BIOTECHNOLOGY '94

Second Conference on Advances in Biochemical Engineering

IChemE
INSTITUTION OF CHEMICAL ENGINEERS

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A three-day symposium organised by the Institution of Chemical Engineers in conjunction with the IChemE — Biochemical Engineering Subject Group, on behalf of the British Coordinating Committee for Biotechnology, and held in Brighton, UK, 4–6 July 1994.

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Please note that the abstracts contained in this volume were not refereed in any form.

Preface

Biochemical engineering is the key discipline in translating exciting advances in the biosciences into profitable production processes. In the more mature areas of biotechnology, where more efficient processes are the key to successful competition, the need for high quality engineering is obvious. However, excellent biochemical engineering is also required for novel, high added-value products, where regulatory concerns dominate the innovation process. In this case, the need is to get the design right at an early stage, which can only be achieved by very good understanding of established and new unit operations, and how they interact and might be controlled. Improvements in understanding can be achieved by both specific research work and from reports on current industrial practice.

The Second Conference on Advances in Biochemical Engineering, held as part of the Second UK Congress of Biotechnology, was a showplace for recent developments in biochemical engineering research and its practice. Most presentations were from the UK, where biochemical engineering is particularly strong, but there was also a welcome leavening of papers from other European countries, and a few from outside Europe. Although the majority of papers were from academic institutions, there were prominent contributions from the industrial sector. This was not an 'ivory tower' conference, and most practising biochemical engineers should find new and useful information and insights in these proceedings.

Colin Thomas

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FERMENTER SCALE-UP CONSIDERATIONS

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Engineering design of large scale production fermenters depends on a clear understanding of microbial requirements and an accurate definition of the operating envelope for efficient bioreaction. Gaining such understanding needs close co-operation across the bioscience-engineering interface and often requires special investigations to develop technical and engineering data at small scale. It is only then that the constants and exponents in engineering scale-up correlations can be evaluated.

The value of experience in scale-up is demonstrated by a careful combination of design techniques, reliable hardware and design margins which allow flexibility of operation to achieve optimum process performance.

Introduction

Bioconversions, in common with many processes, derive economic benefit by operating at large scale. Traditionally, scale-up was attempted by keeping a physical parameter, typically specific power input, oxygen transfer coefficient or mixing time, constant. An additional scale-up tool which has recently been introduced is based on regime analysis whereby a rate limiting envelope is determined by analysis of uptake and generation rates during a fermentation process in order to provide information on how it will react to changing conditions encountered during scale-up[1]. Regime analysis has been given credit as a powerful tool in scale-up of fermentation processes, but physical and microbiological phenomena may change relative to each other in such a way that conventional scale translations may fail.

It is known that metabolic fluxes can be regulated in a matter of seconds and different metabolic activities may not react with the same speed. It is also accepted that scale influences momentum and mass transfer, and by implication kinetics. Consequently, micro-organisms may experience gradients in the chemical and physical environment and the extent of these is scale-dependent. Certainly, concentration gradients inside bioreactors will result from changes in circulation times, producing heterogenous conditions which, in turn, will influence bioreactor performance.

Design Rationale

John Brown has experience in dealing with the uncertainties encountered in fermenter scale-up. The company frequently works with process operating organisations to generate technical and engineering data which allow the design of production fermenters to be undertaken with confidence. In general, investigative programmes commence with a clear understanding of how the bioscience-engineering interface may be overcome. Ideally this is achieved with an expert design workshop attended by fermentation specialists together with acknowledged expert consultants. This critical meeting of researchers, pilot plant development specialists and engineers should lead to the definition of a design rationale or methodology. This may require a series of laboratory scale trials to determine a set of boundary conditions which favour efficient microbiological performance. This may be followed by trials with semi-technical and pilot scale fermenters to confirm operating boundaries and thereby produce accurate process and engineering data. Process information at two scales of operation will allow various scale-up correlations to be evaluated. It will also enable one or more correlations to be chosen as the basis for engineering design of the production fermenters. At this stage, the expert workshop may be reconvened to assess laboratory and

pilot plant data, to consider the relative merits of scale-up correlations, to determine the value of additional investigative work, to evaluate process performance risks and thereby allocate appropriate design contingencies.

Laboratory Scale Investigations

Conventional microbiological strains are more or less tolerant to variations in hydrodynamic pressure, shear, pH, temperature, gas hold-up, nutrient concentrations and biomass concentration. As fermenter scale increases, a cell experiences larger changes to combinations of these conditions as it travels around the vessel. Moreover, residence time distribution in zones which have different combinations of conditions will also change as size and aspect ratio alter.
Transgenic microbiological strains employed for pharmaceutical production are certainly not conventional and they may exhibit severely limited tolerance to variations within a fermenter away from preferred process conditions. Moreover, the prescribed fermentation process may need to be maintained at specific carbon substrate concentration with a critical nutrient limitation and optimum DOT/pH. Very often control of fermenter conditions must be to a predetermined growth curve and with a closely monitored respiratory quotient. These parameters may have been optimised at laboratory scale when they related to the average contents of a research chemostat and relied on instrument sensors at specific locations. It is possible that comparison with semi-technical and pilot plant fermenters may demonstrate substantial differences in performance which may be related to scale. Certainly there will be a larger variation of critical conditions in a pilot fermenter and this will be further amplified in a much larger production unit.

Quantification of macro variations and evaluation of the means to counteract their effect on production performance needs careful measurement and interpretation. If comprehensive information can be made available it might be possible to accurately derive a set of boundary conditions which, in effect, may limit fermenter size. Alternatively, it may be possible to allow considerable relaxation of the control of one variable providing another is strictly controlled.

A series of chemostat trials should be undertaken to determine production sensitivity to the cycling or ramping of values of the following conditions:-

DOT
pH
Temperature
Carbon substrate concentration
Trace element concentrations
Vitamin concentrations
Product concentration
Antifoam concentration
and Shear rate

It may be necessary to conduct a statistically valid number of laboratory fermentation batches for combinations and permutations of fixed and varying values of these measured conditions. It may also be necessary to investigate the dynamic effect of cycling conditions in terms of both frequency and amplitude.

Prediction of the boundary values of fermenter conditions within which the production scale system may be operated can then be attempted. These values must be exceeded during laboratory trials in order to establish confidence in a commercial scale flowsheet and to determine test conditions for trials in semi-technical and pilot scale fermenters.

Pilot Plant Trials

A general purpose pilot plant may need to be modified to include sufficient instrumentation to enable accurate definition of process side mass and heat transfer coefficients, gas hold-up and agitator power consumption. Accurate measurements over a range of conditions are necessary to determine correlations

for transfer coefficients with known values of superficial gas velocity, gas hold-up, agitator tip speed, agitator design and power, broth velocity profiles, over-pressure, antifoam concentration, biomass concentration etc.
A production fermenter design may have to be suitable for exponential and linear growth regimes, for batch, fill and draw, or continuous operation, presterilisation and intermediate operating modes. Demonstration of acceptable process efficiency needs to be established over the expected ranges of process conditions but the scale at which these tests are performed is not always critical. It may be better to carry out a concentrated development programme to generate engineering data on a small highly instrumented test facility (using CMC/PPG mixtures to simulate the range of broth rheological properties) rather than conduct compromised trials in an existing pilot fermenter facility with limited or inaccurate measurements.

The expert design workshop may have addressed problems concerning the definition of operating envelopes for semi-technical and pilot scale fermentation plants. It may also recommend the type of instrumentation necessary to accurately determine process conditions. Certainly demonstration of batch, fill and draw or continuous modes of operation under a set of process conditions which can be directly related to those which will be obtained in the commercial unit must be undertaken. It is also possible that the expert design workshop may make alternative recommendations for investigative work. Moreover, interpretation of the results of laboratory scale or intermediate scale investigations could lead to the conclusion that efficient production can only be achieved in commercial fermenters if major process development is carried out.

Scale-Up Correlations

With hard engineering data derived from measurements at more than one scale of operation, it may be possible to select and modify appropriate scale-up correlations to develop a production fermenter design with a high degree of confidence. This work will include hydrodynamic modelling, process optimisation and design development of fermenter internals.

Having established a productivity requirement, the stoichiometry for a particular bioreaction process will dictate the rate of oxygen demand. Design for oxygen transfer rate (OTR) is determined by the equation;

(1) $OTR = k_L a\,(C^* - C_L)_{log\ mean}$ where

(2) $$(C^* - C_L)_{log\ mean} = \frac{(C_i^* - C_L) - (C_e^* - C_L)}{\ln \frac{(C_i^* - C_L)}{(C_e^* - C_L)}}$$

and $k_L a$ is the oxygen transfer coefficient
C^* is the equilibrium oxygen concentration
C_L is the bulk liquid oxygen concentration
C^*_i is the concentration of oxygen in the liquid film in equilibrium with the inlet gas stream
C^*_e is the concentration of oxygen in the liquid film in equilibrium with the exit gas stream

(3) but $k_L a = A\left(\frac{P_g}{V_L}\right)^m (V_s)^n$ where [2]

P_g is the gassed power
V_L is the ungassed liquid volume
V_S is the superficial gas velocity
A is a characteristic of the fermentation broth
and m and n are constants

For water like broths van t'Riet[2] gives A = 0.026, m = 0.4 and n = 0.5.

However, many fermentation broths include a variety of naturally derived nutrients and contain substantial concentrations of biomass which may release metabolic products and leak proteins. Moreover, to ensure optimum volumetric usage, an antifoam may also be present. All of these species will affect the rheological properties of the broth and, in particular kinematic viscosity, gas bubble size and gas hold-up.

Conventionally gassed power is given by the expression;

$$(4) \qquad P_g = A \frac{(P_o^2 N d^3)^{0.4}}{Q^{0.6}}$$

where A is a characteristic constant (about 1.5)
P_o is the ungassed power
N is the agitator speed
d is the agitator diameter
and Q is the gas flow in the vicinity of the agitator

The exact value of exponents in this equation is affected by broth characteristics.

Ungassed power for each agitator element is given by

$$(5) \qquad P_o = N_p \rho N^3 d^5$$

where N_p is the power number for the style of impeller (5.0 for a Rushton turbine and as low as 0.3 for axial impellers[3]) and ρ is the bulk density of the broth.

Unfortunately, there is often a large difference between calculated and measured gassed power. This is usually due to broth rheology being substantially different to water. Moreover, broth characteristics can change during the course of a fermentation so that non-Newtonian behaviour becomes apparent. Under these circumstances, it is necessary to carry out carefully controlled fermentations at two or more scales of operation - for example semi-technical and pilot plant equipment. The aim is to elucidate the relationship between gas hold-up (H_G) and operational variables in the form;

$$(6) \qquad H_G = f(V_S, N, t)$$

where V_S is superficial gas velocity
N is agitator speed
and t is fermentation operating time

It should then be possible to derive values of constants and exponents in the Equation (4) for P_g, gassed power so that the measured power dissipation and the calculated value coincide.

The modified expression for P_g should certainly take into account gas hold-up and a scale factor, but it should also allow for the particular speed versus power curve of the selected type of agitator element. The conventional design approach to medium scale fermenters is to include a radial impeller above a sparge ring for mass transfer and one or more axial impellers at high elevations for mixing. As fermenter scale increases and the aspect ratio changes, more impellers may be added and each of them will make a contribution to mass transfer and mixing.

Regime analysis work to determine the allowable operating envelope within a large fermenter depends on the estimation of mixing time[1]. This is usually evaluated with the expression;

$$(7) \qquad \Theta_c = \frac{V}{F_l N d^3}$$

where Θ_c is the circulation time
V is the mixed volume (relating to a particular agitator element)

F_l is the flow number of the agitator element.
N is the agitator speed
and d is the impeller diameter

It is then assumed that the mixing time to within 5% of homogeneity[4],[5] is 5 x Θ_c.

Measurement of product and biomass concentrations and exhaust gas analysis should allow fermentation performance to be evaluated. This permits the quantity of oxygen used to be calculated and thence the rate of oxygen mass transfer. Careful comparison of results from two sizes of fermenter with different combinations of gas rates and agitator speeds for a chosen set of values of gassed power should allow exponent m in Equation (3) to be derived. A similar exercise varying agitator power, gas rate and overpressure for chosen values of superficial gas velocity should result in a value for n.

Having achieved a degree of confidence concerning scale-up for oxygen mass transfer, it is possible to postulate a notional fermenter design. Previous experience may indicate a particular configuration for the chosen working volume of broth with a preferred L:D aspect ratio and a particular combination of agitator elements. It is then necessary to assess the effectiveness of this design for heat transfer. An energy balance based on fermentation stoichiometry plus gassed power minus evaporative cooling will give the thermal requirement. Careful heat balances on two sizes of fermenters should enable the designer to evaluate overall heat transfer coefficients during the course of fermentation operations. Heat transfer depends on the liquid film coefficient, the velocity of broth over cooling surfaces and bulk mixing flow patterns. Again variations in gas hold-up and broth rheology can profoundly influence heat transfer. Attenuation of velocity from around the impeller to the vicinity of cooling surfaces depends on power dissipation by the baffles. This in turn is related to fermenter L:D aspect ratio, the number of impellers and the interrelationships between the design of baffles and internal cooling surfaces. A general correlation for heat transfer has the form[6],[7]

(8) $$Nu = B\ Re^{0.67}\ Pr^{0.33}\ (\mu_B/\mu_w)^{0.14}$$

where Nu is the Nusselt number
Re is the Reynolds number
Pr is the Prandtl number
μ_B is the bulk viscosity of the broth
μ_w is the viscosity at the wall
and B is a characteristic of the broth

It is a common failing to assume that the broth will exhibit the same physical properties at all scales of operation and also to assume that h, the heat transfer coefficient, is constant throughout the fermenter. This leads to the approximation

(9) $$Nu \propto Re^{0.67}$$ and therefore

(10) $$h \propto (d^2/N)^{0.67}/D$$

In fact, gas hold-up and broth rheology change during fermentations and, depending on the arrangement of agitator elements flow conditions are very different between the base and at various elevations of the wall and baffles. Moreover, for a particular geometry, scale-up has a profound effect on the ratio of volume (proportional to D^3) and available heat transfer area (proportional to D^2). In fact, as fermenter size increases, it is heat transfer which replaces mass transfer as the rate limiting parameter. To some extent this can be offset by changing aspect ratio and including internal cooling coils although somewhat diminished because broth film heat transfer coefficient is proportional to local broth velocity and this is attenuated as fermenter diameter is increased.
Scale-up for fermentation processes in commercial units exceeding 200 m^3 volume requires the conservative approach which John Brown has so far adopted. Even below this limit there may be process constraints which indicate scale-up should be approached with extreme caution. For example, accurate process control to a predetermined combination of respiratory quotient and exponential growth curve may

require a design with high mixing efficiency and low circulation time. This may preclude a very large fermenter if a limit to agitator tip speed (microbial shear) is imposed. The expert design workshop may be reconvened when laboratory and pilot plant operations have provided sufficient data to allow optimum decisions to be made concerning the selection of appropriate scale-up correlations.

Detailed Engineering

Most fermentations are operated under mono-septic conditions and many require a high degree of sterile integrity and primary containment. This imposes a complex set of design requirements on the mechanical design and fabrication techniques for fermenters and their associated equipment. As scale increases, the requirements for higher dimensional tolerances to ensure adequate seals become more acute and place exacting demands on the designer.

Mono-septic operation is usually ensured by high temperature presterilisation and a strict regime of process sequences which at least are monitored by computer. The requirement for total self draining and venting to prevent low temperature liquid pools and gas pockets means that no horizontal surfaces are permitted. This a stringent requirement in a very large vessel and it is compounded by the necessity to design for high thermal stresses associated with repeated pre-sterilisation cycles.

Conventionally the agitator shaft enters the top of large fermenters through a double mechanical seal with an intermediate sterilising steam chest. This seal should not take substantial static or dynamic loads and should also be of the cartridge type for effective maintenance. The inclusion of a drive mechanism with an inverter type variable speed motor together with high performance external taper bearings and internal steady bearings in a sterile vessel to take static and dynamic loads pose further detailed design complications. Suitably stiff supports for a drive system approaching one megawatt of power which is welded to a sterile and therefore cyclically heated system places high thermal and mechanical loads on the vessel walls.

Careful flow modelling is necessary in order to carry out extensive vibration analysis on the agitator shaft and on internal cooling coils and on baffles which must dissipate drive power through their (sterile design) supports. As scale increases, the thickness of vessel walls and of supporting structures increases. This results in substantial heat sinks during presterilisation and may require dynamic thermal analysis and heating coil compensation.

Most engineering projects involve some aspect of risk management with regard to the design and engineering of very large fermenters, the pragmatic approach is to allow for flexibility of process operation. This may be achieved by including sensible design margins and the opportunity for physical and operational adjustments. Typical safeguards would be to include margins for vessel height (for foam) heat transfer area, agitator motor power air flowrate and overpressure. Small adjustments to agitator speed, elevation of impeller elements and effective diameter of impeller blades allow considerable scope for process optimisation.

Although such design margins and adjustments increase costs, they do allow some relaxation from exact conformance to design requirements where every parameter is closely matched to an optimum solution. Pragmatism is advisable when embarking on the extrapolation of scale-up correlations to regimes outside previous experience. There is always a real chance that when design margins are practically evaluated they can be exploited to achieve improved plant performance.

The economy of scale favours increasing fermenter size to the limit of acceptable technical risk and practical engineering. This engineering limit is presently in the order of $1000m^3$. However, microbial requirements, process limitations and operational constraints currently limit fermenter sizes to about $500m^3$. Consequently, there is still the opportunity for considerable technical development to realise the full potential of fermenter scale-up.

Conclusion

Fermenter design uncertainties often relate to the underlying physiology of a chosen microbiological strain when subjected to large scale operating regimes and to the accuracy of process and engineering data which is currently available. Intuitive judgement is often employed to "design" large scale production fermenters but this can be fraught with uncertainty. There is a powerful argument in favour of arranging operating tests over a range of scales. However, the foregoing arguments suggest that it is critically necessary to generate high quality engineering data in order to reduce design risks. Otherwise, intermediate scale trials may be a hit or miss affair in terms of developing an optimum engineering design. A conventional approach concentrates on the quality and accuracy of technical data in order to provide a sound engineering foundation. It is then possible to quantify risks and incorporate suitable design margins in order to ensure installed equipment meets the desired performance criteria. The basic message of this paper implies that, when a design engineer is given the task of fermenter scale-up, he should firstly concentrate on small scale engineering correlations. Taking this argument to the extreme is the realisation that the ultimate fermenter is a microbial cell, which, when subjected to particular conditions and operations, achieves an acceptable process performance. The basic design requirement for a fermenter is to ensure that optimum conditions are provided for a major proportion of the biomass which it contains.

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PERIODIC FEEDING OF A CONTINUOUS BIOREACTOR

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A stirred tank bioreactor was converted to run as a continuous fermenter. This rig was used to grow *Schizosaccharomyces pombe* on a complex medium supplemented with glucose. Once steady state had been established the feed line was connected to two feed vessels via a solenoid valve. One vessel contained medium with no glucose, while the other contained medium supplemented with glucose at twice the concentration fed to obtain the steady state. The valve was arranged so that it would switch between each vessel at regular intervals, thus imposing a square wave perturbation in nutrient concentration on the system. Furthermore, the overall feed rate of glucose to the fermenter would be the same as that used to achieve steady state. The system exhibited a constant periodic response to the square wave perturbation. This response was broadly sinusoidal in nature and had a period equal to that of the imposed perturbation. Preliminary analysis indicate that the average biomass concentration may be enhanced by up to 11% by this propagation method. These results broadly agree with those obtained from the simulations conducted on **CELCYMUS**. Further studies will establish the reproducibility of this phenomenon.

INTRODUCTION

Studies into the periodic feeding of continuous bioreactors has been limited[1]. The effect of both sinusoidal and square-wave perturbations in dilution rate have been investigated for *Saccharomyces cerevisiae*[2,3]. Square-wave variations in the feed nutrient concentration for cultures of *Escherichia coli* has also been studied[4-6]. These studies did suggest that improvements in productivity and overall performance were possible[5], however the complex responses and the inability of conventional kinetics to describe the results has hindered there further development.

Cell cycle studies in the Department of Chemical and Process Engineering at the University of Surrey have yielded a generic modelling tool, **CELCYMUS**[1] (Cell Cycle Model, University of Surrey). This model has been developed from studies conducted on eukaryotic cells.

This model is capable of simulating a continuous bioreactor subjected to periodic perturbations in the feed-stream. These simulations suggest that these perturbations enhance biomass and product yields by up to 20% and also suggest a possible reason for this increase. It was decided to conduct a short experimental study to confirm these theoretical findings using the yeast *Schizosaccharomyces pombe*; also a eukaryotic organism.

MATERIALS & METHODS

The *Schizosaccharomyces pombe* used in these experiments was obtained from the National Collection of Yeast Cultures. The catalogue number is 132, which corresponds to the Lindner variant; the yeast was isolated from African millet beer.

The growth medium used in all experiments was a peptone/yeast extract base supplemented with glucose and sodium chloride. For conventional continuous operation (as a chemostat) the feed concentration had 5% glucose. For periodically perturbed operation two feeds were used, one had no glucose and the other had 10% glucose.

An LH bioreactor (model CC1500) with a vessel of 2 litres working volume was adapted for continuous operation by the addition of a weir. 20dm^3 Nalgene feed vessels were used for all feedstock. These vessels were convereted to allow a tube to pass aseptically through the lid to the bottom of the vessel and to allow the attachment of a 0.2um bacterial PTFE air filter.

For conventional continuous operation the feed was delivered from the feed vessel via silicone tubing using a Watson Marlow 501Q peristaltic pump. For periodically perturbated operation two feed vessels were used. These were