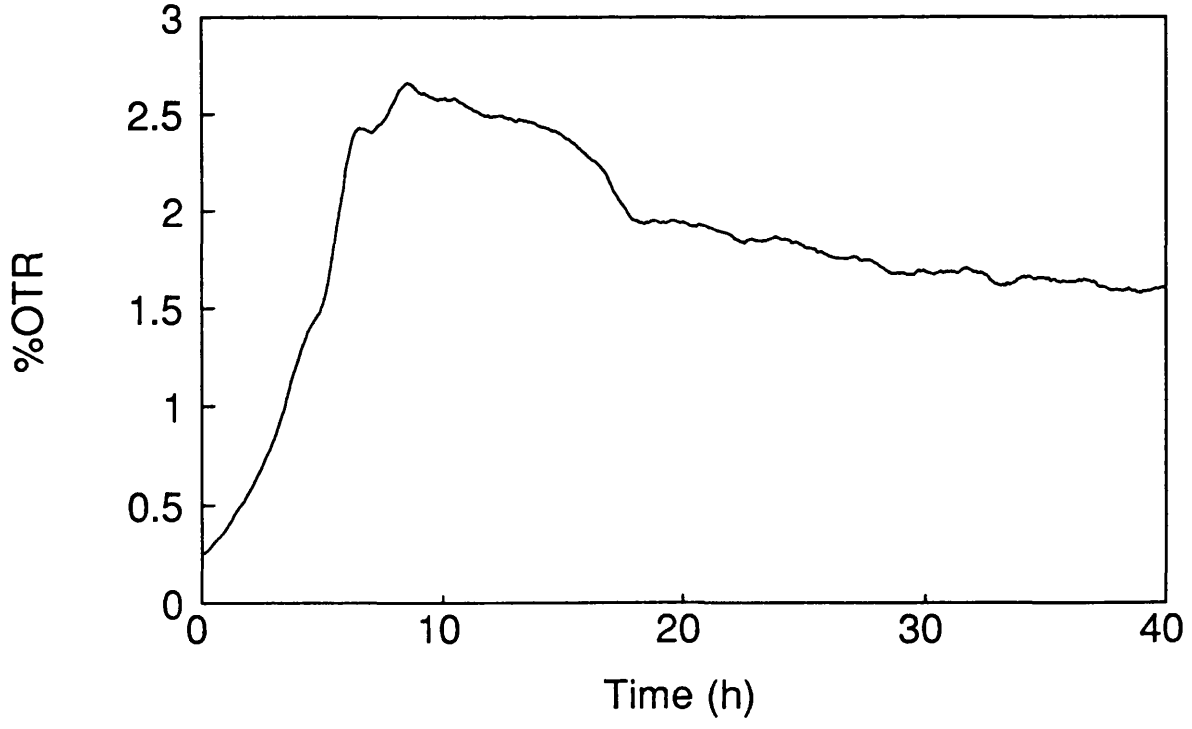


Figure 38: The %OTR after filtering with a time window of 2h (20 points)

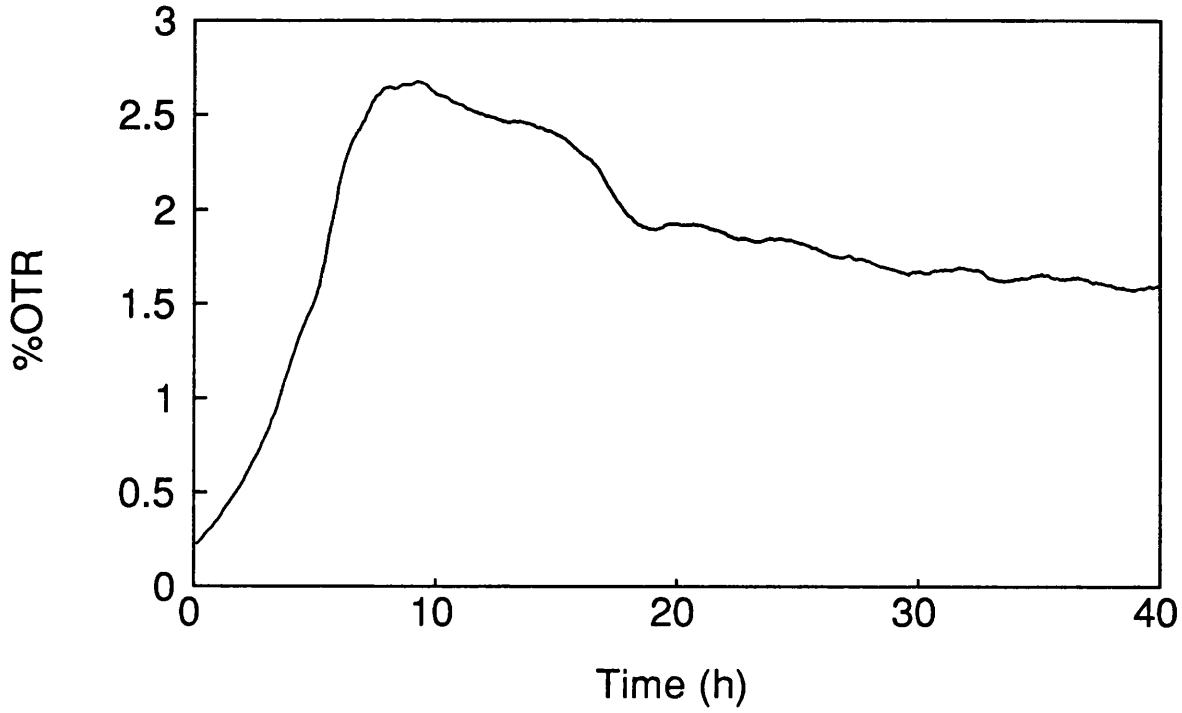


Figure 39: The %OTR after filtering with a time window of 3h (30 points). The non-random disturbance visible in Figure 35 at 7h has been smoothed over

fermentations, with a concomitant fall in the mass transfer coefficient. In Figure 41, the same data are presented after filtering of the %OTR data using the method proposed with a time window of width 1h. There can be seen to be a considerable improvement in the quality of the calculated RQ and K_La with filtering of the %OTR. The disturbances in the RQ remaining after filtering are non-random and associated with the effect of pH control action on the %CTR (Stephanopoulos and San, 1984; Royce, 1992). The noise remaining in the $K_L{}^{O2}a$ data are associated with the measurement of dissolved oxygen. While the filter used is suggested by the nature of the problem, it is by no means the only, or necessarily the best method for filtering noise from the %OTR data of an arbitrary fermentation. For example, use of a linear model requires the data set in the time window to be at least reasonably linear. For fast-growing organisms this may require a narrow time window, which may contain an insufficient number of gas analysis data points for good noise filtering. In such situations, a quadratic or cubic model applied to a wider time window may produce better results. It is however believed that the very simple filter proposed here could be suitably applied to a large number of industrially important fermentations, where relatively low growth rates coupled with the smoothly continuous growth usually generated by the undefined media used will produce %OTR profiles well suited to this type of filter.

This concludes the examination signal of conditioning of fermentation measurements. The principal results obtained are summarized in Table 14.

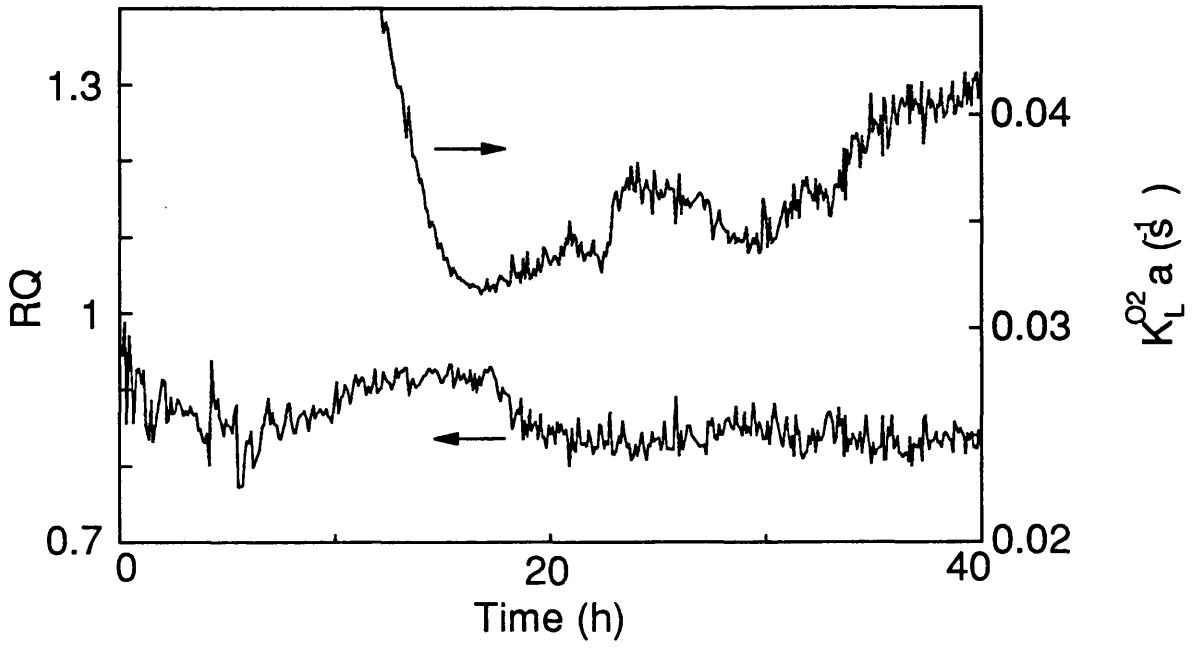


Figure 40: The respiratory quotient, RQ and oxygen mass transfer coefficient, $K_L{}^{O2}a$ for a *S. clavuligerus* fermentation, without filtering of the %OTR data

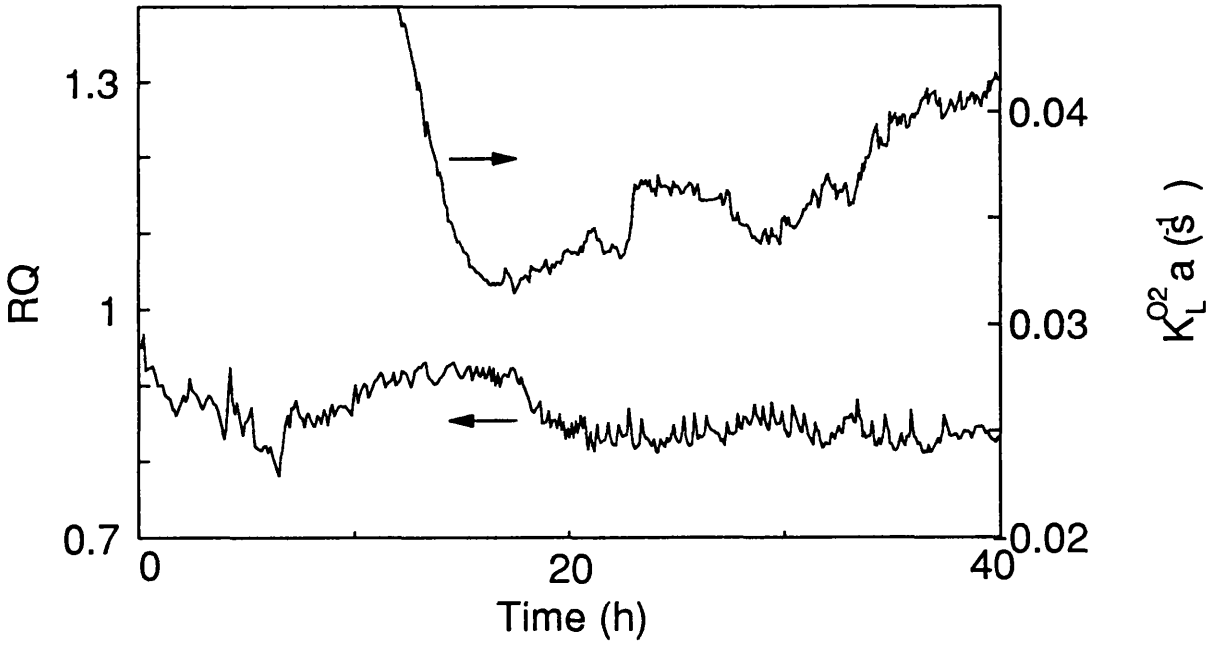


Figure 41: The respiratory quotient, RQ and mass transfer coefficient, $K_L{}^{O2}a$ for a *S. clavuligerus* fermentation, with the %OTR data filtered using a window width of 1h (10 points)

1. Noise in analog fermentation measurements, such as the pH and dissolved oxygen is of a relatively high frequency, and, owing to the high sampling rates possible in the case of analog instrumentation, can be filtered out in the amplifier, or digitally filtered by hardware
2. Noise and bias in raw gas analyses are very low owing to the high quality of analytical instrumentation for gas analyses
3. In spite of the high quality of gas analyses, oxygen transfer rate (OTR) data can contain significant noise and bias because they are calculated from gas analyses as a small difference between two large numbers
4. Bias in OTR data can be important when the difference between the mole percentage of oxygen in the inlet and exit gas streams (%OTR) is less than 0.2, for the gas analysis system at UCL
5. Noise in %OTR data is uncorrelated (ie. random), with Gaussian amplitude probability distribution of absolute standard deviation 0.0145, for the gas analysis system used at UCL
6. Noise in OTR can be reduced by using a least-squares filter (like a chi-square filter) to fit a linear model to a reduced data set containing only the most recent OTR data. Application of such a filter improves the quality of OTR data, as well as that of other derived variables that depend on the OTR, like the $K_L{}^{O2}a$ and RQ

Table 14: Principal results and conclusions arising from a study of the need for signal conditioning of fermentation measurements, presented in Section 4.2

4.3 MONITORING DISSOLVED CARBON DIOXIDE UNDER STEADY-STATE CONDITIONS

Sterilizable sensors for the measurement of the concentration of dissolved carbon dioxide in fermentation broths have been commercially available for several years (Puhar et al, 1980). However, Fox(1984) stated that these sensors cannot be described as an established tool in industry, and this situation does not appear to have changed markedly since then. The sensor is expensive, has only ±10% accuracy when calibrated against standard buffers, has a fairly slow response time and must be calibrated at two points. Such issues may be expected to hamper in particular the application of these sensors in research facilities, where there are generally a large number of small fermentors. An indication of the dissolved carbon dioxide concentration can in any case be obtained by assuming equilibrium between the gas and liquid phases and such an approach has been used in industry for some time (Carleysmith, 1985). Yagi and Yoshida(1977) stated that this assumption of equilibrium will be better for large fermentors than for small ones, because of the generally lower specific aeration rates used.

This section clarifies the basis for using the exit gas analyses to indicate the dissolved CO_2 level. Section 4.3.1 presents semi-theoretical arguments that support the view that carbon dioxide transfer under steady-state conditions can be treated as a purely liquid-film limited physical process. By "steady-state conditions" is meant conditions in which the CO_2 mass balance is not influenced by rapidly changing concentrations of CO_2 in the liquid or gas phases. Having reached this conclusion, Section 4.3.2 derives new generalized correlations that describe the factors which affect the extent to which the partial pressure of dissolved carbon dioxide exceeds the partial pressure of carbon dioxide in the exit gas, the ratio of these partial pressure being referred to here as the "carbon dioxide excess", ξ. It is shown in Section 4.3.3 that experimental measurements of dissolved carbon dioxide using a dissolved CO_2 sensor during *E. coli* fermentations, while not sufficiently accurate to explicitly prove all the theoretical results obtained, are consistent with these results.

The theoretical work described in Section 4.3 was published in Royce and Thornhill(1991) which is presented in Appendix A1.

4.3.1 Carbon dioxide transfer as a liquid-film limited physical process

This section develops new semi-theoretical work which supports the view expressed by some authors that carbon dioxide transfer in fermentation can be treated as a purely liquid-film limited physical process, that can be described in terms of the oxygen mass transfer coefficient, $K_L^{O2}a$. Gas film limitation to CO_2 transfer is shown to be negligible (as in the case of oxygen transfer). A theoretical expression for the extent of mass transfer enhancement by reaction in the liquid film is derived, and shown to indicate negligible enhancement. Finally, a new dependence of the mass transfer coefficient for CO_2 transfer on that for O_2 transfer is put forward.

It was noted in Section 2.2 that, as the cell membrane is relatively impermeable to ionic species (Jones and Greenfield, 1982), the carbon dioxide produced by microbial metabolism enters the broth as dissolved carbon dioxide, $CO_2(l)$. It can then be involved in any of the reversible processes below:

$$CO_2(g) \underset{k_L^{CO2}a}{\overset{k_L^{CO2}a}{\rightleftharpoons}} CO_2(l) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H_2CO_3 \overset{K_{acid}}{\rightleftharpoons} H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

where k_1, k_2 = rate constants for the indicated reactions (s^{-1})

K_{acid} = acid dissociation constant for carbonic acid ($mol.m^{-3}$)

Fermentation processes are operated in the pH range 4-8. At such pH values the concentration of carbonate ions is negligible, complexing of carbon dioxide with amine groups of protein molecules can be ignored, and reaction of dissolved carbon

dioxide with hydroxyl ions is negligible (Sherwood et al, 1976). The concentration of carbonic acid is three orders of magnitude less than that of carbon dioxide. The concentration of bicarbonate ions increases with increasing pH, being equal to that of dissolved carbon dioxide at a pH of about 6.3, and five times that of dissolved carbon dioxide at pH 7, as determined by the constants k_1, k_2 and K_{acid}.

The question of whether transfer of carbon dioxide across the gas-liquid interface is liquid-film limited, as for oxygen transfer, or contains a substantial gas-film limitation will first be considered. It can be shown that the ratio of concentration gradients in the liquid and gas films is around 50 in agitated gas-liquid contactors, for no internal circulation in the gas bubbles (Calderbank, 1959). In practice, there is considerable internal circulation, and this ratio is in reality much higher (Calderbank, 1959). The ratio of partial pressure gradients in the liquid and gas films (partial pressures being the measurements available during fermentations) is thus at least 50.(H/RT), where H, R and T are the Henry's law constant for the component being transferred, the ideal gas constant and the temperature. The respective Henry's law constants for oxygen and carbon dioxide at a typical fermentation temperature of 30°C are 86000 and 3400 $Pa.m^3.mol^{-1}$ (Schumpe and Quicker, 1982). Hence the ratios of the partial pressure gradients in the liquid and gas films at 30°C are at least 1700 for oxygen, and 70 for carbon dioxide, making the gas film resistance negligible for carbon dioxide transfer, as for oxygen transfer.

Having established that the mass transfer limitation is in the liquid film, a second question concerns whether this transfer process is a purely physical process, or is enhanced by reaction in the liquid film. Mass transfer from liquid to gas phase requires that there be a partial pressure gradient of carbon dioxide across the liquid film. Hence the partial pressure of carbon dioxide falls from the boundary of the liquid film to the gas-liquid interface, as shown schematically in Figure 42. For steady-state conditions, the carbon dioxide and bicarbonate ions in the liquid bulk will be in equilibrium. The decrease in the partial pressure of dissolved carbon dioxide across the liquid film means that there will be a net reaction in the liquid

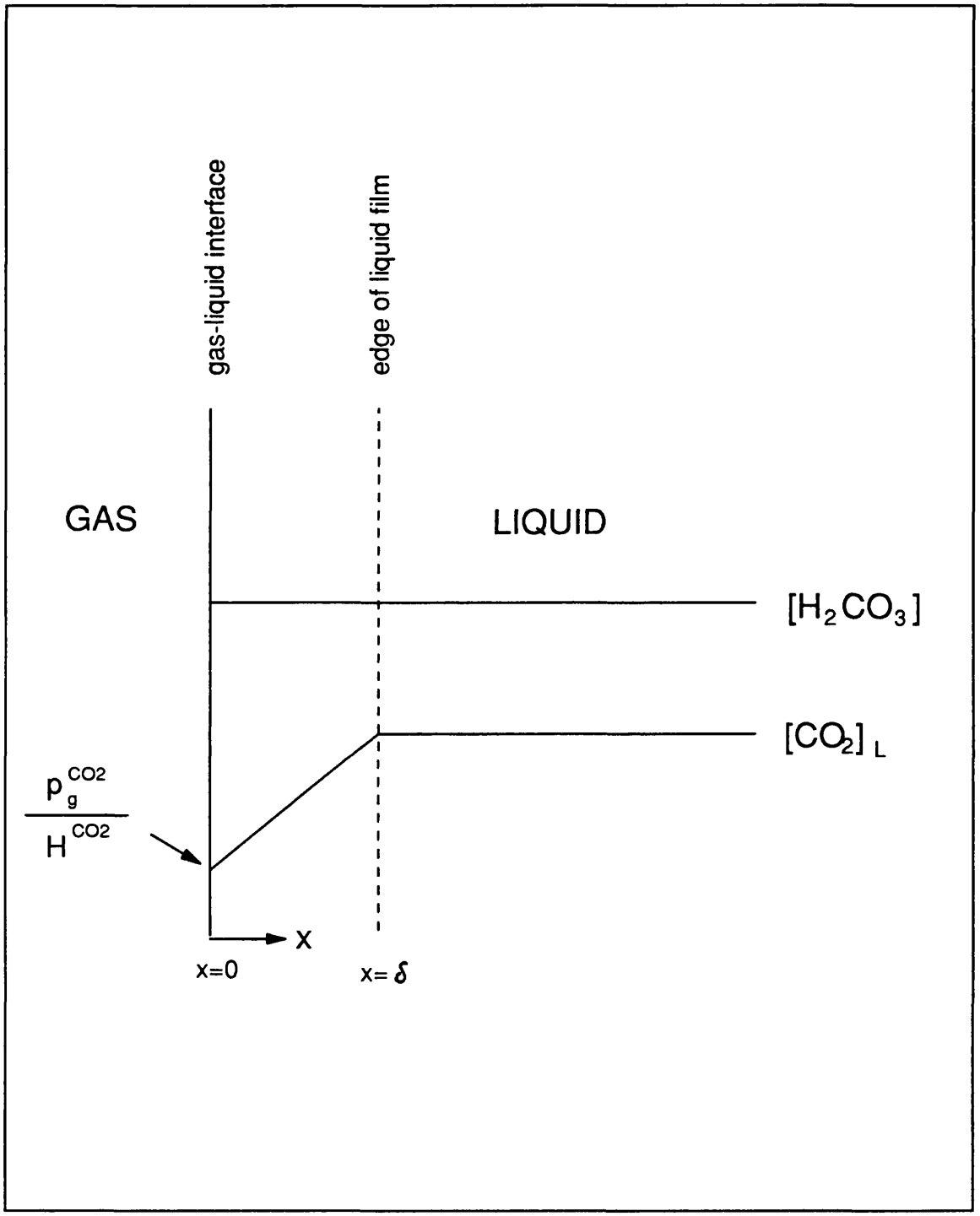


Figure 42: Representation of the concentration profiles of dissolved carbon dioxide and carbonic acid with position in the liquid film at the gas-liquid interface, in terms of boundary-layer theory

film of bicarbonate ions to form carbon dioxide, so as to restore this equilibrium in the liquid film. If this net rate of reaction is significant in comparison with the rate of transfer of carbon dioxide from liquid to gas phase, the effect will be to reduce the partial pressure gradient of carbon dioxide required to achieve a carbon dioxide transfer rate (CTR) from liquid to gas phases equal to the carbon dioxide evolution rate (CER) of biomass in the fermentor - ie. the mass transfer will be enhanced.

If it is initially assumed that the net flux of carbon as a result of reaction in the liquid film (the "film effect") is negligible, then the concentration of H_2CO_3 will be the same at the gas-liquid interface as it is in the bulk broth (Figure 42). This will have the effect of maximizing the concentration gradient driving the film effect. If the expression for the film effect that results indicates that the film effect is negligible, the initial assumption will have been justified.

The problem is solved using boundary layer theory in linear coordinates, as bubble diameters in fermentors are much larger than the thickness of the liquid film. If the interface is at x=0, and the edge of the liquid film at x=δ (Figure 42) then:

$$[CO_2]_L^{x=0} = \frac{p_g^{CO_2,out}}{H^{CO_2}}$$

$$[CO_2]_L^{x=\delta} = \frac{p_g^{CO_2,out}}{H^{CO_2}} + \frac{1}{K_L^{CO_2}} \cdot \frac{CTR}{a \cdot V_d} \qquad (11)$$

$$[H_2CO_3]^{x=0} = [H_2CO_3]^{x=\delta} = \frac{k_1}{k_2} \cdot [CO_2]_L^{x=\delta}$$

where $p_g^{CO2,out}$ = partial pressure of CO_2 in the exit gas (Pa)

H^{CO2} = Henrys' constant for CO_2 (Pa.m3.mol-1)

K_L^{CO2} = film mass transfer coefficient for carbon dioxide ($m.s^{-1}$)

a = gas-liquid interfacial area per unit aerated broth volume (m^{-1})

V_d = aerated broth volume, or dispersion volume (m^3)

$[CO_2]_L$ = concentration of dissolved carbon dioxide CO_2(l) ($mol.m^{-3}$)

$[H_2CO_2]$ = concentration of carbonic acid ($mol.m^{-3}$)

CTR = carbon dioxide transfer rate across the gas-liquid interface ($mol.s^{-1}$)

As the reactions are first order:

$$\begin{aligned} \textit{Film Effect} \\ (mol.s^{-1}) \end{aligned} = \tfrac{1}{2} \cdot (\textit{film effect at } x=0 + \textit{at } x=\delta)$$

$$= \tfrac{1}{2} \cdot (\textit{film effect at } x=0)$$

$$= \tfrac{1}{2} \cdot (k_2 . [H_2CO_3]^{x=0} - k_1 . [CO_2]^{x=0}) . V_{film}$$

$$= \frac{k_1}{2 . K_L^{CO_2}} . \frac{CTR}{a . V_d} . V_{film} \qquad (12)$$

where V_{film} = volume of the liquid film (m^3)

In terms of boundary layer theory, the volume of the liquid film (V_{film}) is:

$$V_{film} = \delta . a . V_d = \frac{D_L^{CO_2}}{K_L^{CO_2}} . a . V_d \qquad (13)$$

Hence:

$$\frac{\textit{Film Effect}}{\textit{Mass Transfer}} = \frac{\left(\frac{k_1}{2 . K_L^{CO_2}} . \frac{CTR}{a . V_d} . \frac{D_L^{CO_2}}{K_L^{CO_2}} . a . V_d \right)}{CTR}$$

$$= \frac{k_1 . D_L^{CO_2}}{2 . (K_L^{CO_2})^2} \qquad (14)$$

At 25°C, k_1 has a value of $0.028s^{-1}$ (Pinsent et al, 1956), and D_L^{CO2} has a value in water of 2.0×10^{-9} $m^2.s^{-1}$ (Incropera and de Witt, 1990). Godbole et al(1984) give values for the film mass transfer coefficient of around 2×10^{-4} $m.s^{-1}$. Hence:

$$\frac{\textit{Film Effect}}{\textit{Mass Transfer}} = 0.0007$$

Therefore, the film effect is negligible, and hence the initial assumption that this is the case was justified. It has been assumed that there is no catalysis of carbon dioxide hydration by the enzyme carbonic anhydrase, as this is an intracellular enzyme, and is present in only a few species of microorganisms.

This shows that carbon dioxide transfer is a purely physical liquid-film limited process, and equations for carbon dioxide transfer analogous to those for oxygen transfer (Equations 7 & 8) can be written, again assuming negligible hydrostatic head.

$$CTR = \frac{G}{100} \cdot \left(\%CO_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} - \%CO_2^{in} \right). \qquad (15)$$

$$= \frac{K_L^{CO_2} a \cdot V_d}{H^{CO_2}} \cdot \left(p_L^{CO_2} - p_g^{CO_2,\,out} \right) \qquad (well\text{-}mixed\ gas) \qquad (16)$$

$$= \frac{K_L^{CO_2} a \cdot V_d}{H^{CO_2}} \cdot \frac{\left(p_g^{CO_2,\,out} - p_g^{CO_2,\,in} \right)}{\ln \left(\dfrac{p_L^{CO_2} - p_g^{CO_2,\,in}}{p_L^{CO_2} - p_g^{CO_2,\,out}} \right)} \qquad (plug\text{-}flow\ gas) \qquad (17)$$

where $K_L{}^{CO2}a$ = average volumetric mass transfer coefficient for CO_2 in the fermentor

The fermentor volumetric mass transfer coefficients for oxygen and carbon dioxide involve the same interfacial area, the same solvent properties, and the same agitation variables. Schneider and Frischknecht (1977) found the volumetric coefficients to be equal, but in view of the complexity of their dynamic approach which involved tracking short-lived transient changes, and their ignoring the extent of gas backmixing and sensor response times, their claimed accuracy seems optimistic. Yagi and Yoshida (1977) recognised the dependence of the film coefficient on the liquid diffusivity, and suggested that from penetration theory, the ratio of the volumetric coefficients should depend on the square root of the diffusivity ratio of oxygen and carbon dioxide, the same exponent as indicated in the "large bubble" correlation of Calderbank(1959), "large" bubbles having a diameter greater than 2.5mm. The work

of Yagi and Yoshida(1977) yielded a value of 0.92 for the mass transfer coefficient ratio. In practice, the high concentration of electrolytes in fermentation media make it quite difficult to generate large bubbles (Calderbank, 1959), and so the "small bubble" correlation (Calderbank, 1959) is more relevant, though the outcome is not very different. Using diffusivity data for water at 25°C (Incropera and de Witt, 1990), this yields:

$$\frac{K_L^{CO_2}a}{K_L^{O_2}a} = \left(\frac{D_L^{CO_2}}{D_L^{O_2}}\right)^{\frac{2}{3}} = \left(\frac{2.0x10^{-9}m^2.s^{-1}}{2.4x10^{-9}m^2.s^{-1}}\right)^{\frac{2}{3}} = 0.89 \qquad (18)$$

where D_L^{O2}, D_L^{CO2} = liquid phase diffusivities of O_2 and CO_2 respectively

Of results obtained from theoretical expressions, 0.89 is closest to the experimental data of Fox (1984), who obtained a ratio of 0.80. While the liquid diffusivities are a function of broth composition (Ho and Ju, 1988) and temperature, their ratio is constant (Wilke and Chang, 1955). Hence the ratio would not be affected by the use of diffusion coefficients in water in place of those in fermentation media. However, it is possible that the presence of large amounts of surface-active agents in fermentation media may affect this mass transfer coefficient ratio by interfering with transfer through the liquid film.

To summarize this section, results obtained support the view that steady-state carbon dioxide transfer in fermentation can be considered to be a purely liquid-film limited physical process, as for oxygen transfer. Semi-theoretical arguments have been presented which show that the mass transfer coefficient for CO_2 transfer should be 0.89 times that for O_2 transfer.

4.3.2 The carbon dioxide excess ratio, ξ, as a function of $K_L^{O2}a$ and the aeration rate

The fermentation industry uses a rule of thumb that the partial pressure of dissolved carbon dioxide is around 10% higher than the partial pressure of carbon dioxide in

the exit gas. In this section, consideration is given to the ratio of the partial pressure of dissolved CO_2 to that of CO_2 in the exit gas, which on the basis of the above rule of thumb, may be expected to have a value slightly above unity. This ratio is called here the carbon dioxide excess, ξ. Practical factors limit how large this ratio can be, and a new correlation is derived which indicates the theoretical maximum value of ξ as a function of the mole fraction of carbon dioxide in the exit gas. In addition, new generalized correlations are derived that describe the sensitivity of the carbon dioxide excess to the assumed extent of gas backmixing, and to fermentation variables.

The concentration of carbon dioxide dissolved in the fermentation broth, $[CO_2]_L$, will in general be higher than the concentration predicted by assuming carbon dioxide in the broth to be in equilibrium with the exit gas from the fermentor, $[CO_2]_{eq}$, as a partial pressure gradient for desorption of carbon dioxide is required. The extent of this difference is limited by two constraints. Firstly, the respiratory quotient (the ratio of the CER to the OUR) is near unity for most fermentations. Hence, under steady-state conditions, the CTR and OTR (being equal to the CER and OUR respectively) are nearly equal. Also, the liquid-phase oxygen partial pressure must be greater than zero to avoid oxygen starvation. Noting also that $\%CO_2^{in}$, being that in air, is very small (around 0.04%), Equations (19) describe the carbon dioxide equilibrium concentration, the maximum CO_2 concentration possible, $[CO_2]_{max}$, and the ratio of these concentrations (called here the maximum carbon dioxide excess, ξ_{max}), in terms of the exit gas composition, for a well-mixed gas phase. It will later be seen that, for a plug-flow gas phase, the dissolved carbon dioxide concentration is much closer to the equilibrium concentration, than for the well-mixed gas case, and so ξ_{max} in Equation (19) is the maximum carbon dioxide excess ratio irrespective of the extent of gas backmixing.

$$[CO_2]_{eq} = \frac{p_g^{CO_2,out}}{H^{CO_2}} = \frac{\%CO_2^{out}}{100} \cdot \frac{(P-p_w)}{H^{CO_2}}$$

$$[CO_2]_{max} = \frac{\%CO_2^{out}}{100} \cdot \frac{(P-p_w)}{H^{CO_2}} + \frac{1}{0.89} \cdot \frac{\%O_2^{out}}{100} \cdot \frac{(P-p_w)}{H^{O_2}} \qquad (19)$$

$$\xi_{max} = \frac{[CO_2]_{max}}{[CO_2]_{eq}} = 1 + \frac{1}{0.89} \cdot \frac{H^{CO_2}}{H^{O_2}} \cdot \frac{\%O_2^{out}}{\%CO_2^{out}}$$

where P = operating (head space) pressure (Pa)

p_w = partial pressure of water in the exit gas (Pa)

$\%O_2^{out}$ = mole percentage of O_2 in exit gas from fermenter (dry basis)

$\%CO_2^{out}$= mole percentage of CO_2 in exit gas from fermenter (dry basis)

$p_g^{CO2,out}$= partial pressure of CO_2 in exit gas (Pa)

H^{O2} = Henrys' constant for O_2 (Pa.m3.mol-1)

H^{CO2} = Henrys' constant for CO_2 (Pa.m3.mol-1)

Values for the Henry's law constants for water are available in the literature (eg Schumpe and Quicker, 1982). Their dependence on temperature (in K) is indicated below:

$$H^{O_2} = \exp\{12.74 - \frac{133.4}{(T - 206.7)}\}$$

$$H^{CO_2} = \exp\{11.25 - \frac{395.9}{(T - 175.9)}\} \qquad (20)$$

At 30°C, H^{O2} and H^{CO2} are 86000 and 3400 $Pa.m^3.mol^{-1}$ respectively.

Figure 43 plots the equilibrium and maximum concentrations of dissolved carbon dioxide indicated by Equation (19), for operation at atmospheric pressure, and 30°C, assuming the inlet air to contain 21% oxygen. The ratio of these concentrations, ξ_{max}, is shown in Figure 44. It can be seen in Figure 43 that the absolute difference between the maximum and equilibrium carbon dioxide concentrations is a fairly constant quantity. The extent of this difference cannot be very large because of the

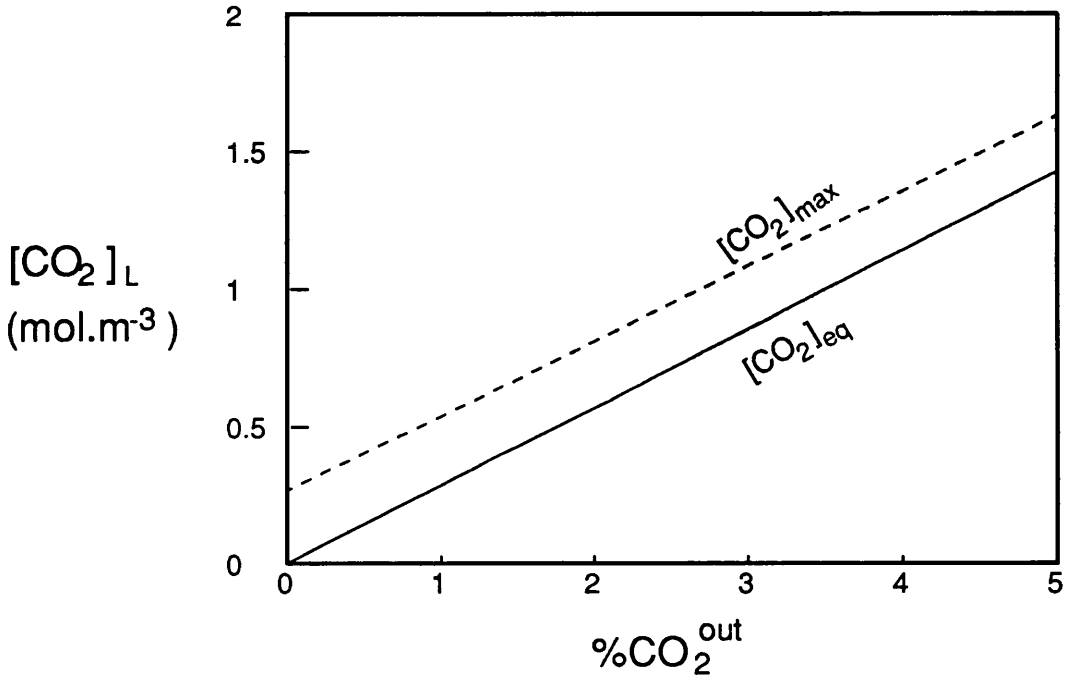


Figure 43: The maximum and equilibrium concentrations of dissolved carbon dioxide calculated from Equation (19), a function of the exit gas composition, for a well-mixed gas phase (30°C, RQ=1)

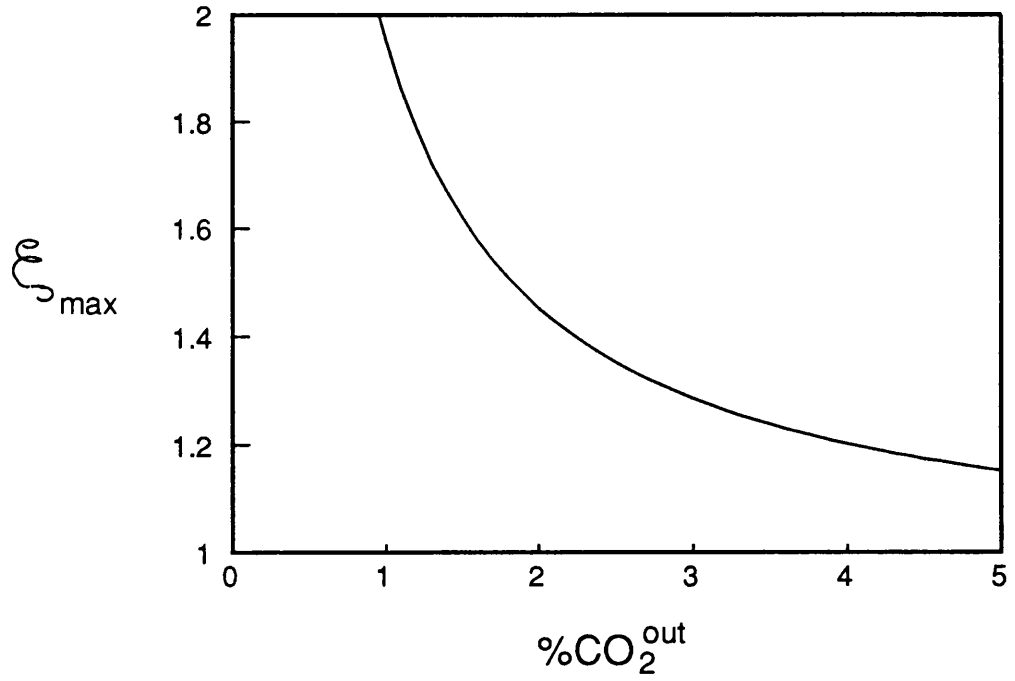


Figure 44: The ratio of the maximum to the equilibrium concentrations of dissolved carbon dioxide shown in Figure 43, ξ_{max}, calculated from Equation (19), a function of the exit gas composition, for a well-mixed gas phase (30°C, RQ=1)

need to work at oxygen mass transfer coefficients that will maintain the concentration of the sparingly soluble oxygen above a level that affects respiration. The difference becomes a smaller proportion of the total dissolved carbon dioxide concentration with increasing $\%CO_2^{out}$, causing ξ_{max} to decrease in Figure 44. While this means that the equilibrium assumption becomes more accurate, the organism will be more sensitive to a given error at the higher dissolved carbon dioxide concentration.

The exact value of the carbon dioxide excess (rather than its maximum value) can be evaluated from Equation (21), again for a well-mixed gas.

$$\xi = \frac{[CO_2]_L}{[CO_2]_{eq}} = 1 + \frac{CTR.H^{CO_2}}{(K_L^{CO_2}a).V_d.p_g^{CO_2,out}}$$

$$= 1 + \frac{Q.(1-\varepsilon).H^{CO_2}}{60RT_0.K_L^{CO_2}a}.\left(\frac{P_0}{P-p_w}\right).\left(\frac{\%CO_2^{out}.\frac{\%N_2^{in}}{\%N_2^{out}} - \%CO_2^{in}}{\%CO_2^{out}}\right)$$

$$\approx 1 + \frac{1}{\left(\frac{0.89.K_L^{O_2}a.P}{H^{CO_2}.Q.(P_0/60RT_0)}\right)} \qquad (well\text{-}mixed\ gas) \qquad (21)$$

where Q = specific aeration rate at standard temperature and pressure

T_0 = standard temperature (298K)

P_0 = standard pressure (1.01×10^5 Pa)

R = ideal gas constant (8.314 $J.mol^{-1}.K^{-1}$)

ε = gas void fraction

ξ = ratio of the dissolved CO_2 partial pressure to the CO_2 partial pressure in the exit gas

The specific aeration rate, Q, is normally based on the unaerated volume, and so the void fraction, ε, enters the expression. The void fraction is typically less than 10%, while p_w is normally around 5% of the operating pressure, and so these variables can

be considered to cancel out with little error. The term involving gas analyses falls away as it is close to unity.

For a given operating pressure, the value of the carbon dioxide excess, ξ, by Equation (21), is therefore determined mainly by the values of Q and $K_L{}^{O2}a$. It should be noted that these variables are not wholly independent. While Westerterp et al(1963) suggest that they are independent, most authors have found a weak power law dependence of $K_L{}^{O2}a$ on the superficial gas flowrate, and hence on Q. They are treated here as approximately independent ($K_L{}^{O2}a$ depending mainly on the agitation rate) to clarify the discussion. The ranges of these variables are quite limited in practice and so the range of ξ for most practical cases can be plotted as a function of these two variables. Figure 45 plots Equation (21) for operation at 30°C and atmospheric pressure. The resulting curves are relatively insensitive to the temperature, and hence give a first approximation for operation at other temperatures.

In Figure 45, it can be seen that the error in the equilibrium assumption becomes relatively smaller as the volumetric mass transfer coefficient rises, or the specific aeration rate falls. The typical error in the equilibrium assumption will be 20-30%. At high aeration and low agitation, the error can be more than 40%. Such conditions will tend to coincide with a low value of the $\%CO_2{}^{out}$, and hence of $[CO_2]_L$ (Figure 43), and so this error may be of relatively little importance. Under opposing conditions of low aeration and high agitation, the error in the equilibrium assumption will be only 10-20%. Such conditions will in general be associated with a high value of the $\%CO_2{}^{out}$, and hence of $[CO_2]_L$. At high dissolved carbon dioxide concentrations, organisms are relatively more sensitive to variations in the dissolved carbon dioxide concentation, and hence even a small error in the equilibrium assumption may be of importance. Figure 45 allows the fermentation technologist to establish whether the carbon dioxide excess above the equilibrium assumption is important or not, and, where important, to calculate more accurate values for the dissolved carbon dioxide level.

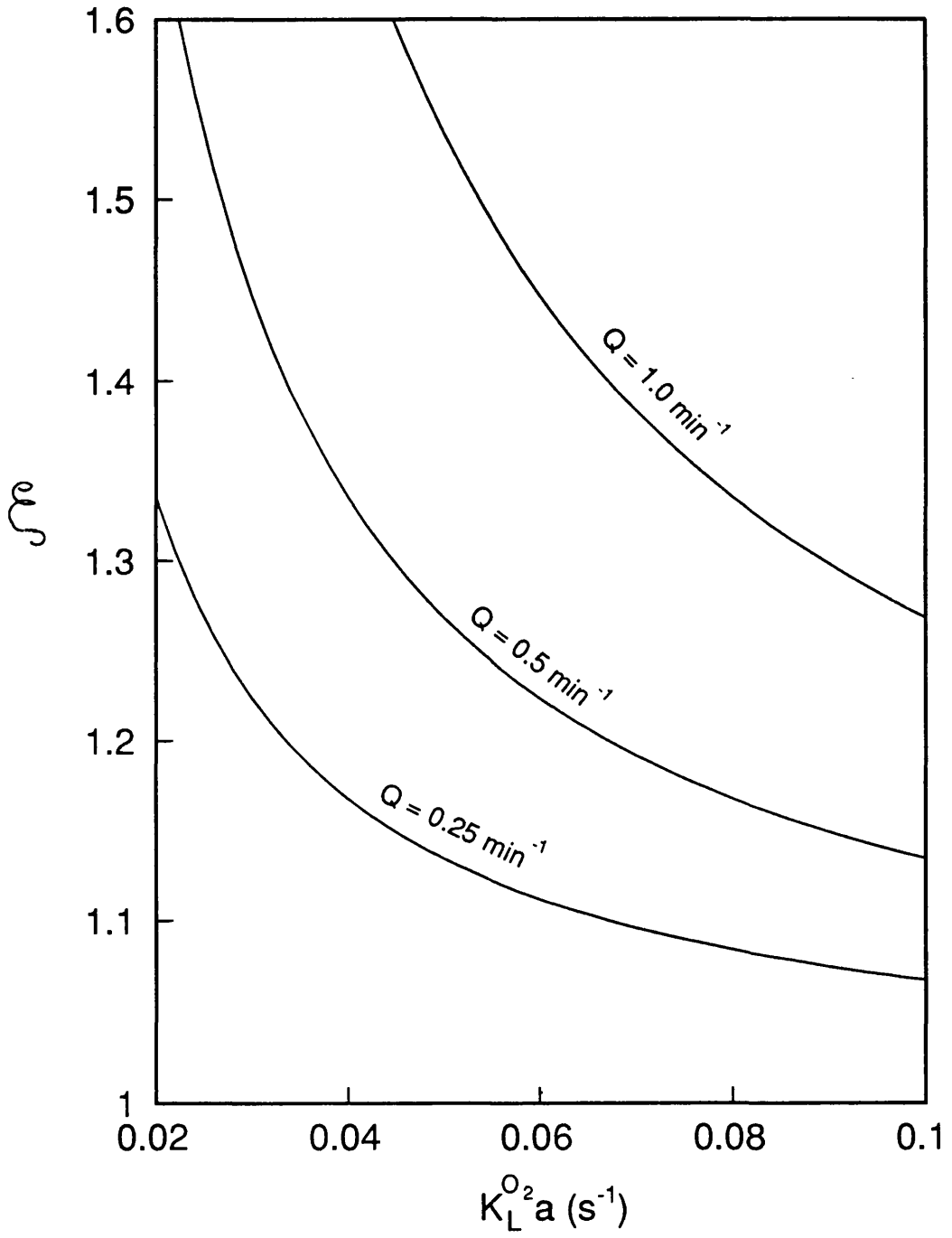


Figure 45: Variation of the carbon dioxide excess with the specific aeration rate, Q, and the volumetric mass transfer coefficient, $K_L^{O2}a$, for a well-mixed gas phase (30°C)

A result analogous to that in Equation (21) can be obtained for the plug-flow gas case:

$$\xi = \frac{[CO_2]_L}{[CO_2]_{eq}}$$

$$\approx 1 + \frac{1}{\exp\left(\frac{0.89 K_L^{O_2} a.P}{H^{CO_2}.Q.(P_0/60RT_0)}\right) - 1} \quad (plug\text{-}flow\ gas) \quad (22)$$

In Figures 46, 47 & 48, the well-mixed and plug flow gas cases (Equations 21 & 22) are compared graphically at aeration rates of ⅓, ½ and 1vvm. These graphs show that while the carbon dioxide excess is sensitive to the aeration rate and the mass transfer coefficient in the well-mixed gas case, there is an almost exact approach to equilibrium in the plug-flow gas case. The reason for this is that, due to the high solubility of carbon dioxide, the concentration gradient required for meeting the CTR translates into a relatively small partial pressure gradient (i.e. the Henrys' constant for CO_2 is relatively small). If there is a substantial degree of plug-flow character in the gas phase, as may be expected on larger fermentors, large partial pressure gradients will exist at the bottom of the fermentor, as the inlet air has a very low carbon dioxide content. The dissolved carbon dioxide concentration cannot vary greatly in the axial direction, as it is typically a few times that of dissolved oxygen, and, as has already been noted, fermentation broths are relatively well-mixed with regard to dissolved oxygen. Hence, most carbon dioxide transfer will occur at the bottom of the fermentor where high partial pressure gradients exist, and the gas will be in carbon dioxide equilibrium with the broth as it reaches the broth surface.

Yagi and Yoshida (1977) pointed out that on large fermentors, the approach to equilibrium will be better than in small fermentors, as they noted that with increasing scale, the specific aeration rate, Q, must be reduced in order to maintain the gas superficial velocity (and hence, the gas void fraction, ε) within practical limits. Figure 45 shows that a decrease in the specific aeration rate at constant $K_L{}^{O2}a$ improves the relative accuracy of the equilibrium assumption. Such reductions of

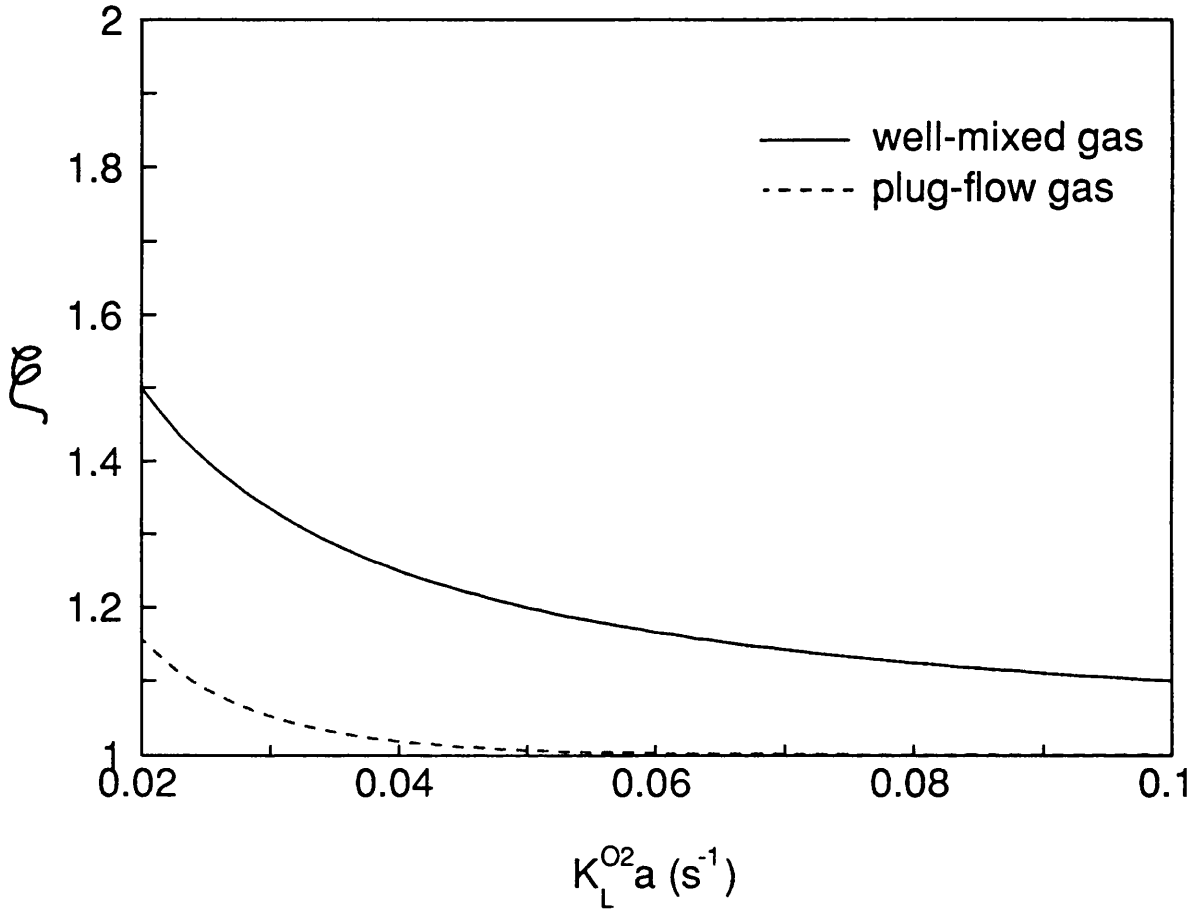


Figure 46: Effect of extent of gas backmixing on the carbon dioxide excess for an aeration rate of ⅓vvm (37°C)

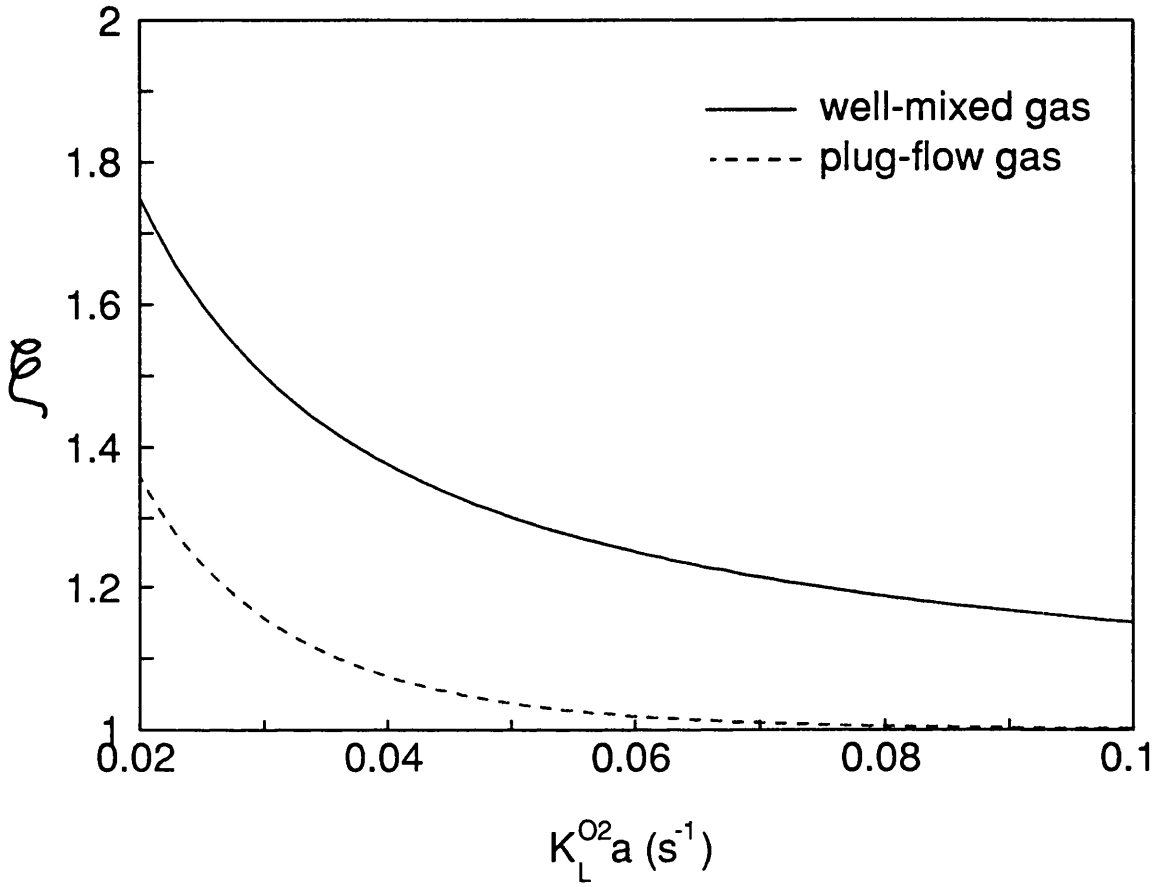


Figure 47: Effect of extent of gas backmixing on the carbon dioxide excess for an aeration rate of ½vvm (37°C)

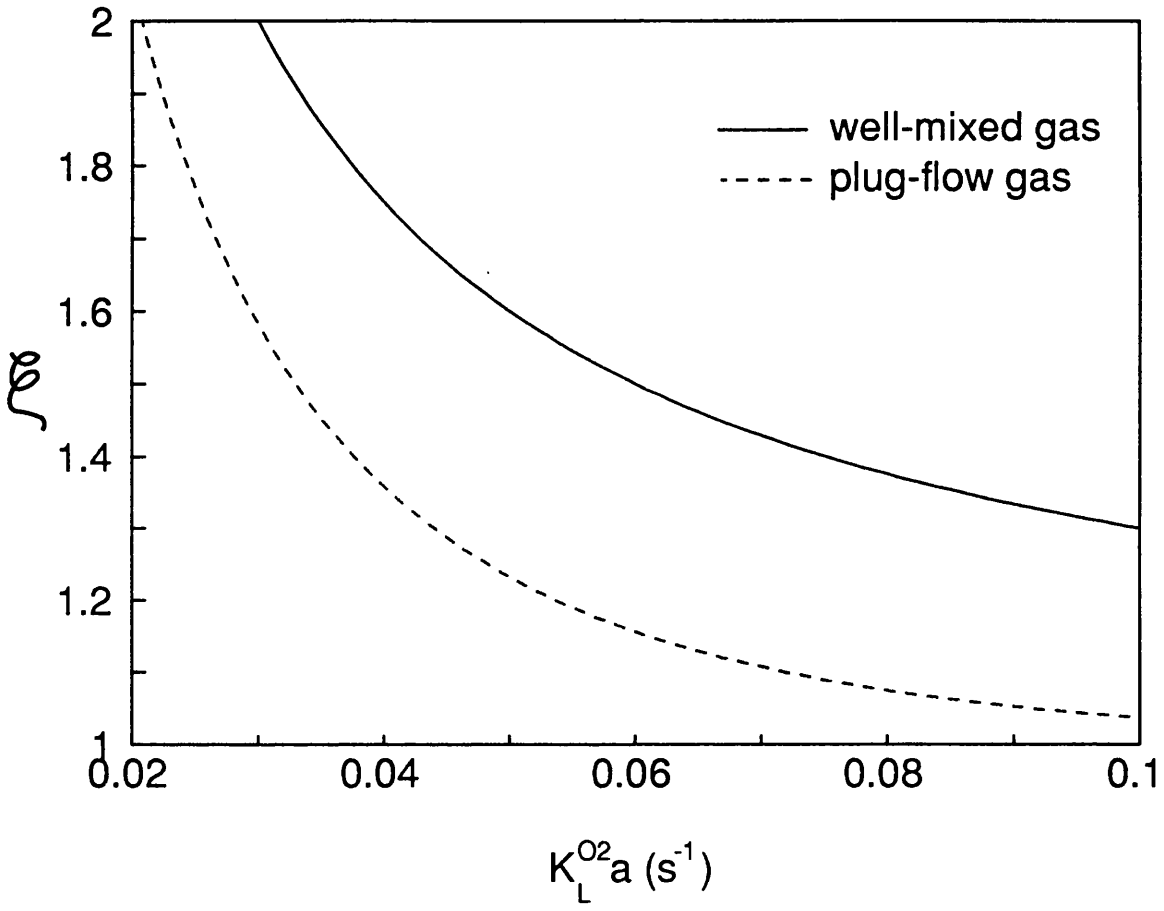


Figure 48: Effect of extent of gas backmixing on the carbon dioxide excess for an aeration rate of 1vvm (37°C)

aeration rate with scale are, however, limited by the increasing $\%CO_2^{out}$ which ultimately causes severe inhibition in most aerobic organisms, and as a result, the minimum specific aeration rates used are around 0.2 min^{-1}, even for very large fermentors (Sittig, 1982). Hence, while Yagi and Yoshida(1977) were correct in expecting a better approach to equilibrium on large fermentors, this result may be mainly expected to the due to an increased component of plug-flow character is gas flow at large scales, and only to a smaller extent due to the low aeration rate. Indeed, it will be seen in the experimental work reported in Section 4.3.3 that, even with comparatively low aeration rates, there is a substantial excess of carbon dioxide above the equilibrium assumption in a small fermentor.

It was noted in Section 2.2 that while most microorganisms are sensitive to dissolved carbon dioxide rather than bicarbonate ions, there are some organisms in which these sensitivities are reversed. For fermentations employing such organisms, the objective would be to monitor the concentration of bicarbonate ions. This concentration can, under steady-state conditions, be deduced directly from the concentration dissolved carbon dioxide:

$$[HCO_3^-] = \frac{k_1 \cdot K_{acid}}{k_2 \cdot 10^{-pH}} \cdot [CO_2]_L \qquad (23)$$

The values for the constants in the above equation are available in the chemical engineering literature (Sirs, 1958; Pinsent et al, 1956; Arrua et al, 1990).

4.3.3 Measurements of dissolved carbon dioxide in *E. coli* fermentations

Prior to examining the time profile of the dissolved carbon dioxide concentration during an entire fermentation, the effect of the agitation rate on the dissolved CO_2 is first considered in isolation. During the fed-batch phase of an *E. coli* fermentation, step changes to the agitation rate were made (700rpm → 500rpm → 400rpm → 360rpm), at a constant aeration rate (Q=1/3 min^{-1}) and glucose feedrate (3.3 $ml.min^{-1}$), which produced an exit gas carbon dioxide content of about 1.75%

on a molar basis. In Figure 49 are shown the calculated $K_L^{O2}a$ and ξ resulting from these changes, plotted against time. The $K_L^{O2}a$ were calculated assuming a well-mixed gas phase, but this assumption has little effect on the numerical result, as the change in gas phase oxygen mole fraction across the fermentor is small. The significant amount of noise in the ξ data in particular are caused by the noise introduced into the CTR by pH control action (Buckland et al, 1985), and also reflect the resolution limit of the dissolved carbon dioxide probe. In Figure 50 the average value of ξ in Figure 49 at each agitation rate is plotted against the corresponding average $K_L^{O2}a$ for this agitation rate. Also plotted are the theoretical results for ξ for the well-mixed gas and plug-flow gas cases (Equations 21 & 22), with H^{CO2}=4000 $Pa.m^3.mol^{-1}$, corresponding to the operating temperature of 37°C. There is good agreement between the experimental results and the theoretical results for a well-mixed gas phase, indicating that in the 42L fermentor used, the gas is highly backmixed for all agitation rates of practical interest, as might be expected in a small fermentor.

In Figure 51 are presented the time profiles of the partial pressures of CO_2 in the liquid phase and exit gas during the course of an *E. coli* fermentation. Also presented is the oxygen mass transfer coefficient, $K_L^{O2}a$, which only varied slightly during the course of the fermentation, from $0.04s^{-1}$ to $0.055s^{-1}$. As the mass transfer coefficient only varied slightly, and the aeration rate was constant throughout the fermentation, the theoretical work already presented in Section 4.3.2 would suggest that the carbon dioxide excess (the ratio of the partial pressures of dissolved CO_2 to that in the exit gas) should be constant throughout the fermentation. This ratio is evaluated in Figure 52, and is indeed fairly constant during the course of the fermentation, with a value around 1.25. Early on in the fermentation, however, the ratio is close to unity. This may be because, at the low p_L^{CO2} encountered early in the fermentation, the dissolved CO_2 probe has a slow response time, at a time when $p_g^{CO2,out}$ is rising fast.

The ratio of p_L^{CO2} to $p_g^{CO2,out}$ is even constant during periods of unsteady-state carbon dioxide transfer. In Figure 53, unsteady-state CO_2 transfer was generated between 4h and 9h, by changes in the pH. The partial pressures of carbon dioxide in the liquid

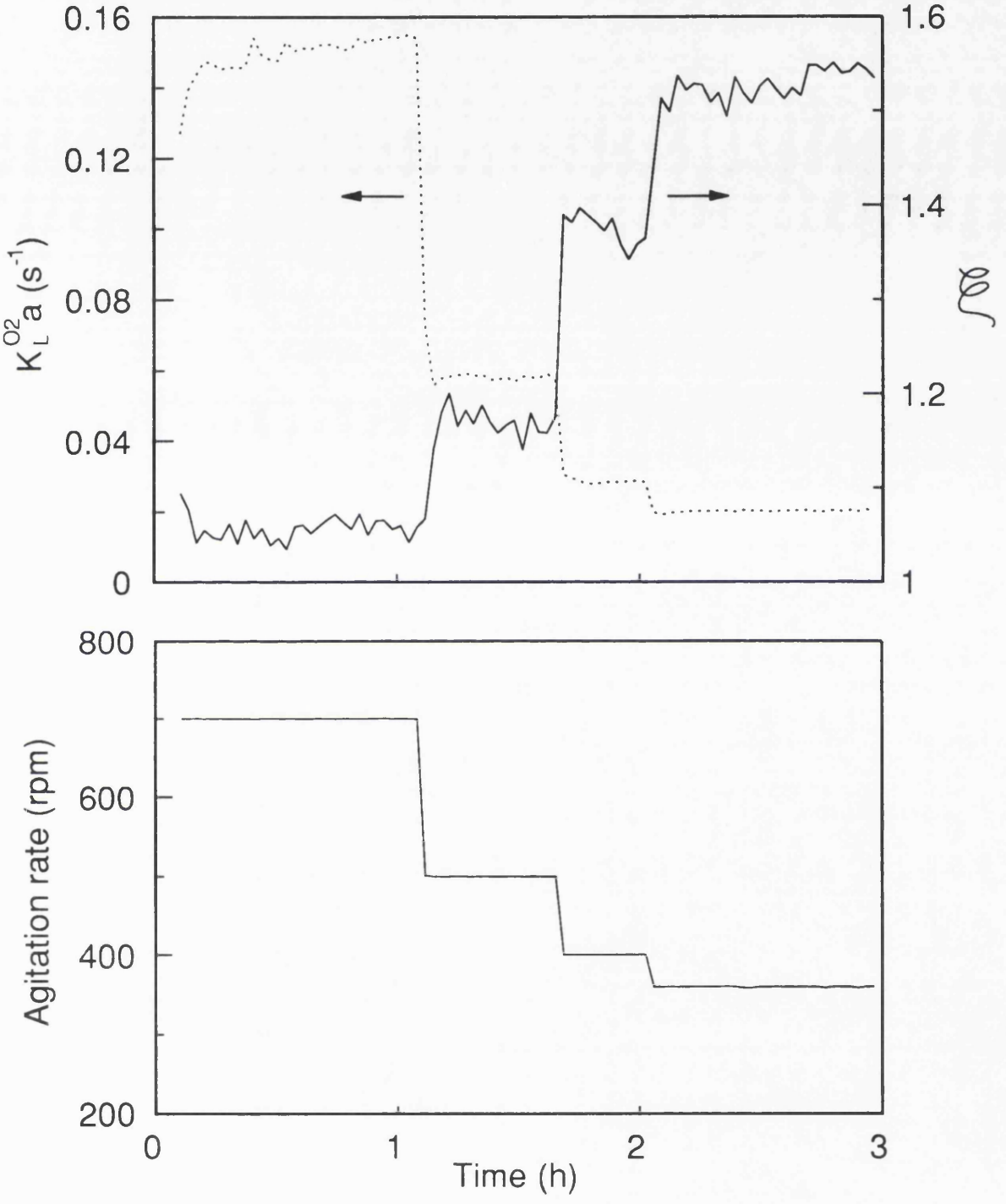


Figure 49: Effect of changes in the agitation rate on the mass transfer coefficient, $K_L^{O2}a$, and the carbon dioxide excess, ξ, during the fed-batch phase of an *E. coli* fermentation (⅓vvm, 37°C)

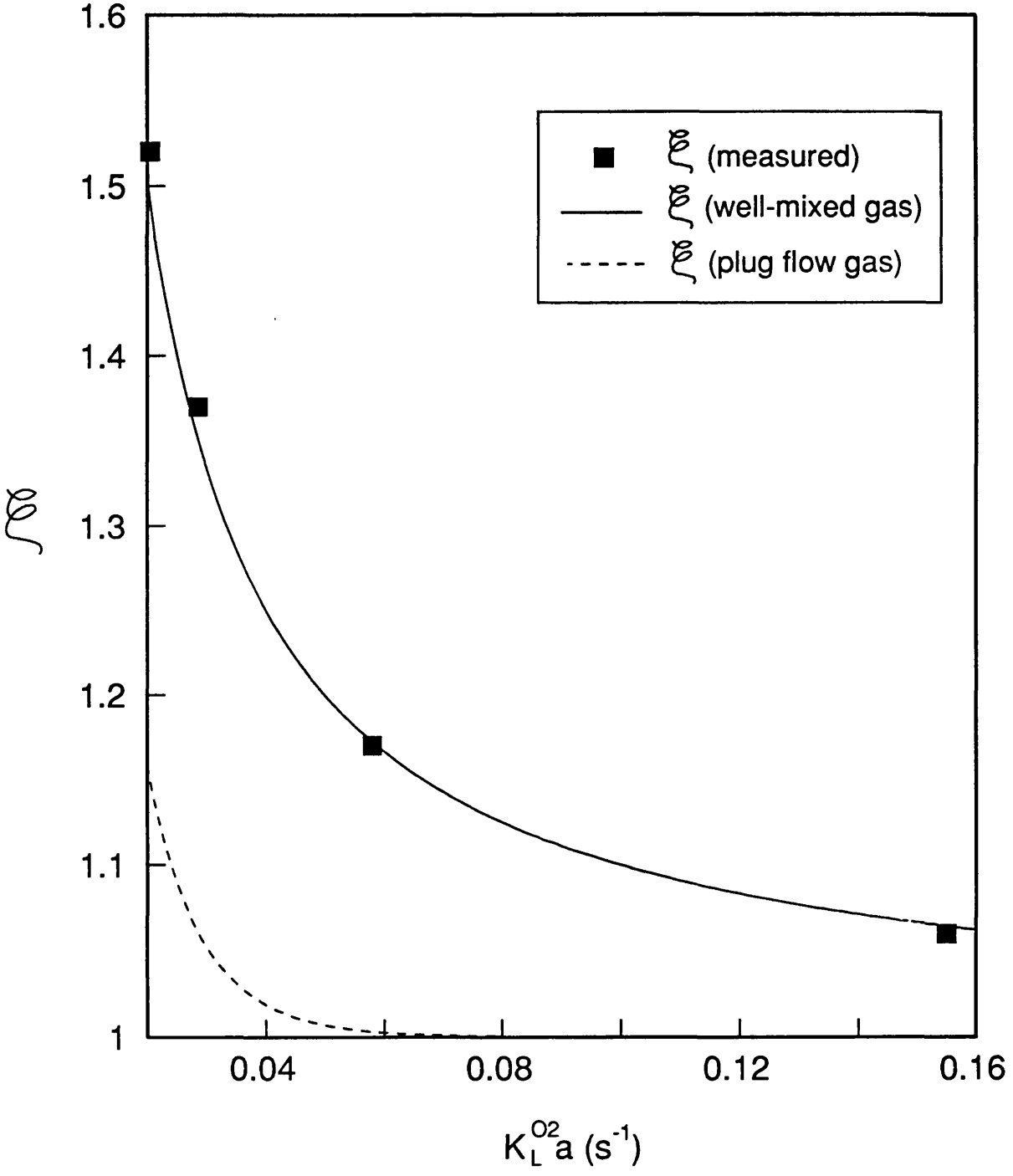


Figure 50: Plot of theoretical (well-mixed gas) and experimental (Figure 49) carbon dioxide excess, ξ, against the mass transfer coefficient, $K_L^{O2}a$ (⅓vvm, 37°C)

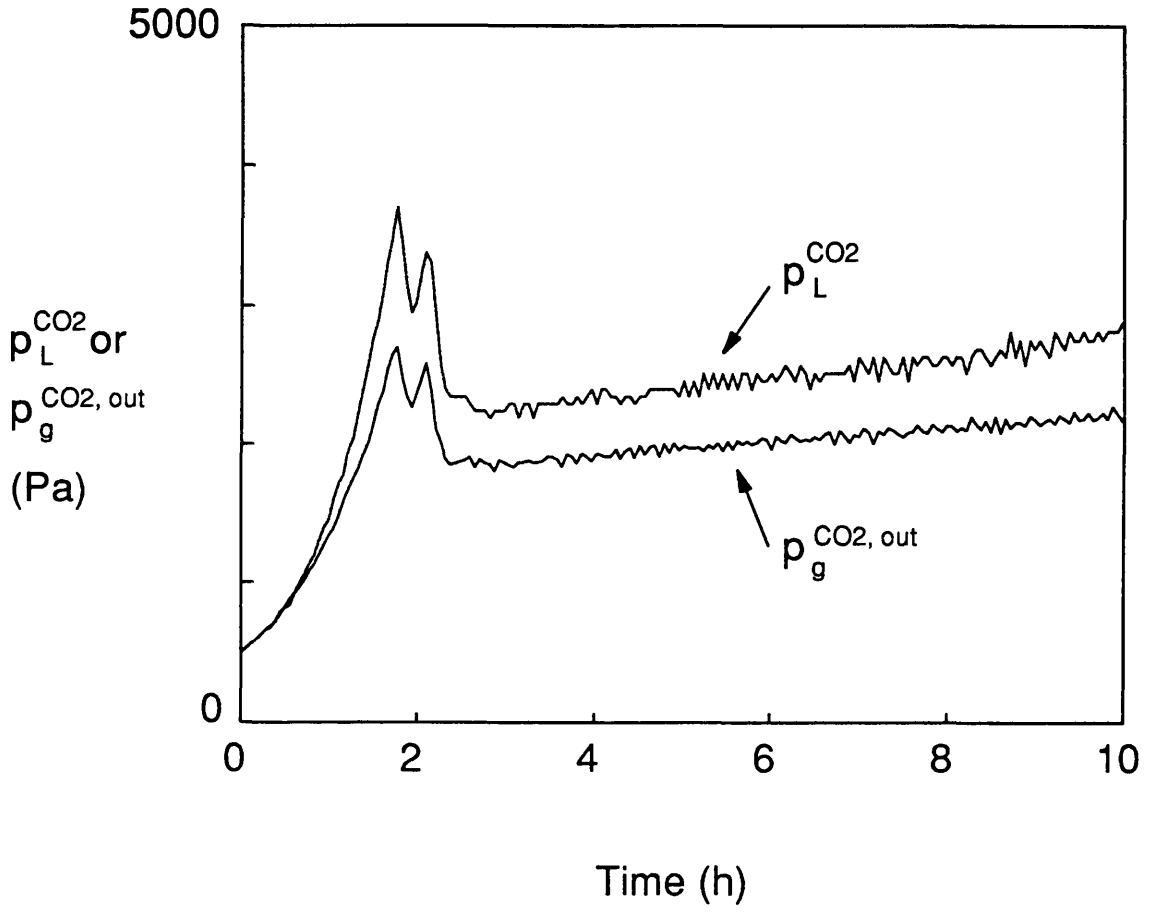


Figure 51: The partial pressure of dissolved carbon dioxide, p_L^{CO2} and of carbon dioxide in the exit gas, $p_g^{CO2,out}$ for an *E. coli* fermentation run at constant pH (⅓vvm, 37°C)

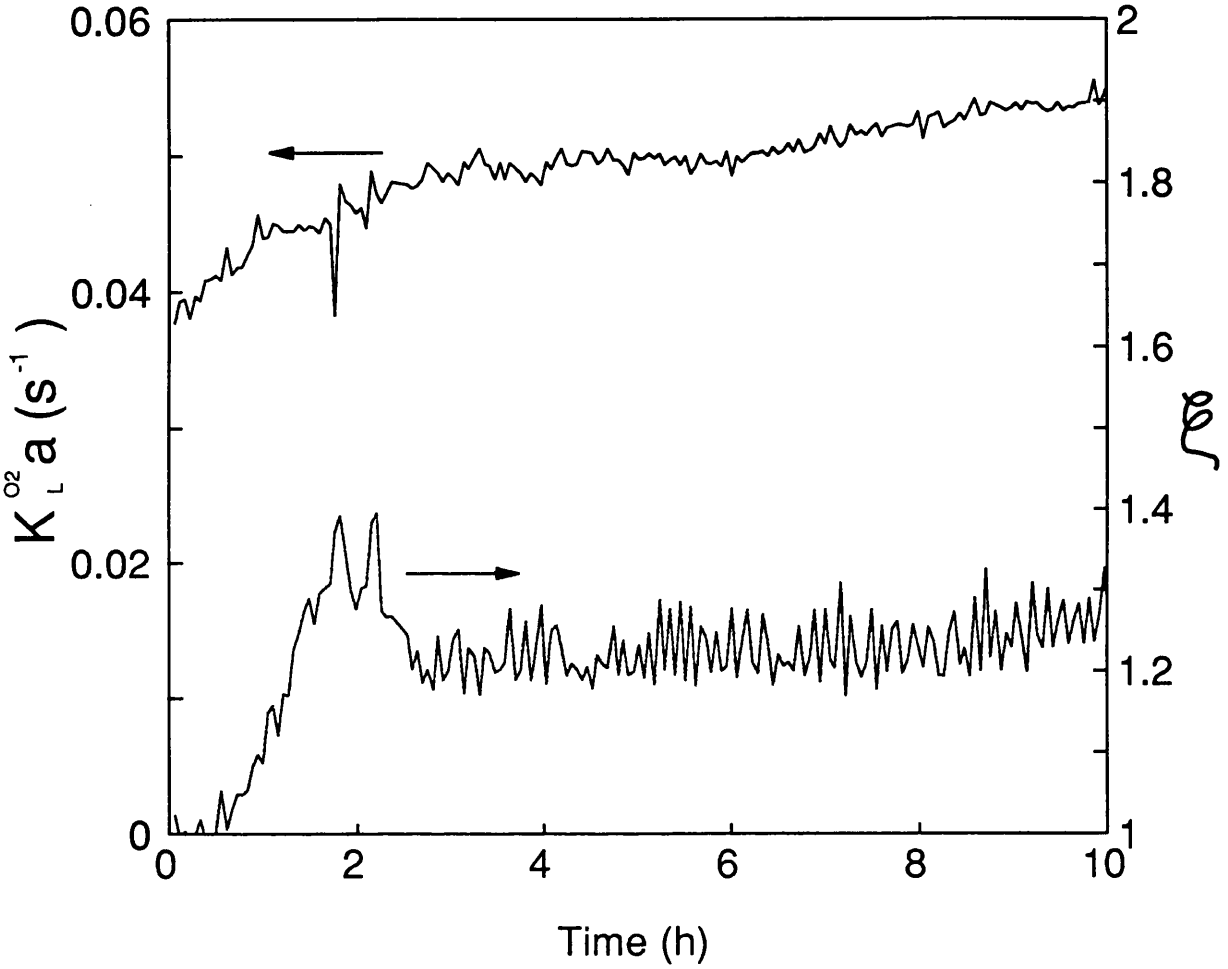


Figure 52: The mass transfer coefficient, $K_L^{O2}a$ and the carbon dioxide excess, ξ for the *E. coli* fermentation data presented in Figure 51

and gas phases can be seen to fluctuate as a result of the changing pH, but their ratio appears qualitatively to be approximately constant. This is confirmed in Figure 54 which presents the carbon dioxide excess, ξ, for this fermentation.

The limited accuracy of p_L^{CO2} measurements precludes the explicit validation of the mass transfer coefficient ratio proposed in Equation (18). However, the values of the carbon dioxide excess obtained experimentally, being in fair agreement with the theoretical predictions of Section 4.3.2, are at least consistent with a mass transfer coefficient ratio of around 0.89.

4.3.4 Summary of Section 4.3

Table 15 summarizes the main results and conclusions from Section 4.3. To support the view that carbon dioxide transfer at steady state is a purely liquid-film limited physical process, it was shown that the resistance in the liquid-film is at least seventy times that in the gas film. Further, an expression describing the extent of any mass transfer enhancement by reaction in the liquid film was derived, and shown to indicate negligible enhancement. A new ratio of the mass transfer coefficients for carbon dioxide to that of oxygen of 0.89 was obtained, being equal to the diffusion coefficient ratio raised to the two-thirds power, and suggested by noting that bubbles in agitated fermentors are "small" (i.e. <2.5mm diameter). The above results were implicitly used to investigate the accuracy of the commonly-made assumption that the partial pressure of dissolved carbon dioxide is approximately equal to that in the exit gas. A new theoretical expression was obtained which shows that the maximum relative error in this assumption can be large, but only when the dissolved carbon dioxide partial pressure is small (and therefore of little concern). The exact error is typically in the range 20-30% for fermentors in which the gas phase is well backmixed (i.e. small fermentors) but considerably smaller when the gas phase is in plug flow (corresponding larger fermentors). This error decreases with decrease in aeration rate, or with an increase in the oxygen mass transfer coefficient.

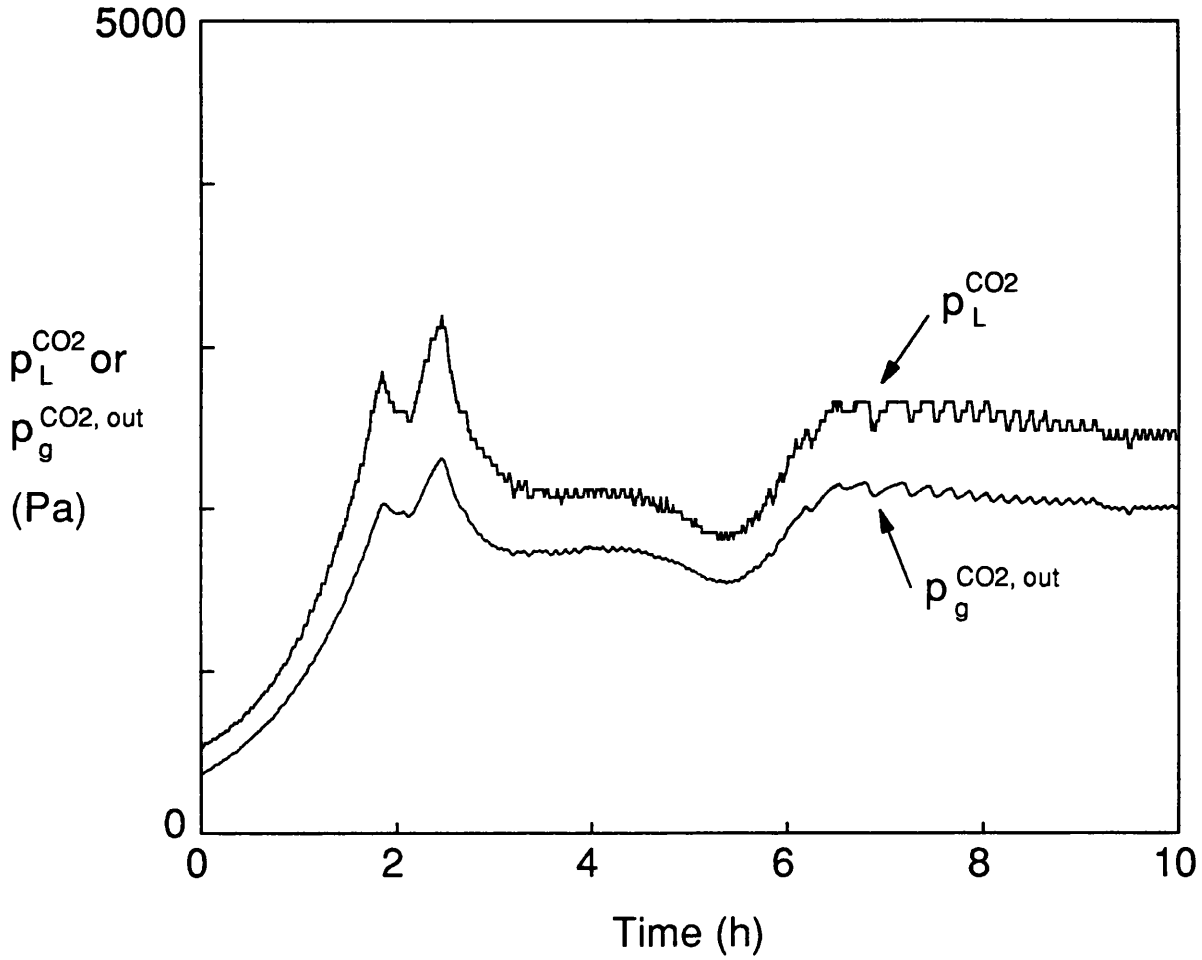


Figure 53: The partial pressure of dissolved carbon dioxide, p_L^{CO2} and of carbon dioxide in the exit gas, $p_g^{CO2,out}$ for an *E. coli* fermentation (⅓vvm, 37°C). Unsteady-state CO_2 transfer generated between time 4h and 9h by changing the pH doesn't appear to affect the relationship between p_L^{CO2} and $p_g^{CO2,out}$

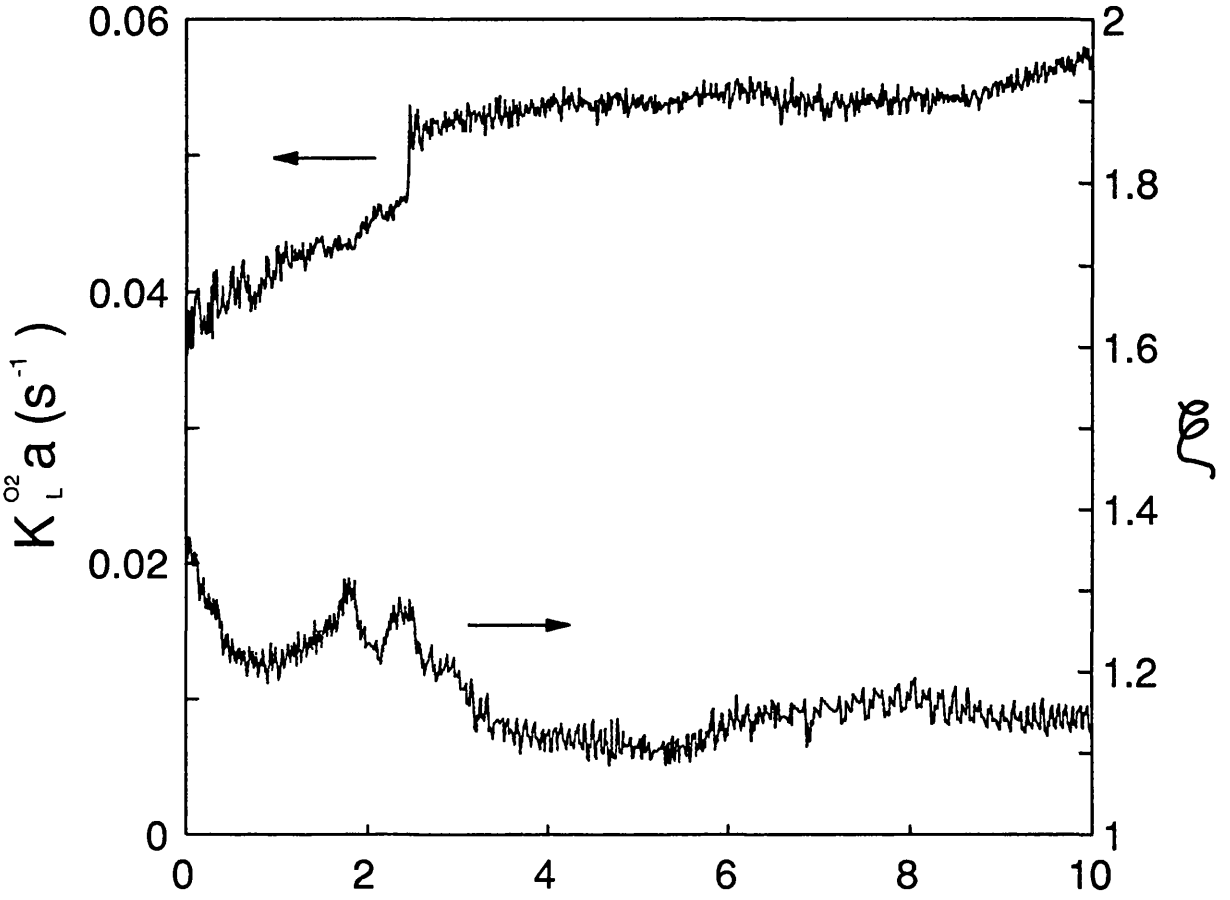


Figure 54: The mass transfer coefficient, $K_L^{O2}a$ and the carbon dioxide excess, ξ for *E. coli* fermentation data presented in Figure 53

1. The ratio of the partial pressure gradient in the liquid film to that in thae gas film is at least 1700 for oxygen transfer and 70 for carbon dioxide transfer, indicating the mass transer of both these components is liquid-film limited
2. A theoretical expression for the extent of enhancement of carbon dioxide mass transfer by reaction in the liquid film was derived and shown to indicate negligible enhancement
3. As bubbles in fermentors are small (<2.5mm diameter as defined by Calderbank, 1959), the ratio of the mass transfer coefficients for carbon dioxide and oxygen depends on the ratio of their liquid diffusivities to the two-thirds power, yielding a coefficient ratio of 0.89 in water
4. The maximum error in the assumption of equilibrium between dissolved CO_2, and CO_2 in the exit gas is limited by practical constraints
5. The exact value of the error in the equilibrium assumption increases with increase in aeration rate, or decrease in $K_L{}^{O2}a$, and is typically around 20-30% when the gas phase is well mixed (small fermentors) and less when the gas phase is in plug flow
6. Experimental measurements using a sterilizeable dissolved CO_2 probe are consistent with theoretical results

Table 15: Principal results and conclusions arising from a study of steady-state CO_2 transfer presented in Section 4.3

4.4 THE DEVELOPMENT AND EXAMINATION OF THE BEHAVIOUR OF A MODEL OF UNSTEADY-STATE CO_2 TRANSFER

By "unsteady-state" transfer of carbon dioxide is meant transfer under conditions where the partial pressure of carbon dioxide in the liquid or gas phase is changing with time. The need to describe unsteady-state transfer of CO_2 arises because of observations made by several authors and noted earlier, concerning the effect of pH control action on the carbon dioxide evolution rate (CER), and the anomalously low values of the respiratory quotient (RQ) during the early stages of a fermentation. This section develops a model to describe unsteady-state CO_2 transfer in fermentation, that uses information available in the chemical engineering literature concerning the reaction rate constants and equilibria for the reactions of carbon dioxide in aqueous solution. The model is used to examine the sensitivity of unsteady-state effects to fermentation variables like the pH.

While the oxygen uptake rate (OUR) and CER, in being biological fluxes, may generally be expected to be fairly smooth functions of time, some authors have noted for example, the effect of pH control action on carbon dioxide evolved from the fermentor. In fermentations for the production of efrotomycin, the application of RQ control is made difficult by the large amount of CO_2 liberated each time the acid is added to the broth to control the pH (Buckland et al, 1985). For fermentations producing avermectin where the pH is not controlled and hence varies between pH 5 and 7, the OUR is used in preference to the CER for biomass estimation, because of the latters distortion by the changing pH (Buckland et al, 1985). Changes in the pH cause changes in the amount of CO_2 evolved which may be interpreted incorrectly as changes in the rate of growth of the organism (Stephanopoulos and San, 1984). Such disturbances do not reflect real changes in the microbial CER, but are physical effects, and hence a method for their removal is desirable.

The OUR is related to the oxygen transfer rate (OTR) are related by means of a simple mass balance involving the concentration of oxygen dissolved in the fermentation broth:

$$OTR = OUR + V_L . \frac{d[O_2]_L}{dt} \qquad (24)$$

where V_L = unaerated broth volume (m^3)

$[O_2]_L$ = concentration of dissolved oxygen in the liquid phase ($mol.m^{-3}$)

OUR = oxygen uptake rate ($mol.s^{-1}$)

OTR = oxygen transfer rate ($mol.s^{-1}$)

Oxygen is sparingly soluble in aqueous solutions, and so the differential term involving the dissolved oxygen concentration is usually very small, so that the OUR and OTR are in practice equal (Heinzle, 1987; Stephanopoulos and San, 1984). This result is of importance as it means that the OUR which is the variable of greater interest to fermentation technologists, can be deduced directly from gas analyses (which indicate the OTR).

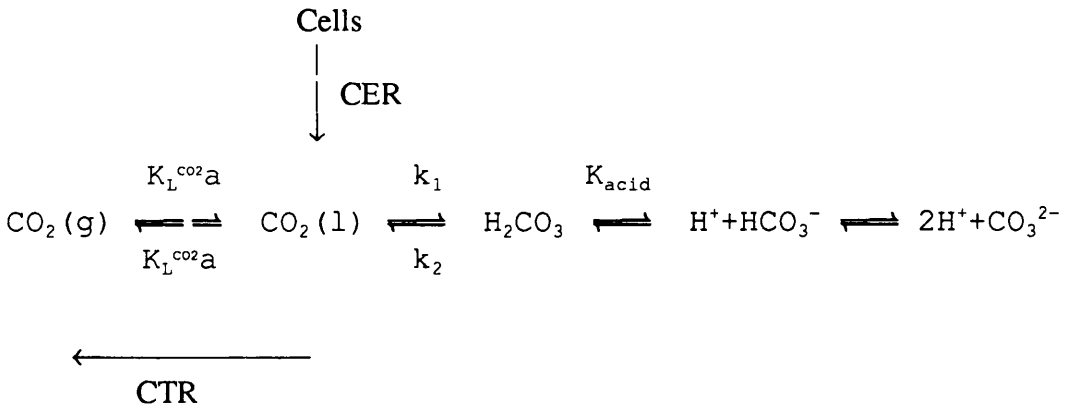
It cannot, however, necessarily be assumed that the CO_2 evolution rate (CER) of biomass in the fermentor, and the CO_2 transfer rate (CTR) across the gas-liquid interface are equal, as carbon dioxide has a much higher solubility than oxygen, a solubility that is enhanced by its reaction with water to form bicarbonate ions. Changes in the concentrations of dissolved carbon dioxide and bicarbonate ions can cause the CTR (available from gas analyses) and CER (unmeasureable) to differ. The subject of this section is to elucidate the relationship between the CER and CTR, and to examine the effects of this relationship on the measured respiratory quotient of aerobic fermentations.

4.4.1 Model development and definition of the transfer quotient, TQ

It has already been noted that the gas analyses indicate the CTR and the OTR (or OUR, as the OTR and OUR are equal). The ratio of the CTR to the OTR is the "measured respiratory quotient" as apparent from gas analyses. However, while

fermentation technologists use this "measured respiratory quotient" to indicate the RQ (being the ratio of the CER to the OUR), in reality, this is only an approximation, as the CER and hence the RQ are, strictly speaking unmeasureable. It will be shown in later sections that there are several circumstances under which the CER and CTR are not equal, causing the "measured respiratory quotient" apparent from gas analyses to differ from the real, underlying RQ. It is the RQ, in reflecting the biological fluxes (CER and OUR) that is of interest to fermentation technologists, but it is the "measured respiratory quotient" reflecting the ratio of the transfer rates (CTR and OTR) which can be measured. It was therefore considered necessary in this project to define a mathematical quantity that reflects this ratio of transfer rates, and this quantity was called the "transfer quotient" or TQ. It will be seen later in this section that this new variable, which differs from the RQ under unsteady state conditions, forms a useful basis on which to examine unsteady-state transfer effects.

Carbon dioxide diffuses through the cell membrane (Jones and Greenfield, 1982) to be involved in the following reversible reactions:



where k_1, k_2 = rate constants for the indicated reactions (s^{-1})

K_{acid} = carbonic acid dissociation constant ($mol.m^{-3}$)

$K_L^{CO2}a$ = volumetric mass transfer coefficient for carbon dioxide transfer (s^{-1})

It was noted in Section 4.3.1 that in the liquid phase, only $CO_2(l)$ and HCO_3^- are

present in significant concentrations. Hence the mass balance for carbon dioxide is:

$$CER = CTR + V_L.\left(\frac{d[CO_2]_L}{dt} + \frac{d[HCO_3^-]}{dt}\right) \qquad (25)$$

where CTR = carbon dioxide transfer rate across gas-liquid interface ($mol.s^{-1}$)

CER = carbon dioxide evolution rate by biomass in the fermentor ($mol.s^{-1}$)

$[CO_2]_L$ = concentration of dissolved carbon dioxide, $CO_2(l)$ ($mol.m^{-3}$)

$[HCO_3^-]$ = concentration of bicarbonate ions ($mol.m^{-3}$)

As there is no sink for bicarbonate ions other than by dehydration, the following equation describes the dynamics of the bicarbonate ion concentration:

$$\frac{d[HCO_3^-]}{dt} = k_1.[CO_2]_L - k_2.[H_2CO_3] \qquad (26)$$

The timescale of changes in fermentation are sufficiently long that the reactions involved in Equation (26) are often close to equilibrium. The rate constants associated with carbonic acid dissociation are so large that this reaction can be considered to be at equilibrium (Ishizaki et al, 1971):

$$[H_2CO_3] = \frac{10^{-pH}.[HCO_3^-]}{K_{acid}} \qquad (27)$$

Some fermentation broths may have high ionic strength which can affect this equilibrium (Arrua et al, 1991), but in most cases the use of concentrations rather than ionic activities will be sufficiently accurate. Although pH probes are sensitive to hydrogen ion activity, they are calibrated against buffers of known hydrogen ion concentration, and so pH can be used to indicate this concentration.

It was shown in Section 4.3.1 that the transfer of carbon dioxide across the gas-liquid interface is a purely physical process as for oxygen, which is liquid-film limited, and which, for a well-mixed gas phase yields:

$$CTR = K_L^{CO_2}a.V_d.([CO_2]_L - p_g^{CO_2,out}/H^{CO_2}) \qquad (28)$$

The next equation introduces the interdependence of the partial pressure of carbon dioxide in the gas leaving the fermentor, $p_g^{CO2,out}$, and the CTR, via the $\%CO_2^{out}$:

$$p_g^{CO_2,\,out} = (P - p_w) \cdot \frac{\%CO_2^{out}}{100} \qquad (29)$$

where P is the head-space pressure in Pa, and p_w the partial pressure of water in the exit gas. The partial pressure of water has to be taken into account because the gas analyses are taken to be on a dry basis, as this is what is produced by mass spectrometers, which are increasingly widely used in fermentation gas analysis.

The relationship between the CER and CTR can be seen from Equation (25) to depend critically on the concentrations of dissolved carbon dioxide and bicarbonate ions. The concentration of dissolved carbon dioxide is dominated by its solubility (Fox, 1984). While the partial pressure of carbon dioxide is typically an order of magnitude less than that of oxygen, its solubility is 25 times that of oxygen. Further, as carbon dioxide transfer is from liquid to gas, by contrast with oxygen, the concentration of dissolved carbon dioxide is typically a few times that of oxygen. The concentration of bicarbonate ions becomes an increasing multiple of the concentration of dissolved carbon dioxide as the pH rises. The fermentation industry derives its primary income from antibiotic fermentations, which are run in the pH range 6.5-8. At pH 7, for example, the bicarbonate concentration is around 5 times that of carbon dioxide, rising to 50 times at pH 8 . Hence, for pH>6.5, the total concentration of carbon dioxide in solution, both in the form of carbon dioxide and bicarbonate ions, can be one to two orders of magnitude greater than that of oxygen. Hence a small relative rate of change with time in the concentration of dissolved carbon dioxide or bicarbonate ions may mean that the differential terms in Equation (25) cannot be ignored.

The differential terms in Equation (25) can be generated during the course of a fermentation by changes in one of several variables. Changes in agitation and aeration rates affect $K_L^{CO2}a$ while changes in the aeration rate and head-space pressure affect $p_g^{CO2,\,out}$ in Equation (28). Such changes are in most practical

situations, relatively rare events and transient in nature, taking place perhaps only once or twice during the course of an entire fermentation. Of greater interest are changes that generate differential terms in the mass balance, that occur continually during the course of a fermentation, such as changes in the CER (Equation 25) and the pH (Equation 27). Changes in the CER are associated in particular with the exponential growth phase in fed-batch fermentations. Where the pH is uncontrolled, changes in the pH arise where the micro-organism consumes or produces an acidic or basic compound (of which carbon dioxide itself is one). Where pH is controlled, pH changes are associated either with an open-loop control strategy to improve productivity (Andreyeva and Biryukov, 1973; Constantinides et al, 1970), such as pH ramping, or with small steps in pH associated with on-off pH control action when the pH is controlled to a constant value.

It has already been noted that the OUR and OTR are equal. The effect of changes in the pH and CER is to cause the CER and CTR to differ. A quantity analogous to the respiratory quotient can be defined, which reflects the relative rates of transfer of carbon dioxide and oxygen across the gas-liquid interface, and is called here the transfer quotient (TQ).

$$RQ = \frac{CER}{OUR} \qquad TQ = \frac{CTR}{OTR}\left(= \frac{CTR}{OUR}\right) \qquad (30)$$

The TQ is therefore the "measured respiratory quotient", that is to say, the respiratory quotient that is apparent from gas analyses. Clearly, it is the RQ that is the fundamental derived variable that is of interest to fermentation technologists, but it is the TQ that can be measured.

In the following sections, the sensitivity of the time profile of the TQ or "measured respiratory quotient" to changes in the pH, CER, and agitation & aeration rates, is investigated by modelling, assuming that the "real" RQ is constant with a value, RQ=1. Although effects in the TQ are caused by effects in the CTR according to Equation (30), only the TQ, and not the CTR is examined, as the TQ has a limited range tied to the range of the RQ in microbial systems, and doesn't vary as greatly as the CTR during fermentations. It is hence easier to illustrate unsteady-state CO_2

transfer effects in the TQ. Solution of equations (25)-(30) requires numerical integration in time, as the CER profile is not known *a priori*. A fourth-order Runge-Kutta scheme was used here, and the C program written to perform this numerical integration is presented in Appendix A3. The parameter and variable values used for modelling are tabulated in Table 16. In modelling work, the fermentor head space, which acts as a response function on the CER, was treated as a well-mixed tank with a residence time of 2 minutes. The RQ was assumed to have a constant value of RQ=1.

4.4.2 Modelling of the effect of pH changes on the TQ

In Figures 55 & 56, the effect of a step change in pH on the TQ is examined, assuming the CER to be constant with a value corresponding to an average exit gas carbon dioxide content of 2%. A step increase or decrease in the pH by 0.03 units is intended to represent a single action of an on-off pH controller. As already noted, the RQ is fixed at a value of 1. In spite of the small pH step, there is a significant transient change in the TQ that becomes more significant with increasing pH. If pH control during a fermentation is by on-off addition of acid or base to counter the drifting pH caused by the organism's production or consumption of acidic or basic compounds, a saw-tooth pH profile results, and so there will be regular fluctuations in the TQ. Such effects are modelled in Figures 57 & 58. The fluctuations in the TQ (caused by corresponding fluctuations in the CTR) can be seen to be a smooth cycling, and not random noise. However, this distinction may not be apparent during real fermentations, because it must be remembered that gas analyses are only available at the relatively low rate of 0.1-0.2min^{-1}, a rate that is asynchronous with pH control action. Asynchronously sampling of the TQ data in Figures 57 & 58 may produce a data set that appears to be noisy in a random way.

In Figure 59, the effect of a constant rate of change in the pH on the TQ is examined, again assuming the real RQ=1. If the pH is increasing, the respiratory quotient as apparent from gas analyses (ie. the TQ) will be smaller than the real RQ,

PARAMETER / VARIABLE	VALUE
$K_L^{CO2}a$	$0.89K_L^{O2}a$ (ref. 14) = 0.89 x 0.05 s^{-1} = 0.045s^{-1}
T	310K
k_1	0.058s^{-1} (ref. 13)
k_2	80.6s^{-1} (ref.18)
K_{acid}	0.68 mol.m^{-3} (ref. 21)
H^{CO2}	4000Pa.m^3.mol^{-1} (ref. 16)
V_d	V_L/0.95 (ie. gas void fraction 5%)
V_L	0.03m^{-3}
G	0.0068mol.s^{-1} (ie. ⅓ vvm based on V_L)
P	1.02x10^5 Pa
p_w	6260 Pa
$\%CO_2^{in}$	0.038 (that of air)
$\%CO_2^{out}$	approximately 2
RQ	1

Table 16: Parameters values used in modelling of unsteady-state CO_2 transfer (unless otherwise indicated in figure captions)

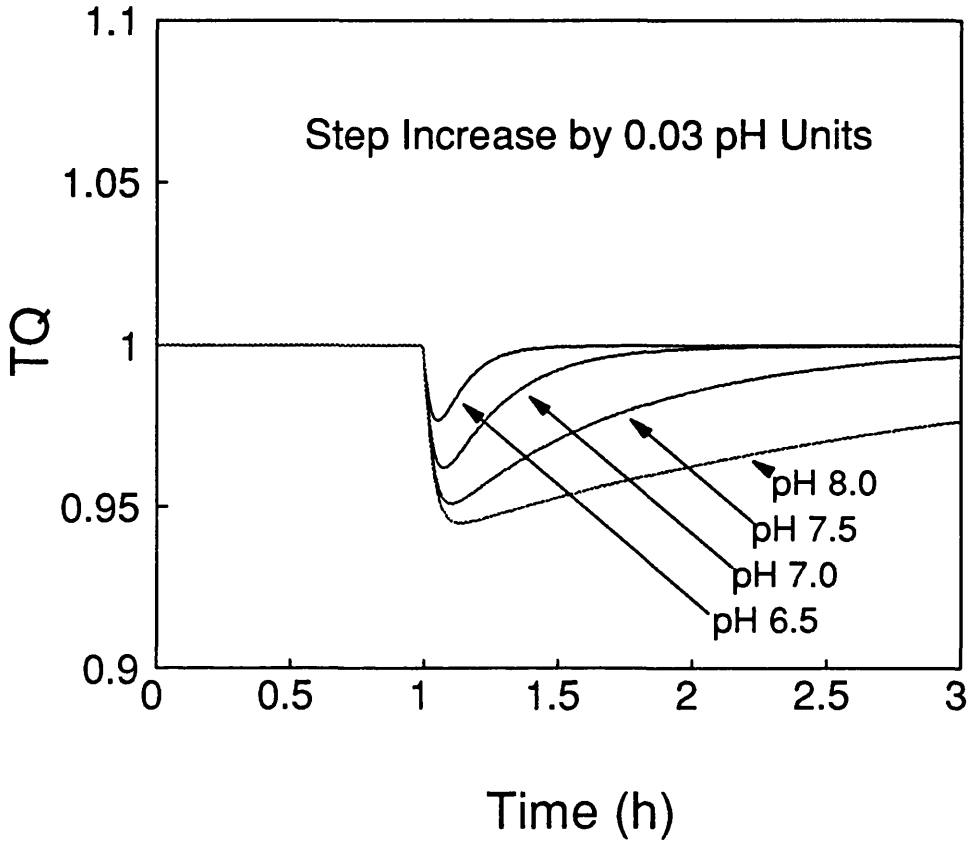


Figure 55: Predicted effect on the transfer quotient, TQ, of a step increase in the pH of 0.03 units (RQ=1)

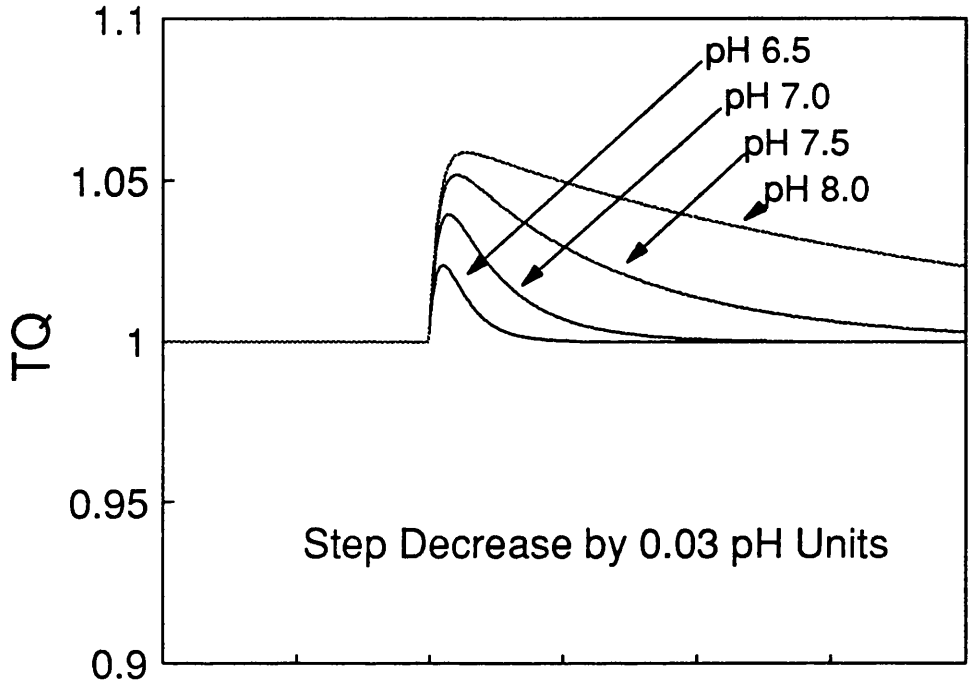


Figure 56: Predicted effect on the transfer quotient, TQ, of a step decrease in the pH of 0.03 units (RQ=1)

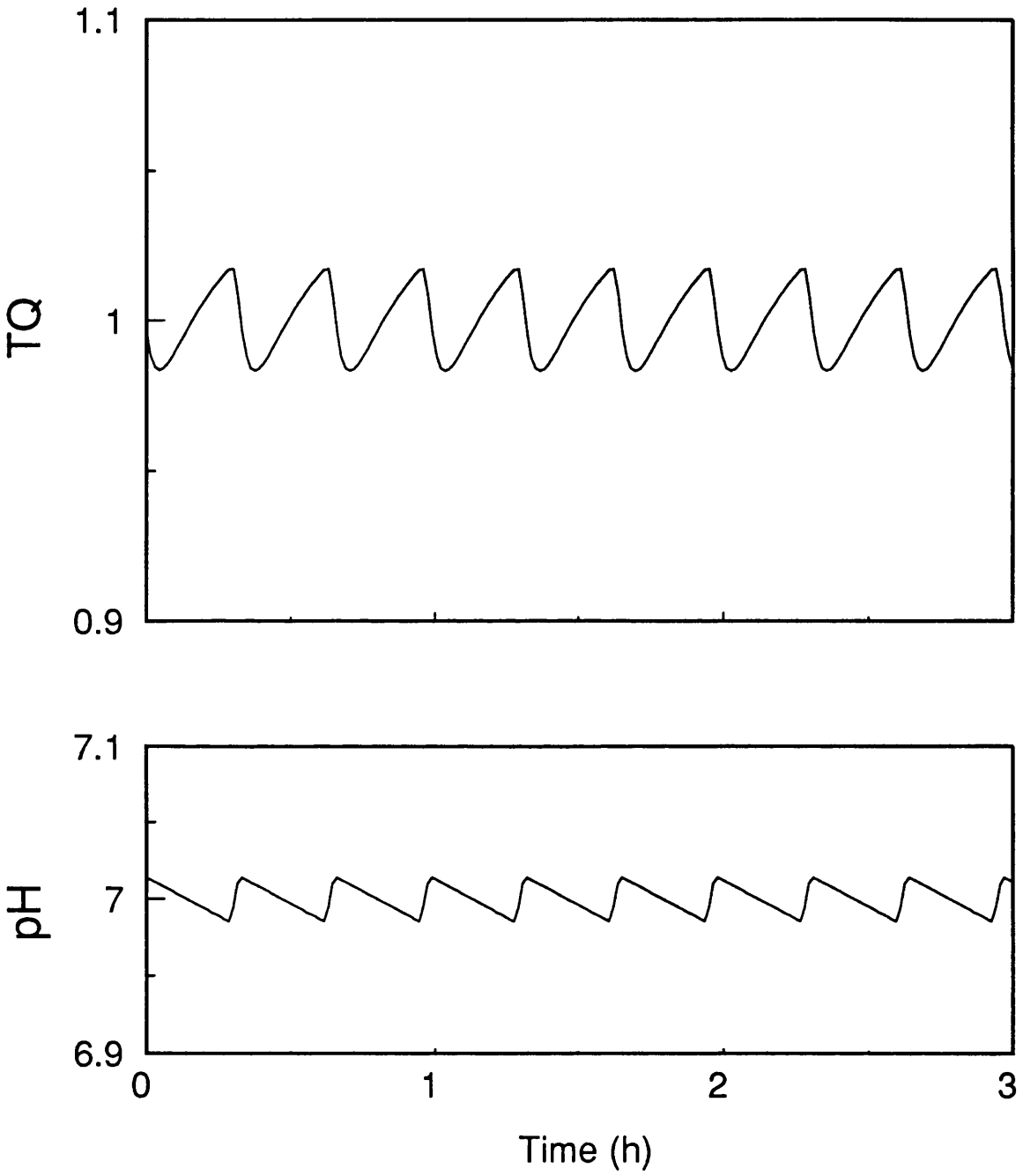


Figure 57: Predicted effect on the transfer quotient, TQ, of pH control to pH 7, by on-off base additions, to compensate for acid production by the fermentation (RQ=1). The fluctuations in the TQ are synchronous with pH control action

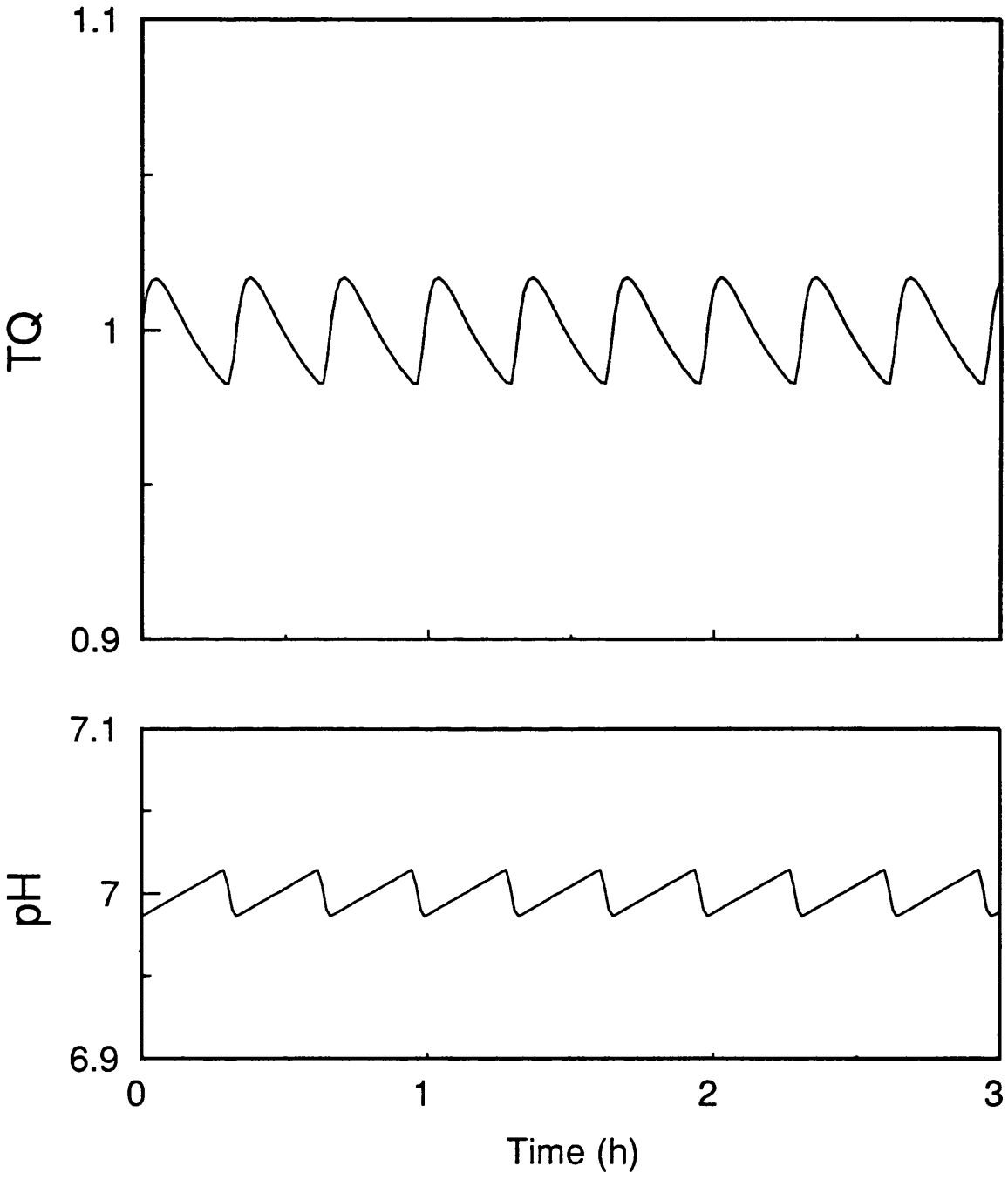


Figure 58: Predicted effect on the transfer quotient, TQ, of pH control to pH 7, by on-off acid additions, to compensate for base production by the fermentation (RQ=1). The fluctuations in the TQ are synchronous with pH control action

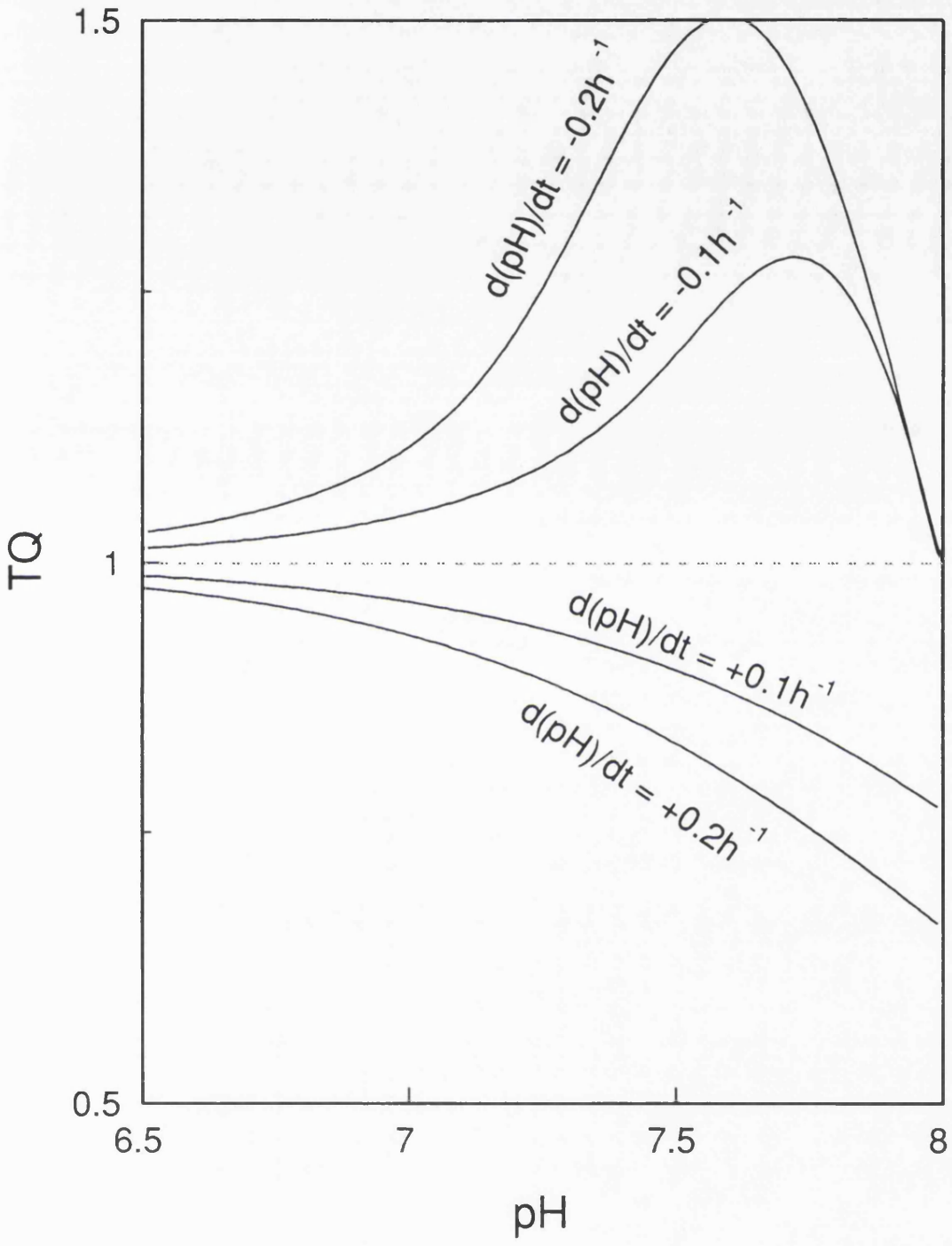


Figure 59: Predicted effect on the TQ of a constant rate of change of pH, as a function of the pH (RQ=1). For the case of decreasing pH, where the result is strongly influenced by the initial conditions, the pH was assumed to have been constant at pH 8 prior to decreasing

and *vice versa*. The case of decreasing pH is strongly influenced by the history of pH changes, and in Figure 59, the curve displays a maximum as the initial conditions assumed were that the pH was constant at pH 8.0, prior to decreasing linearly. The extent of the difference between the TQ and RQ can be seen to increase with increasing pH and increasing rate of change of pH, because both these variable changes serve to make the differential terms in Equation (25) larger.

The way that the information contained in Figure 59 translates into unsteady-state effects in the TQ as a result of a specific fermentation pH profile is modelled in Figure 60 & 61. In Figure 60, the pH is initially constant at pH 7, then ramped at 0.2 units.h^{-1} to pH 7.4, where it remains constant. This could represent an identified optimal open-loop pH control strategy for a particular fermentation. The TQ is initially equal to the RQ for constant pH=7, but deviates from RQ=1 once the pH starts changing. Once the pH has stopped changing at pH 7.4, the deviation between the TQ or "measured respiratory quotient" and the real RQ falls and ultimately disappears. In Figure 61, the pH is again initially constant at pH 7, and then follows a more complex pH profile than in Figure 60, with the pH initially smoothly rising, turning and then falling at a linear rate. This pH profile could represent the pH changes in a fermentation in which the pH is not controlled. The effect of these pH changes on the TQ or "measured respiratory quotient", for RQ=1 can be seen to be significant.

4.4.3 Modelling the effect of CER changes on the TQ

Even when the pH is constant, differences between the TQ and RQ can arise because of changes in the CER. The rate of change in the CER is particularly pronounced during the exponential growth phase of fermentations. The exponential growth phase can be described in terms of a growth rate, μ:

$$CER(t) = CER(t_0) \cdot e^{\mu \cdot (t - t_0)} \qquad (31)$$

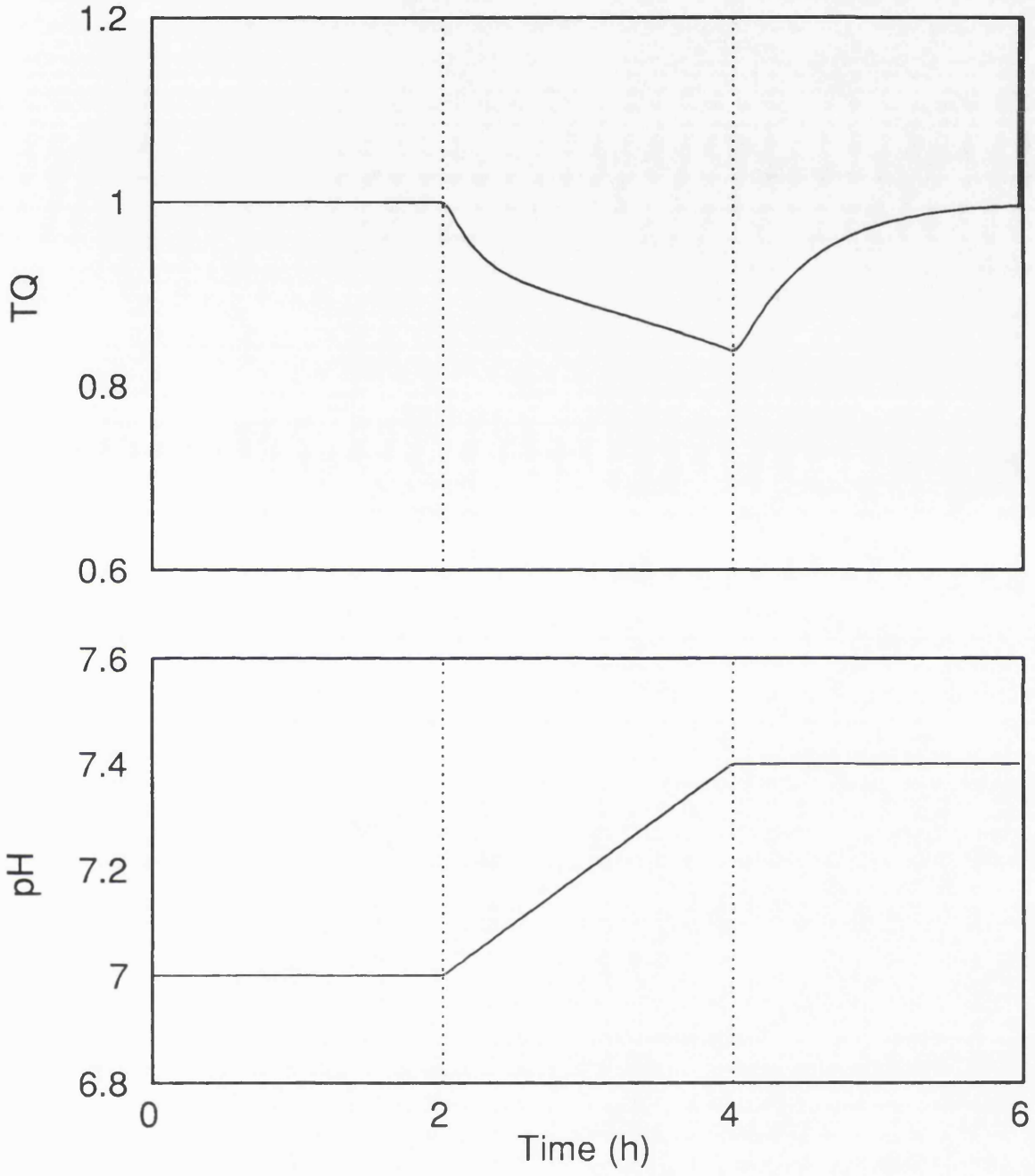


Figure 60: Predicted response of the TQ to a ramping of the pH (RQ=1)

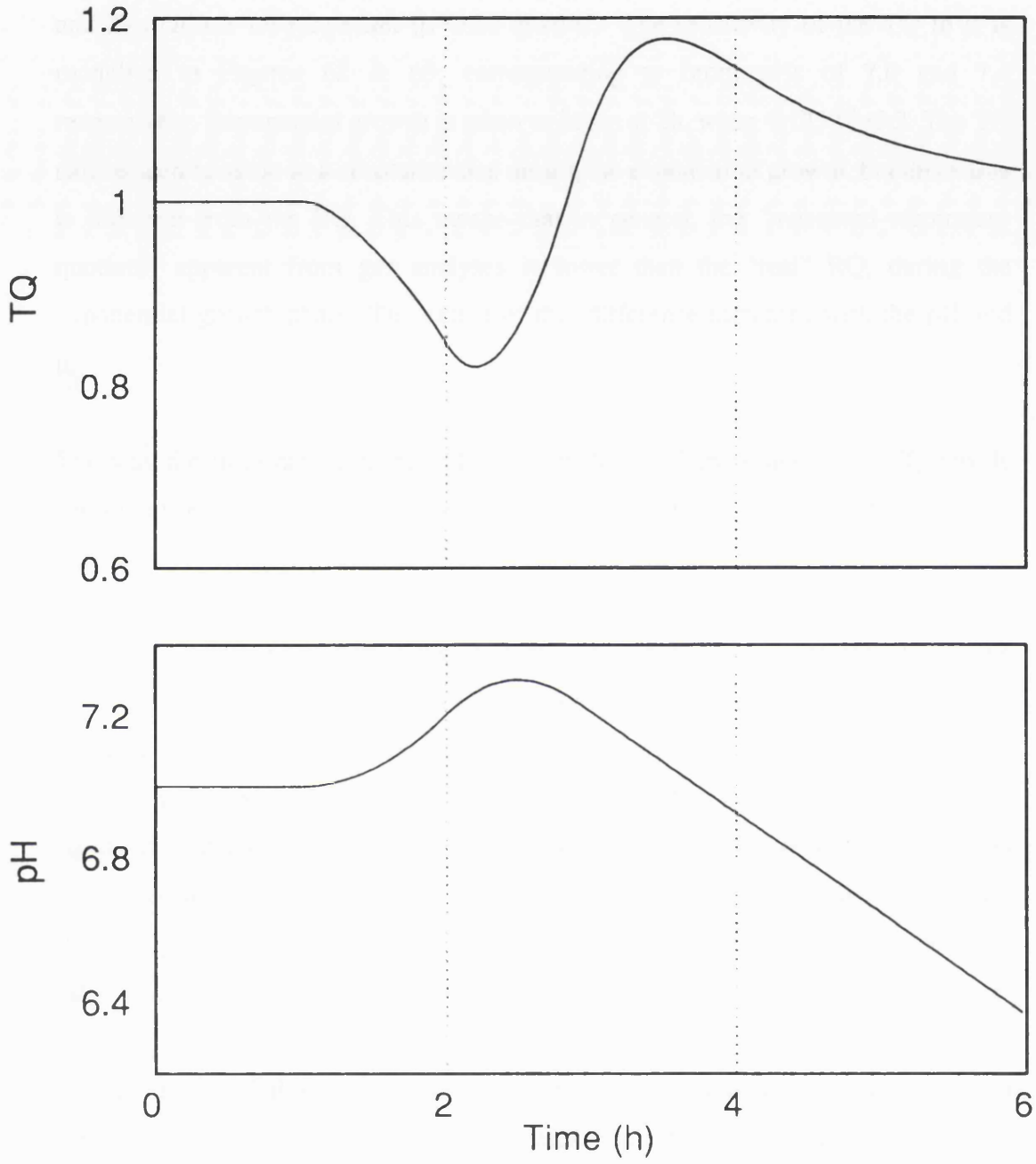


Figure 61: Predicted response of the TQ to uncontrolled drifting in the pH (RQ=1)

An example of a fast-growing organism is *Escherichia coli* with μ=1.0 h^{-1} at 37°C, while slower growing organisms include *Streptomyces clavuligerus* ($\mu \approx 0.30$ @26°C) and *Penicillium chrysogenum* ($\mu \approx 0.20$ @26°C). The sensitivity of the TQ to μ is modelled in Figures 62 & 63, corresponding to broth pHs of 7.0 and 7.4 respectively. Exponential growth is taken to begin at 2h, when $\%CO_2^{out}$=0.2. The TQ can be seen to settle to a constant value during the exponential growth, but this value is different from the RQ. This means that in general, the "measured respiratory quotient" apparent from gas analyses is lower than the "real" RQ, during the exponential growth phase. The extent of this difference increases with the pH and μ.

The way the information contained in Figure 62 & 63 translates into a TQ profile for an entire fermentation is modelled in Figure 64. The CER profile for an entire fermentation is taken to consist of an exponential growth phase (with a growth rate of $0.5h^{-1}$), followed by a reduction in CER corresponding to a substrate limitation, and then a steady CER during the production phase. It can be seen that during the exponential growth phase, the TQ or "measured respiratory quotient" is constant but less than the RQ, which is taken to have a value of one throughout the fermentation. During the period of falling CER, there is a peak in the TQ, and once the CER is steady during the production phase, the TQ approaches the value of the RQ, as unsteady-state effects become less important. Since the TQ is often taken to indicate the RQ by fermentation technologists, there is a danger that these physico-chemical effects could be wrongly interpreted as indicating metabolic changes in the culture.

Changes in the CER are driven not only by phases associated with microbial growth (and death) but also by changes in feed rates. If the feedrate of carbon source to a fermentation is ramped linearly while maintaining substrate limitation, then there will be an approximate linear ramping in the CER as a result. The effect of this ramping of the CER on the TQ can be seen in Figure 65.

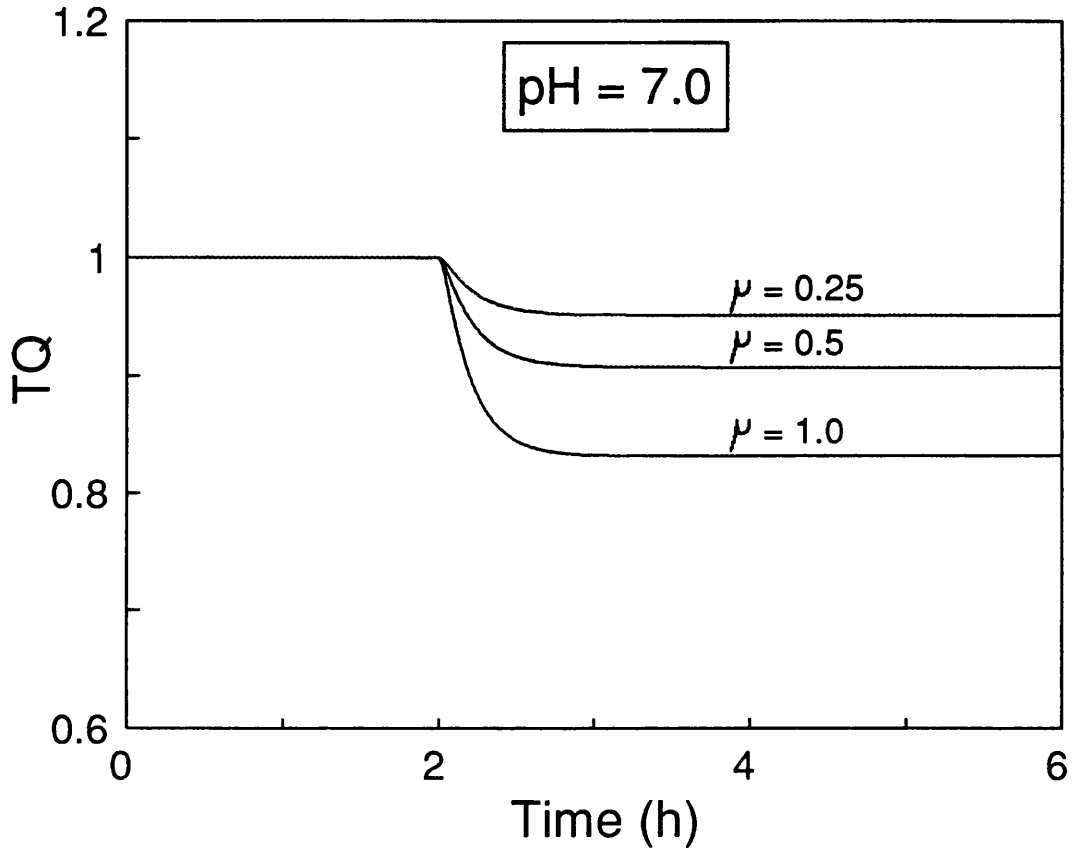


Figure 62: Predicted effect on the TQ of an exponentially changing CER starting at time 2h when $\%CO_2^{out}$=0.2 (pH 7)

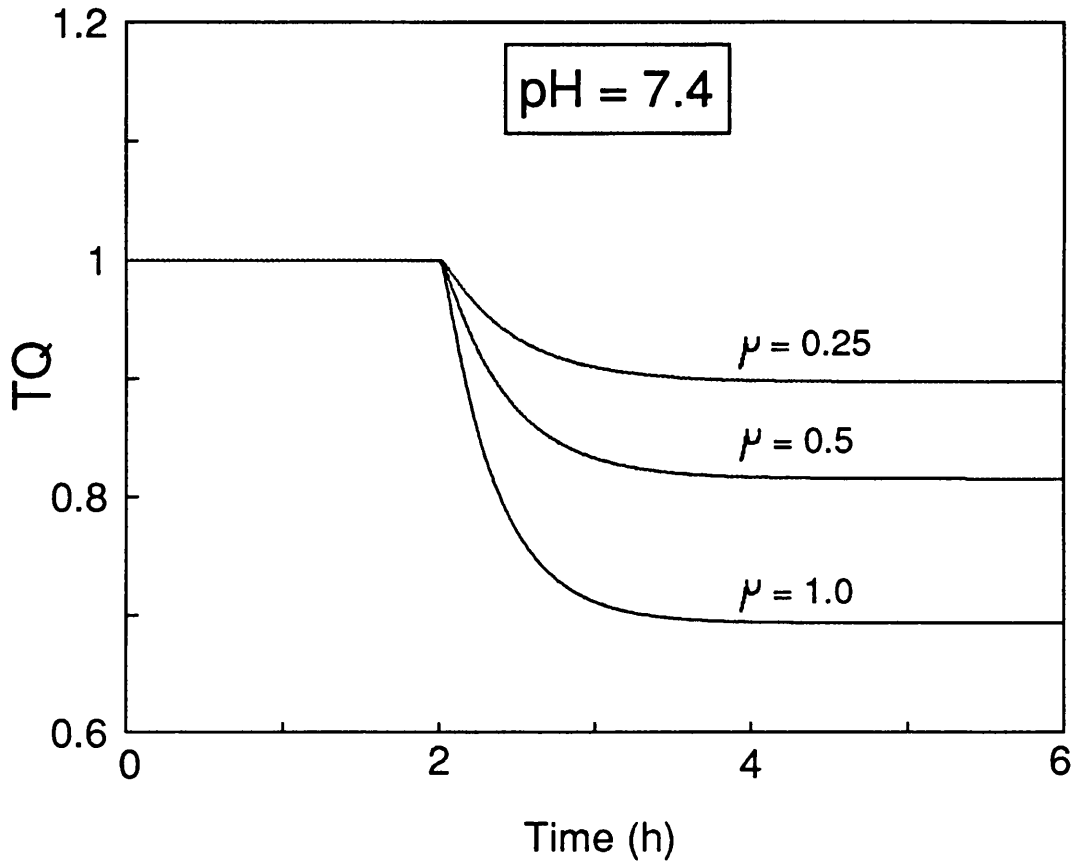


Figure 63: Predicted effect on the TQ of a exponentially changing CER starting at time 2h when $\%CO_2^{out}$=0.2 (pH 7.4)

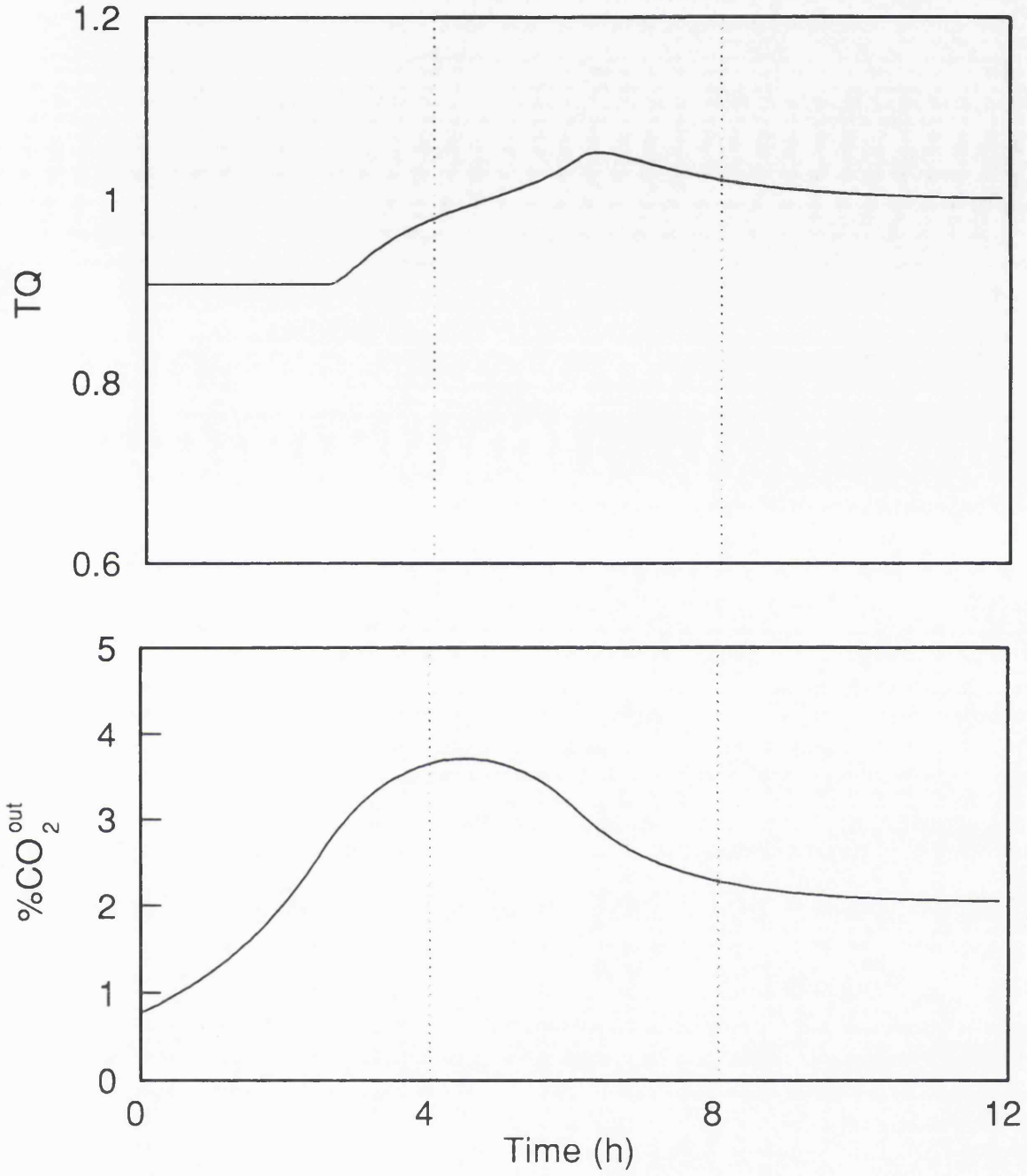


Figure 64: Predicted response of the TQ to a typical CER profile (as indicated by $\%CO_2^{out}$) during the course of a fermentation at pH 7. The growth rate during the exponential growth phase was $0.5h^{-1}$, and RQ=1 throughout

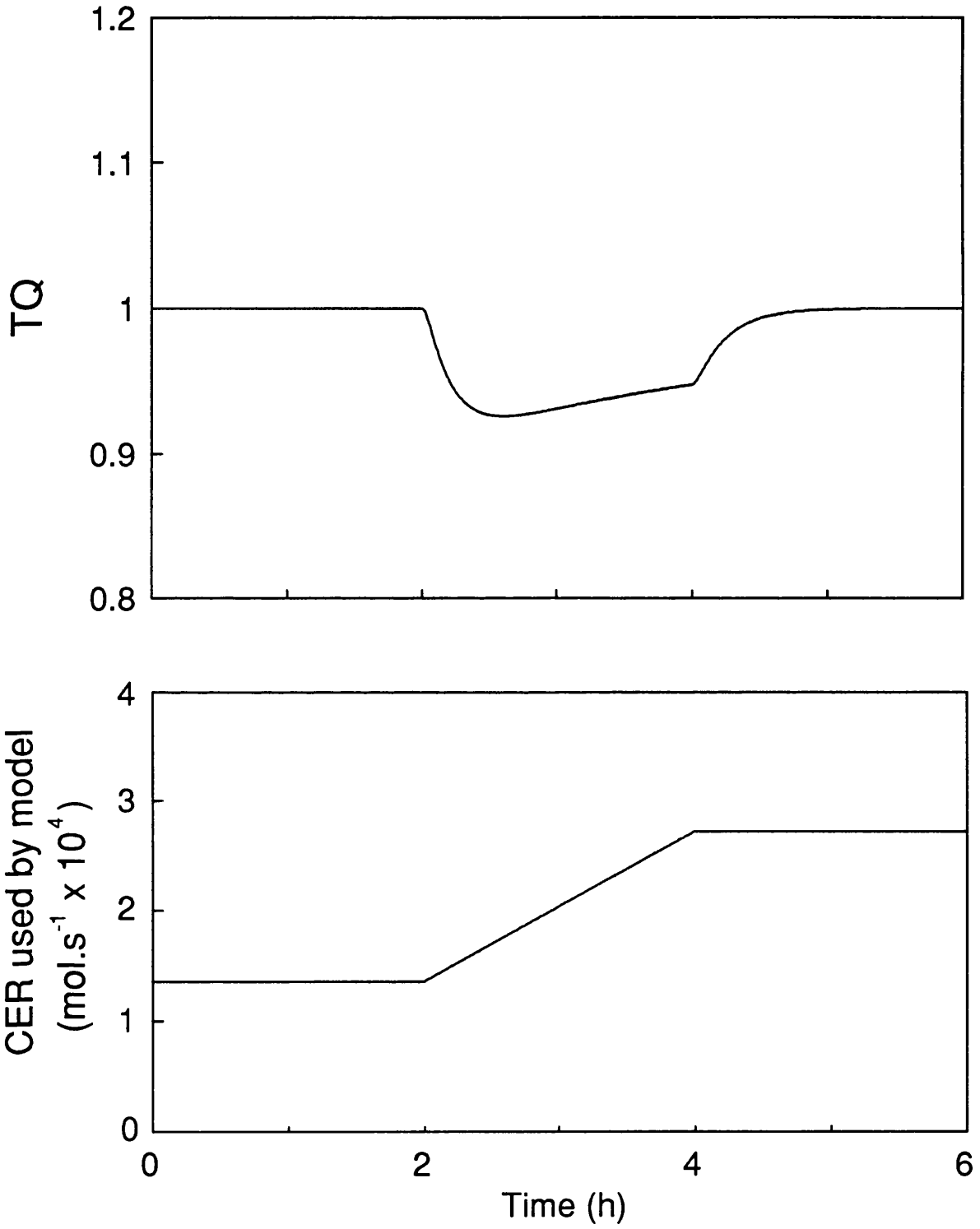


Figure 65: Predicted effect on the TQ of a ramping of the feedrate of growth-limiting substrate, so as to produce a ramping in the CER (RQ=1)

4.4.4 Modelling the effect of changes in agitation and aeration rates on the TQ

Changes in agitation and aeration rates during fermentations are relatively rare events, if occuring at all, and hence their influence on the TQ is of relatively little importance. A step change in agitation rate affects $K_L^{O2}a$, the exponent of the agitation rate in a power law correlation for $K_L^{O2}a$ being, typically, 2.5. Such a change in $K_L^{O2}a$ is mirrored in $K_L^{CO2}a$ (Equation 18), which causes unsteady-state carbon dioxide transfer as a result of the appearance of $K_L^{CO2}a$ in Equation (28). The effect of a step change in agitation on the TQ is modelled in Figure 66.

A step change in aeration rate interacts with the model of unsteady-state CO_2 transfer in a somewhat more complex way. A change in aeration affects $K_L^{O2}a$ (and hence $K_L^{CO2}a$), and $p_g^{CO2,\,out}$, and hence enters the model through Equations (28) and (29). The exponent of the aeration rate (normally expressed as the superficial gas velocity) in a power law correlation of $K_L^{O2}a$ is typically 0.5, as can be seen from Table 6 in the Chapter 2. The effect of a step change in the aeration rate on the TQ is modelled in Figure 67.

This concludes the examination of unsteady-state effects by means of the model developed. The conclusions from modelling can be seen summarized in Table 17. In the next section, experiments using *E. coli* and *S. clavuligerus* are reported which seek to test whether the predictions of the model are observed in practice.

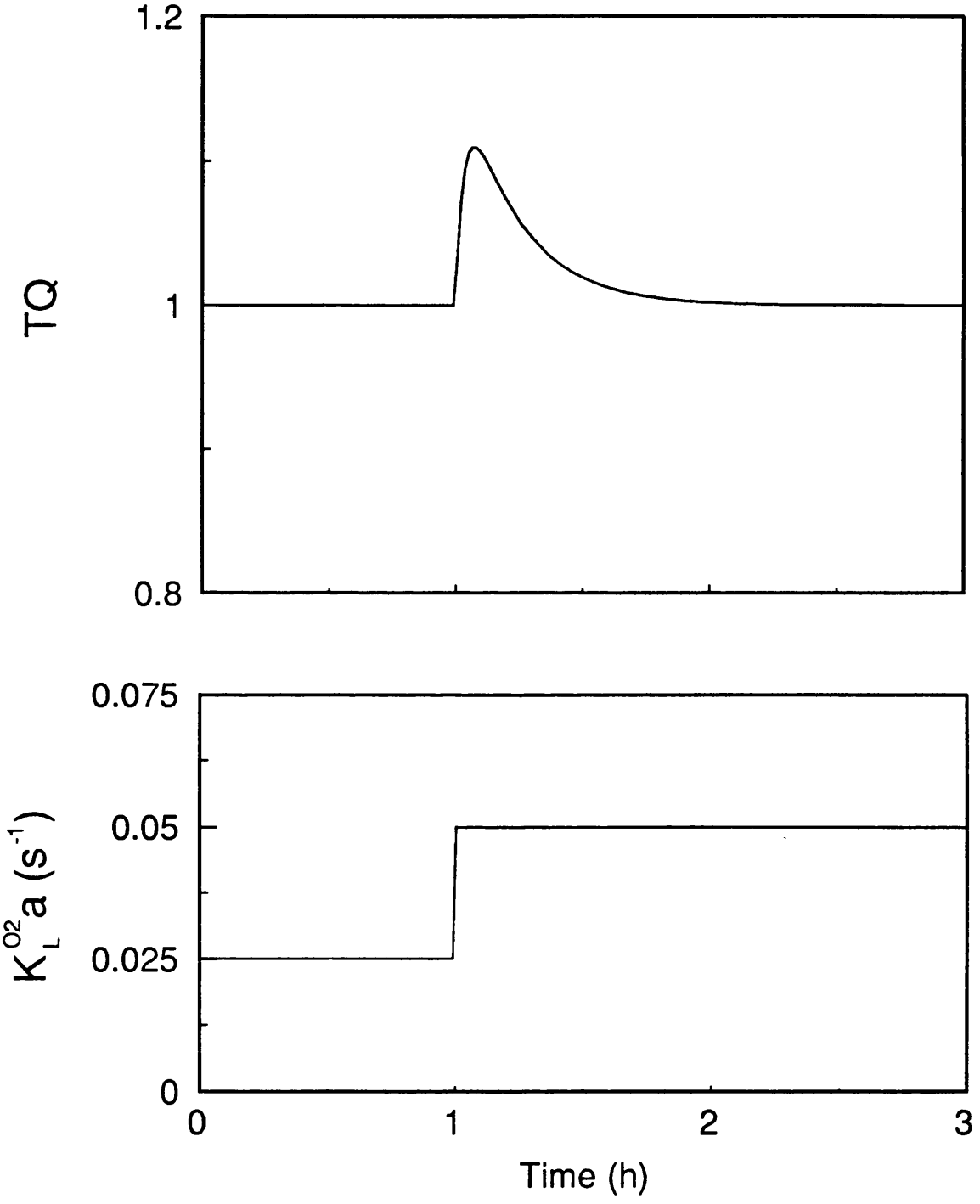


Figure 66: Predicted effect on the TQ of a step change in the mass transfer coefficient, $K_L^{O2}a$ (RQ=1)

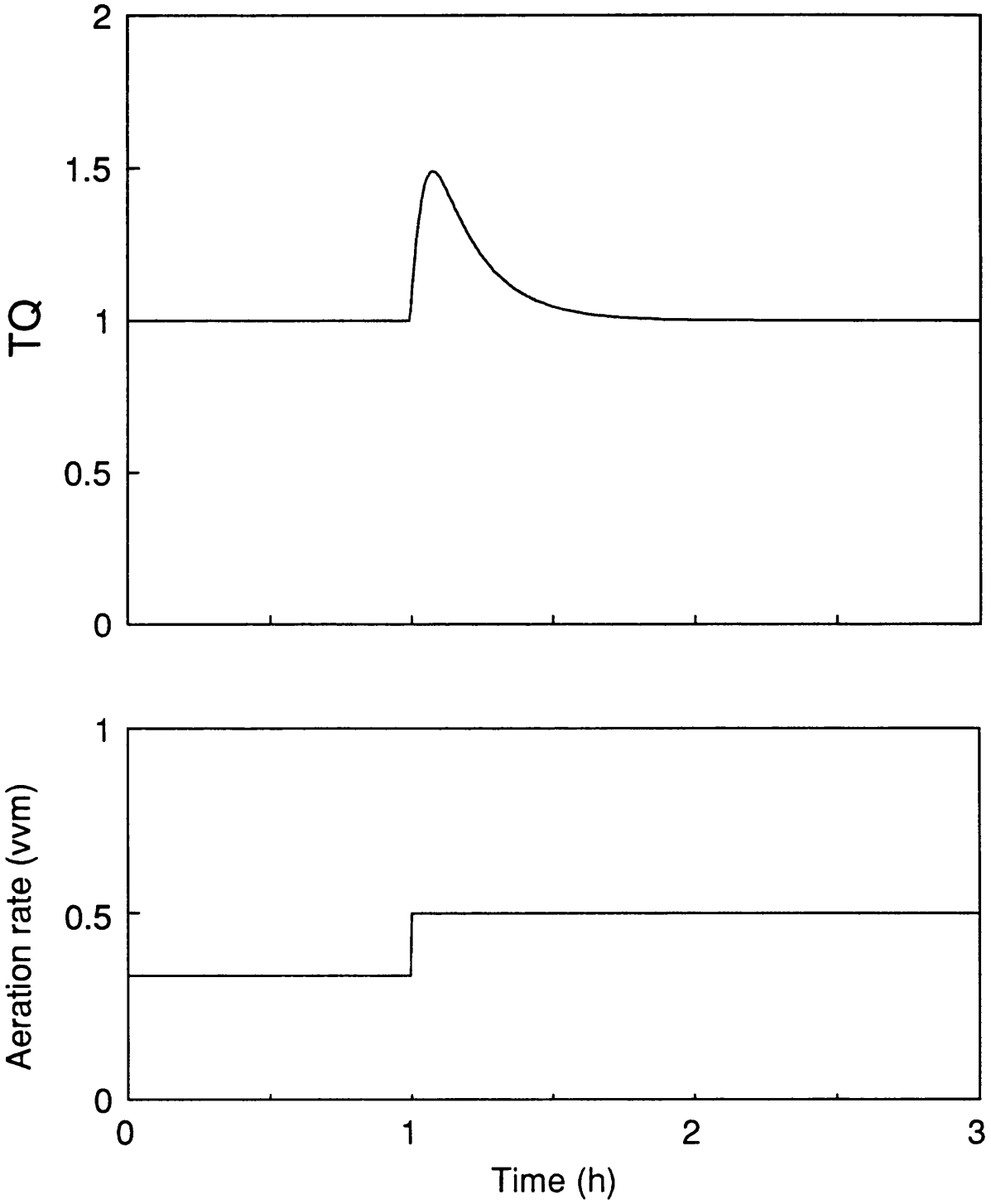


Figure 67: Predicted effect on the TQ of a step change in the aeration rate, from ½ to ⅓vvm (RQ=1)

TQ Changes in some fermentation variables (like the pH and CER) cause changes in the concentration of dissolved carbon dioxide which can affect the respiratory quotient as a apparent from gas analyses (the "measured respiratory quotient"). A model to predict these effects was developed, and used to predict the circumstances under which these effects will be important. The "measured respiratory quotient" being the ratio of the CTR to the OTR, was called the transfer quotient (TQ) to distinguish it from the real underlying RQ (to which the TQ is not always equal)
pH effects 1. A given step change in the pH causes a transient changes in the TQ (for constant RQ) which increases in magnitude and duration with increase in pH. The effect is only significant above pH 6.5 2. The use of on-off acid or base additions to control pH tends to result in a saw-tooth time profile of the pH, which has the effect of introducing regular fluctuations into the TQ (for constant RQ). As the TQ is available only infrequently (from gas analyses), these smooth fluctuations may not be apparent, the TQ appearing instead to be noisy in a random way 3. When the pH changes linearly with time, this generates a sustained difference bewtween the TQ and RQ which increases with increase in pH, and rate of change of pH
CER effects Changes in the CER, whether arising due to microbial growth (e.g. as during the exponential growth phase) or because of changes to the feedrate to a substrate-limited fermentation, generate a sustained difference between the TQ and RQ. The magnitude of this difference increases with pH and with rate of change of CER. During the exponential growth phase, the TQ is smaller than the RQ
Other effects Step changes in agitation and aeration rates, which are occasionally necessary during the course of a fermentation (for dissolved oxygen control, for example) lead to transient changes in the TQ (for constant RQ)

Table 17: Principal results and conclusions from the modelling work concerning unsteady-state CO_2 transfer presented in Section 4.4

4.5 EXPERIMENTAL OBSERVATIONS OF UNSTEADY-STATE CO_2 TRANSFER IN FERMENTATIONS OF *E. coli* AND *S. clavuligerus*

The section describes experimental work to validate the model presented in Section 4.4. The predictions from modelling in Section 4.4 were used to design experiments in which unsteady-state transfer effects could be anticipated. The predicted effect that changes in the pH and CER have on the CTR and TQ were all observed in practice. An important conclusion was that the "measured respiratory quotient" (TQ) during the exponential growth phase is less than the real RQ by an amount that depends on the microbial growth rate and the pH. A simple estimator was proposed and tested, which is able to calculate the true RQ from the TQ recursively, and could therefore be applied on-line in a supervisory system.

Explicit validation of the model presented in Section 4.4 is difficult as it requires an independent way of determining the (unmeasureable) CER, or requires a carbon dioxide mass balance, incorporating rapid analysis of liquid samples. Instead, the model is tested implicitly, by firstly identifying the characteristic fermentation profiles for the model fermentation systems chosen and then investigating how these profiles are modified by conditions shown in Section 4.4 to generate unsteady-state CO_2 transfer. Results are presented from seven *E.coli* fermentations and from one *S. clavuligerus* fermentation.

Standard fermentation time profile for *E. coli*

In Figure 68 are presented typical time profiles for the *E. coli* fermentation, with the pH controlled to pH 7 throughout, by base additions to neutralize the acetic acid produced by the fermentation (for operating conditions described in Section 3.3.2 on Page 124). The gas analysis results were available with a frequency of approximately $0.15min^{-1}$, a typical rate in industrial fermentation facilities where expensive mass spectrometers are multiplexed between several fermentors (Buckland et al, 1985).

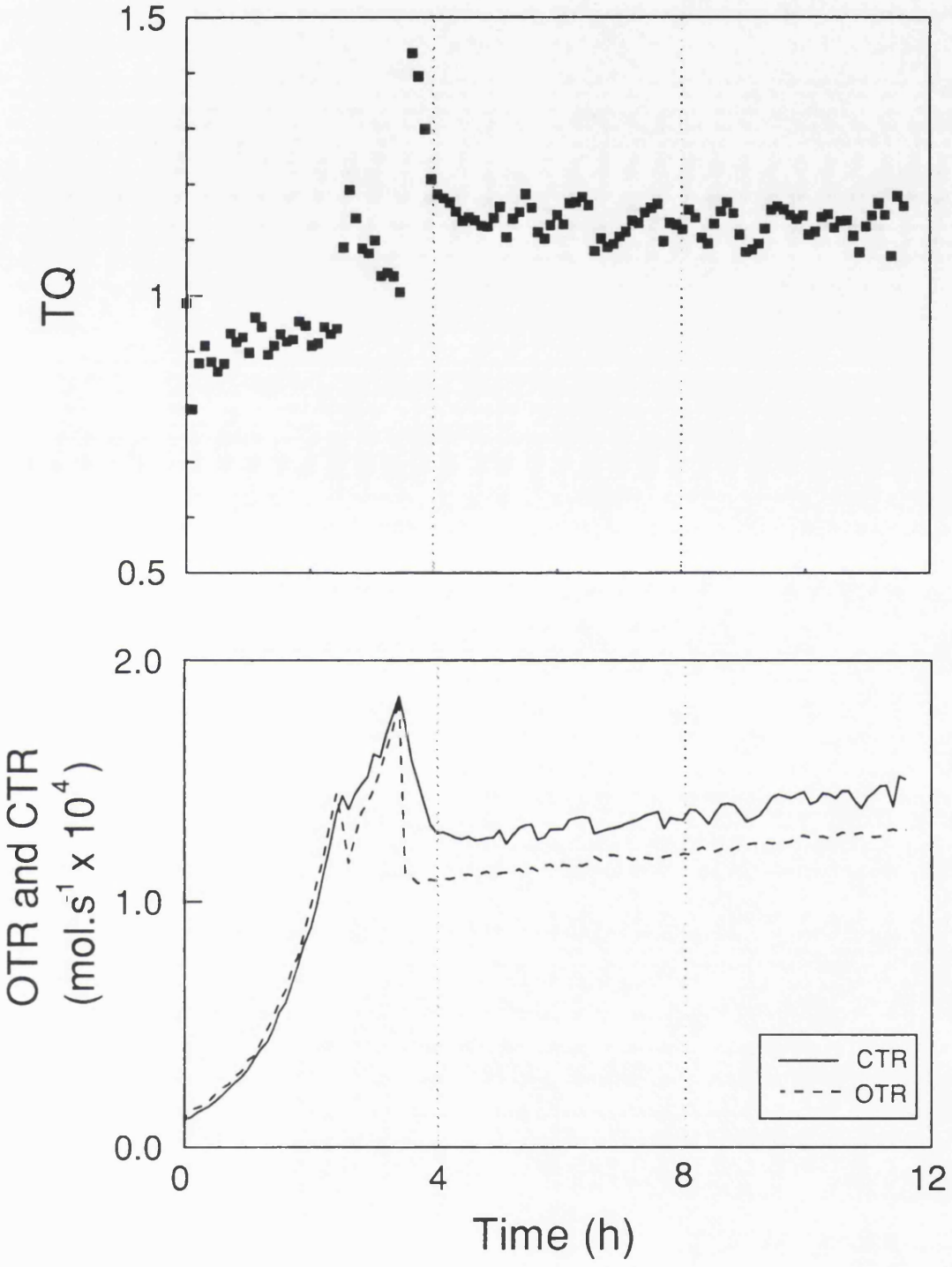


Figure 68: The CTR, OTR and TQ profiles for a standard *E. coli* fermentation run at pH 7

The TQ settles to an average value of 0.94 during the exponential growth phase. The slighty lower value of the TQ in the earliest stages of the exponential growth phase is due to bias in the oxygen analyses being a larger proportion of the (very low) OTR, as noted in Section 4.2.2. Glucose feeding (at 3.3ml.min^{-1}) begins just after 2h, and between 2.5h and 4.5h, there is a period of adjustment to the fed-batch conditions. During the fed-batch phase from 4.5h onwards, the CTR and OTR rise very slightly, while the TQ remains constant at an average value of 1.13. In an identical fermentation run a week after that presented in Figure 68, the values of the TQ during the exponential growth phase and fed-batch phase were again 0.94 and 1.13, indicating that the TQ or "measured respiratory quotient" under a given set of operating conditions is very characteristic of the fermentation and highly reproducible. It is only during the period 4.5h to 12h that both the pH and CER are fairly constant, and hence only during this period that the TQ may be expected to be equal to the RQ. The following may therefore be deduced from the analysis of Section 4.4 concerning the nature of the TQ and RQ:

Phase	**Period**	**TQ**	**RQ**	**Relationship**
Exponential	0.0 - 2.5 h	constant	constant	TQ ≠ RQ
Transition	2.5 - 4.5 h	varying	varying?	TQ ≠ RQ
Glucose-fed	4.5 - 12 h	constant	constant	TQ = RQ

The phase of constant glucose feeding, in involving steady-state carbon dioxide transfer, forms a useful basis from which unsteady-state effects may be measured. A series of fermentations were run, which were prepared in the same way as that in Figure 68. In each case, once the fermentation had adjusted to fed-batch conditions with a glucose feed rate of 3.3 ml.min^{-1}, as indicated by the TQ being steady at a value of 1.13, changes were made to the pH or glucose feed rate in order to generate unsteady-state transfer of CO_2. The observed changes in the TQ were compared with those predicted by the model proposed in Section 4.4.

4.5.1 Effect of pH changes on the CTR and TQ

This section examines the experimentally-observed effects of pH variations on the "measured respiratory quotient" (TQ) apparent from gas analyses, and shows that the observed effects are physico-chemical in nature and can be accurately accounted for by the model proposed in Section 4.4.

With a constant feed rate of glucose, the CER is forced to be substantially constant, so that the effect of a changing pH on the TQ can be examined in isolation. Prior to examining the effect on the TQ of changes in the pH occuring over extended periods, the possible link between noise in the CTR and TQ, with pH control action is examined. Figure 68 shows that most of the noise in the TQ originates in the CTR rather than the OTR. Section 4.4 predicted (Figures 57 & 58) that the saw-tooth pH profile produced by on-off pH control action will cause recurring fluctuations in the TQ. Figure 69 shows a magnified section of data from the fed-batch phase of an *E. coli* fermentation, during which the gas analysis data were available at a high frequency (1.25 min^{-1}). The CTR data points from the fermentation can be seen to cycle synchronously with pH control action, indicating that pH control action is responsible for this cycling. Gas analysis data in industrial facilities are normally available at a much lower frequency of around 0.1 min^{-1}, and in such an instance, the cycling may not be apparent, and the CTR and TQ data may simply appear to be noisy in a random way, as in Figure 68. It can also be seen in Figure 69 that if the model of Section 4.4 is provided with the actual pH data from the fermentation, it is able to quantitatively predict the fluctuations that will be observed in the CTR data. The model output presented in Figure 69 (and in later figures in this section) was generated assuming the head space in the 42L fermentor (which has a volume of 12L) to act as a CSTR with residence time 1¼ minutes. The response time of the pH probe and residence time of gas held up in the fermentation broth were both sufficiently small to be ignored. The data presented in Figure 69 clearly show that fluctuations present in the CTR are purely physical effects, and do not reflect fluctuations in the CER. The fluctuations will however reflect in the measured

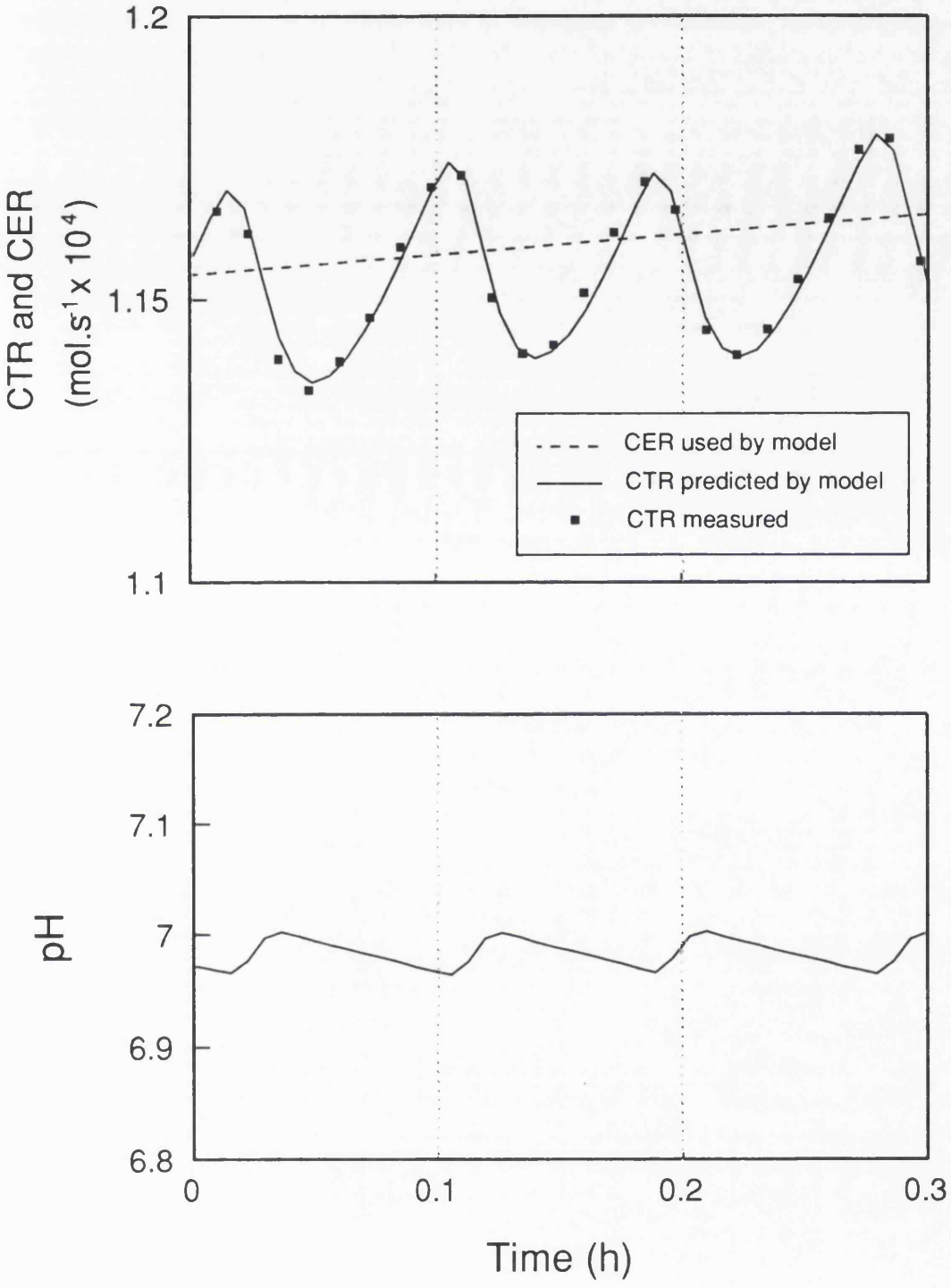


Figure 69: Measured effect of pH control action on the CTR of an *E. coli* fermentation, compared with the effect predicted by the model. The model results used the actual fermentation pH profile, and assumed the CER to be a smooth function of time

respiratory quotient (TQ), even though the underlying RQ doesn't contain such fluctuations. Removal of these effects is achieved implicitly in the estimation algorithm which will be shown in Section 4.5.3, which allows the feedback estimation of the RQ from the TQ data.

Such cycling of the CTR as a result of pH control action, is also apparent during the *S.clavuligerus* fermentations. Figure 70 shows a section of data from a *S.clavuligerus* fermentation, and it can be again seen that fluctuations in the CTR and hence in the TQ, are synchronized with pH control action.

Figure 71 shows the fed-batch phase of an *E. coli* fermentation in which the pH was ramped from 7.0 to 7.4 over a period of two hours, while the glucose feed rate was maintained at a constant 3.3 $ml.min^{-1}$. The discrete points indicate the TQ calculated from gas analyses initially starts at 1.13, already established as being characteristic of this fermentation. As the pH begins to rise, this TQ falls. If the measured respiratory quotient apparent from gas analyses (TQ) were assumed to directly reflect the real underlying respiratory quotient (RQ), an assumption normally made by fermentation technologists, Figure 71 would imply that a changing pH is causing metabolic changes that result in a variation in the RQ. In reality, it can be seen that this is not the case, as the starting value of the TQ of 1.13 is restored once the pH reaches 7.4 and has ceased to change with time. This restoration is not surprising for, as long as the process is operated within a pH range suited to the organism, there is no reason to expect fundamental changes in the underlying metabolic processes. It can be seen in Figure 71 that the continuous line, which is the TQ predicted by the model described in Section 4.4 (using a constant RQ of 1.13, and using the actual pH data) agrees well with the observed changes in the TQ. It may therefore be concluded that in Figure 71, the RQ has remained unchanged throughout, and has a value of 1.13. The "noise" in the simulated TQ is a time-compressed version of the fluctuations arising from pH control action, described earlier.

In Figure 72, the effect of a maximum in the pH profile is examined. At the turning

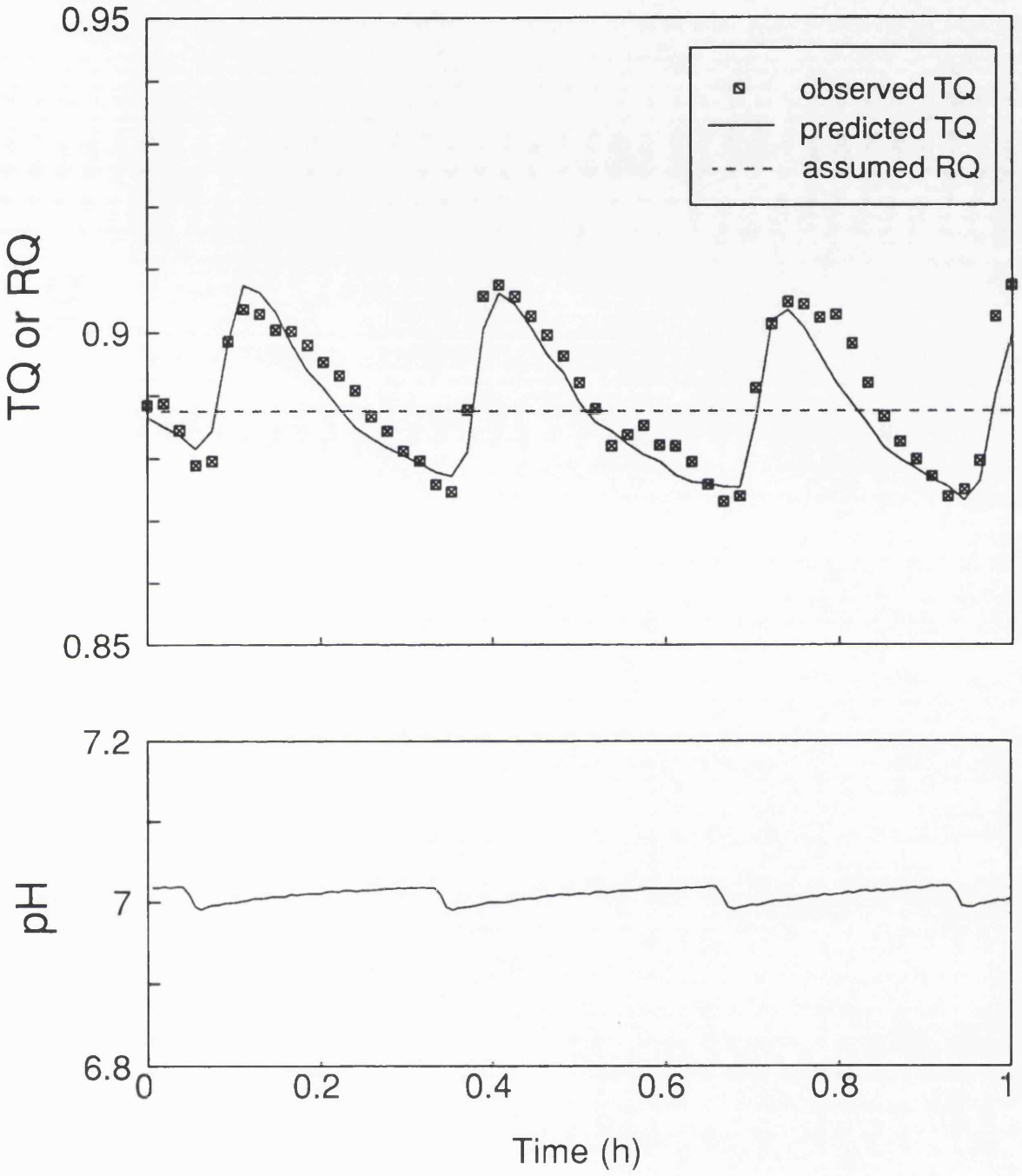


Figure 70: Measured effect of pH control action on the CTR of an *S. clavuligerus* fermentation, compared with the effect predicted by the model. The model results used the actual fermentation pH profile, and assumed the CER to be a smooth function of time

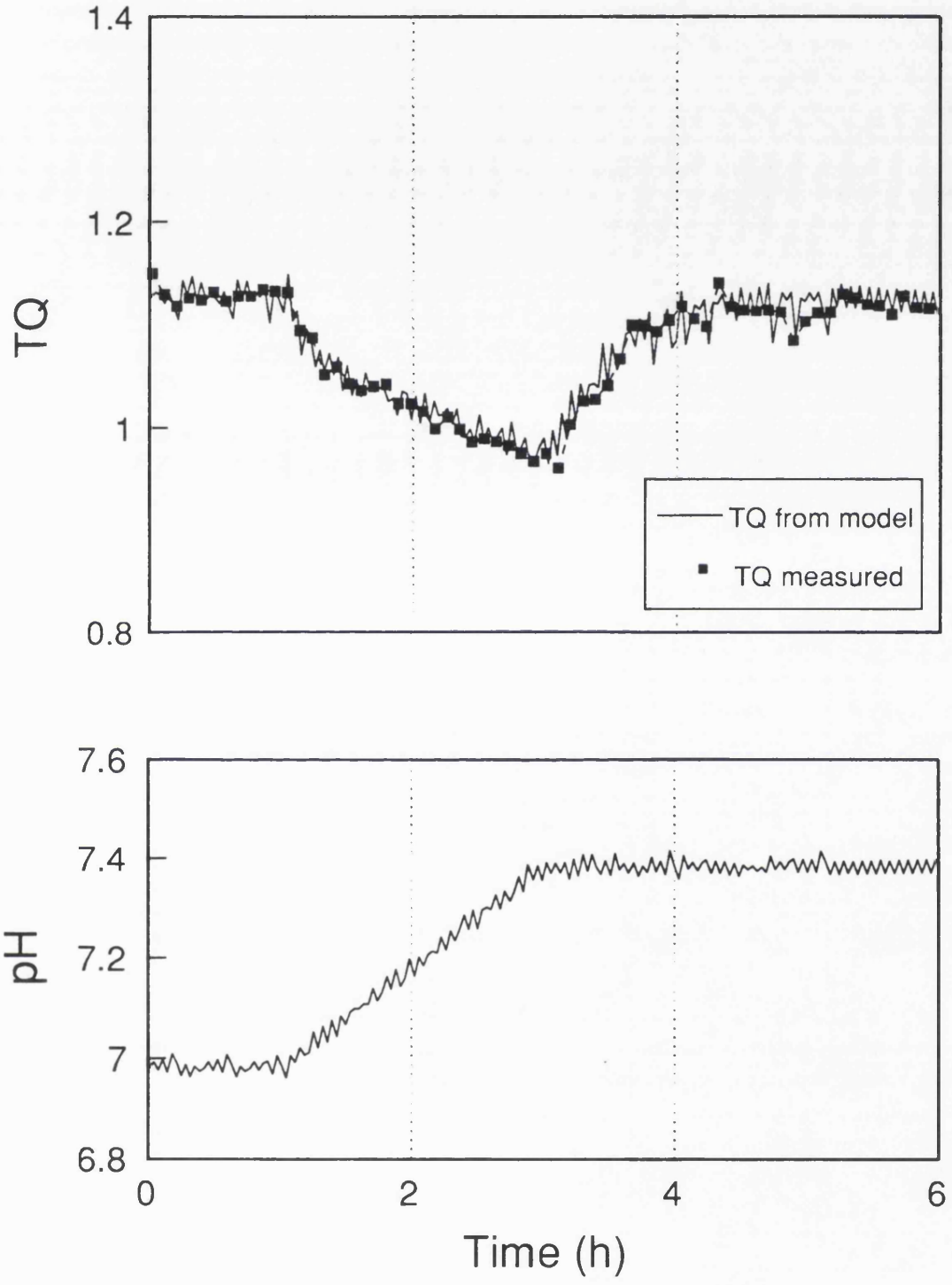


Figure 71: Measured effect of a pH ramp on the TQ during the fed-batch phase of an *E. coli* fermentation, compared with that predicted from the model (RQ=1.13)

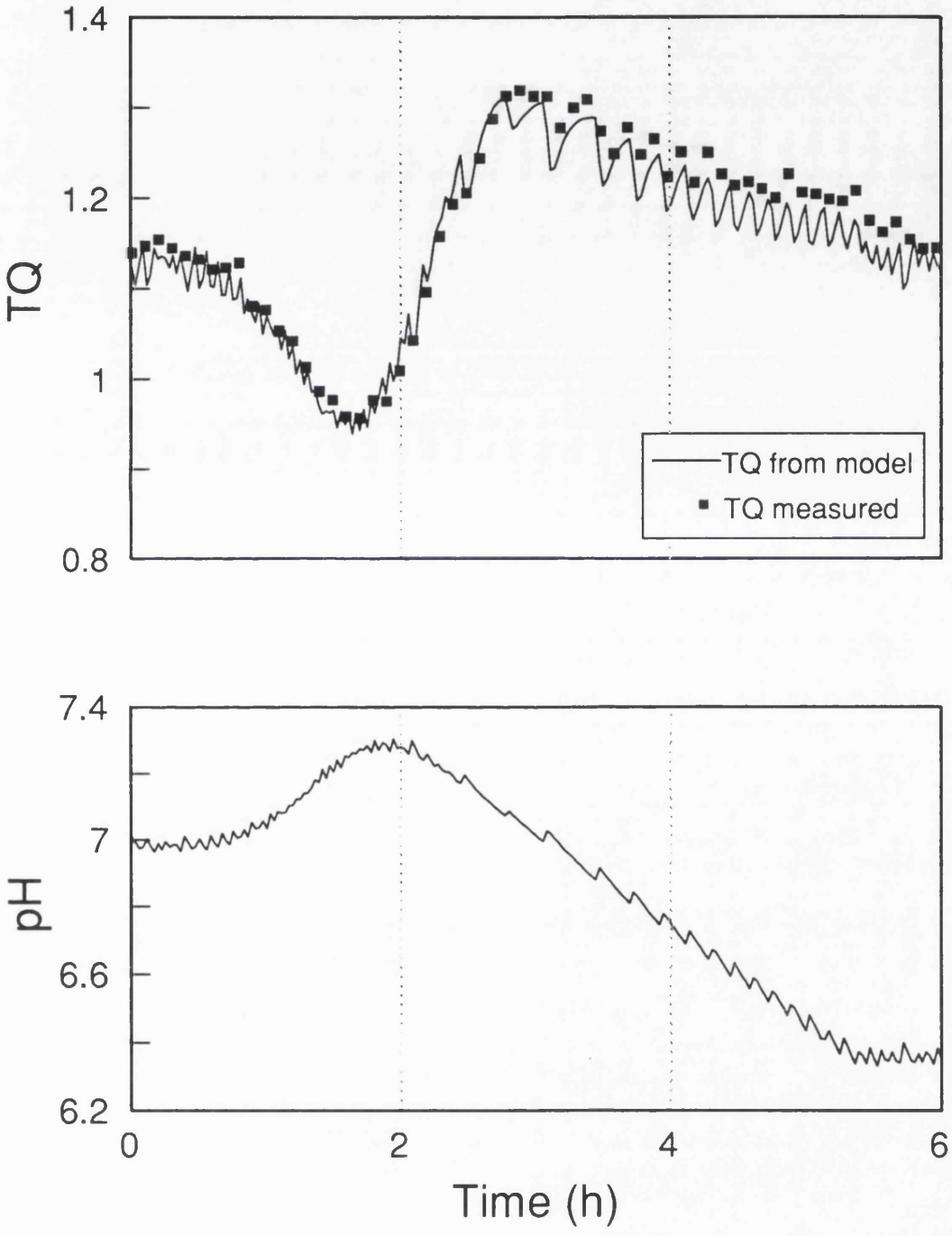


Figure 72: Measured effect of a complex pH profile on the TQ during the fed-batch phase of an *E. coli* fermentation, compared with that predicted from the model (RQ=1.13)

point in the pH, the experimentally measured TQ (indicated by the discrete points) changes from being less than the starting value of 1.13, to being more than this starting value. As the pH falls linearly, the TQ approaches its starting value of 1.13, and once the pH is constant at 6.4, the TQ returns to this starting value. The continuous line indicates the TQ predicted by the model, assuming RQ=1.13 throughout, and using the actual pH profile. All the observed changes in the TQ or "measured respiratory quotient" are predicted by the model, indicating that again, the real RQ for this fermentation has in reality remained fixed throughout at a value of 1.13.

It is clear from Figures 71 and 72 that a changing pH generates an unsteady-state carbon dioxide transfer problem which causes the TQ or "measured respiratory quotient" to change with time even when the real underlying RQ is constant. Clearly, this has important implications for fermentations monitoring and control, as there is a danger of misinterpreting information from fermentations if the TQ is used blindly as a measure of the underlying RQ.

4.5.2 Effect of changes in the CER on the TQ

In this section, it is demonstrated experimentally that a changing CER causes the TQ and RQ to differ. Such effects are observed irrespective of whether the change in the CER is due to microbial growth (e.g. the exponential growth phase) or due to changes to the substrate feedrate to a substrate-limited fermentation. It is concluded that the "measured respiratory quotient" (TQ) apparent from gas analyses is smaller than the real underlying RQ during the exponential growth phase in all fermentations, by an amount that depends on the exponential growth rate and pH.

Varying the feedrate of glucose to the fermentation has the effect of causing the CER to vary. In Section 4.4 it was predicted that a varying CER will generate a difference between the TQ and the RQ. While a varying CER arises as a matter of course during the exponential growth phase of the *E. coli* fermentation, the RQ is

not known *a priori* during this period, and so the model predictions can't be clearly validated. Hence in this work the varying CER is initially generated "artificially" by changing the glucose feedrate after the steady-state fed-batch TQ of 1.13 associated with the constant glucose feed rate of 3.3ml.min^{-1} has become established. In this way, the initial value of the RQ is known to be 1.13.

Figure 73 illustrates the TQ resulting from a linear ramping of the glucose feedrate from 3.3 to 6.6 ml.min^{-1} over two hours. The discrete points indicate the changes in the experimentally measured TQ, calculated from gas analyses. The continuous line indicates the TQ predicted by the model, assuming RQ=1.13 throughout. The CER required by the model was taken to be indicated by the product of the actual fermentation OTR with the RQ, and is indicated in Figure 73. It can be seen that the model was substantially correct in predicting the effect of the changing feedrate on the TQ, although the initial average value of the observed TQ, being 1.10, is slightly lower than in previous fermentations. The data in Figure 73 suggest that the RQ has in reality changed slightly during the course of the period indicated. This is not unexpected as, at a higher glucose feedrate, the cell is able to divert more of its energies to growth rather than maintenance, and so a change in carbon fluxes in the underlying metabolic processes is likely. The important result in Figure 73 is the experimental evidence for the effect on the measured respiratory quotient (TQ) that a changing CER has.

Two points emerge from Figure 73. Firstly, the effect of a changing CER on the TQ as predicted by the model of Section 4.4 is observed in practice. This means that, during any period of changing CER, such as the exponential growth phase of an organism, the respiratory quotient apparent from gas analyses (TQ) will in general be different from the real underlying RQ. Secondly, for the model of Section 4.4 to be used in the removal of unsteady-state CO_2 transfer effects so as to expose the underlying (unmeasureable) RQ, a feedback estimator is required so that real changes in the RQ during the course of a fermentation can be revealed.

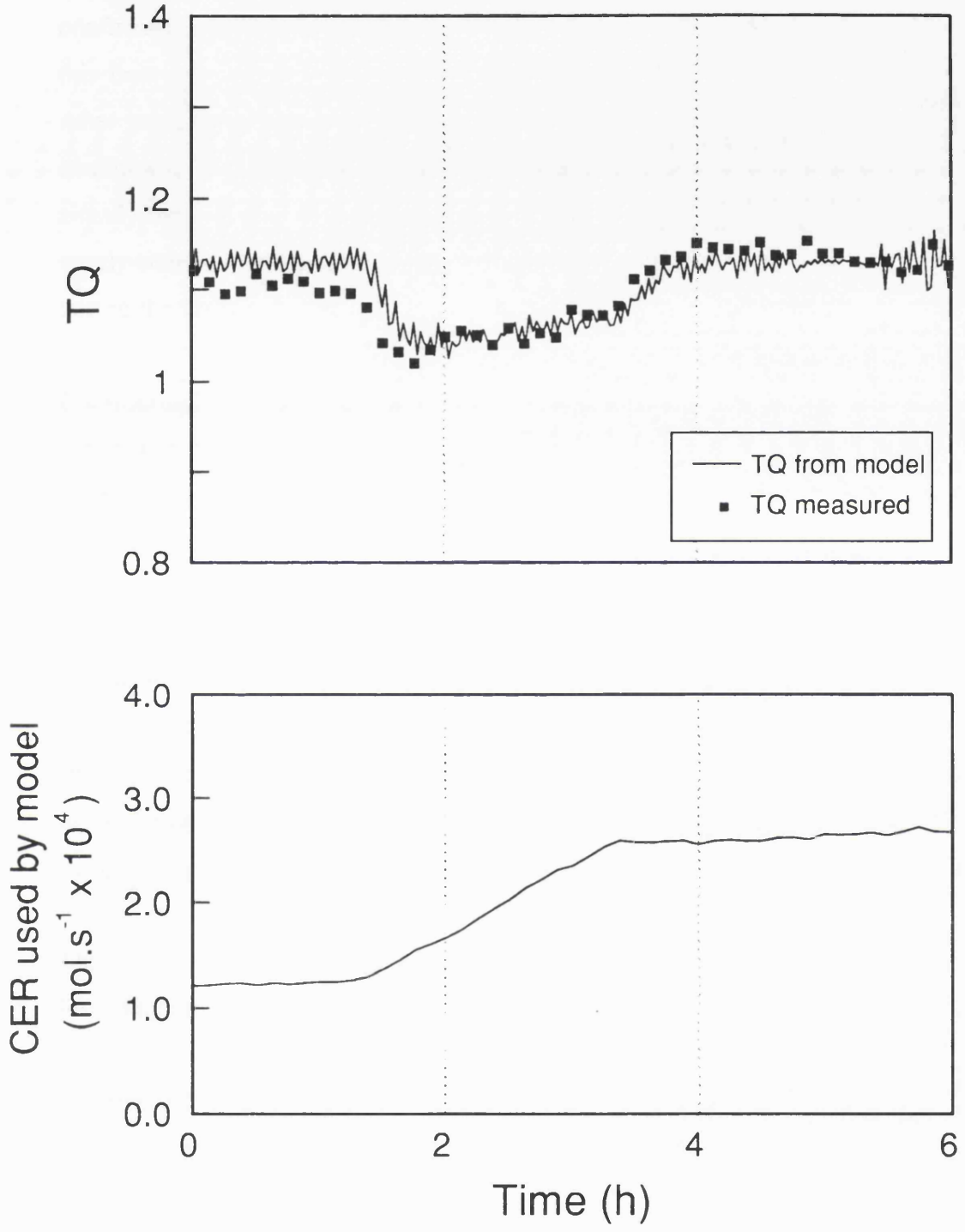


Figure 73: Measured effect of a ramping in the CER on the TQ of an *E. coli* fermentation, compared with the model predictions. The CER data required as input to the model were indicated by the product of the actual fermentation OTR with the RQ (RQ=1.13)

The first of these above points, concerning the dependence of the TQ during the exponential growth phase on the pH of the fermentation, for a given RQ, is easily confirmed experimentally. The same fermentation as shown in Figure 68 is run, but this time at a pH of 7.4, instead of at pH 7. The results are shown in Figure 74. After exponential growth (growth rate μ=1.0 h^{-1}) and adjustment to fed-batch conditions, the TQ during the fed-batch phase is again 1.13, as for Figure 68. It has already been noted that the fed-batch phase at constant glucose feedrate produces a steady-state CO_2 problem during which the TQ and RQ are equal. Hence the RQs during the fed-batch phases in Figures 68 and 74 are equal, at 1.13.

Considering now the exponential growth phase in Figure 68 and 74, the TQ during the exponential growth phase can be seen to be reduced, from 0.94 in Figure 68, to 0.79 in Figure 74. It was predicted from modelling work earlier (Figures 62 & 63) that for a growth rate of 1.0 h^{-1} (that of *E. coli* in fermentations presented here) the TQ during exponential growth will be 0.83 at pH 7, and 0.69 at pH 7.4, if the underlying RQ=1. Assuming the ratio of the TQ to RQ to be independent of the RQ over a small range, then the underlying RQ during the exponential phases in Figure 68 and 74 must have been 1.13 in both cases. This is a very interesting result as it shows that the RQ during both the exponential growth phase and the fed-batch phase of the fermentations reported in Figures 68 and 74 was 1.13, a result that would not be apparent if the TQ was blindly assumed to indicate the RQ.

There is circumstantial evidence for the same effect in the *S.clavuligerus* fermentations, although, as the growth rate, at around 0.3h^{-1}, is much less than in the case of *E. coli*, the unsteady-state effects are less pronounced. Figure 75 shows the TQ profile during the exponential growth phase of *S. clavuligerus*, during which the agitation rate is changed from 400→650rpm. At each agitation rate the TQ is fairly steady, but there is a shift in the TQ after the change in agitation rate, and this shift is maintained at the new agitation rate. When the agitation rate is changed, this

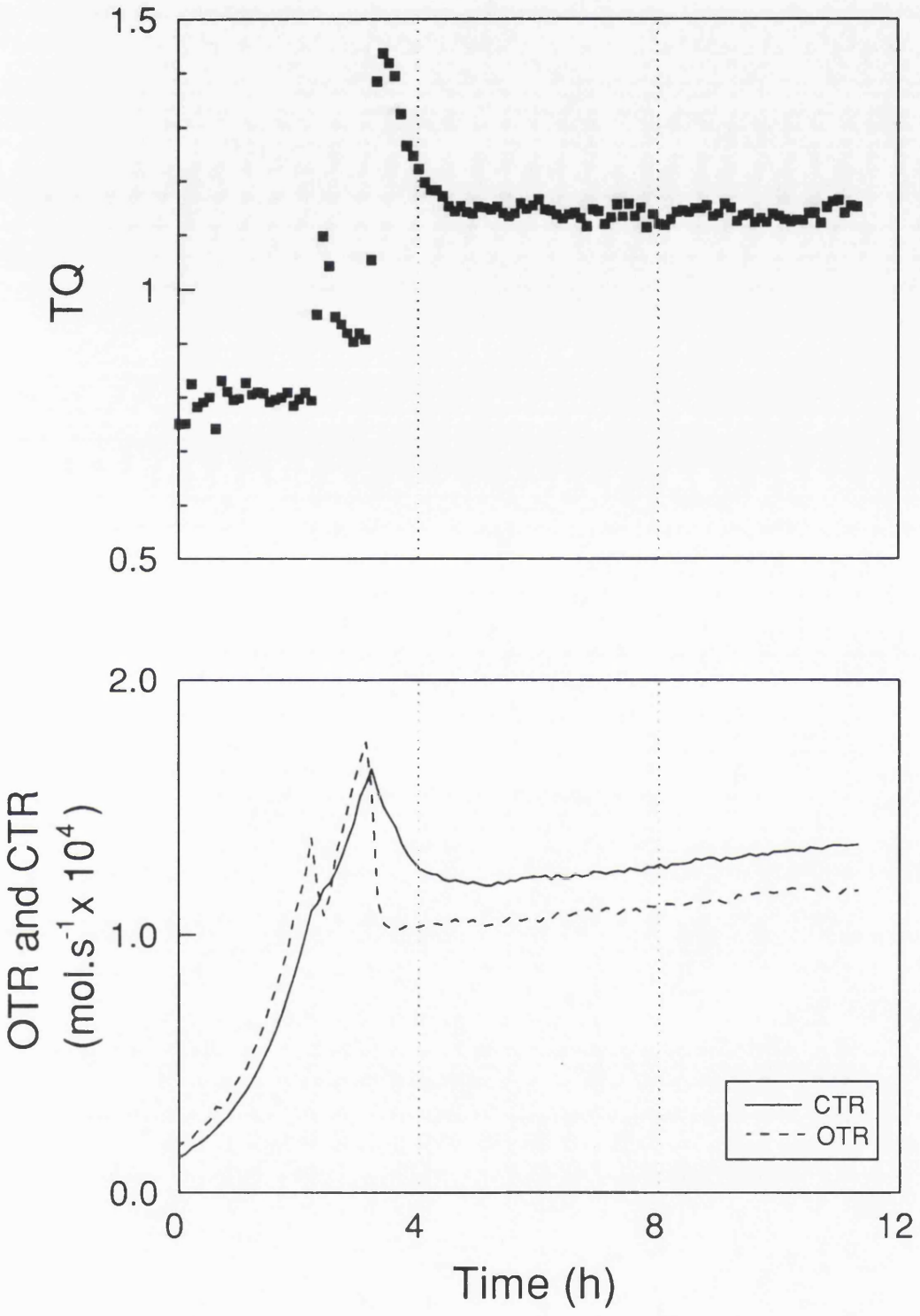


Figure 74: The CTR, OTR and TQ profiles for an *E. coli* fermentation run at pH 7.4

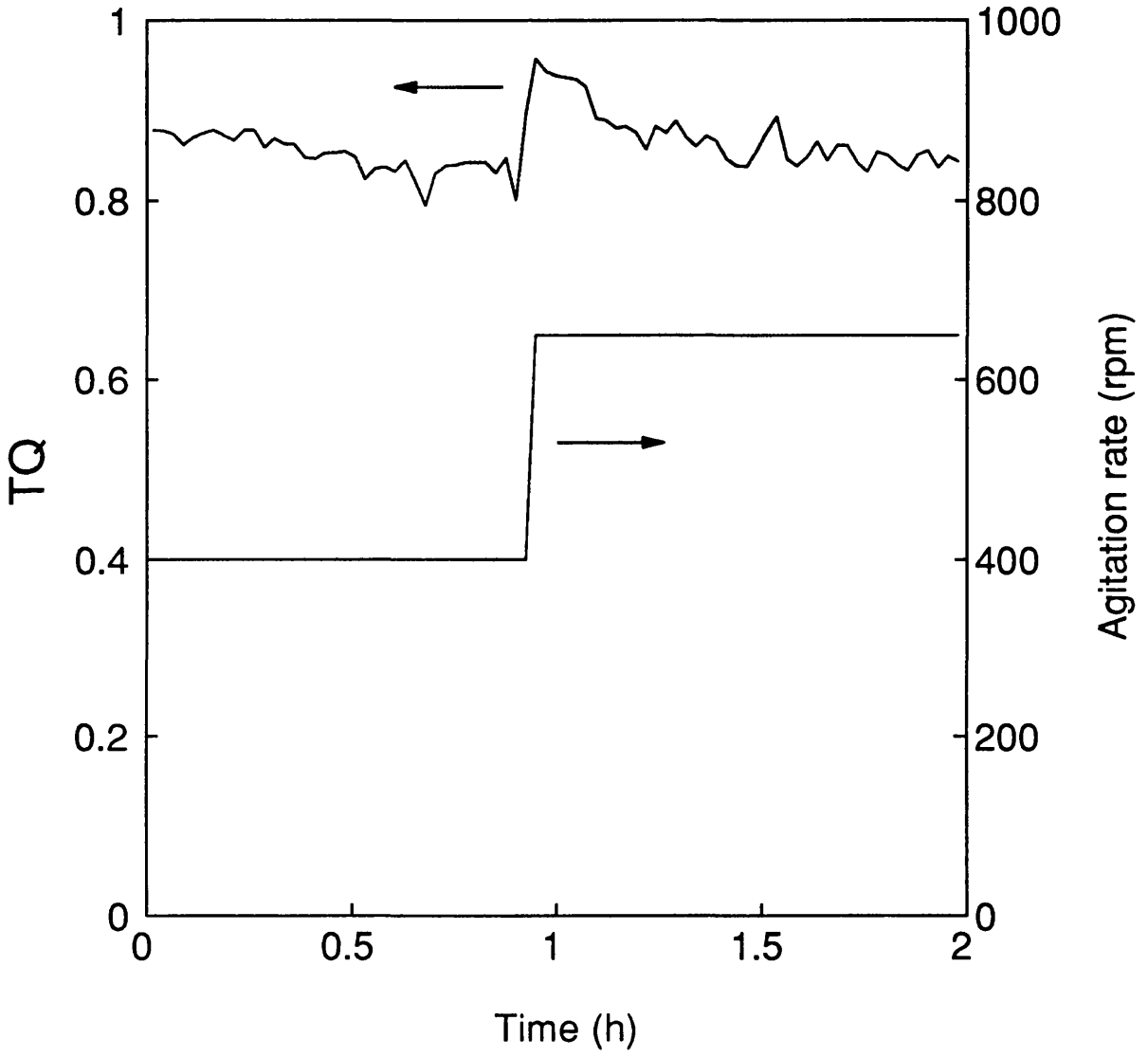


Figure 75: Circumstantial evidence for the effects of a changing CER on the TQ of a *S. clavuligerus* fermentation, during the exponential growth phase. A change in the agitation rate can be seen to cause a sustained change in the TQ

affects $K_L{}^{CO2}a$ in Equation (28), and so modifies the discrepancy between the CTR and CER (and hence between the TQ and RQ), during periods when the CER is changing.

4.5.3 A recursive method to estimate the RQ from the TQ during an *E. coli* fermentation

Having shown that the "measured respiratory quotient" (TQ) apparent from gas analyses can differ significantly from the real underlying RQ, there is a need for a method to estimate the RQ recursively, that could be applied on-line for fermentation monitoring. This section proposes such an estimator.

Figure 76 indicates the results of the application of an algorithm to recursively estimate the RQ from the experimentally measured TQ data, using the fermentation data previously displayed in Figure 68. The estimation algorithm was intentionally very simple, in order to illustrate the ease with which the model is able to remove most of the unsteady-state CO_2 transfer effects. The initial conditions assumed to start the numerical integration were steady-state CO_2 transfer, and chemical equilibrium in the liquid phase. The initial estimate of the RQ resulting from these assumptions was updated during the fermentation by proportional feedback of ⅓ of the error between the predicted and measured TQ. This estimator can be represented in the form of a difference equation as follows:

$$RQ(t) = RQ(t-1) + \tfrac{1}{3}.(TQ(t) - TQ(t-1))$$

In spite of the simplicity of the estimator, the initial value of the estimated RQ in Figure 76 is quickly adjusted to a value close to 1.13, and remains close to this result throughout the fermentation. Figure 76 also shows that the peaks appearing in the TQ over the time period 2.5 to 4.5h are consistent with a relatively constant RQ over this period. Figure 76 clearly shows that for this fermentation, the profile of the measured respiratory quotient (TQ) indicated by gas analyses is much more complex

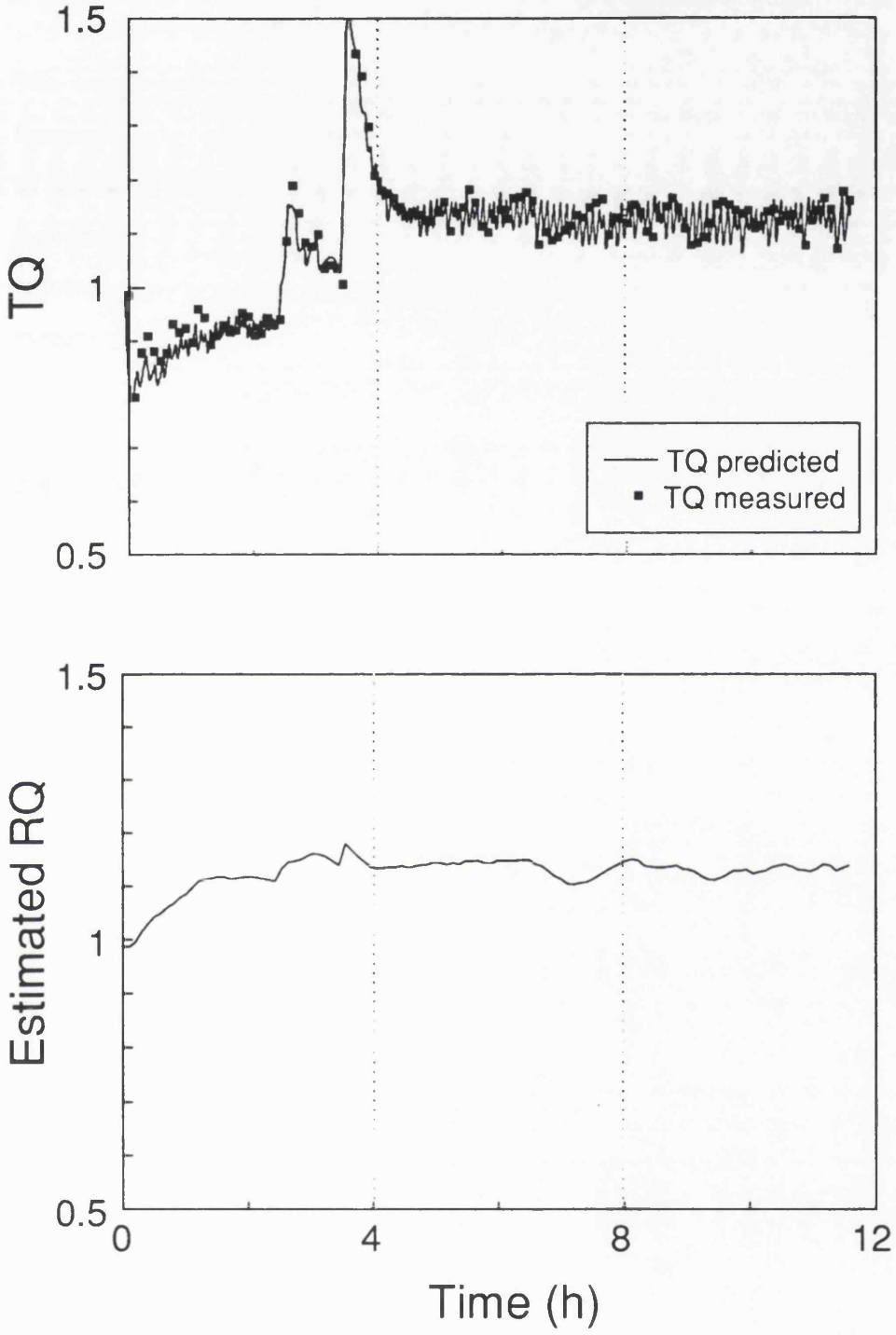


Figure 76: Recursive estimation of the RQ during an *E. coli* fermentation, by feedback of ⅓ of the error between the predicted and measured TQs

than the real RQ (which is substantially constant throughout the fermentation at 1.13).

The estimation algorithm described requires pH data to be available at a high frequency (say once per minute) but requires gas analyses less frequently. In practise, as the pH is an analog measurement dedicated to a fermentor, while gas monitoring equipment is usually shared between several fermentors, this situation is normally existent. Hence the estimator proposed could be readily encoded into a supervisory system so as to compute values close to the true RQ (and CER).

4.5.4 Effect of changes in agitation and aeration on the CTR and TQ

It has already been noted that, as changes in agitation and aeration during fermentations are normally rare events, their effect on the TQ is of lesser importance than the effect of CER and pH changes on the TQ, and they are therefore dealt with only briefly here. Figure 77 indicates the effect of a series of changes in agitation (250 → 350 → 500rpm) and aeration (5 → 10 → 20SLPM) rates on the CTR, during the exponential growth phase of an *E. coli* fermentation. The media used for this fermentation were different from those used in other *E. coli* fermentations reported in this section in not containing antifoam, and having an initial concentration of glucose of $5g.L^{-1}$. This difference simply reflects the fact that the fermentation shown in Figure 77 was run at a different stage in the project than other *E. coli* fermentations. The $K_L{}^{O2}a$ information required by the model to predict unsteady-state effects in the TQ were generated from a power law correlation involving the agitation and aeration rates, that had been fitted to the actual $K_L{}^{O2}a$ data:

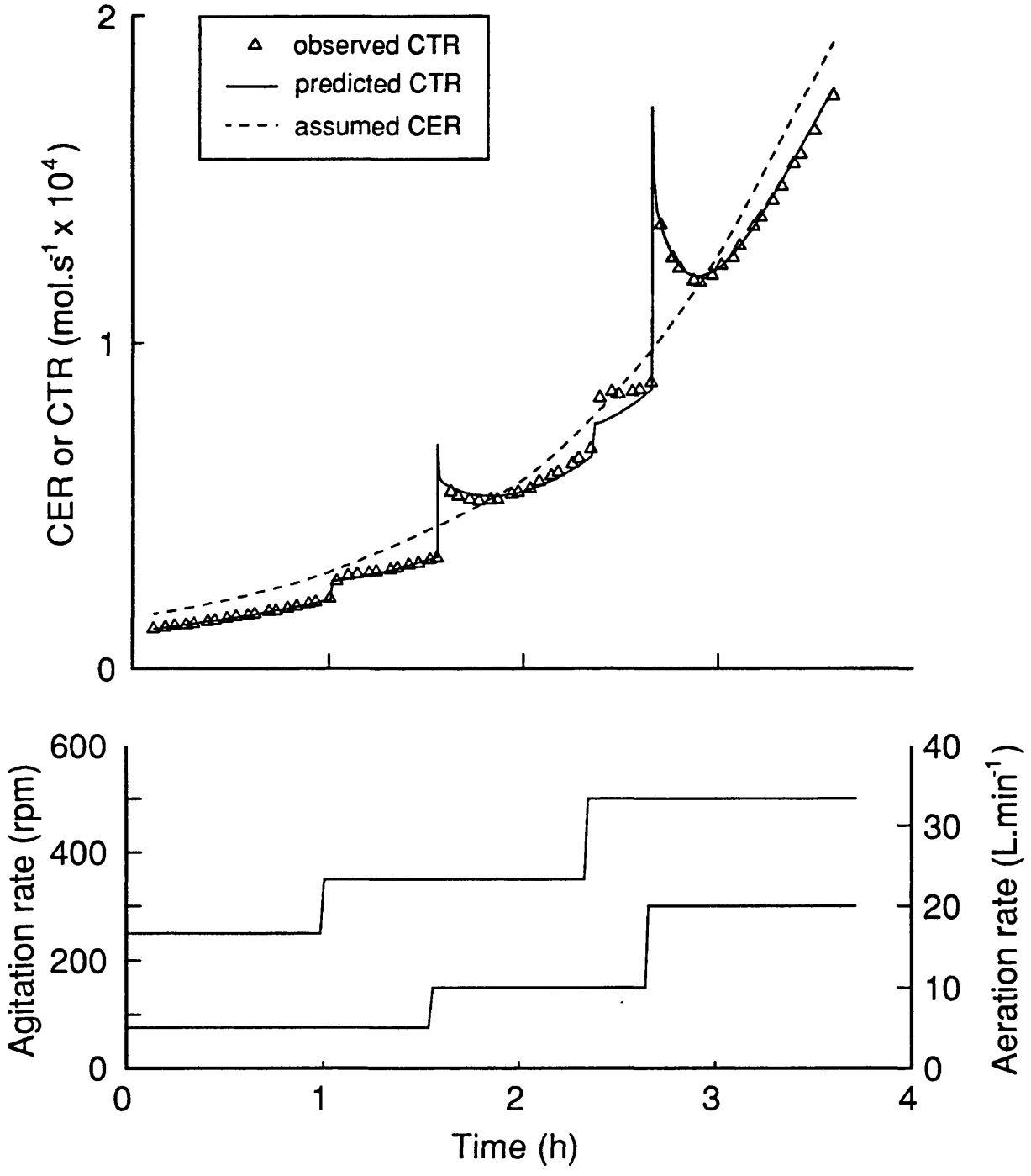


Figure 77: Measured effect of changes in the agitation and aeration rates on the CTR during an *E. coli* fermentation, compared with those predicted (assuming an exponentially changing CER)

$$K_L^{O_2}a = 2.28x10^{-8}.N^{2.5}.G^{0.25}$$

where N = agitation rate (rpm)

G = aeration rate ($mol.s^{-1}$)

It can be seen in Figure 77 that the predicted fluctuations in the CTR arising as a result of changes in the agitation and aeration rates are consistent with those actually observed, indicating that the underlying CER is a smoothly exponential function.

4.5.5 Detection of pH probe failure using the analytical redundancy provided by unsteady-state transfer effects

It has been shown that sudden changes in some fermentation variables can cause corresponding transient changes in derived variables available from gas analyses that can be accurately predicted. The fact that the effect on gas analyses can be both measured and predicted indicates an element of redundant information that could be used to detect certain kinds of faults. This section examines the application of this "analytical redundancy" to detect the simulated failure of a pH sensor.

The most realistic example of such a possibility concerns the failure of pH probes. When a pH probe fails, the pH probe output suddenly changes to the probes' electical zero. The simplest way to detect this failure would be to incorporate circuitry that detects a sudden change in the noise characteristics of the probe output. If this approach is not adopted, an alternative concerns the use of the analytical redundancy arising from unsteady-state CO_2 transfer effects. The need for such an approach arises because in some instances, it would not necessarily be obvious from the time profile of the pH probe output that failure had occurred. A pH probe's electrical zero is in the vicinity of pH 7. Hence, if the fermentation broth pH is near 7, the change in pH when the probe fails will be small. Further, this change may not appear inconsistent with changes in pH associated with pH control, particularly if, as is often the case in industrial fermentations, pH control is not particularly tight (typically being to ± 0.1 units).

It was proved in Section 4.5.1 that step changes in pH are accompanied by fluctuations in the CTR and TQ. If a step change in the pH is not accompanied by a fluctuation in the CTR or TQ, this could indicate that the pH probe has failed. That detection of pH probe failure in this way is possible is shown in Figure 78, which shows the detection of a simulated failure of the pH probe, within the CTR data set previously shown in Figure 77. The pH output is assumed to change suddenly from pH 7 to pH 7.2, as a result of failure, at time 2h. The unsteady-state CO_2 model

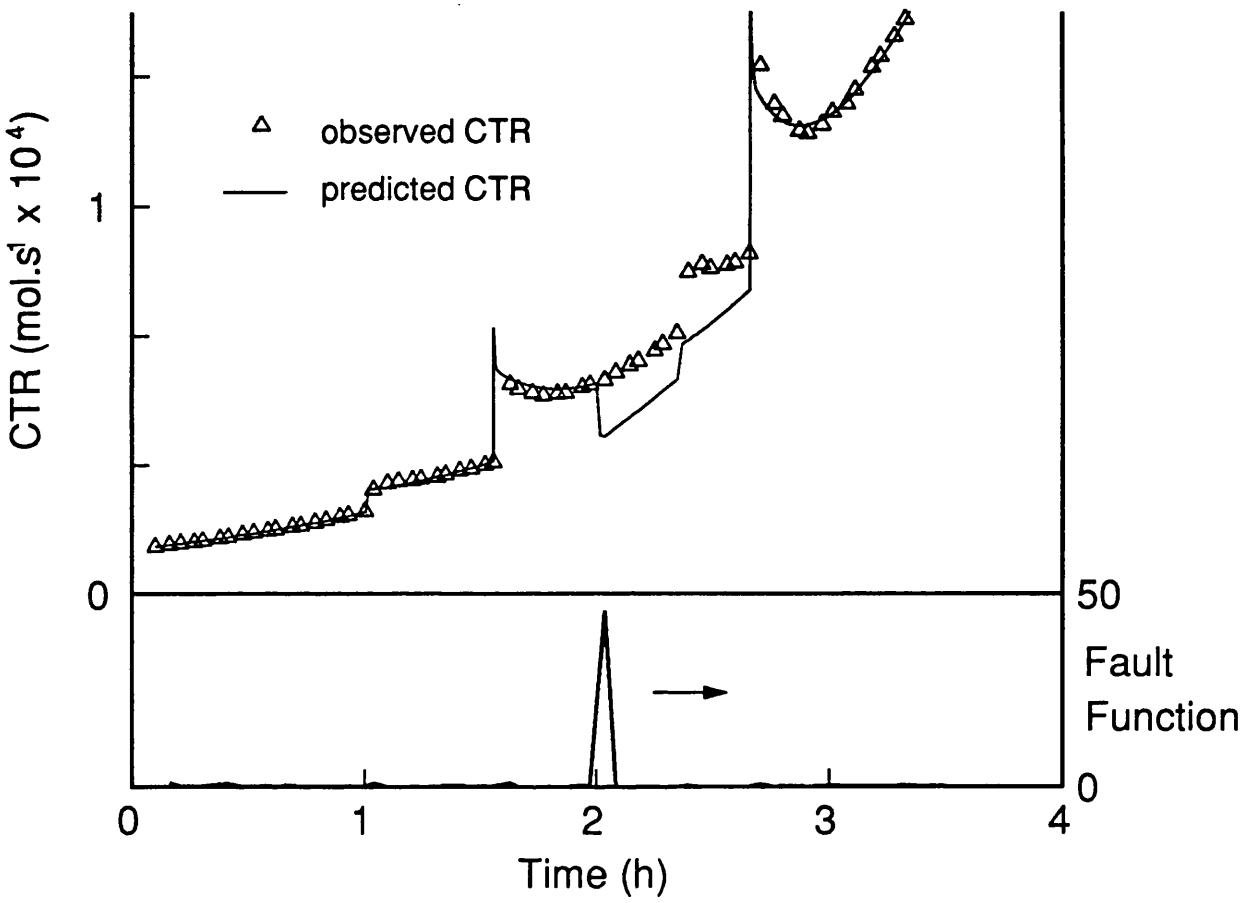


Figure 78: Detection of a simulated failure of a pH probe at time 2h during an *E. coli* fermentation. A change in the pH probe output, from 7 (indicating broth pH) to 7.2 (indicating the probe electrical zero) at time 2h, is not mirrored by a change in in the CTR, indicating the pH probe to have failed. The "fault function", being the ratio of the change over the sampling period of the predicted CTR, to that in the measured CTR, detects the discrepancy which identifies the failure, at 2h.

predicts a fluctuation in the CTR, which is not mirrored by a fluctuation in the actual fermentation CTR data. A "fault function" can be defined which is the ratio of the change in the predicted CTR compared with that in the measured CTR over the period from one gas analysis to the next. Clearly, in the case of a pH probe failure, this fault function will have an anomolously high value, as there will be a large change in the predicted CTR (caused by the change in pH output of the failed pH probe) but no (immediate) corresponding change in the measured CTR. This "fault function" can be seen to identify the simulated pH probe failure in Figure 78. The fault function is not influenced by the fluctuations in the CTR associated with changes in agitation and aeration rates noted in Figure 77, as these are consistent with the predicted change in the CTR.

While fault detection by the method proposed is possible, it is a relatively complex approach, and it would therefore be preferable, in the authors view, to detect such failure by a simpler method, such as by means of the identification of a changes in the noise characteristics of the probe output. Fault detection by analytical redundancy was not pursued further in this project.

This completes the experimental examination of unsteady-state transfer in fermentations. Table 18 summarizes the main results and conclusions from this section (Section 4.5).

pH effects 1. Figures 68, 69 & 70: The CTR data in the *E. coli* fermentations reported appears to be much noisier than the OTR data. This "noise" is not random, but instead arises from the discrete sampling of what is, in reality, a smooth cycling of the CTR caused by on-off pH control action. This effect of pH control action on the CTR is also observed in *S. clavuligerus* fermentations 2. Figures 71 & 72 (*E. coli*): When the pH changes smoothly with time over an extended period, a difference is generated between the TQ and RQ, which is sustained as long as the pH is changing, and above pH 6.5
CER effects 1. Figure 73: Ramping of the feedrate of glucose to a substrate-limited *E. coli* fermentation over a two-hour period caused a ramping of the CER. This changing CER had the effect of generating a sustained difference between the TQ and RQ, which was maintained as long as the CER was changing. 2. Comparing Figures 68 & 74: The TQ during the exponential growth phase of a fermentation of *E. coli* was found to be 0.94 for operation at pH 7, and 0.79 for operation at pH 7.4. It was shown that these results were consistent with the underlying RQ being the same in both fermentations, with a value of 1.13.
RQ estimation Figure 76: For fermentations where the RQ is not indicated directly by the TQ, such as in the case of the *E. coli* fermentations, the RQ can be estimated on-line using a recursive estimator. When applied to data from an *E. coli* fermentation, it was shown that the real underlying RQ had a value of 1.13 throughout the fermentation, although its value as apparent from gas analyses (TQ) varied considerably.
Other effects Figure 77: Changes to the agitation and aeration rates during an *E. coli* fermentation caused transient changes in the CTR.
Fault detection Figure 78: The ability both the predict and measure unsteady-state CO_2 transfer effects provides an analytical redundancy that in principle could be used to detect faults. This possibility was demonstrated by the detection of a simulated pH probe failure during an *E. coli* fermentation.

Table 18: Principal results and conclusions from experimental work concerning the effect of changes in fermentation variables on CO_2 transfer and hence on the TQ, as presented in Section 4.5

4.6 AUTOMATIC CONTROL OF DISSOLVED CARBON DIOXIDE

In Sections 4.3, 4.4 and 4.5, the monitoring of dissolved carbon dioxide under both steady-state and unsteady-state conditions was fully described. The current section considers the problem of controlling this concentration. In Section 4.6.1, general points are made about the problem, and it is shown that a proportional-integral-derivative (PID) feedback control scheme can easily be developed to achieve such control. PID control of highly non-linear processes like fermentation can, however, result in unstable control. To alleviate such a danger, Section 4.6.2 shows that approximate control of dissolved carbon dioxide can be achieved by means of a very simple ratio controller. It is pointed out that such dissolved CO_2 control not only implies a more stable environment for microbial growth and the elimation of the risk of CO_2 inhibition to growth and productivity, but has the further economic advantage of achieving these benefits while minimizing the demand for aeration in multi-fermentor facilities.

4.6.1 Observations on control of dissolved CO_2

An examination of the effect of dissolved carbon dioxide on micro-organisms, in Section 2.2, found that the growth and productivity of aerobic organisms is adversely affected if the dissolved carbon dioxide concentration is too high, and fermentations using such organisms may be better operated by controlling the dissolved CO_2 to below some limiting value. A few organisms are sensitive to bicarbonate rather than dissolved carbon dioxide suggesting the need for bicarbonate concentration control, but if steady-state CO_2 transfer can be assumed, the bicarbonate concentration is proportional to the dissolved CO_2 concentration (Equation 23) and the control problem remains one of controlling dissolved CO_2.

While it was pointed out in Section 2.2 that too low a concentration of carbon dioxide can adversely affect growth, this minimum CO_2 concentration will normally be met as a result of the net evolution of CO_2 arising from microbial respiration.

Hence the problem of controlling the dissolved carbon dioxide concentration will normally amount to a problem of limiting the dissolved carbon dioxide partial pressure below a certain maximum throughout the fermentation. It is possible that the optimum value of this maximum may vary according to the growth phase (exponential, production etc.). This possibility is not examined explicitly here, but could be easily implemented from the work described.

It was noted in Section 1.2.3 that the ability to reliably monitor a variable is the principal hurdle to be overcome in the closed-loop control of that variable. It has been demonstrated in this project that the dissolved carbon dioxide concentration can be reliably monitored. It has been further demonstated that the carbon dioxide excess, ξ, being the ratio of the dissolved carbon dioxide concentration to the concentration in equilibrium with the exit gas, is never much greater than 1. Hence, the problem of controlling the dissolved carbon dioxide concentration can be achieved by manipulating the partial pressure of carbon dioxide in the exit gas. The exit gas CO_2 partial pressure depends on the CER and the aeration rate, and, clearly, it is the latter than can be easily manipulated to effect control of dissolved CO_2. A feedback control scheme for the control of dissolved CO_2 to a setpoint ($[CO_2]_L$ setpt.) is shown in Figure 79, wherein the error between the calculated concentration of dissolved CO_2 and the desired setpoint is transformed through the proportional, integral and derivative constants into a control action to manipulate the aeration rate.

Changes to the aeration rate associated with dissolved carbon dioxide control will also affect the dissolved oxygen concentration. It is however, possible to independently control the concentrations of these dissolved gases. This is because the dissolved CO_2 is sensitive to the aeration rate, but only weakly sensitive to the agitation rate, while for dissolved O_2, these sensitivities are reversed.

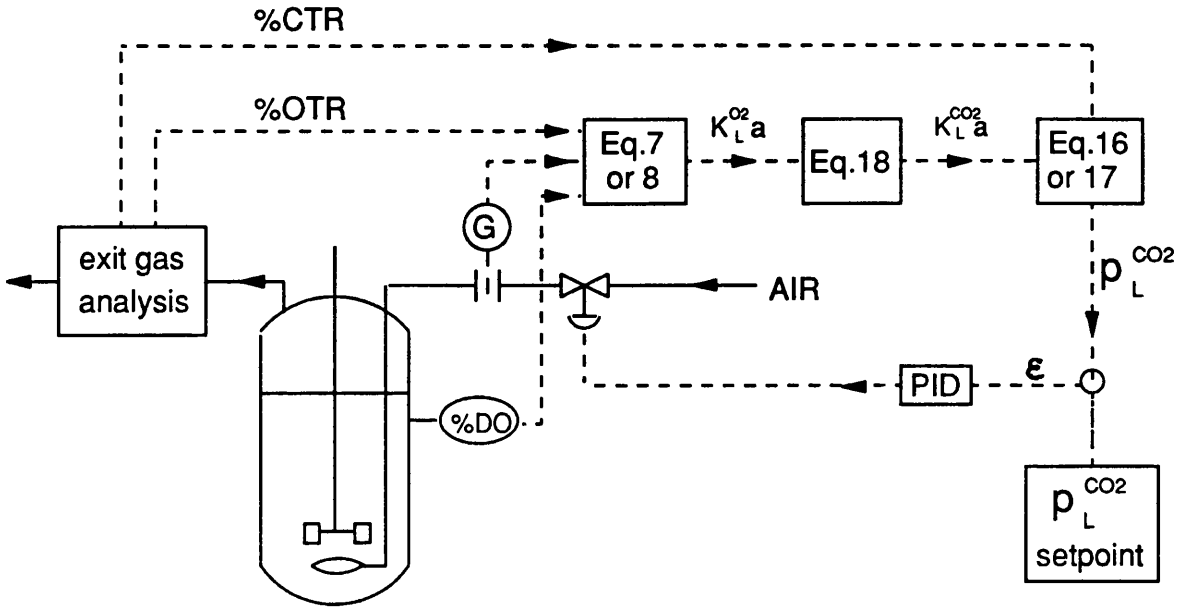


Figure 79: Scheme for the PID feedback control of dissolved carbon dioxide

4.6.2 Ratio control of dissolved CO_2 during an *E.coli* fermentation

PID feedback control can be unstable when applied to processes that are highly non-linear, like fermentation. To avoid this danger, a simple ratio controller is arrived at in this section by re-examining the equations presented in earlier sections.

Previously, in Figures 46, 47 & 48 it was shown that when the gas phase in the fermentor is in plug flow, the concentration of dissolved carbon dioxide is almost equal to the equilibrium concentration over the entire range of mass transfer coefficients of interest in industrial fermentation. Hence in large fermentors, control of dissolved carbon dioxide is simply a problem of control of the equilibrium concentration i.e. of the exit gas carbon dioxide partial pressure, $p_g^{CO2,out}$, and the oxygen mass transfer coefficient has no effect on this result. While the approach to equilibrium in the case of a well-mixed gas phase (i.e. small fermentors) is not as close, it can be seen in Figure 45 that the absolute dissolved CO_2 concentration is relatively insensitive to small changes in the oxygen mass transfer coefficient. Admittedly, in the case of mycelial fermentations, the change in mass transfer coefficient during the fermentation may be quite large (Figure 26), but for many other fermentations, such as for the *E. coli* fermentations already reported, the change is small. Hence, for many fermentations, quite tight control of dissolved CO_2 can be achieved simply by controlling the exit gas carbon dioxide pressure, $p_g^{CO2,out}$, to a constant value. This simpler control problem will now be examined.

Equation (15) in Section 4.3.1 can be approximated for most systems as:

$$CTR \sim G.\frac{\%CO_2^{out}}{100} \sim G.\frac{p_g^{CO_2,out}}{(P - p_w)} \qquad (32)$$

In most of the fermentations reported in this thesis so far, the aeration rate, G, and fermentor operating pressure ($P-p_w$) have been constant. From Equation (32) it can be seen that the effect is to cause $p_g^{CO2,out}$ (on which p_L^{CO2} depends) to be proportional to the CTR, as was seen in Figures 51 and 53. In order to control the dissolved carbon dioxide partial pressure, p_L^{CO2} to a constant value (by controlling

$p_g^{CO2,out}$ to a constant value), the aeration rate must be:

$$G \propto CTR.(P-p_w) \qquad (33)$$

This result is represented diagrammaticaly in Figure 80. If the head space pressure is constant during the fermentation, as was the case in fermentation work reported in this thesis, the control simplifies to requiring the aeration rate, G, to be proportional to the CTR, making the result a simple ratio controller.

The control algorithm proposed in Figure 80 was tested during an *E. coli* fermentation. The exact control rule used to update the aeration rate setpoint with time was:

$$G(t) = 0.6 \times (\%CO_2^{out}(t)) \times G(t-1) \qquad (34)$$

This rule was expected to control the dissolved CO_2 partial pressure to a constant value below about 2000 Pa. The exact value of this constant level was not known *a priori* as the oxygen mass transfer coefficient (which affects dissolved CO_2 in small fermentors) was not monitored. The effect of this simple control rule on the measured dissolved CO_2 concentration during this fermentation is shown in Figure 81. Equation (34) was not implemented until it indicated an aeration rate of above 5 SLPM (0.17vvm) as aeration rates less than this are not used in fermentation. Hence, during the first hours of the fermentation, the aeration rate was constant at 5 SLPM, and the dissolved carbon dioxide partial pressure, p_L^{CO2}, increased with the CTR. Once Equation (34) indicated the need for an aeration rate greater than 5 SLPM, the aeration rate setpoint was changed accordingly, and this control scheme was used over the period 2h to 6h in Figure 81. Over the period when the CTR was changing rapidly (from 2h to 3.5h) there was poor control of dissolved CO_2, with large oscillations being apparent. However, when the CTR was only changing slowly (during the period 3.5h to 6h) very good control of dissolved CO_2 was achieved with Equation (34). The reason for the control being poor during the period 2h to 3.5h is that the rapidly changing CTR implies the need for a rapidly changing aeration rate, G, and this generates unsteady-state CO_2 transfer effects, as was noted earlier in, for

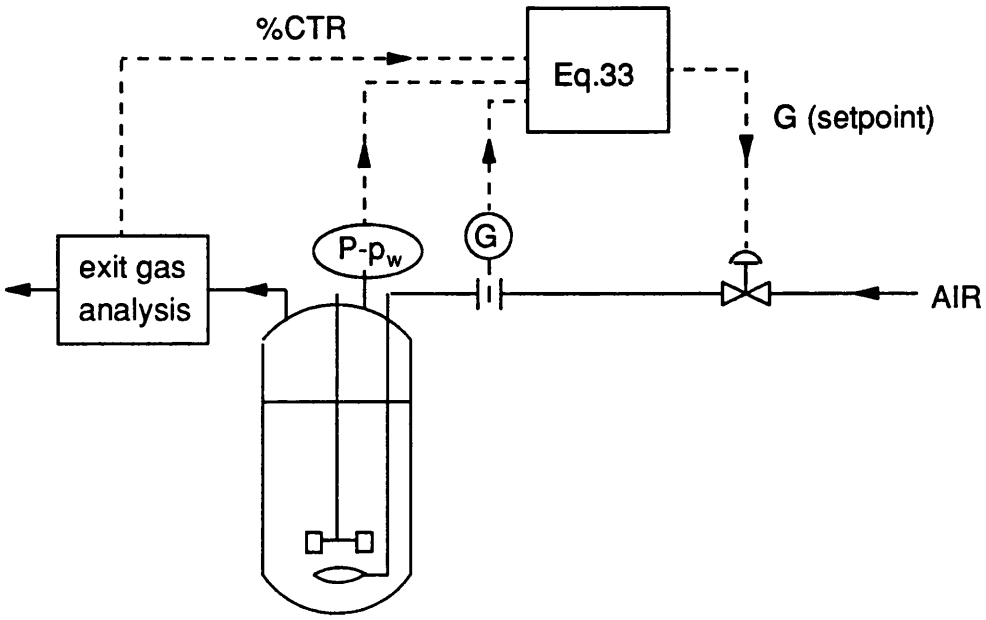


Figure 80: Scheme for the control of dissolved carbon dioxide to an approximately constant value, by means of a ratio controller

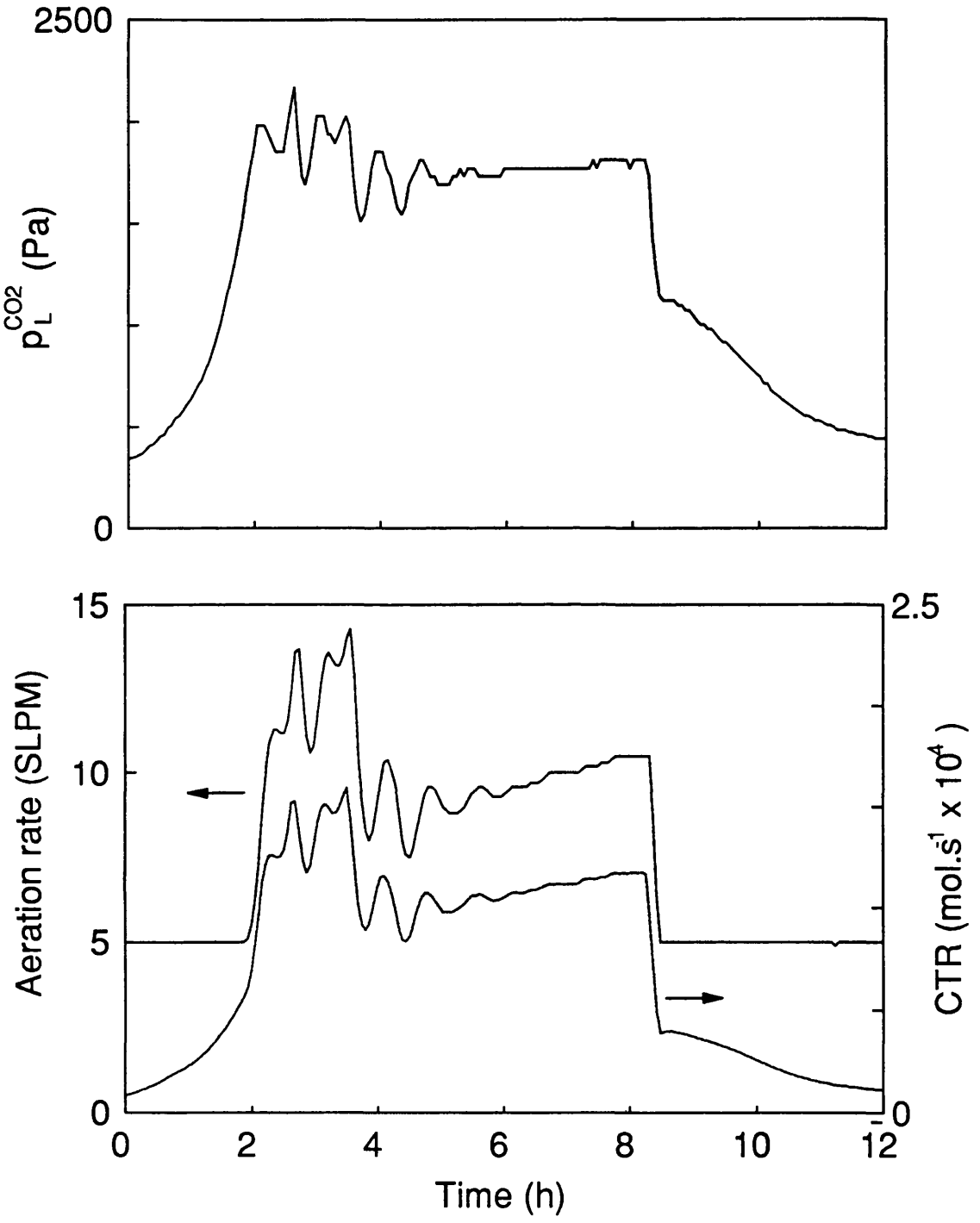


Figure 81: Application of a ratio controller for control of the dissolved CO_2 partial pressure, p_L^{CO2} to an approximately constant value, during an *E. coli* fermentation, by making the aeration rate a constant ratio of the CTR

example, Figure 77. Hence the simple control rule suggested by Equation (34) can give good control of dissolved CO_2, but only in fermentations where the CTR does not change very rapidly. To achieve good dissolved CO_2 control when the CTR is changing rapidly would require the incorporation of the model of unsteady-state CO_2 transfer developed in Section 4.4 into the control algorithm.

It has already been noted that, for most systems, what is required in terms of dissolved carbon dioxide control is likely to be little more than control of its value below a certain level above which dissolved CO_2 inhibition results. Such a requirement can clearly be met within the framework of a constant aeration rate that is simply made high enough so that the maximum $p_g^{CO2,out}$ during the fermentation doesn't result in inhibition. No work to the authors knowledge has set out to examine whether there are benefits accrueing to controlling dissolved CO_2 to a constant level, by virtue of the effect of the more stable environment on micro-organisms. There are however, economic reasons for dissolved CO_2 control that don't concern CO_2 inhibition directly. Fox(1984) noted that, to reduce compression costs in industrial fermentation, the aeration rate is reduced to the minimum possible that doesn't give rise to dissolved CO_2 inhibition. Clearly, the control scheme proposed in Figure 80 allows that the average aeration rate during the course of a fermentation be minimized, while avoiding carbon dioxide inhibition by keeping the dissolved carbon dioxide below a specified level throughout the fermentation. Such large changes in aeration rate would not be satisfactory in a single-vessel installation, as there is usually limited flexibility in the operation of air compressors. However, in a multi-fermentor facility, each fermentor would be at a different stage in the fermentation, and hence have a different aeration requirement, while the total aeration requirement for the facility as a whole would be substantially constant. As such facilities are usually supplied from a single compressed air supply, the scheme of Figure 80 would have the effect of minimizing the compressed air requirement for multi-fermentor facilities.

5. CONCLUSIONS

Information from measurements made by on-line sensors are, directly or indirectly, critical to strategies for improved monitoring and control of industrial fermentations. Over the past 20 years, a large body of research has, with relatively little success, attempted to expand the library of on-line measurements routinely used in industrial fermentation. Partly as a result, research efforts have increasingly been targeted at the incorporation of existing on-line measurement data into biological models that yield data for important fermentation states that cannot be measured directly.

Due to the complexity of most of these models, industrial applications have largely been limited to the simplest of empirical models. These have been based around "derived fermentation variables", which are physically meaningful fermentation variables that derive directly from one or more on-line measurements, most of them associated with gas exchange. This project sought to improve the conditioning, analysis and application of gas exchange data.

Consideration was first given to the evaluation of the oxygen mass transfer coefficient, $K_L{}^{O2}a$, as this variable is widely monitored in the fermentation industry, and its evaluation was required for later work. It was shown that a single value of the mass transfer coefficient for a fermentor, if defined as the volume integral of the local mass transfer coefficient, retains some of the fundamental physical properties of this latter variable. It was shown that an approximate value for this integral can be obtained from an expression not involving the local mass transfer coefficient (which is never, in practical situations, known). The expression for $K_L{}^{O2}a$ can be quite simple, as simplifying assumptions can be made. These assumptions include assuming the liquid phase to be well mixed (which leads to at most a 10% error in the calculated $K_L{}^{O2}a$), and ignoring the variation of hydrostatic pressure with axial position but instead using the average hydrostatic pressure (which leads to at most a 3% error). The fermentor $K_L{}^{O2}a$ calculated from the resulting expression was found to be quantitatively reproducible from one fermentation to the next.

It was shown that the oxygen transfer rate (OTR) is unique amongst on-line variables (both measured and derived) in having a significant component of random noise that could not simply be digitally filtered, owing to the relatively low frequency of gas analyses. The noise arises because the OTR is calculated as a small difference between two large numbers. The noise was found to be uncorrelated, Gaussian noise. A simple way to remove this noise was shown to be by using a least-squares filter, such as a chi-square filter, to fit a linear model to a reduced data set containing a 'time window' of the most recent OTR data points. This approach was tested on data from a *S. clavuligerus* fermentation. The optimum time window was qualitatively found to be one hour (involving the ten most recent OTR data points). Application of this noise filter was shown to improve the quality of the OTR data, and of other derived variables that depend on the OTR, such as the respiratory quotient (RQ) and oxygen mass transfer coefficient ($K_L{}^{O2}a$).

The transfer of carbon dioxide in aerobic fermentations is more complex than that of oxygen because carbon dioxide is highly soluble in fermentation media, and in addition reacts chemically with water to form bicarbonate ions. Semi-theoretical work showed that there is negligible gas-film limitation of carbon dioxide transfer, as for oxygen transfer. Further, a theoretical expression was derived, which was used to show that there is negligible mass transfer enhancement of carbon dioxide transfer as a result of reaction in the liquid film. Both these results support the view that carbon dioxide transfer can be treated as a purely liquid-film limited physical process, as for oxygen. It was proposed that the ratio of the mass transfer coefficient for CO_2 transfer to that for O_2 transfer should depend on the ratio of their liquid diffusivities raised to the two-thirds power, yielding a coefficient ratio of 0.89.

Using these conclusions, an examination was made of the accuracy of assuming that the partial pressure of dissolved carbon dioxide is equal to the its partial pressure in the exit gas. It was shown that practical issues mean that the error in this assumption can never be very large, and these practical issues were summarized in a mathematical expression for the maximum error (for a well-mixed gas phase). A further correlation was derived to illustrate the sensitivity of the accuracy of the

equilibrium assumption to fermentation operating conditions. It was found that the error in the equilibrium assumption is small where the gas phase is in plug flow. The error can be considerably larger in fermentors where the gas phase is well mixed. These theoretical results were supported with experimental data from *E. coli* fermentations.

It was shown that the concentration of dissolved carbon dioxide in fermentation media is one to two orders of magnitude greater than that of dissolved oxygen, for pH 6.5 and above. In the case of oxygen transfer, pseudo-steady state can be assumed, meaning that the oxygen transfer rate (OTR) available from gas analyses, can be assumed equal to the (unmeasureable) oxygen uptake rate (OUR) of biomass in the fermentor. This same assumption is not necesarily valid in the case of carbon dioxide transfer. Where the pH or CER is changing, the carbon dioxide transfer rate (CTR) available from gas analyses can differ substantially from the (unmeasureable) carbon dioxide evolution rate (CER) of biomass in the fermentor. The result is that the "measured respiratory quotient" apparent from gas analyses, being the ratio of the CTR to the OTR, can be different from the real underlying (and unmeasureable) RQ. A model to predict the circumstances under which these unsteady-state CO_2 transfer effects would be important was developed. The predicted effects were matched very closely by those observed in practice during fermentations of *E. coli* and *S. clavuligerus*. It was shown that an estimator incorporating the model of unsteady-state transfer can recursively estimate the real RQ from its value as apparent from gas analyses (which was called the transfer quotient, or TQ). Such an estimator could be applied on-line within a supervisory system for fermentation monitoring.

It was shown that, arising from theoretical work, a simple ratio controller can be used to automatically control the partial pressure of dissolved CO_2 to an approximately constant value during a fermentation. This was demonstrated by application to an *E. coli* fermentation, in which the ratio controller involved making the aeration rate a constant ratio of the CTR. Good control was achieved when the CTR was changing slowly, but control was poorer when the CTR was changing

rapidly, as is the case during the exponential growth phase. The reason for this poorer control was that a rapidly changing CTR implied the need for a rapidly changing aeration rate, which led to the generation of transient unsteady-state CO_2 transfer effects.

6. RECOMMENDATIONS FOR FUTURE WORK

The tools developed in this project were designed to be easily implemented on-line within a supervisory system for fermentation monitoring. It is recommended that these tools, namely the evaluation of the oxygen mass transfer coefficient with simplifying assumptions, noise filtering of OTR data, estimation of dissolved CO_2 partial pressures, filtering of unsteady-state CO2 transfer effects for evaluating the true RQ and CER, and the control of dissolved CO_2, be incorporated into the new fermentation supervisory systems currently being developed at UCL.

While good agreement was found between the predicted partial pressure of dissolved carbon dioxide, and that measured in the fermentation systems used in this project, it was noted that high concentrations of surface-active components in (other) fermentation media may affect dissolved CO_2 levels. This possibility should be examined as part of a wider study of the importance of dissolved CO_2 on microorganisms, and in particular, on the productivity of the highly selected strains used in the secondary metabolite industry.

It is further recommended that a study of the benefits accrueing to dissolved CO_2 control during fermentations, in terms of microbial growth and productivity be evaluated, now that a simple method for automatically and routinely controlling dissolved CO_2 during fermentations is available. In view of the fact that in secondary metabolite fermentations, product synthesis tends to occur after the exponential growth phase has ended, it would be interesting to investigate whether dissolved CO_2 control during the exponential growth phase, has a beneficial effect on product synthesis during the production phase. As a control problem, it would also be informative to examine the ease with which independent control of dissolved oxygen and dissolved carbon dioxide can be achieved.

Other recommendations for further work arose from the survey of fermentation operation presented in Chapter 1, concerning areas most requiring further research to advance industrial fermentation operation, and these can be seen summarized in

Table 1. The conclusions relating to research with relevance to production-scale fermentations included, in particular, the need for improved storage and analysis of previous batch data, automated modelling and identification, and optimized temperature control of fermentation.

NOMENCLATURE

a = average gas-liquid interfacial area per unit aerated broth volume ($m^2.m^{-3}$)

$(a)_l$ = local gas-liquid interfacial area per unit aerated broth volume ($m^2.m^{-3}$)

CER = carbon dioxide evolution rate by the micro-organisms ($mol.s^{-1}$)

CTR = carbon dioxide transfer rate across the gas-liquid interface ($mol.s^{-1}$)

%CTR = difference between the mole percentage of carbon dioxide in the inlet and exit gas streams

$[CO_2]_L$ = concentration of dissolved carbon dioxide, $CO_2(l)$ ($mol.m^{-3}$)

$[CO_2]_{eq}$ = concentration of dissolved carbon dioxide assuming equilibrium between the exit gas and fermentation broth ($mol.m^{-3}$)

$[CO_2]_{max}$ = maximum possible concentration of dissolved carbon dioxide ($mol.m^{-3}$)

$\%CO_2^{in}$, $\%CO_2^{out}$ = gas-phase mole percentages of carbon dioxide in the inlet (in) and exit (out) gas streams (dry basis)

D_L = liquid-phase diffusivity

G = inlet air flowrate (dry basis) ($mol.s^{-1}$)

H = Henry's law constant ($Pa.m^3.mol^{-1}$)

H^{CO2} = Henry's law constant for carbon dioxide ($Pa.m^3.mol^{-1}$)

H^{O2} = Henry's law constant for oxygen ($Pa.m^3.mol^{-1}$)

$[HCO_3^-]$ = concentration of bicarbonate ions in the liquid phase ($mol.m^{-3}$)

$[H_2CO_3]$ = concentration of carbonic acid in the liquid phase ($mol.m^{-3}$)

k_1, k_2 = rate constants governing carbon dioxide hydration (s^{-1})

K_{acid} = carbonic acid dissociation constant ($mol.m^{-3}$)

K^*_{acid} = pseudo-dissociation constant for carbon dioxide ($mol.s^{-1}$)

$K_L^{CO2}a$ = average volumetric mass transfer coefficient in the fermentor for carbon dioxide transfer (s-1)

K_L^{O2} = liquid film mass transfer coefficient for oxygen ($m.s^{-1}$)

$K_L^{O2}a$ = average volumetric mass transfer coefficient in the fermentor for oxygen transfer(s^{-1})

$(K_L^{O2}a)_l$ = local volumetric mass transfer coefficient (s^{-1})

N = molar flux across an interface ($mol.s^{-1}$)

$\%N_2^{in}$, $\%N_2^{out}$ = mole percentages of nitrogen in the inlet (in) and exit (out) gas streams respectively (dry basis)

$\%O_2^{in}$, $\%O_2^{out}$ = mole percentages of oxygen in the inlet (in) and exit (out) gas streams respectively (dry basis)

OTR = oxygen transfer rate across the gas-liquid interface ($mol.s^{-1}$)

%OTR = difference between the mole percent of oxygen in the inlet and exit gas streams

OUR = oxygen uptake rate by the micro-organisms ($mol.s^{-1}$)

$[O_2]_g$ = concentration of oxygen in the gas phase ($mol.m^{-3}$)

$[O_2]_L$ = concentration of oxygen in the liquid phase ($mol.m^{-3}$)

P = total pressure in fermentor head space (Pa)

P_0 = standard pressure ($1.01x10^5$ Pa)

$p_g^{CO2,in}$ = partial pressure of carbon dioxide in the inlet gas stream to the fermentor (Pa)

$p_g^{CO2,out}$ = partial pressure of carbon dioxide in the exit gas stream from the fermentor (Pa)

p_g^{O2} = partial pressure of oxygen in the gas phase (Pa)

$p_g^{O2,in}$ = partial pressure of oxygen in the inlet gas to the fermentor (Pa)

$p_g^{O2,out}$ = partial pressure of oxygen in the exit gas from the fermentor (Pa)

p_L^{CO2} = partial pressure of dissolved carbon dioxide (Pa)

p_L^{O2} = partial pressure of dissolved oxygen (Pa)

p_w = partial pressure of water in the exit gas (Pa)

Q = aeration rate in vvm, the volumetric aeration rate at standard temperature and pressure per unit unaerated broth volume (min^{-1})

R = ideal gas constant ($8.314\ J.mol^{-1}.K^{-1}$)

RQ = respiratory quotient, the ratio of CER to the OUR (-)

T = temperature of broth (K)

T_0 = standard temperature (298K)

TQ = transfer quotient or "measured respiratory quotient", the ratio of the CTR to the OTR (or OUR) (-)

t = time (h or s)

t_0 = initial or reference time (h)

V_d = aerated broth volume or dispersion volume (m^3)

V_{film} = liquid film volume (m^3)

V_L = volume of the liquid phase in the fermentor (m^3)

Greek

δ = thickness of the liquid film (m)

ε = gas holdup as a fraction of the aerated broth volume (-)

ξ = dissolved carbon dioxide excess, the ratio of the concentration (or partial pressure) of dissolved carbon dioxide to that at equilibrium (ie. that in the exit gas)

ξ_{max} = maximum dissolved carbon dioxide excess, defined in Equation (19) on Page 180

μ = growth rate during the exponential growth phase (h^{-1})

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APPENDICES

A1: PUBLISHED PAPERS RELATING TO THIS PROJECT

Estimation of Dissolved Carbon Dioxide Concentrations in Aerobic Fermentations

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Dissolved carbon dioxide and bicarbonate ions in fermentation broths can (independently) inhibit or promote microbial growth and productivity. In research facilities with a large number of fermenters, dissolved carbon dioxide sensors tend not to be used, and as a result this variable will generally go unmonitored, making the meaningful analysis of data more difficult. For aerobic fermentations, mass transfer of carbon dioxide can be described in an analogous way to oxygen transfer. The mass transfer coefficient for carbon dioxide is 0.89 times that for oxygen. The maximum dissolved carbon dioxide concentration as a function of exit gas composition is compared with the concentration obtained by assuming equilibrium between the broth and exit gas. The difference between these two concentrations is typically 20–40% of the equilibrium concentration. In large fermenters, a degree of plug flow behavior in the gas and the generally lower specific aeration rates will serve to produce a better approach to equilibrium than for research fermenters.

Introduction

Sterilizable sensors for the measurement of the concentration of dissolved carbon dioxide in fermentation broths have been commercially available for several years (Puhar et al., 1980). These sensors, however, cannot be described as an established tool in the industry (Fox, 1984). The sensor is expensive, has only ±10% accuracy when calibrated against standard buffers, has a fairly slow response time, and must be calibrated at two points. Such issues may be expected to hamper in particular the application of these sensors in research facilities, where there are generally a large number of small fermenters. An indication of the dissolved carbon dioxide concentration can in any case be obtained by assuming equilibrium between the gas and liquid phases, and such an approach has been used in the industry for some time (Carleysmith, 1985). For plant-scale fermentors, where the specific aeration rates are generally lower than for research fermenters and there is a significant degree of plug-flow behavior in the gas phase, this may be a good assumption (Yagi and Yoshida, 1977). For research and pilot-scale fermenters, the assumption will be poorer.

The concentration of dissolved carbon dioxide is of particular interest in the case of mycelial fermentations, affecting both the morphology and the productivity of the fermentation (Ho and Smith, 1986). Pirt and Mancini (1975) found that the penicillin synthesis rate of *P. chrysogenum* was decreased by 33% at a carbon dioxide partial pressure of 0.05 atm and by 50% at 0.08 atm compared to the control case. Belmar-Campero (1988) found a 35% decrease in the clavulanic acid production of *S. clavuligerus* for a carbon dioxide partial pressure of 0.08 atm. Inosine production by *B. subtilis* was found to be inhibited above 0.03 atm (Ishizaki et al., 1973). Bicarbonate ions, whose concentration during fermentations depends (at steady state) on the dissolved carbon dioxide concentration and pH, can inhibit or promote microbial growth and reproduction independent of dissolved carbon dioxide (Repaske et al., 1974).

The dissolved carbon dioxide concentration is, therefore, of importance in determining the productivity of a fermentation, in some cases as important as the concentration of dissolved oxygen (Nyiri and Lengyel, 1968). Fermentations are run under conditions that produce dissolved carbon dioxide concentrations that do not seriously affect microbial growth or productivity, without process operators knowing explicitly what these concentrations are. Variations in the (unmonitored) dissolved carbon dioxide concentration will contribute to batch-to-batch variability in fermentation productivity, so rendering

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meaningful analysis of data from research facilities more icult.

his article describes how, for aerobic fermentations, the centration of dissolved carbon dioxide can be calculated tinely on-line from existing fermentation measurements ıout the need to resort to the use of a dissolved carbon xide sensor or assuming gas-liquid equilibrium. The contration of bicarbonate ions can consequently also be calated.

eory

ygen mass transfer

Most of the resistance to oxygen transfer in aerobic fermntations lies in the liquid film near the gas-liquid interface r example, Steel and Maxon, 1966). Only the liquid-film ss transfer coefficient, $K_L^{O_2}$, is needed to describe the oxygen nsfer across this interface, as the gas-film mass transfer fficient is quite high in agitated vessels, leading to negligible -phase resistance (Fair et al., 1973).

$$d(OTR) = \frac{K_L^{O_2} \cdot a}{H^{O_2}} \cdot (p_g^{O_2} - p_L^{O_2}) \cdot dV \tag{1}$$

ere OTR is the oxygen transfer rate, $p_L^{O_2}$ and $p_g^{O_2}$ are the ygen partial pressures in the liquid and gas phases, respecely, V is the aerated broth volume, a is the interfacial area r unit aerated broth volume and H^{O_2} is the Henry's law nstant for oxygen. The output from an oxygen sensor is oportional to the liquid-phase oxygen partial pressure. The oth volume V is taken by convention to be the aerated volıe. If a load cell measurement rather than level measurement available, it may be more convenient to define the interfacial ea on the basis of the unaerated broth volume.

For research fermenters, the environment in the fermenter relatively homogeneous. Steel and Maxon (1966) found no riation in dissolved oxygen concentration with position, in 15-m^3 novobiocin fermentation, suggesting that the assumpon of a well mixed liquid may be good even for quite large rmenters. Gas circulation rates are commonly an order of agnitude higher than the supply rates in small, agitated, rated vessels (Westerterp et al., 1963). Hence, the gas phase n be considered completely backmixed, and the gas-phase ygen partial pressure of interest is the exit gas partial presıre.

Both a and $K_L^{O_2}$ are functions of specific power input and nce of position (Lopes de Figueiredo and Calderbank, 1979). his functionality need not be determined as the integral of ieir product can simply be defined to be the fermenter volmetric oxygen mass transfer coefficient:

$$OTR = \frac{K_L^{O_2} a}{H^{O_2}} \cdot (p_g^{O_2}(\text{out}) - p_L^{O_2}) \cdot V \tag{2}$$

here $K_L^{O_2}a$ is the fermenter volumetric oxygen mass transfer oefficient and $p_g^{O_2}(\text{out})$ is the oxygen partial pressure in the xit gas.

The oxygen transfer rate can be calculated from gas analyses. Inder the pseudo-steady-state conditions normally applicable during fermentations, it is equal to the oxygen uptake rate by the organisms:

$$OTR = \frac{G}{100} \cdot \left(\%O_2^{in} - \%O_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} \right) \approx OUR \tag{3}$$

where OUR is the oxygen uptake rate, G is the inlet air flow rate, and the compositions of the inlet and exit gas streams are mole percentages. In this work, gas analyses (and hence the aeration rate, G) are assumed to be on a dry basis, such as produced by mass spectrometers, which are increasingly widely used in fermentation facilities.

Carbon dioxide mass transfer

As the cell membrane is relatively impermeable to ionic species (Jones and Greenfield, 1982), the carbon dioxide produced by microbial metabolism enters the broth as dissolved carbon dioxide. It can then be involved in any of the reversible processes below:

$$CO_2(g) \underset{k_L^{CO_2}a}{\overset{k_L^{CO_2}a}{\rightleftharpoons}} CO_2(aq) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H_2CO_3 \overset{K_{ac}}{\rightleftharpoons} H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

where

k_1, k_2 = rate constants for the indicated reactions, s^{-1}
K_{ac} = carbonic acid dissociation constant, $mol \cdot m^{-3}$

Fermentation processes are operated in the pH range 4–8. At such pH values, the concentration of carbonate ions is negligible, complexing of carbon dioxide with amine groups of protein molecules can be ignored, and reaction of dissolved carbon dioxide with hydroxyl ions is negligible (Sherwood et al., 1976). Of the chemical species in the liquid phase, only dissolved carbon dioxide, $CO_2(aq)$, contributes to the transfer of carbon dioxide across the gas-liquid interface. The concentration of carbonic acid is three orders of magnitude less than that of carbon dioxide. The concentration of bicarbonate ions increases with increasing pH, being equal to that of dissolved carbon dioxide at a pH of about 6.3. This bicarbonate concentration, however, has no impact on carbon dioxide mass transfer for pseudo-steady-state conditions (that is, static or slowly varying pH and carbon dioxide evolution rate) which are applicable during most fermentations. Unsteady-state conditions can be accommodated where necessary (Royce et al., 1989). The concentration of dissolved carbon dioxide, $[CO_2]$, will settle at a value that provides a concentration gradient such that the carbon dioxide transfer rate, CTR, will be equal to the carbon dioxide evolution rate, CER, by the organism.

The principal resistance to transfer of carbon dioxide across the gas-liquid interface again lies in the liquid film, with the gas film resistance being negligible. It can be shown that the ratio of concentration gradients in the liquid and gas films is around 50 in agitated gas-liquid contactors for no internal circulation in the gas bubbles (Calderbank, 1959). In practice, there is considerable internal circulation, and this ratio is in reality much higher (Calderbank, 1959). The ratio of partial pressure gradients in the liquid and gas films (which are the measurements available during fermentations) is thus at least $50.0(H/RT)$, where H, R and T are the Henry's law constant

for the component being transferred, the ideal gas constant, and the temperature. The respective Henry's law constants for oxygen and carbon dioxide at a typical fermentation temperature of 30°C are 86,000 and 3,400 $Pa \cdot m^3 \cdot mol^{-1}$ at 30°C (Schumpe and Quicker, 1982). Hence, the ratios of the partial pressure gradients in the liquid and gas films at 30°C are at least 1,700 for oxygen and 70 for carbon dioxide, making the gas film resistance negligible in both cases. Carbon dioxide transfer can therefore be described in an analogous way to oxygen transfer:

$$CTR = \frac{G}{100} \cdot \left(\%CO_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} - \%CO_2^{in} \right) \quad (4)$$

$$= K_L^{CO_2} a \cdot \left([CO_2] - \frac{p_g^{CO_2}(out)}{H^{CO_2}} \right) \cdot V \quad (5)$$

$$\approx CER$$

where $K_L^{CO_2}a$ is the fermenter volumetric carbon dioxide mass transfer coefficient.

The fermenter volumetric mass transfer coefficients for oxygen and carbon dioxide involve the same interfacial area, the same solvent properties, and the same agitation variables. Schneider and Frischknecht (1977) found the volumetric coefficients to be equal; however, in view of the complexity of their fast transient approach and their ignoring the extent of gas backmixing and sensor response times, their claimed accuracy seems optimistic. Yagi and Yoshida (1977) recognized the dependence of the film coefficient on the liquid diffusivity and suggested that the ratio of the volumetric coefficients should depend on the square root of the diffusivity ratio, yielding a value of 0.92, which corresponds to the large bubble correlation of Calderbank (1959). In practice, the high concentration of electrolytes in fermentation media makes it quite difficult to generate large bubbles (Calderbank, 1959), so the small bubble correlation (Calderbank, 1959) is more relevant, though the outcome is not very different. Using diffusivity data for water at 25°C (Incropera and de Witt, 1990), this yields:

$$\frac{K_L^{CO_2}a}{K_L^{O_2}a} = \left(\frac{D_L^{CO_2}}{D_L^{O_2}} \right)^{2/3} = \left(\frac{2.0 \times 10^{-9} m^2 \cdot s^{-1}}{2.4 \times 10^{-9} m^2 \cdot s^{-1}} \right)^{2/3} = 0.89 \quad (6)$$

where $D_L^{O_2}$ and $D_L^{CO_2}$ are the liquid-phase diffusivities of oxygen and carbon dioxide. This result agrees better with the experimental data of Fox (1984), who obtained a ratio of 0.80. While the liquid diffusivities are a function of broth composition (Ho and Ju, 1988) and temperature, their ratio is constant (Wilke and Chang, 1955).

For there to be no mass transfer enhancement of the desorption of CO_2 by reaction in the liquid film, the following inequality must be satisfied:

$$\frac{k_1 \cdot D_L^{CO_2}}{2 \cdot (K_L^{CO_2})^2} \ll 1 \quad (7)$$

Appendix 1 shows the derivation of this inequality and calculates the value of the expression to be of order 10^{-3}, indicating that there is negligible mass transfer enhancement.

Discussion

Dissolved carbon dioxide excess

The concentration of carbon dioxide dissolved in the fermentation broth, $[CO_2]$, will in general be higher than the concentration predicted by assuming carbon dioxide in the broth to be in equilibrium with the exit gas from the fermenter, $[CO_2]_{eq}$, as a partial pressure gradient for desorption is required. The extent of this difference is limited by two constraints. First, the respiratory quotient (the ratio of the CER to the OUR) is near unity for most fermentations. Hence, the CTR and OTR are nearly equal. Also, the liquid-phase oxygen partial pressure must be greater than zero to avoid oxygen starvation. Noting also that $\%CO_2^{in}$, being that in air, is very small (typically 0.035%), Eqs. 8 describe the carbon dioxide equilibrium concentration, the maximum concentration possible, $[CO_2]_{max}$, and the ratio of these concentrations (called here the carbon dioxide excess, ξ_{max}), in terms of the exit gas composition.

$$[CO_2]_{eq} = \frac{p_g^{CO_2}(out)}{H^{CO_2}} = \frac{\%CO_2^{out}}{100} \cdot \frac{(P - p_w)}{H^{CO_2}}$$

$$[CO_2]_{max} = \frac{\%CO_2^{out}}{100} \cdot \frac{(P - p_w)}{H^{CO_2}} + \frac{1}{0.89} \cdot \frac{\%O_2^{out}}{100} \cdot \frac{(P - p_w)}{H^{O_2}} \quad (8)$$

$$\xi_{max} = \frac{[CO_2]_{max}}{[CO_2]_{eq}} = 1 + \frac{1}{0.89} \cdot \frac{H^{CO_2}}{H^{O_2}} \cdot \frac{\%O_2^{out}}{\%CO_2^{out}}$$

where P is the operating pressure, and p_w the partial pressure of water in the exit gas.

Values for the Henry's law constants for water are available in the literature (for example, Schumpe and Quicker, 1982). Their dependence on temperature is indicated below.

$$H^{O_2} = \exp\left\{ 12.74 - \frac{133.4}{(T - 206.7)} \right\}$$

$$H^{CO_2} = \exp\left\{ 11.25 - \frac{395.9}{(T - 175.9)} \right\}$$

At 30°C, H^{O_2} and H^{CO_2} are 86,000 and 3,400 $Pa \cdot m^3 \cdot mol^{-1}$, respectively. Figure 1 plots Eqs. 8 for operation at atmospheric pressure and 30°C, assuming the inlet air to contain 21% oxygen.

From Figure 1 it is clear that the difference between the maximum and equilibrium carbon dioxide concentrations is a fairly constant quantity. The extent of this difference cannot be very large because of the need to work at oxygen mass transfer coefficients that will maintain the concentration of the sparingly soluble oxygen above a level that affects respiration. This difference becomes a smaller proportion of the total dissolved carbon dioxide concentration with increasing $\%CO_2^{out}$, and hence ξ_{max} is seen to decrease. While this means that the equilibrium assumption becomes relatively more accurate, the organism will in most cases be more sensitive to a given error at the higher dissolved carbon dioxide concentration.

Figure 1 indicates that the carbon dioxide excess may be important and can be evaluated more precisely from Eq. 9.

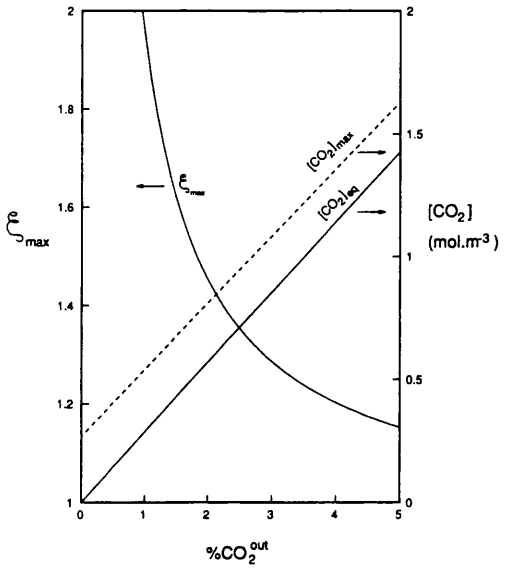

Figure 1. Maximum and equilibrium concentrations of dissolved carbon dioxide and their ratio as a function of the exit gas composition at 30°C and 1 atm for an $RQ = 1$.

$$\xi = \frac{[CO_2]}{[CO_2]_{eq}} = 1 + \frac{CTR \cdot H^{CO_2}}{(K_L^{CO_2}a) \cdot V \cdot p_g^{CO_2}(\text{out})}$$

$$= 1 + \frac{Q \cdot (1-\epsilon) \cdot H^{CO_2}}{60RT_0 \cdot K_L^{CO_2}a} \cdot \left(\frac{P_0}{P - p_w}\right) \cdot \left(\frac{\%CO_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} - \%CO_2^{in}}{\%CO_2^{out}}\right)$$

$$\approx 1 + \frac{Q \cdot H^{CO_2}}{60RT_0 \cdot (0.89 \cdot K_L^{O_2}a)} \cdot \left(\frac{P_0}{P}\right) \quad (9)$$

where Q is the specific aeration rate at standard temperature and pressure, T_0 and P_0, are the standard temperature (298 K) and pressure (1.01×10^5 Pa), R is the ideal gas constant (8.314 J·mol^{-1}·K^{-1}), ϵ is the gas void fraction, and ξ is the dissolved carbon dioxide excess over the equilibrium assumption. The specific aeration rate Q is normally based on the unaerated volume, so the void fraction ϵ enters the expression. The void fraction is typically less than 10%, while p_w is normally around 5% of the operating pressure, so these variables can be considered to cancel out with little error. The term involving gas analyses falls away as it is close to unity.

For a given operating pressure, the value of the carbon dioxide excess ξ by Eq. 9 is therefore determined mainly by the values of Q and $K_L^{O_2}a$. It should be noted that these variables are not wholly independent. While Westerterp et al. (1963) suggest that they are independent, most authors have found a weak power law dependence of $K_L^{O_2}a$ on the superficial gas flow rate, and hence on Q. They are treated here as approximately independent ($K_L^{O_2}a$ depending mainly on the agitation rate) to clarify the discussion. The ranges of these variables is quite limited in practice, so the range of ξ for most practical cases can be plotted as a function of these two variables. Figure 2 plots Eq. 9 for operation at the atmospheric pressure and a temperature of 30°C.

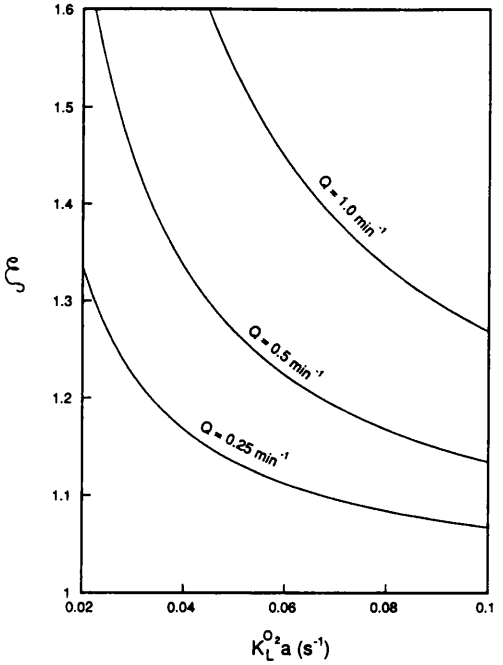

Figure 2. Variation of the carbon dioxide excess with specific aeration rate and volumetric mass transfer coefficient at 30°C and 1 atm.

Figure 2 shows that the error in the equilibrium assumption becomes relatively smaller, as the volumetric mass transfer coefficient rises or as the specific aeration rate falls. The typical error in the equilibrium assumption will be 20–40%. At high aeration and low agitation, the error can be more than 40%. Such conditions will tend to coincide with a low value of the $\%CO_2^{out}$ and hence of $[CO_2]$ (Figure 1), and so this error may be of relatively little importance. Under opposing conditions of low aeration and high agitation, the error in the equilibrium assumption will be only 10–20%. Such conditions will in general be associated with a high value of the $\%CO_2^{out}$ and hence of $[CO_2]$. At high dissolved carbon dioxide concentrations, organisms are relatively more sensitive to variations in the dissolved carbon dioxide concentration, hence even a small error in the equilibrium assumption may be of importance.

The accuracy required is clearly determined by the sensitivity of the organism to the dissolved carbon dioxide concentrations involved. The assumption of equilibrium may be inadequate where the organism is sensitive to the dissolved carbon dioxide concentration, particularly if the aeration rate is high or the volumetric mass transfer coefficient is low. A low mass transfer coefficient could be caused not only by a highly viscous broth,

which would have the effect of lowering the film mass transfer coefficient $K_L^{O_2}$, but also by low agitation rates.

The carbon dioxide concentration can be calculated on-line during the fermentation using Eqs. 2 to 6. The concentration of bicarbonate ions in the bulk broth under steady-state conditions may be obtained from this:

$$[HCO_3^-] = \frac{k_1 \cdot K_{ac}}{k_2 \cdot 10^{-pH}} \cdot [CO_2] = \frac{K_{ac}^*}{10^{-pH}} \cdot [CO_2] \qquad (10)$$

where K_{ac}^* is the pseudo-dissociation constant for carbon dioxide, which has a value of 4.7×10^{-4} mol·m^{-3} at 30°C in water (Arrua et al., 1990). While pH electrodes are sensitive to hydrogen ion activity, they are calibrated against hydrogen ion concentrations in buffers, and hence $[H^+]$ can be obtained from the pH measurement directly. Arrua et al. (1990) provide data for cases where the ionic strength affects the equilibrium.

The above discussion explains why the equilibrium assumption will be better for large fermenters than for small ones. Part of the reason was pointed out by Yagi and Yoshida (1977), who noted that with increasing scale, the specific aeration rate Q must be reduced to maintain the gas superficial velocity (and hence, the gas void fraction, ϵ) within practical limits. Figure 2 shows a decrease in the specific aeration rate at constant $K_L^{O_2}a$ to improve the relative accuracy of the equilibrium assumption. Such reductions, however, are limited by the increasing $\%CO_2^{out}$ which ultimately causes severe inhibition in most aerobic organisms. As a result, the minimum specific aeration rates used are around 0.2 min^{-1} even for large fermentors (Siggig, 1982).

A more important reason for the good approach to carbon dioxide equilibrium in large fermenters concerns the extent of gas backmixing. The high solubility of carbon dioxide means that the concentration gradient required for meeting the CTR translates into a relatively small partial pressure gradient. If there is a substantial degree of plug-flow character in the gas phase, as may be expected on larger fermenters, large partial pressure gradients will exist at the bottom of the fermenter, as the inlet air has a very low carbon dioxide content. The dissolved carbon dioxide concentration cannot vary greatly in the axial direction, as it is an order of magnitude greater than the dissolved oxygen concentration. Hence, the CTR for the fermenter will be met rapidly at the bottom of the fermenter, and the gas will be in carbon dioxide equilibrium with the broth as it reaches the broth surface.

Conclusions

The absolute difference between the actual and equilibrium carbon dioxide concentrations is limited for aerobic fermentations by practical considerations. Figure 1 can provide an *a priori* assessment of the maximum value of this difference. If significant, the precise value of the excess carbon dioxide concentration over the equilibrium assumption can be evaluated from the curves presented in Figure 2. The data presented are for operation at 30°C, but as the dependence on temperature is rather weak, they can provide a first approximation at other temperatures.

For systems where the aeration rate is high and the mass transfer coefficient is low, the excess over the equilibrium assumption may be more than 40%. For opposing conditions of small aeration rates and large mass transfer coefficients, the concentration of dissolved carbon dioxide will be only 10–20% greater than the equilibrium assumption. Under such circumstances, the accuracy of the equilibrium assumption may be better than that of a dissolved carbon dioxide probe. In large fermentors, the approach to carbon dioxide equilibrium between the broth and exit gas will be better than that in research fermentors for the reasons discussed.

The equations presented can be used to monitor routinely the concentrations of dissolved carbon dioxide and bicarbonate ions in fermentation broths. This ability means that the concentrations of dissolved oxygen and carbon dioxide can be independently controlled. Stricter control of these variables may facilitate more meaningful comparisons to be made between different strains and operating conditions during fermentations in research facilities, and improve productivities in production systems.

Notation

a = interfacial surface area per unit volume of aerated broth, m^2·m^{-3}
CER = carbon dioxide evolution rate, mol·s^{-1}
CTR = carbon dioxide transfer rate, mol·s^{-1}
$[CO_2]$ = concentration of dissolved carbon dioxide, CO_2(aq), mol·m^{-3}
$[CO_2]_{eq}$ = concentration of dissolved carbon dioxide assuming equilibrium between the exit gas and fermentation broth, Eq. 8, mol·m^{-3}
$[CO_2]_{max}$ = maximum possible concentration of dissolved carbon dioxide assuming $RQ = 1$, mol·m^{-3}
$D_L^{CO_2}$ = liquid diffusivity of carbon dioxide, m^2·s^{-1}
$D_L^{O_2}$ = liquid diffusivity of oxygen, m^2·s^{-1}
G = inlet air flowrate (dry basis), mol·s^{-1}
H^{CO_2} = Henry's law constant for carbon dioxide, Pa·m^3·mol^{-1}
H^{O_2} = Henry's law constant for oxygen, Pa·m^3·mol^{-1}
$\%i^{in}$ = mole percentage of component i in inlet air to fermenter (dry basis)
$\%i^{out}$ = mole percentage of component i in exit gas from fermenter (dry basis)
k_1, k_2 = rate constants governing carbon dioxide hydration, s^{-1}
K_{ac} = carbonic acid dissociation constant, mol·m^{-3}
K_{ac}^* = pseudo-dissociation constant for carbon dioxide defined in Eq. 10, mol·s^{-1}
$K_L^{CO_2}$ = liquid film mass transfer coefficient for carbon dioxide, m·s^{-1}
$K_L^{CO_2}a$ = fermenter volumetric carbon dioxide mass transfer coefficient, s^{-1}
$K_L^{O_2}$ = liquid film mass transfer coefficient for oxygen, m·s^{-1}
$K_L^{O_2}a$ = fermenter volumetric oxygen mass transfer coefficient, s^{-1}
OTR = oxygen transfer rate, mol·s^{-1}
OUR = oxygen uptake rate, mol·s^{-1}
P = total pressure in fermenter, Pa
P_0 = standard pressure, 1.01×10^5 Pa
$p_g^{O_2}$ = partial pressure of oxygen in the gas phase, Pa
$p_g^{CO_2}$(out) = partial pressure of carbon dioxide in the exit gas, Pa
$p_g^{O_2}$(out) = partial pressure of oxygen in the exit gas, Pa
p_w = partial pressure of water in the exit gas, Pa
Q = specific aeration rate, volumetric aeration rate at standard temperature and pressure per unit unaerated broth volume, min^{-1}
R = ideal gas constant, 8.314 J·mol^{-1}·K^{-1}
RQ = respiratory quotient, the ratio of CER to the OUR
T = temperature of broth, K
T_0 = standard temperature, 298 K
V = aerated broth volume, m^3
V_{film} = liquid film volume, m^3

Greek letters

ϵ = gas void volume as a fraction of the aerated broth volume

ξ = dissolved carbon dioxide excess, the ratio of the dissolved CO_2 concentration to the equilibrium concentration, Eqs. 8

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Appendix: Mass Transfer Enhancement by Liquid-Film Reaction

The liquid-phase reactions involving carbon dioxide have already been discussed. At pseudo-steady state (static or slowly changing *CER* and pH), chemical equilibrium can be assumed in the bulk broth. In the liquid film, however, $[CO_2]$ drops from its value in the bulk broth to an interfacial value dependent on the gas-phase composition. At the interface, there will therefore be a gradient driving the flux of carbon from H_2CO_3 to CO_2(aq). If this flux is significant in comparison to the *CTR*, it will have the effect of enhancing mass transfer.

If it is initially assumed that the film effect is negligible, then the concentration of H_2CO_3 will be the same at the interface as it is in the bulk broth. This will have the effect of maximizing the concentration gradient driving the film effect. If the resulting expression for the film effect indicates that it is negligible, the initial assumption will have been justified.

The problem is solved using boundary layer theory in linear coordinates, as bubble diameters in fermenters are much larger than the thickness of the liquid film. If the interface is at $x=0$ and the edge of the liquid film at $x=\delta$, then:

$$[CO_2]^{x=0} = \frac{p_g^{CO_2}(\text{out})}{H^{CO_2}}$$

$$[CO_2]^{x=\delta} = \frac{p_g^{CO_2}(\text{out})}{H^{CO_2}} + \frac{1}{K_L^{CO_2}} \cdot \frac{CTR}{a \cdot V}$$

$$[H_2CO_3]^{x=0} = [H_2CO_3]^{x=\delta} = \frac{k_1}{k_2} \cdot [CO_2]^{x=\delta}$$

As the reactions are first order:

$$\begin{aligned}\text{film effect } (\text{mol} \cdot \text{s}^{-1}) &= \frac{1}{2} \text{ at } x=0+x=\delta \\ &= \frac{1}{2} \text{ at } x=0 \\ &= \frac{1}{2}\left(k_2 \cdot [H_2CO_3]^{x=0} - k_1 \cdot [CO_2]^{x=0}\right) \cdot V_{\text{film}} \\ &= \frac{k_1}{2 \cdot K_L^{CO_2}} \cdot \frac{CTR}{a \cdot V} \cdot V_{\text{film}}\end{aligned}$$

where V_{film} is the volume of the liquid film. In terms of boundary layer theory, the volume of the liquid film (V_{film}) is:

$$V_{\text{film}} = \delta \cdot a \cdot V = \frac{D_L^{CO_2}}{K_L^{CO_2}} \cdot a \cdot V$$

Hence,

$$\frac{\text{film effect}}{\text{mass transfer}} = \frac{\left(\frac{k_1}{2 \cdot K_L^{CO_2}} \cdot \frac{CTR}{a \cdot V} \cdot \frac{D_L^{CO_2}}{K_L^{CO_2}} \cdot a \cdot V\right)}{CTR} = \frac{k_1 \cdot D_L^{CO_2}}{2 \cdot (K_L^{CO_2})^2}$$

At 25°C, k_1 has a value of 0.028 s^{-1} (Pinsent et al., 1956), and D_{CO_2} has a value in water of 2.0×10^{-9} $m^2 \cdot s^{-1}$ (Incropera and de Witt, 1990). Godbole et al. (1984) give values for the film mass transfer coefficient of around 2×10^{-4} $m \cdot s^{-1}$. Hence,

$$\frac{\text{film effect}}{\text{mass transfer}} = 0.0007$$

Therefore, the film effect is negligible, and assumption *a* above is justified. It has been assumed that there is no catalysis of carbon dioxide hydration by the enzyme carbonic anhydrase, as this is an intracellular enzyme and is present in only a few micro-organisms.

Manuscript received Apr. 17, 1991, and revision received Aug. 26, 1991.

BB 40(5) pp 634-637 (August 20)

Communications to the Editor

Analysis of Noise and Bias in Fermentation Oxygen Uptake Rate Data

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Received December 10, 1991/Accepted March 27, 1992

The calculation of many derived fermentation variables such as the respiratory quotient (RQ) and mass transfer coefficient (K_La) requires the differences between the molar percentages of oxygen and carbon dioxide in the fermentor inlet and exit gas, called the %OUR and %CER. Noise and bias in %CER data is of order that in the exit gas carbon dioxide analysis. However, the relative amount of noise in the %OUR is one to two orders of magnitude greater than the noise in the raw oxygen analyses because the %OUR is calculated as a small difference between two large quantities. The noise in the %OUR is white with a Gaussian amplitude probability distribution of absolute standard deviation 0.0145. A chi-square filter of the %OUR data is shown to considerably improve the quality of the calculated RQ and K_La for a fermentation of *S. clavuligerus*. © 1992 John Wiley & Sons, Inc.

Key words: mass spectrometer • fermentation • oxygen uptake rate • noise • bias • filter

INTRODUCTION

Instruments available for the analysis of fermentation gases are both highly accurate and reliable. For example, an accuracy of gas analyses of ±0.1% and calibration drift of less than 1% per month have been reported[2] for a mass spectrometer with a magnetic sector of fixed field strength used in a fermentation facility. The fermentation variables of greatest interest depend on the difference between the molar percentages of oxygen and carbon dioxide in the inlet and exit gas streams, called here the %OUR and %CER, respectively:

$$\%\mathrm{OUR} = \%\mathrm{O}_2^{\mathrm{in}} - \%\mathrm{O}_2^{\mathrm{out}} \cdot \left(\frac{\%\mathrm{N}_2^{\mathrm{in}}}{\%\mathrm{N}_2^{\mathrm{out}}}\right)$$

$$\%\mathrm{CER} = \%\mathrm{CO}_2^{\mathrm{out}} \cdot \left(\frac{\%\mathrm{N}_2^{\mathrm{in}}}{\%\mathrm{N}_2^{\mathrm{out}}}\right) - \%\mathrm{CO}_2^{\mathrm{in}}$$

The superscripts indicate the inlet (in) and exit (out) gas stream analyses. Fermentation variables such as the carbon dioxide evolution rate (CER), oxygen uptake rate (OUR), mass transfer coefficient (K_La), and respiratory quotient (RQ) require the %CER and %OUR for their calculation:

$$\mathrm{CER} = \frac{\%\mathrm{CER}}{100} \cdot G \qquad \mathrm{OUR} = \frac{\%\mathrm{OUR}}{100} \cdot G$$

$$\mathrm{RQ} = \frac{\mathrm{CER}}{\mathrm{OUR}} = \frac{\%\mathrm{CER}}{\%\mathrm{OUR}} \qquad K_La \propto \%\mathrm{OUR}$$

where G is the molar aeration rate.

Air fed to a fermentor contains about 0.04% CO_2 and 21% O_2. At most one-fourth of the oxygen fed is consumed.[3] As the RQ is generally around 1, the %CER is approximately equal to $\%CO_2^{out}$, while the %OUR is calculated as a small difference between two large numbers. Gas analyses contained an element of noise and fixed bias, which is inevitably reflected to some extent in the calculated %CER and %OUR. As $\%CO_2^{in}$ is close to zero, the noise and bias in the %CER will be of order that in the $\%CO_2^{out}$ analyses and should therefore be very small. The purpose in this article is to examine the extent of noise and bias reflected in the %OUR, on which the most important on-line derived fermentation variables depend.

MATERIALS AND METHODS

Fermentation gas analyses were provided by a mass spectrometer with a scanning magnetic field and single Faraday cup detector (Model MM8-80, VG Gas Analysis, Cheshire, UK). The system is multiplexed to several fermentors and configured to analyze for N_2, O_2, Ar, and CO_2, with a settling time of 20 s per fermentor gas analysis. The fermentation gases analyzed here were from a spore-inoculated fermentation of *S. clavuligerus* (ATCC 27064) grown on soluble complex media[1] at 26°C and pH 7.

RESULTS AND DISCUSSION

Bias in the %OUR

If the cause of bias affects the instrument slope (i.e., sensitivity) or zero point, then the proportion of bias in

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the %OUR will be no greater than that in the raw oxygen analyses and may even be less. If, however, a source of bias affects the inlet or exit gas stream analyses independently of one another, then this fixed error will become an increasing proportion of the %OUR as the %OUR falls.

This effect can be observed by examining gas analysis data during the early part of a fermentation. Figure 1 presents RQ and %OUR data at the beginning of a fermentation of *S. clavuligerus*. Initially the RQ data are considerably above 1, settling to a value of 0.9 at 2 h, at which time the %OUR is around 0.2. The high values of the RQ during the earliest stages of the fermentation are due to bias in the %OUR because the direction of settling to RQ = 0.9 is not reproducible from one fermentation to the next. As the %OUR is much greater than 0.2 during most fermentations, bias in the %OUR will not normally be a problem for the gas analysis system used here. For other systems this threshold may be higher.

Noise in the %OUR

Noise in the $\%O_2^{in}$ analysis for the mass spectrometer used here has a standard deviation of 0.04% of the signal. The exit gas oxygen content is only slightly lower than the inlet content. The absolute standard deviation of noise in the %OUR will therefore be independent of the %OUR. This noise will become a larger proportion of the %OUR as the %OUR falls. This can be seen in Table I, which tabulates the standard deviation of noise in samples of 10 %OUR data points during a fermentation of *S. clavuligerus*. For %OUR <0.2, the %OUR data are dominated by noise. At higher values of the %OUR, the noise will significantly corrupt derived variables.

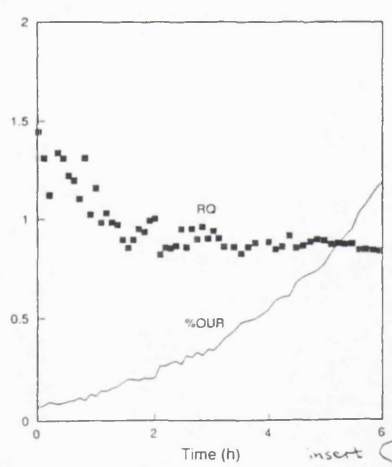

Figure 1. The RQ and %OUR during the early stages of a fermentation of *S. clavuligerus*.

Table I. Standard deviation of noise in 10-point sets of %OUR data as a percentage of the signal during a fermentation of *S. clavuligerus*.

%OUR	Standard deviation of noise as percentage of %OUR
2.0	0.8
1.0	1.5
0.5	2.1
0.2	8.5
0.1	22.0

Characterization of noise in a signal requires that the noise amplitude be measured relative to some smoothed signal baseline. Where the signal is known to be constant, this baseline is easily generated. Where the signal is varying, the method used to generate a baseline may influence the resulting analysis of noise. In this work, the noise data set were generated from a 500-point set of %OUR data available at the relatively high frequency of 0.67 min^{-1} and which were continuous and substantially constant, being from the glycerol-based growth phase of a fermentation of *S. clavuligerus*. This meant that the method used to generate the baseline should have little impact on the conclusions of the noise analysis, and this was confirmed by using both chi-square and moving average filters to generate the baseline and comparing the results.

Figure 2 presents the autocorrelation and amplitude probability distribution for this 500-point noise data set. A criterion for white noise[4] is that the autocorrelation at

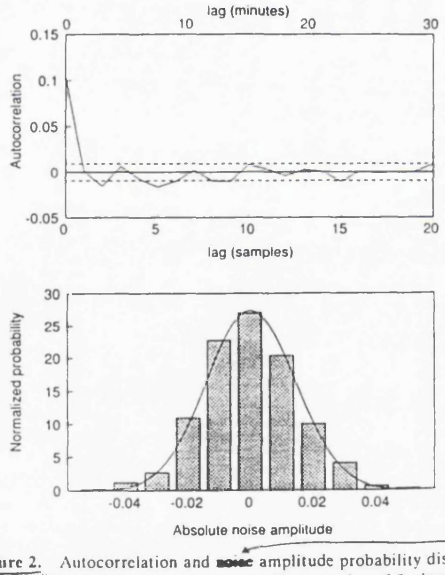

Figure 2. Autocorrelation and noise amplitude probability distribution of 500 %OUR data points from a fermentation of *S. clavuligerus*. The smooth curve in the bar graph is the normal distribution with standard deviation 0.0145.

nonzero lags as a fraction of that at zero lag should be less than $\pm 2/\sqrt{N}$, where N is the number of samples of noise. For a 500-point data set, this requires the autocorrelation at nonzero lags to be less than 9% of that at zero lag. This criterion is indicated in Figure 2. It is slightly exceeded at lags of two and five samples, but the data are not strongly correlated. Analysis of other %OUR noise data sets showed similar results, although the lags at which the criterion was exceeded were different, again indicating the noise to be effectively white.

The probability distribution of noise amplitude for the %OUR data set can be seen in Figure 2 to be approximately Gaussian, with an amplitude standard deviation of 0.0145. This reflects the individual contributions of noise in the inlet and exit gas analyses. The standard deviation of noise in the $\%O_2^{in}$ and $\%O_2^{out}$ analyses is 0.008 (0.04% of the signal). When $\%O_2^{out}$ is based on the inlet aeration rate, its noise standard deviation increases to 0.012 due to noise in the nitrogen analyses. The standard deviation of noise in the %OUR is the square root of the sum of the inlet and exit gas oxygen analysis noise variances.

Removal of Noise in %OUR

Since the noise in the %OUR is approximately white, a model-based method for noise removal rather than a spectral method is required. As the noise amplitude probability distribution is Gaussian, a least-squares method such as a chi-square filter will give a maximum-likelihood estimate of the %OUR and model parameters. The %OUR profile for an entire fermentation is commonly made up of two or more growth phases, and it is rare for a good model to be available to describe this profile. It is more practical to use a convenient class of function, such as polynomials, applied to a reduced data set. The simplest polynomial is a constant function, which could be used to produce a moving average of the most recent %OUR data points. This is not a suitable filter for real-time application to %OUR data, as the estimate applies to the midpoint of the time range covered by the data. Gas analyzers for fermentation applications, being expensive instruments, are multiplexed between several fermentors, which means that gas analyses for a given fermentor are typically only available at a relatively low frequency of around 0.1–0.2 min^{-1}. Hence a 10-point moving average, for example, would produce %OUR estimates around 40 min old. The next simplest polynomial is a linear model, which was used in this work. To obtain a real-time estimate of the %OUR, a chi-square filter is used to fit a straight line to a time window of %OUR data less than 1 h old, in Figure 3. Clearly, the time window has to be narrow enough so that the data within the window are at least reasonably linear, while at the same time containing a sufficient number of data points for the parameter estimation. The gas analyses in Figure 3 were available every 6 min

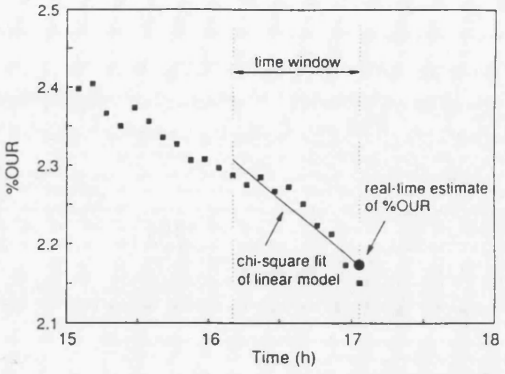

Figure 3. Real-time estimation of the %OUR by a chi-square fit of a linear model to a time window of %OUR data less than 1 h old.

(frequency 0.17 min^{-1}), so that a 10-point data set was contained within the time window. For a linear model, a window width of 1/2–3 h was found to give good filter performance.

In Figure 4, the unfiltered %OUR, RQ, and K_La data for a fermentation of *S. clavuligerus* are presented. During the first 10 h of the fermentation, the broth displayed Newtonian rheology, giving very high mass transfer coefficients (off-scale) at the chosen agitation rate. Once the concentration of mycelial biomass had risen sufficiently, broth rheology became plastic as is the case for most mycelial fermentations, with a concomitant fall in the

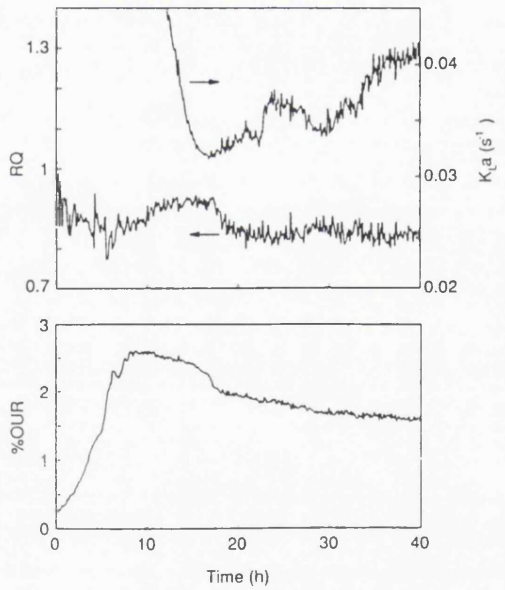

Figure 4. The %OUR, RQ, and K_La during a fermentation of *S. clavuligerus* without filtering of the %OUR data.

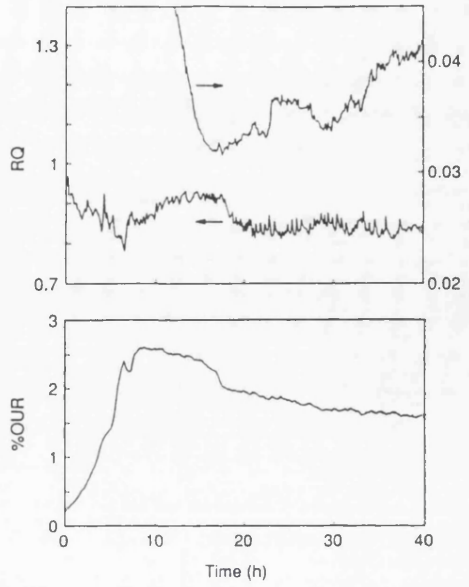

Figure 5. The %OUR, RQ, and K_La during a fermentation of *S. clavuligerus* with filtering of the %OUR data.

mass transfer coefficient. In Figure 5, the same data are presented after filtering of the %OUR data using the method proposed. There can be seen to be a considerable improvement in the quality of the calculated RQ and K_La with filtering of the %OUR. The disturbances in the RQ remaining after filtering of the %OUR data are nonrandom and associated with the effect of pH control action on the %CER.[5,6] The noise remaining in the K_La data are associated with the measurement of dissolved oxygen. While the filter used is suggested by the nature of the problem, it is by no means the only, or necessarily the best, method for filtering noise from the %OUR data of an arbitrary fermentation. For example, use of a linear model requires the data set in the time window to be at least reasonably linear. For fast-growing organisms this may require a narrow time window, which might may contain an insufficient number of gas analysis data points for good noise filtering. In such situations, a quadratic or cubic model applied to a wider time window might may produce better results. It is however believed that the very simple filter proposed here could be suitably applied to a large number of industrially important fermentations, where relatively low growth rates coupled with the smoothly continuous growth usually generated by the undefined media used will produce %OUR profiles well suited to this type of filter.

The authors gratefully acknowledge the financial support of the SERC.

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corrections: Royce, P.N.C. 1992. Effect of pH changes on the measured RQ of fermentations. In: F. Vardar-Sukan and S. Suha Sukan (eds), Recent Advances in Biotechnology, Nato-ASI Series E: Applied Scien Vol. 210, Kluwer Academic, The Netherlands, pp 527-528.

operating conditions of a production run deviating beyond acceptable limits. Most importantly, they are opening up the field for biosensor applications. This has been closed for direct on-line sampling because of lack of sensor robustness. Finally, the new analytical technologies of MS and CE can be coupled to R&D process development for monitoring the increasingly complex therapeutic proteins now being made.

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forum

A need to refocus research in the operation of fermenters?

workshop

Although fermentation reactions probably constitute the oldest face of biotechnology, the efficient operation of fermenters remains a key factor in determining productivity and cost of most industrial bioprocesses.

The recent surge of interest in bioprocess design and operation has produced a large body of research data describing novel fermenter configurations, and measurement, modelling and control techniques. However, the range of biochemical-engineering unit operations actually practised on an industrial scale, particularly those associated with large-scale fermentation, is still relatively small[1].

Understandably, industry is reluctant to invest heavily in new systems until long-term productivity/cost benefits are proven. However, by examining the needs of industrial-scale operations, particularly with regard to large-scale aerobic fermentations in which production costs are an important factor, it may be possible to refocus research to address these needs.

Features of industrial fermentation

The majority of industrial fermentations are run in stirred tanks[2] of a fairly standard design of up to 200 m^3 working volume. The impeller shaft has three to five impellers, and on pilot- and production-scale fermenters, specific power inputs are in the range 1–4 kW m^{-3} (Ref. 3), the temperature range is 25–40°C, and the pH range is 4.0–8.0. Aerobic fermentations represent the most important industrial fermentation processes, with aeration rates in the range 0.2–1.0 vvm (volume per volume per minute). However, although most industrial microorganisms thrive under these conditions, new systems will have to be developed for exploitation of organisms whose unusual metabolic activities represent a new resource for biotechnology. To date, extremophiles have seen little application in industry due to their low growth rates, low cell yields and high shear sensitivity.

Monitoring

On-line monitoring of industrial fermentation involves measuring physico-chemical parameters such as pH and gas analyses, whereas off-line measurements can be used for most types of biochemical analysis. Since off-line measurements require manual sampling, they are taken infrequently, and the results are often delayed in time. Hence off-line measurements are most commonly used only for post-event evaluation of a process.

Feed strategy

Batch, fed-batch or continuous operation?

Batch operations (except for the production of certain pharmaceutical products)[4], are not an economic prospect and are relatively uncommon because of the advantages of fed-batch operation. The fed-batch mode of operation is the most widely used in industrial fermentations. Fed-batch processes are particularly well-suited for fermentations that exhibit substrate inhibition, catabolite repression, toxic precursors or the glucose effect.

What is perhaps surprising at first sight is the relative scarcity in industry of continuous fermentation processes. Although much research concerning yeast, bacteria and fungi in continuous fermentation has been published, with a few exceptions (Table 1), full-scale production in continuous fermentation systems is rare. Continuous operation has been employed as a research tool[5], since steady states, or small excursions from steady states can be more readily measured in continuous operation than in batch or fed-batch operation. Continuous fermentation offers other important advantages (in particular by reducing manpower costs and capital costs, and avoiding peak demands in utility consumption), but has the requirement that the strain of the organism used is one that is stable over a large number of generations. This makes continuous fermentation less suited for the production of intermediate and secondary metabolites, where genetic drift is a problem. While continuous operation may minimize capital and operating costs, it does not, in general, lead to conditions for optimal productivity within the fermenter. As a result, most of the examples of continuous fermentations in industry reported in Table 1 concern primary metabolism.

In addition, the technical difficulty of maintaining sterility over long periods of time means that continuous fermentation processes tend to be ones that don't require strict sterility, or in which the maintenance of sterility is assisted by the operating conditions, such as low pH.

Widening of the range of processes that are operated continuously will require developments allowing the producer strain to be given a competitive advantage over faster-growing mutants or contaminants. This

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may be achieved by combination of genetic manipulation with control of the operating conditions and broth composition. However, in view of the (increasing) dominance of the fed-batch fermentation, a greater emphasis could be placed on researching the design and optimization of fed-batch control methods. Since raw materials (together with utilities) account for the largest part of production costs[4], research in this direction is particularly relevant.

Fermenter design and operating conditions

The need for adequate oxygen transfer limits the size of aerobic fermenters to about 200 m^3. Anaerobic fermenters tend not to be much larger, since there is a strong economic incentive to reduce the peak demand for utilities required intermittently, such as steam for sterilization. At such scales, there is little economic incentive to avoid mechanical agitation, and hence the large majority of fermenters are mechanically agitated. It is only when the scale of operation is very much larger (e.g. 1500 m^3 SCP [single-cell protein] fermentation, and 30 000 m^3 activated-sludge fermentations) that alternative bioreactor designs that avoid mechanical agitation (such as the bubble-column and airlift fermenters) offer significant economic advantage. The isolation of ever more productive strains for secondary-metabolite production may mean that the optimum scale of production fermenter is decreasing. Mechanical agitation offers an additional degree of freedom in accommodating changes in broth rheology and oxygen demand that occur not only during the course of fed-batch fermentations but also as a result of the application of new producer strains. For high-viscosity broths, mechanical agitation appears to be the only solution[2].

The degree of freedom provided by mechanical agitation is likely to become more, rather than less important, in view of the pressure towards process flexibility and multiproduct plants. Such pressure is generated not only by the large number of new products being developed by the biotechnology industry, but also by increases in titres in established products promised by recombinant DNA methods[5]. Even so, process flexibility is rarely a consideration in the design of novel bioreactors by the academic community. Stirred-tank fermenters are likely to continue to be the workhorse of the fermentation industry. There may be more mileage in developing better variants of the stirred-tank fermenter, than in developing alternatives to it.

In spite of the widespread use of the stirred-tank fermenter, little research has examined the effect on microorganisms of the heterogeneous nature of heat, mass and momentum transfer in stirred tanks. In addition, these processes are poorly described for non-Newtonian fluids, which include the majority of fermentation broths. Microorganisms are subject to rapidly varying shear rates (see E. T. Papoutsakis, *TIBTECH* 9, 427–437, 1991), and cycling of the dissolved oxygen concentration, and of substrate or precursor concentrations in the case of fed-batch fermentations. In the case of aerobic fermentations, the extent to which aeration rates, and hence compression costs, can be reduced, is limited by the need to avoid inhibition by dissolved carbon dioxide[6]. Studies of dissolved-carbon-dioxide controlled fermentations are scarce.

Instrumentation, modelling and control

Few variables that describe the state of the fermentation can be measured on-line. However, the desire to avoid increased risk of contamination to production fermenters often leads to well-instrumented R&D fermenters, while production fermenters are the least well instrumented[7]. Is the academic community perhaps overestimating the interest the fermentation industry has in implementing new on-line measurements in production facilities?

The development of novel on-line sensors will need to take into account features of on-line instrumentation currently in use (Table 2) if they are to meet the criteria required for application by industry. Some novel sensors are beginning to see industrial application, such as the use of radio-frequency dielectric permittivity for the measurement of biomass concentration, which has been used in yeast fermentations in particular. Many ideas for on-line instrumentation, however, meet few, if any, of the criteria listed in Table 2. Automated analytical techniques, such as on-line HPLC, are too expensive and unreliable for application in production facilities[8] (B. Buckland, 4th Annual Biochemical Engineering Lecture, University College London, UK, 1989), although they may be useful as development tools. In secondary-metabolite production, for example, on-line analytical techniques might speed the introduction of new production strains by allowing the rapid dynamic optimization of operating conditions in the pilot plant. For some fermentations, however, there are relatively few degrees of freedom for refined control of the fermentation, and hence the ability to measure a particular fermentation state on-line does not automatically ensure its use in closed-loop control strategies, without which there is little justification for on-line measurement.

There is a great deal of scope for the better use of existing on-line and

Table 1. Examples of fermentation products according to feeding strategy

Continuous
Yeast, single-cell protein (SCP) (biomass)
Ethanol (primary metabolite)
Beer, vinegar, fermentation of milk (fermented foods)
Activated-sludge process for sewage and industrial wastes (biodegradation)
Glucose isomerase
Fed-batch
Secondary metabolites (e.g. antibiotics, vitamins)
Most other fermentation products including most of those also produced in continuous and batch fermenters
Batch
Some pharmaceutical preparations

Table 2. Features of most commonly used on-line fermenter instrumentation

Performance indicators
Work reliably for >80% of fermentations
Accuracy 1–2% of full scale
Low maintenance
Calibration simple and fast
Steam-sterilizable (*in-situ* probes)
Cross-sensitive only to other on-line measurements
Drift <1–2% of full scale over period of fermentation
Cost-benefit considerations
Information provided is of use in controlling fermentation to increase profitability
Instruments cost < UK£3000 per fermenter

off-line measurements in monitoring and controlling fermentations. Examples of developments required include:

- more robust dissolved oxygen sensors or fault-tolerant dissolved oxygen control algorithms;
- improved automatic analysis of previous batch data;
- automated synthesis and identification of 'physiological' or 'black box' models (i.e. models which are not based on understanding of the underlying mechanisms of growth and production);
- more-structured monitoring of derived variables (e.g. unsteady carbon-dioxide transfer can affect the respiratory quotient, calculation of meaningful mass-transfer coefficients will require detailed knowledge of liquid and gas backmixing).

Greater emphasis in academic research could be placed in development of physiological models which use only on-line measurements and derived variables. While the number and diversity of multiparameter biological models is ever increasing, their application in industry is still limited*. For secondary-metabolite fermentations, where regular changes to the production strain requires the repeated re-identification of the model, complex multiparameter models are not cost effective.

Solutions looking for problems?

The disparity between current academic research and industrial problems was apparent in the proposed titles for the debates at the ICCAFT5 fermentation conference*, whose topics included 'Futuristic methods versus mundane needs', 'Models in biotechnology: daydreaming in the ivory towers?' and 'Computers in industry: killing flies with hammers?'. A particularly strong theme at the conference (in the oral as well as the poster sessions) was the application of artificial neural networks (ANNs) to fermentation development, analysis and control by the academic community. One industry participant commented, however, that there was a feeling of 'solutions looking for problems'. Another commented that little attempt was being made to compare the performance of new techniques (such as ANNs) with established techniques. While a function of academic research in engineering is to develop novel ideas, such ideas clearly must not lose sight of the reasons for the existing state of industrial fermentation, the kinds of problems being considered by industry, and the criteria by which solutions are judged.

* ICCAFT5 (5th International Conference on Computer Applications to Fermentation Technology), together with IFAC-BIO2 (2nd International Federation of Automatic Control Symposium on Modelling and Control of Biotechnical Processes), was held in Keystone, Colorado, USA, 29 March – 2 April, 1992.

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A2: PROGRAM WRITTEN BY THE AUTHOR, AND BY P. MAVRIKIS, FOR DISSOLVED CO_2 CONTROL AS DESCRIBED IN SECTION 4.6.2

The program listed here is a merging of two earlier programs. One of these was SER0317.C written by P. Mavrikis (Mavrikis, 1992), which handled data capture from the mass spectrometer. The second was TCSIO.C written by the author, which handled bidirectional communications with the TCS 6358 controller and 6432 signal processor. Only certain of the subroutines in both these programmes were required in the program listed here for achieving the feedforward control proposed in Section 4.6.2.

```
#include <stdio.h>
#include <string.h>
#include <dos.h>
#include <time.h>
#include <stdlib.h>
#include <math.h>
#include <bios.h>
#include <time.h>
#include <ctype.h>
#include <conio.h>

#define COMINT 0x14

#define STX '\002'
#define ETX '\003'
#define EOT '\004'
#define ENQ '\005'
#define ACK '\006'
#define ID8L '6'          /* 8-loop ID number   */
#define IDSP '7'          /* sig.proc. ID number */
#define BLOCKSP '8'         /* specify 8-channel block number on 32-ch s.proc. */

#define TCSLENG 5           /* number of characters in a TCS numeric variable eg. PV= 018-6 */
#define SEND8L 8           /* number of chars. in prompt to 8-loop channel */
#define SENDSP 9           /* number of chars. in prompt to sig. proc.     */
#define RECEIV8L 10         /* number of characters in 8-loop response*/
#define RECEIVSP 11         /* no. of chars. in sig. proc. resp.      */
#define START8L 4          /* parse start position of numeric data in tcs response */
#define STARTSP 5
#define CHANGE8L 15         /* number of characters in set-point change instruction */

#define PORTTCS 4          /* COM1=0, COM2=1 etc.  */
#define TCSDELAY 0.1        /* time delay between reading of successive channels */
```

```
void  delay(float),
      set8L(float,float),
      readSP(float*),
      floattotcs(char*,float,int);
float tcstofloat(char*),
      read8L(float*);
char checksum(char*,int),comms(unsigned int, unsigned int);
int tcscomset();

struct port_set{
   char *ah,
         *baud,
         *parity,
         *stopbits,
         *databits;
};

struct loop {
   char loopID,
         varsID1,
         varsID2,
         *name;
}

static struct loop tcs8L[]={          /* variables to be addressed on 8-loop  */
   {'8','P','V',"pH",},
   {'9','P','V',"Temp",},
   {'A','P','V',"%DO",},
   {'B','P','V',"Agit.",},
   {'C','P','V',"Gas1",},
   {'D','P','V',"Gas2",},
   {'E','P','V',"DCO2",},
   {'F','P','V',"Press.",},
};

static struct loop tcsSP[]={          /* variables to be addressed on signal proc  */
   {'2','P','V',"Load1",},
   {'3','P','V',"Load2",},
   {'4','H','R',"Tube D",},
   {'6','H','R',"Pump V",},
};

#define VARS8L (sizeof(tcs8L)/sizeof(tcs8L[0]))
#define VARSSP (sizeof(tcsSP)/sizeof(tcsSP[0]))

#define PORT3 0x330
#define COMINT 0x14

union REGS com3port;
char incoming_data[200];
void initialise_port3();
void initialise_structure();
void menu();
void delay();
void delay2();
```

```
void synchronize_clocks();
int no_errors();
void receive_a_record();
int fermenter_id();
void save_last_ms();
void read_desired();
void read_time();
void read_concentrations();
int read_inlet();
void save_desired();
FILE *fp,*fp1;
int clocks_synchronized=0;
int tcs_buffer_lines=10;
int records_received=0;
int records_to_receive;
int records_in_file=2;
int inlet_monitored=25;
int first_inlet_to_receive=25;
float time_in_seconds;
float delay_in_seconds=120.0;
float m_8l[10][8],approx_8l[8];
float m_sp[10][2],approx_sp[2];
struct fermenter_data {
char inlet[3];
char hours[3];
char minutes[3];
char seconds[3];
char n2[9];
char co2[9];
char o2[9];
char ar[9];
};
char name_of_mass_spec[8]="m_spec";
char name_of_drive[3]="a:";
struct dostime_t;

/*-------------------------------------------------------------------------*/
main()
{
static float data8L[VARS8L],dataSP[VARSSP];
int the_inlet;
float newspd, newg, oldg, co2num;              /* new aeration rate setpoint */
struct fermenter_data fermenter;
initialise_port3();
menu();

start:
initialise_structure(&fermenter);
receive_a_record();
if(!no_errors()) goto start;
printf("---------------------------------------\n");
the_inlet=fermenter_id();
printf("Fermenter:%d\n",the_inlet);
if(the_inlet==first_inlet_to_receive)
{
```

```
  if(first_inlet_to_receive==inlet_monitored) first_inlet_to_receive=10;
  else first_inlet_to_receive=inlet_monitored;
  printf("Record:%d  No of records to stop:%d\n\n",\
  records_received+1,(records_to_receive-records_received-1));
  save_last_ms();
  read_desired(&fermenter);
printf("inlet:%s\n",fermenter.inlet);
printf("%s:%s:%s\n",fermenter.hours,fermenter.minutes,fermenter.seconds);
printf("N2:%s CO2:%s O2:%s Ar:%s\n",fermenter.n2,fermenter.co2\
,fermenter.o2,fermenter.ar);
  save_desired(&fermenter); /* should be 10 or 25 */



                /* tcs data acquisition */

if(the_inlet==25){
   printf("At this point, address tcs\n");
   oldg=read8L(&data8L[0]);
   newspd=500.0;
   co2num=(float)atof(fermenter.co2);
   newg=0.5*oldg*co2num;
   if(newg<=5.0)newg=5.0;

   set8L(newspd, newg);
   printf("We are in main here: oldg=%f, %CO2=%f, newg=%f\n\n", oldg,co2num,newg);
   }
                /* ____________________ */


  records_received+=1;
}

if(records_received<records_to_receive) goto start;
printf("\nEnd of run.");
}



/*-------------------------------------------------------------------------*/
void menu() {
char name[20],series[3],c; int serial_number;
system("cls");
printf("Enter number of records to receive: ");
scanf("%d",&records_to_receive);

strcpy(name,name_of_drive);
strcat(name,name_of_mass_spec);
strcat(name,".num");

if((fp=fopen(name,"r"))==NULL){
printf("Cannot read .num file in menu()\n"); exit(1); }
series[0]=getc(fp); series[1]=getc(fp); series[2]='\0';
fclose(fp);
if( (series[0]==('9') ) && (series[1]==('9')) )
```

```
{series[0]='0'; series[1]='1'; goto label;}
if( series[1]==('9') )
{series[1]='0'; series[0]=series[0]+1; goto label;}
series[1]++;
label:
printf("New serial number: %s",series);
if((fp=fopen(name,"w"))==NULL){
printf("Cannot write .num file in menu()\n"); getch(); exit(1); }
putc(series[0],fp);
putc(series[1],fp);
fclose(fp);
end:
printf("\nNow running...\n\n"); }
/*-------------------------------------------------------------------------*/
void initialise_port3()
{
com3port.h.ah=0;
com3port.x.dx=2;
com3port.h.al=131;
int86(COMINT,&com3port,&com3port);
}
/*-------------------------------------------------------------------------*/
int no_errors()
{
int f;
f=fermenter_id();
return(f);
}
/*-------------------------------------------------------------------------*/
void delay(int f)
{
time_t ltime;
long int initial;
time(&ltime);
initial=ltime;
loop:
time(&ltime);
if( abs(ltime-initial)<f ) goto loop;
}
/*-----------------------------------------------------*/
void receive_a_record()
{
int k=0,i,record_arrived=0;
int s;
char c;
small_loop:
s=_bios_serialcom(_COM_STATUS,2,0);
if( (s&512) ) goto small_loop;
if( !(s&256) ) goto small_loop;
collect_data:
 c=_bios_serialcom(_COM_RECEIVE,2,0);
 if(c=='a') {
 if(record_arrived) k=0;
 record_arrived=1;  }
 if(record_arrived) incoming_data[k]=c;
```

```
 else goto small_loop;
 printf("%c",incoming_data[k]);
if(k>0) {
          if((incoming_data[k-1]==10)&&(incoming_data[k]==13))
          goto data_now_collected;
         }
k=k+1;

small_loop2:
s=_bios_serialcom(_COM_STATUS,2,0);
if( (s&512) ) goto small_loop2;
if( !(s&256) ) goto small_loop2;
goto collect_data;

data_now_collected:
/* printf("%d",k+1); k=83 */
printf("\n");
for(i=k+1;i<200;i++)
incoming_data[i]=0;
}
/*-------------------------------------------------------------------------*/
int fermenter_id()
{
int f,i,sm_colon=0,digit_1=0,digit_0=0;
for(i=0;i<90;i++)
{
/* printf("%c",incoming_data[i]); */
if(incoming_data[i]==';')
sm_colon=sm_colon+1;
if(sm_colon==9) {
digit_1=incoming_data[i+1]-'0';
digit_0=incoming_data[i+2]-'0';
sm_colon+=1; }
}
f=10*digit_1+digit_0;
if( (f>25)||(f<10) ) f=0;
if( (records_received==0)&&(f==10) ) {
printf("Fermenter 10 skip\n"); f=0;}
return(f);
}
/*-------------------------------------------------------------*/
void save_last_ms()
{
int i,f; char name[20];
lst:
f=fermenter_id();
if (f==10) strcpy(name,"a:m_spec10.lst");
if (f==inlet_monitored) strcpy(name,"a:m_spec25.lst");
if((fp=fopen(name,"w"))==NULL) {
printf("Write error in save_last_ms()");  goto  lst; }
printf("opened a:m_spec**.lst\n");
for(i=0;i<84;i++) /* length is 84 */
putc(incoming_data[i],fp);
fclose(fp);
}
```

```
/*-------------------------------------------------------------------------*/
void read_desired(struct fermenter_data *f)
{
struct fermenter_data fermenter;
read_time(&fermenter);
strcpy((*f).hours,fermenter.hours);
strcpy((*f).minutes,fermenter.minutes);
strcpy((*f).seconds,fermenter.seconds);   /* before,clear (*f) */
read_concentrations(&fermenter);
strcpy((*f).n2,fermenter.n2);
strcpy((*f).co2,fermenter.co2);
strcpy((*f).o2,fermenter.o2);
strcpy((*f).ar,fermenter.ar);
read_inlet(&fermenter);
strcpy((*f).inlet,fermenter.inlet);
}

/*-------------------------------------------------------------------------*/

void read_time(struct fermenter_data *frm)
{
int i,sm_colon=0,hours,minutes,seconds;
int d3,d2,d1,d0;
float d4;
char h[3],m[3],s[3];
for(i=0;i<85;i++)
{
  if(incoming_data[i]==';')
  sm_colon+=1;              /* send time as a struct */
  if(sm_colon==8)
  {
    d0=(incoming_data[i-2]-'0');
    d1=10*(incoming_data[i-3]-'0');
    d2=100*(incoming_data[i-4]-'0');
    d3=1000*(incoming_data[i-5]-'0');
    d4=1000*(incoming_data[i-6]-'0'); d4=d4*10;
    d4=(d4+d3+d2+d1+d0);
    hours=(d4/(864.0*100.0) )*24.0;
    d4=( ( d4/(864.0*100.0) )*24.0-hours)*60.0;
    minutes=d4;
    seconds=(d4-minutes)*60.0;
    sm_colon+=1;
  }
}
printf("\n%d:%d:%d\n",hours,minutes,seconds);
time_in_seconds=3600.0*hours+60.0*minutes+seconds-delay_in_seconds;
printf("Time in seconds: %0.4f\n",time_in_seconds);
if (clocks_synchronized==0) synchronize_clocks(hours,minutes,seconds);
else printf("Clocks are synchronized\n");
h[0]=(char) (hours/10)+'0';
h[1]=(char) (hours-(hours/10)*10)+'0';
h[2]='\0';
m[0]=(char) (minutes/10)+'0';
m[1]=(char) (minutes-(minutes/10)*10)+'0';
m[2]='\0';
```

```
s[0]=(char) (seconds/10)+'0';
s[1]=(char) (seconds-(seconds/10)*10)+'0';
s[2]='\0';
strcpy((*frm).hours,h);
strcpy((*frm).minutes,m);
strcpy((*frm).seconds,s);
}
/*-----------------------------------------------------------------*/
void read_concentrations(struct fermenter_data *frm)
{
int i,j,sm_colon=0;
char n2[9],co2[9],o2[9],ar[9];
for(i=0;i<90;i++)
{
  if(incoming_data[i]==';')
  sm_colon+=1;
  if(sm_colon==11)
     {
     for(j=i+1;j<i+9;j++)
     n2[j-(i+1)]=incoming_data[j];
     n2[7]='\0';
     sm_colon+=1;
     }
   if(sm_colon==14)
     {
     for(j=i+1;j<i+9;j++)
     co2[j-(i+1)]=incoming_data[j];
     co2[7]='\0';
     sm_colon+=1;
     }
   if(sm_colon==17)
     {
     for(j=i+1;j<i+9;j++)
     o2[j-(i+1)]=incoming_data[j];
     o2[7]='\0';
     sm_colon+=1;
     }
   if(sm_colon==20)
     {
     for(j=i+1;j<i+9;j++)
     ar[j-(i+1)]=incoming_data[j];
     ar[7]='\0';
     sm_colon+=1;
     }
}
strcpy((*frm).n2,n2);
strcpy((*frm).co2,co2);
strcpy((*frm).o2,o2);
strcpy((*frm).ar,ar);
}
/*-----------------------------------------------------------------*/
int read_inlet(struct fermenter_data *frm)
{
int f;
f=fermenter_id();
```

```
if(f==inlet_monitored) { strcpy((*frm).inlet,"25"); return(0); }
if(f==10) { strcpy((*frm).inlet,"10"); return(0); }
strcpy((*frm).inlet,"**");
}
/*-------------------------------------------------------------*/
void save_desired(struct fermenter_data *f)
{
char series[3],name[20];   int f_id;
f_id=fermenter_id();
ms:
if((fp=fopen("a:m_spec.num","a+"))==NULL){
printf("Cannot open num file in save_desired()"); goto ms; }
series[0]=getc(fp); series[1]=getc(fp); series[2]='\0';
fclose(fp);
strcpy(name,name_of_drive);
strcat(name,name_of_mass_spec);
strcat(name,series);
strcat(name,".ms");
ms1:
if((fp=fopen(name,"a+"))==NULL) {
printf("Read error in save_desired()"); goto ms1; }
printf("opened %s\n",name);
if(f_id==inlet_monitored) putc('\n',fp);

fprintf(fp,"%s:%s:%-4s",(*f).hours,(*f).minutes,(*f).seconds);
/*fputs((*f).hours,fp);
fputs(":",fp);
fputs((*f).minutes,fp);
fputs(":",fp);
fputs((*f).seconds,fp);
*/
if(f_id==10) { fputs(" ",fp);  fputs((*f).n2,fp);}
else { fputs(" ",fp); fputs((*f).n2,fp); }
fputs(" ",fp); fputs((*f).co2,fp);
fputs(" ",fp);  fputs((*f).o2,fp);
/* fputs(" ",fp);  fputs((*f).ar,fp); */
if(f_id==inlet_monitored) fputs("   ",fp);
fclose(fp);
}
/*-----------------------------------------------------------*/

void initialise_structure(struct fermenter_data *frm)
{
int i;
(*frm).inlet[0]='\0'; (*frm).inlet[1]=0; (*frm).inlet[2]=0;
(*frm).n2[0]='\0';
(*frm).co2[0]='\0';
(*frm).o2[0]='\0';
(*frm).ar[0]='\0';
for(i=1;i<9;i++)
  {
  (*frm).n2[i]=0;
  (*frm).co2[i]=0;
  (*frm).o2[i]=0;
  (*frm).ar[i]=0;
```

```
  }
}
/*-------------------------------------------------------*/
void synchronize_clocks(hour,minute,second)
int hour,minute,second; {
struct dostime_t time;
_dos_gettime(&time);
time.hour=hour; time.minute=minute; time.second=second;
printf("Time set to %d:%d:%d\n",time.hour,time.minute,time.second);
_dos_settime(&time);
clocks_synchronized=1;
}
/*-------------------------------------------------------*/


/*-----------------------------------------------------------------*/


void delay2(float wait)                    /* routine introduces a delay of wait */
{                                        /* seconds measured against some     */
                                                 /* characteristic time timefn (secs)  */
   float initial,present;

   initial = present = (float)clock()/CLK_TCK;
   while(present < initial + wait)present=(float)clock()/CLK_TCK;
}


/*-----------------------------------------------------------------*/


char comms(dx,ax)                  /* COMINT indicates the bios interrupt  */
                                       /* for serial port routine. DX (dxval)  */
unsigned int dx,ax;                /* shows which port is to be read, and  */
{                                /* AX (ah & al) status controls action  */
   union REGS com1port;

   com1port.x.dx=dx;
   com1port.x.ax=ax;
   int86(COMINT,&com1port,&com1port);
   return(com1port.h.al);
}

/*-----------------------------------------------------------------*/

int tcscomset()                    /* define port setup variables   */
{
   struct port_set tcs;
   char *string,*stopstring;
   int ul;

   tcs.ah="00000000";
   tcs.baud="110";
   tcs.parity="11";
   tcs.stopbits="0";
```

```
   tcs.databits="10";

/*   strcat(string,strcat(tcs.ah,strcat(tcs.baud,strcat(tcs.parity,
           strcat(tcs.stopbits,tcs.databits)))));
   ul=(int)strtoul(string,&stopstring,2);  */
   ul=218;
   return(ul);
}

/*-------------------------------------------------------------------------*/


float tcstofloat(char *addrtcs)
{                                  /* routine converts TCS to float format */
   static char buf[5],val[6];
   int i,ptpos;

   for(i=0;i<=TCSLENG-1;i++)
   {
      buf[i]=*addrtcs;
      addrtcs+=1;
   }

   if(strchr(buf,'-'))             /* test if number is negative */
   {
      ptpos=((int)strchr(buf,'-')-(int)&buf[0])/sizeof(char);
      for(i=1;i<=5;i++)val[i]=buf[i-1];
      val[0]='-';
      val[ptpos+1]='.';
   }
   else if(strchr(buf,'.'))        /* test if number positive     */
   {
      for(i=1;i<=5;i++)val[i]=buf[i-1];
      val[0]=' ';
   }
   else                       /* if no '.' or '-', variable cannot be phys. quantity */
   {
      val[0]=val[1]=' ';
      for(i=2;i<=5;i++)val[i]='0';
   }
   return((float)atof(val));
}


/*-------------------------------------------------------------------------*/


void floattotcs(char *addrtcs,float setpoint,int tcsplaces)
{
   char *string;                   /* routine converts float to TCS format */
   int i,decimal,sign;

   string=ecvt((double)setpoint,TCSLENG-1,&decimal,&sign);

   for(i=0;i<=TCSLENG-1;i++)
```

```
  {
    if(i<=TCSLENG-tcsplaces-2)
    {
        if(decimal-(TCSLENG-1-tcsplaces)+i<0) *addrtcs='0';
        else *addrtcs=string[decimal-(TCSLENG-1-tcsplaces)+i];
    }

    if(i==TCSLENG-1-tcsplaces)
    {
        if(sign==0) *addrtcs='.';
        else *addrtcs='-';
    }

    if(i>=TCSLENG-tcsplaces) *addrtcs=string[decimal-(TCSLENG-1-tcsplaces)+i-1];

    addrtcs+=1;
  }
}


/*-------------------------------------------------------------------------------------------*/


float read8L(float *addrdata)          /* routine reads channels on TCS   */
                                       /* 8-loop specified   */
                                       /* in include file tcs.h   */
{
  static char prompt8L[]={EOT,ID8L,ID8L,'0','0','A','A',ENQ};
  static char return8L[RECEIV8L],looppv[RECEIV8L-5];
  int iloop,ichar;
  float pv[8];
  FILE *fp_tcs;
  fp_tcs=fopen("tcs.dat","a");


  for(iloop=0; iloop<=VARS8L-1;iloop++)
  {
    prompt8L[3]=prompt8L[4]=tcs8L[iloop].loopID;    /* define tcs prompt by specifying */
    prompt8L[5]=tcs8L[iloop].varsID1;               /* loop and variable options in the */
    prompt8L[6]=tcs8L[iloop].varsID2;               /* string prompt8L[]   */

    for(ichar=0;ichar<=SEND8L-1;ichar++)       /* send prompt8L string   */
    {
        comms(PORTTCS,(256+(int)prompt8L[ichar]));
                                                /*   while(!(inp(PORTBUSY) & 0x20));
   */
                                                /*   outp(PORTTCS,prompt8L[ichar]);    Ian
Holwills method */
    }
                                                                          /              *
for(ichar=0;ichar<=SEND8L-1;ichar++)printf("%c",prompt8L[ichar]); */

    delay2(TCSDELAY);                          /* give TCS time to respond before sending */
                                               /* next instruction                        */
    for(ichar=0;ichar<=RECEIV8L-1;ichar++)
```

```
    {
        return8L[ichar]=comms(PORTTCS,512);
    }

    for(ichar=0;ichar<=RECEIV8L-6;ichar++)looppv[ichar]=return8L[ichar+3];
    pv[iloop]=(float)atof(looppv);

    printf("%f\n", pv[iloop]);

  }

  for(ichar=0;ichar<=7;ichar++)fprintf(fp_tcs,"%9.3f",pv[ichar]);
  fprintf(fp_tcs,"\n");
  fclose(fp_tcs);
  return(pv[4]);
}


/*-------------------------------------------------------------------------*/


void set8L(float dummy, float SLloopC)      /* routine reads channels on TCS   */
                                            /* 32-ch sig.proc. specified   */
                                            /* in include file tcs.h   */
{
  static char loopCstr[]={EOT,ID8L,ID8L,'C','C',STX,'S','L',
                          '0','0','.','0','0',ETX,'0'};
  int ichar;
  char ack;

  floattotcs(&loopCstr[8], SLloopC, 1);
  loopCstr[CHANGE8L-1]=checksum(&loopCstr[6],8);

  for(ichar=0;ichar<=CHANGE8L-1;ichar++)        /* send setpoint string  loop C */
  {
    comms(PORTTCS,(256+(int)loopCstr[ichar]));
  }
  delay2(TCSDELAY);                             /* give TCS time to respond */
  ack=comms(PORTTCS,512);
}

/*-------------------------------------------------------------------------*/

char checksum(char character[],int count)
{
int i,sum;
char check;
/* The checksum is calculated by successively exclusively or-ing the */
/* character codes presented to the function in the form of an array */
sum = (int)character[0];

for (i=1; i<=count-1; i++) sum ^= (int)character[i];
check = toascii(sum); /* this converts the sum into a character type */
return(check);
}
```

A3: PROGRAM WRITTEN FOR MODELLING UNSTEADY-STATE CO_2 TRANSFER

```
/* PROGRAM WRITTEN 9/8/91 BY PATRICK ROYCE - it assumes a data file called */
/* "data.dat" is provided, which contains COLS number of columns of data    */
/* including the CER (rather than CTR), SS, G, P, pH, V against time (h)   */
/* and uses a kinetic model (Royce et al, 1989) to predict how the measured */
/* CTR will be affected by changes in kla, G, pH and CER. The kla is calc   */
/* using a correlation that takes the SS and G data                         */
/* Integration of the differtial equations describing the two state [CO2]   */
/* and [HCO3-] is performed by the rk4 program from Numerical Recipes       */

#include <stdio.h>
#include <math.h>
#include "c:\qc\nr\nr.h"
#include "c:\qc\nr\nrutil.h"

#define N 4                  /* number of states y[1]=[CO2] y[2]=[HCO3-] */
#define COLS 7               /* columns in "data.dat" t,%CER,pH,SS,Q L/min,P,V */
#define TCONS 5              /* number of constant that depend on temp only */
                             /* ie. k1, k2, Kac, HCO2, pw */

char datain[]="datain.dat",dataout[]="dataout.dat";          /* name of data file */

float temp=299.15, fco2air=0.00038, taumix, voidage=0.03;    /* taumix is residence time in */
float rq=0.8875;                                             /* secs in well mixed gas spaces */
float line[2][COLS], rate[TCONS],current[COLS];              /* current contains data interpolated
*/
FILE *fp_in,*fp_out;                                         /* between line[0][COLS] and line[1][COLS]
*/
                                                             /* required by rk4 routine */
void rateconst();
void readline();
void interpol();
void derivs();
void response();


float mtc(float ss, float vs)                /* mass transfer corr */
{
float klo2a;
klo2a=2.89E-7*(float)pow((double)ss,2.4)*(float)pow((double)vs,0.5);
return(klo2a);
}


void derivs(x,y,dydx)                        /* routine calcs derivs of concs of CO2  */
                                             /* and bicarb  */
float x, y[], dydx[];
{
int i;
float klo2a,pgco2,vs;                        /* pgco2 gas phase pCO2  */
```

```
interpol(x);

vs=current[4]*8.314*temp/current[5]/(3.142/4.0*0.25*0.25);
klo2a=mtc(current[3],vs);
printf("%f\n",klo2a);
pgco2=(0.89*klo2a*(current[6]/(1.0-voidage))*y[1]+fco2air*current[4])/
      (current[4]/(current[5]-rate[4])+0.89*klo2a*(current[6]/(1.0-voidage))/rate[3]);

dydx[2]=rate[0]*y[1]-rate[1]/rate[2]*current[2]*y[2];
dydx[1]=(current[1]/100.0*current[4]-0.89*klo2a*(current[6]/(1.0-voidage))*
          (y[1]-pgco2/rate[3]))/current[6]-dydx[2];
dydx[3]=(0.89*klo2a*(current[6]/(1.0-voidage))*(y[1]-pgco2/rate[3])/current[4]*100.0-y[3])/taumix;
  /* effect of cstr on %ctr */
dydx[4]=(current[1]/rq-y[4])/taumix;                /* describes effect of cstr on %otr */
}


main()
{
int i;
float *y,*dydx,*yout,h,klo2a,vs,pgco2,tq,ph;

y=vector(1,N);                  /* state vector at time t       */
dydx=vector(1,N);               /* vector of state derivatives */
fp_in=fopen(datain,"r");       /* name of data file containing G/SS etc */
fp_out=fopen(dataout,"w");

rateconst(temp);
readline(&line[0][0]);


vs=line[0][4]*8.314*temp/line[0][5]/(3.142/4.0*0.25*0.25);
klo2a=mtc(line[0][3],vs);
y[1]=line[0][1]/100.0*line[0][4]/(0.89*klo2a)/(line[0][6]/(1.0-voidage))+
     (line[0][1]/100.0+fco2air)*(line[0][5]-rate[4])/rate[3];   /* initialise */
y[2]=rate[2]*rate[0]/rate[1]*y[1]/line[0][2];                   /* concs mol/m3!! [H+] too and  */
pgco2=(0.89*klo2a*(line[0][6]/(1.0-voidage))*y[1]+fco2air*line[0][4])/
      (line[0][4]/(line[0][5]-rate[4])+0.89*klo2a*(line[0][6]/(1.0-voidage))/rate[3]);
y[3]=0.89*klo2a*(line[0][6]/(1.0-voidage))*(y[1]-pgco2/rate[3])/line[0][4]*100.0;       /* %ctr */
y[4]=line[0][1]/rq;                                                                     /* %otr */
dydx[1]=dydx[2]=dydx[3]=dydx[4]=0.0;

while(!feof(fp_in))
   {
   readline(&line[1][0]);
   h=line[1][0]-line[0][0];
   response();
   rk4(y,dydx,N,line[0][0],h,y,derivs);
   derivs(line[1][0],y,dydx);
   for(i=0;i<=COLS-1;i++)line[0][i]=line[1][i];

   ph=-log10(line[0][2]/1.0E3);
   tq=y[3]/y[4];
   fprintf(fp_out,"%f\t %f\n",ph,tq);
   }
```

```
fclose(fp_in);
fclose(fp_out);
free_vector(y,1,N);
free_vector(dydx,1,COLS);
}


        void rateconst(temp)            /* routine calcs rate constants governing */
                                        /* CO2 hydration, henry                   */
                                        /* constant for CO2, dissoc constant      */
                                        /* for carbonic acid and pwater, using kelv */
        float temp;
        {
        float kco2,kh2co3,kac,hco2,pw,*kinet;

        kinet=rate;

        kco2=exp(759.51-110.54*log(temp)-39755/temp);     /*  /s  */
        *kinet=kco2;
        ++kinet;
        kh2co3=exp(32.943-8855.7/temp);                   /*  /s  */
        *kinet=kh2co3;
        ++kinet;
        kac=(-8.22E-6+5.29E-8*temp-8.0E-11*temp*temp)*kh2co3/kco2*1.0E3; /* mol/m3 */
        *kinet=kac;
        ++kinet;
        hco2=exp(11.25-395.9/(temp-175.9));
        *kinet=hco2;
        ++kinet;
        pw=exp(18.304-3816.4/(temp-46.13))/760.0*1.013E5;
        *kinet=pw;
        }



        void readline(adrline)    /* reads one line in the data file   */
                                  /* and converts to SI units          */
        float *adrline;
        {
        float buffer;

        fscanf(fp_in,"%f",&buffer);
        *adrline=3600.0*buffer;                    /* conv time in h to secs    */
        ++adrline;
        fscanf(fp_in,"%f",adrline);                /* %CER    */
        ++adrline;
        fscanf(fp_in,"%f",&buffer);
        *adrline=exp(-2.303*buffer)*1.0E3;         /* conv ph to [H+] in mol/m3  */
        ++adrline;
        fscanf(fp_in,"%f",adrline);                /* ss in rpm */
        ++adrline;
        fscanf(fp_in,"%f",&buffer);
        *adrline=buffer/60000.0*(1.013E5/8.314/298.0);   /* conv G l/min to mol/s  */
```

```
        ++adrline;
        fscanf(fp_in,"%f",adrline);                 /* press in Pa  */
        ++adrline;
        fscanf(fp_in,"%f",adrline);                 /* vol in m3  */
        }


void interpol(time)                 /* calcs current values of */
                                            /* variables by interpol data pts */
float time;
{
int i;
float timestep, *adrcurrent;

adrcurrent=current;
timestep=(time-line[0][0])/(line[1][0]-line[0][0]);
*adrcurrent=time;
for(i=1;i<=COLS-1;i++)  {++adrcurrent;
                         *adrcurrent=line[0][i]+(line[1][i]-line[0][i])
                         *timestep;
                         }
}


void response()             /* calcs res time in head space */
{
float vvessel=.042;         /* total volume of fermentor */

taumix=(vvessel-line[0][6])/(line[0][4]*.0245);
taumix=66.0;
}
```