Application of Computational Fluid Dynamics to Micro-titre Plate Scale Bioreactors

A thesis submitted to University of London for degree of Doctor of Philosophy

by

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"Commit your blunders on a small scale
and make your profits on a large scale”

--------H. Baekeland, the inventor of Bakelite, 1916
Abstract

The manufacture of a new drug requires process development at the early stage in the development cycle of the drug to reduce cost and time. Current approaches based on laboratory and pilot-plant operations require large amount of materials which may not be available. A new bioprocess development method based on a miniature bioreactor is proposed. The engineering parameters of the miniature bioreactor are obtained in this study with the aid of computational fluid dynamics (CFD) and the results are compared with data obtained for other small scale systems including micro-titre plates and shake flasks.

A new miniature bioreactor with a diameter equal to that of a single well of a 24-well plate is described and its engineering performance as a fermenter assessed. Mixing in the miniature bioreactor is provided by a set of three impellers mechanically driven via a micro-fabricated electric motor and aeration is achieved with a single tube sparger. Parameter sensitive fluorophors are used with fibre optic probes for continuous monitoring of dissolved oxygen tension and an optical based method is employed to monitor cell biomass concentration during fermentation.

The local gas and liquid velocity, gas volume fraction and energy dissipation rate are derived from an analysis of the multiphase flow in the miniature bioreactor using CFD. The predictions are compared to experimental observations from the literature. Volumetric mass transfer coefficients are predicted using Higbie's penetration model with the contact time obtained from the CFD simulations of flow in the bioreactor. Comparative measurements are provided from parallel experiments carried out in a 20L (15L working volume) conventional fermenter. Measured volumetric mass transfer coefficients are in good agreement in the miniature bioreactor and 20L bioreactor although the CFD simulation data for the miniature bioreactor are underpredicted. The values of $k_{La}$ from experiments and simulations in the miniature bioreactor are in the range 100 hr$^{-1}$ to 400 hr$^{-1}$, typical of those reported for large-scale fermentation.

Mixing in the shake flask is analysed with the aid of CFD. The gas-liquid interface changes are mapped as the shake flask moves in a rotary shaking platform. Local liquid velocity and energy dissipation rate are obtained. The average power input is obtained
by integrating the local energy dissipation rate over the entire working volume and results are compared to experimental measurements from literature. It was shown that power input is more sensitive to shaking diameter than to the shaking frequency, providing new insight into the optimisation of shake flask operations. The volumetric mass transfer coefficient obtained from Higbie's penetration model is in the range of the experimental results (30~100 hr\(^{-1}\)).

The shaken micro-titre plates play an important role in the drug discovery process and have the potential to provide information for process design and development. CFD analysis of flow is made in a 24-well and a 96-well reactor. The predicted flow patterns in the 24 well are very different to those in the 96-well reactor operating under the same shaking conditions. Flow patterns, average power input and volumetric mass transfer coefficients, obtained from CFD, are much more sensitive to changes in shaking diameter rather than shaking frequency. Based on equal power consumption, the volumetric mass transfer rate in the microwell reactor is higher than that in the shake flask.

Analysis of the results and comparison with data obtained from the laboratory scale operation indicate that while the mass transfer in different small scale systems (miniature bioreactor, micro-titre plate and shake flask) may be correlated through the use of the concept of equal energy dissipation per unit volume, the fermentation and cell culture growth in these systems are more difficult to relate to each other. In conclusion, based on the results the miniature bioreactor offers the best option for scale-up to laboratory and pilot-scale because of similarity in engineering properties of the two systems.
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<td>2D</td>
<td>Two dimension</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimension</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge coupled device</td>
</tr>
<tr>
<td>CFD</td>
<td>Computational fluid dynamics</td>
</tr>
<tr>
<td>HTS</td>
<td>High throughput screening</td>
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<tr>
<td>IBC</td>
<td>Imposed boundary conditions</td>
</tr>
<tr>
<td>IO</td>
<td>Inner-outer technique</td>
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<tr>
<td>LDA</td>
<td>Laser-doppler anemometer</td>
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<tr>
<td>LED</td>
<td>Light emitting diode</td>
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<tr>
<td>MRF</td>
<td>Multiple frames of reference technique</td>
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<tr>
<td>MUSIG</td>
<td>Multiple size group</td>
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<tr>
<td>OTR</td>
<td>Oxygen transfer rate</td>
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<tr>
<td>PIV</td>
<td>Particle image velocimetry</td>
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<tr>
<td>SG</td>
<td>Sliding grid technique</td>
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<tr>
<td>SP</td>
<td>Snapshot technique</td>
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<tr>
<td>SS</td>
<td>Source-sink technique</td>
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<td>VOF</td>
<td>Volume of fluid</td>
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Chapter 1 Introduction

The realisation that there are approximately 30,000 genes in the human genome has shifted drug discovery research significantly towards proteomics and away from genomics. Currently, the available drugs on the market target nearly 500 of the estimated hundreds of thousands of human proteins, with the expectation that this number will increase by a factor of 10 to 100 in the next few years. A major challenge for drug discovery now is to elucidate the relationship between proteins produced by each gene and disease. In this respect, advances in proteomics and automated high-throughput screening based on the shaken microwell plate system have provided the technology platform for a significant increase in the number of potential drug candidates that are likely to come forward. A related challenge that is yet to be addressed is the need to define the conditions for the translation of results from the microwell system to conventional laboratory scale. For example, in the case of \textit{E. coli} fermentation, while a number of discovery companies now routinely run fermentation in a 24-well plate to produce small quantities of proteins for crystallographic studies, scale-up to laboratory fermentation, typically up to 10L, is proving difficult to achieve.

The ability to scale-up such information and the capacity to generate process data from unit operations carried out at the microwell scale have become important issues in the development pathway of a new drug. This forms the basis of this thesis which is a study on microwell scale bioprocessing. The following section examines the role of process development within a new drug development cycle. The challenges of the current process design and development is described in section 1.2. Section 1.3 introduces some micro-scale bioreactors and in section 1.4 a new development method is proposed for new process development. The success of the new approach requires an understanding of the engineering flow environment in these reactors. It is shown that this may be achieved with the aid of Computational Fluid Dynamics (CFD), which are discussed in section 1.5. The last section provides the scope of the thesis.

1.1 Drug discovery process challenges new bioprocess development and design

The development of a new drug follows the overall stages of discovery, preclinical development, clinical trials and approval. Process development plays an important role
in the development cycle of a new drug.

1.1.1 Process development within the production development cycle

The overall drug development cycle in the biopharmaceutical industry is outlined in Fig.1-1. Drug discovery forms the beginning of the development cycle. Advances in genomics, proteomics, bioinformatics, and metabolomics combined high throughput screening techniques have greatly accelerated the discovery process, resulting in promising leads. However, only about 20 out of every 10,000 drug candidates warrant further investigation (Jackson, 1996). If the target genes and the mechanism for treatment of the disease have been identified through small in-vitro or in-vivo experiments, methods for expressing the protein need to be developed, initially for clinical trials and subsequently for large-scale use. Usually the target genes are cloned into various host cells such as *E. coli*, yeast, CHO, BHK etc. Then a huge amount of different culture conditions has to be investigated to obtain the factors which play a crucial role in turning these genes on. The culture conditions have to enable the production of the drug candidates repeatedly and reproducibly.

![Fig.1-1 overall drug development cycle and process development in the biopharmaceutical industry.](image-url)
If a drug candidate is worthwhile for further exploration, it is transferred into preclinical development. The main activities at this stage are the preliminary evaluation of toxicity in animal models, an assessment of the pharmacological and pharmokinetic properties of the compound and studies of potential delivery routes. Usually process development starts at this stage, the critical factors in the high throughput screening platform have to be repeated, optimised and scaled up to a laboratory scale. Enough quantities of the drug candidates have to be provided to support the above main activities.

Clinical trials proceed through three phases. Side effects, dosage and efficacy of the drug candidate are evaluated and hundreds or thousands of patients are involved over a period of time which may be as long as several years. Large quantities of material needed to satisfy the demands of clinical trials require significant processing capacities. Usually, during phase II, a process needs to be established for production at full manufacturing scale and process data are generated for submission to the regulatory authorities. Following successful completion of Phase III clinical trials, the accumulated data both on clinical effects and the validation of the manufacturing process is submitted to the regulatory authorities for approval.

1.1.2 New challenges in process development in biopharmaceutical industry

At the stage of drug discovery, little attention is paid to process-related issues as small quantities of the drug candidate is needed for initial evaluation. But at preclinical development, the pressure on process development can become intense since enough quantities of material have to be supplied for preclinical research in a very short time. However, conventional bioprocess development from shake flasks to laboratory scale bioreactors is very complicated and currently this poses a bottleneck in process. Details are discussed in the section 1.2.

There is a contradiction for process development at the clinical trial stage. While the drug candidate stands a very high risk of failure due to unexpected medical or pharmacological behaviour, a process design for manufacturing scale does not start until the completion of Phase I or II of clinical trials. However, the regulatory authorities such as the Food and Drug Administration (FDA) require all drugs manufactured for use in humans follow the published Good Manufacturing Practice (GMP) guidelines (21 CFR 21). The process used to produce clinical trial materials set the final manufacturing process because the manufacturer usually does not want to go back into clinical trials to
re-validate a manufacturing process different from that for the production of clinical trial materials, which may cause long delays in time-to-market. This means a full manufacturing process has to be developed before the start of the clinical trials. As current biopharmaceutical process development is based on empirical rules, the process development is becoming a major bottleneck.

There are two key issues that need to be addressed: How to obtain process information at a small scale with a high throughput capacity and how to scale up such information and put the products into market in a short time. The conventional approaches to process design are presented in the following section.

1.2 Conventional bioprocess development

Development of a new bioprocess for a drug candidate from a laboratory scale to a production scale is usually carried out in four steps as shown in Fig.1-2: (1) shake flask for parallel studies of cell growth culture, media screening and cell expansion in the screening stage; (2) bench scale for screening suitable operation conditions in a laboratory fermenter whose geometry is similar to plant scale; (3) pilot scale where operational conditions are checked; (4) plant scale where the process is bought to an economically favourable level. This development is often complicated, costly and time-consuming. Especially long delays occur from step one to step two because all parameters in shaking flasks have to be reestablished in a laboratory bioreactors.

![Fig.1-2 Conventional fermentation process development](image)
1.2.1 Shake flask culture

High throughput screening using the shaken micro-titer plates, and new emerging technologies based, for example, on miniature bioreactors and parallel processing (Lamping et al., 2003; Kostov et al., 2001) have the potential to provide additional process information rapidly from milliliter quantities of material, but these techniques are in their infancy, as far as process operation is concerned and it will be many years before such systems become available on the market. Currently, shake flasks offer the best alternative approach. They have the advantage of being readily available for different sizes at low cost. Shake flask fermentations have been used routinely for nearly five decades for laboratory scale fermentation and cell growth culture experiments, media and strain screening and cell expansion, as well as studies on biotransformation and metabolic pathways. Shake flask fermentation requires relatively small amounts of material, typically between 50 mL and 500 mL. They are sealed with closures like cotton plugs, foam pads, silicone sponge plugs, loose-fitting stainless steel, or plastic closures. These closures prevent a convective gas exchange between the interior of the shaking flasks and the environment, and thus contamination after the sterilization. Hundreds of shaking flasks in a temperature-controlled incubator can be fixed on a horizontal platform, which is orbitally moved with an apparatus-specific eccentricity (2.5-5 cm) at rotation rates of up to more than 300 min⁻¹ to induce bulk mixing of the broth and to promote oxygen dissolution by surface aeration. Oxygen transfer, cell growth and product yield in the shake flask are determined by bulk mixing. The energy input through the vessel wall during this rotational movement serves for mixing the reactants as well as for heat and mass transfer.

In order to improve mixing, cell growth and oxygen transfer, design improvements to the shake flask have been made during the recent years. For example, inserts are used including one or more baffles from the lip of the flask (McDaniel and Bailey, 1969; Tunac, 1989). Bull et al. (1998) proposed controlling the motion of the platform in the orthogonal directions as well as the use of combination of motion of the platform. And the Erlenmeyer flask is replaced by a cylindrical or a square reaction vessel provided with a thin sterile membrane at the top for gas exchange with the environment across the entire circumference (Kato and Tanaka, 1998). Due to the larger exchange area and shorter diffusion path compared to the conventional shake flask, the gas exchange is much improved. Because of the motion of these vessels, convective mass transfer
through the thin sterile membrane is achieved. Recently, “instrumented” shake flasks have become available, allowing limited process conditions, e.g. pH and temperature, to be established (Kato et al., 1999; Buchs, 2001; Maier and Buchs, 2001; Rhodes and Gaden, 1957).

Shake flask fermentation practice, however, has remained essentially unchanged for nearly 50 years. Shake-flask methods are laborious and monitoring of cell growth and product yields is still not possible and the conditions of nutrient and oxygen transfer are poorly defined.

Great efforts have been made to relate process conditions in shaking flasks to the laboratory stirred reactor. The important engineering differences between the simple shake flask and a stirred tank are the low ratio of maximum local energy dissipation in the shaking flask to the averaged power input, a lack of control of important process parameters such as pH and pO₂ in the shaking flask, and low volumetric oxygen transfer coefficient KLa due to surface aeration (Henzler and Schedel, 1991). Work is in progress to establish mixing and oxygen transfer rates in shake flask operations and develop relationships between these parameters and cell growth and product yields (Weuster-Botz et al., 2001). Anderlei et al. (2000) published a new method considering the rate of removing volatile compounds out of fermentation broth as an additional scale up criterion from shaking flasks to stirred tanks. Buchs et al. (2000a and b) presented empirical equations to calculate the specific power consumption for different flasks. However, a recent survey (Buchs, 2001) concluded that while nearly 90% of all laboratory cell culture and fermentation experiments are performed in shake flasks only less than 2% of all the relevant publications in the area dealt with engineering aspects of shake flask operation. For example, it is recognized that oxygen transfer rate in shake flasks is controlled by sharp changes of the gas-liquid interface, however basic hydrodynamics information representing conditions at the interface is still unavailable and only limited measurements have been reported on the volumetric mass transfer coefficient and interfacial area (Maier and Buchs, 2001; van Suijdam et al., 1978; Henzler and Schedel, 1991). Equally important is the effect of power input on volumetric mass transfer in the shake flask, but few reliable techniques exist for the determination of power input (Buchs et al., 2000a, b; Sumino et al., 1972). Measurements of the power input to the motor contains mechanical and electrical losses which are significantly higher than the power dissipated in the liquid, making reliable
measurements difficult to carry out. As a result, it is seldom possible to compare mixing and oxygen transfer rate data, and fermentation and cell culture performance, obtained from the shake flask to those from a stirred tank bioreactor. No direct scale-up relationships have been established between the two scales, necessitating significant additional time-consuming experimental work in laboratory scale bioreactors. The information about full-scale process operation and scale-up is currently obtained through additional experiments using laboratory scale reactors, typically 2 to 10 litre in volume, and geometrically similar pilot-scale fermentors. Manpower and facility space requirements are high and, progress is slow and the "time-to-market" is delayed because of the large number of such fermentation runs that are needed.

1.2.2 Fermentation process from bench to production scale

The basic concept of scale up is that the optimum physiological conditions that are obtained in the small scale studies should be maintained on the large scale by controlling engineering environmental conditions (Humphrey, 1998). But the physiological state of cells and its relation to growth and product formation is the result of a complex interaction between the engineering environment and the cellular reactions (metabolic pathways). Engineering environment in the fermentation process may be described at different scales, but the cellular reactions are far more difficult to define.

Some empirical rules of scale up from laboratory scale to pilot and production scales have been put forward and met with success for specific cases. For example, for a stirred bioreactor, constant ratio of power to liquid volume, constant mass transfer coefficient and constant impeller tip speed, and equal mixing time have been used. Table 1.1 shows that the most common scale up rules in fermentation industry.

<table>
<thead>
<tr>
<th>Scale up criteria</th>
<th>Percentage of industries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant P/V</td>
<td>30</td>
</tr>
<tr>
<td>Constant k_L,a</td>
<td>30</td>
</tr>
<tr>
<td>Constant tip speed</td>
<td>20</td>
</tr>
<tr>
<td>Constant partial pressure of O_2</td>
<td>20</td>
</tr>
</tbody>
</table>
Shear stress is believed to affect the performance at a large scale and constant shear stress is also used as a criterion. Under conditions of turbulent flow, the shear stress is proportional to \((ND)^2\), which results in a constant value of \(ND\) as a scale up criterion. But the effect of turbulent shear stress on cell growth and its metabolic activities is also dependent on the exposure time to the shear stress. One should expect \((ND)^{N}\) to be important at different scales, this has the same effect as keeping constant \(P/V\).

Scaling-up of aerobic bioprocesses is often based on the rule of identical oxygen transfer rate or volumetric mass transfer coefficient \((k_{L}a)\) because in the aerobic culture of microorganisms, which have a high demand for dissolved oxygen concentration, the rate-limiting step is the mass transfer at the gas-liquid interface. This criterion has been used for production of penicillin and streptomycin (Karow et al., 1953). The working volume varied from 0.005 m\(^3\) to 57 m\(^3\), covering four orders of magnitude. The product yield was maximal and remained nearly constant when \(K_{aw}*P\) (The oxygen transfer rate =\(K_{aw}*P*(y*-y)\), \(y\) is the molar ratio of oxygen in the air, \(P\) is the total pressure) reached 0.5 mol/(L.hr). This corresponds to a maximum \(k_{L}a\) of 0.1 s\(^{-1}\) and is within the average value of 0.07–0.1 s\(^{-1}\) normally observed in typical fermentation process (Perry and Green, 1998). The successful translation from the laboratory to a production scale by Karow et al. (1953) was also obtained by Bylinkina et al. (1976) for the fermentation of penicillin and streptomycin. Four fermentors of 0.015 m\(^3\), 0.1 m\(^3\), 3m\(^3\) and 63m\(^3\) were used and the scale-up was based on the volumetric mass transfer coefficient at the gas-liquid interface.

But Bartholomew (1960) found that in the bacterial production of Vitamin B12, the yield reduced as oxygen transfer rate increased. And this effect was found to be dependent on the fermentor size, so that the oxygen transfer rate could not be used for scale up. Keeping constant \(k_{L}a\) can also lead to a better performance than expected (Taguchi, et al., 1968). The gluco-amylase production yield (relative enzymatic activities) was measured in fermentors of 0.06m\(^3\), 3m\(^3\), and 30m\(^3\) and it was found that the yield at a large scale was higher than at a small scale based on the same \(k_{L}a\) value. But Takei et al. (1975) observed a worse performance than expected for protease production by Streptomyces sp. from 0.03 m\(^3\) to a 0.2 m\(^3\) using constant \(k_{L}a\) as a scale-up criterion.

One third of the scale-up translations performed in the fermentation industry employ the
scale up criteria of constant specific power consumption. The rule of equal specific power input was first proposed by Zwietering (1958). He suggested that the power requirement decreased with the increasing dimension of the bioreactor because the ratio between its surface to volume decreased. The main reason for its industrial success probably is the fact that all hydrodynamics and mass transfer rate are correlated with the dissipated energy. Experience indicates that scale-up on this basis overestimates the size of the motor (speed of agitation required) leading to a safe design.

In the fermentation process of penicillin, the constant specific power input has been used as a criterion for scale-up. Gaden (1961) described the production concentration as a function of $P_g/V$ and the value of 1.5-3.0 kW/m$^3$ was used for scale-up. Humphrey (1964) carried out scale-up at a $P_g/V$ of 1-2.5 kW/m$^3$ for penicillin production and $P_g/V$ of 1.5 kW/m$^3$ for scale-up of streptomycin production.

But scale up based on the constant power consumption does not take into account the gas sparging. Assuming the aeration rate is kept constant as 1.0 vvm (volume of air/volume of liquid/minute) for most fermentation processes, the superficial gas velocity varies from 0.6 cm/s to 13.3 cm/s corresponding to vessels of 10L to 100 m$^3$ with an aspect ratio of twice the liquid depth to vessel diameter. But impeller flooding occurs when the superficial air velocity approaches 25-50% of the bubble rise velocity, which is averaged to around 22 cm/s. Increasing power input can alleviate the problem, but the more economic and practical means is reducing the aeration rate. Wegrich and Shurter (1953) kept the same power consumption and the same gas superficial velocity (not the same aeration rate) and successfully scaled up a penicillin production process from 8 m$^3$ to 100 m$^3$ fermentors. According to the Van’Riet’s correlation (1979), maintaining constant $P_g/V$ in non-Newtonian fluids leads to the same volumetric mass transfer coefficients.

However, bioreactors typically operate well only in a very narrow operating window of temperature, pH, and biomass concentration. Therefore, simple scale-up rules should be used with great caution. Typical scale-up problems result from insufficient interphase mass transfer or inadequate removal of heat from the reactor, or from non-uniform temperature and concentration profiles in the reactor. The reduction of mixing efficiency and mass transfer capacity during the scale-up of bioreactors can result in cells at production scale experiencing fluctuating environments of substrate, oxygen, pH
and nutrient concentration (Lily, 1983). This in turn can affect biomass yields and productivity (Sweere et al., 1988). Such concentration fluctuations may influence the physiology of microorganisms, leading to strain degradation and to a decrease of process yield (Bylund et al., 1998). The multiparameter flow cytometric techniques developed by Hewitt et al. (2003) were used in fed-batch fermentation process at two scales, production (20,000 L) and bench (5L). The technique demonstrated that a changing microenvironment with respect to substrate concentration (glucose and dissolved oxygen tension) has a profound effect on cell physiology and hence on viable biomass yield. A low biomass yield but a high cell viability (with respect to cytoplasmic membrane permeability) throughout the fermentation process was found in the production scale to be associated with poor mixing conditions. While a high biomass yield with the lowest cell viability was found in a well mixed bench bioreactor. The work challenged the assumptions that the physiological state of a cell population is independent of the scale of cultivation. New and reliable prediction of biomass yield and production synthesis on scale-up is required.

The fluctuations of substrate concentration and pH in the large-scale bioreactor have been studied at small scale using several small reactors operating at different conditions. The scale down approach aims to re-establish the process conditions of large reactors at small scale while maintaining scalability. For example, a scale down reactor with two compartments was designed to study the effect of oscillating substrate concentration on biomass yield based on the key parameters of residence time in the feed zone (Bylund et al., 1999). Amaullah et al. (2001) proposed a scale-down model consisting of a stirred tank reactor and a recycle loop containing a plug flow reactor to investigate pH variations in large-scale bioreactors. Construction of scale down apparatus can be a powerful complement to mathematical models, scale-up rules and fractional pilot plant operation. However, it is difficult to build such a protocol at a small scale which can be also recommended for use in large scale while maintaining the physical principles of mixing, mass transfer and growth for the two systems.

Due to a lack of detailed knowledge of both fluid dynamics and physiology, scale-up is perhaps more an art than an exact science (Humphrey, 1998). A model which includes fluid hydrodynamics, interphase mass transfer and cell growth and metabolic kinetics can produce a more scientific basis for scale up. Computational Fluid Dynamics (CFD) models can be utilised to establish mixing and it can also incorporate mass transfer and
cell growth kinetics models. The flow analysis allows the effect of mixing on the concentration and temperature gradients to be established. CFD can also be used to evaluate conditions within a large-scale vessel prior to process start-up to ensure that they are similar to those experienced in the pilot or laboratory reactor. The techniques can reduce cost, time-to-market, and production delays associated with physical trials.

Gupta et al. (2000) developed a liquid gas circulation zone model to describe the hydrodynamics in a bubble column reactor which has a number of zones for each phase. This model has been successfully validated using tracer data from a methanol synthesis pilot plant which does not require any adjustable parameters. Banka et al. (2003) described CFD models developed for pilot-scale and production-scale fermentors. The pilot-scale reactor was validated by using laser doppler anemometry (LDA) data, while the production-scale reactor was validated using base-addition-response data. Good correlation was obtained in both cases. CFD models may also be used for elucidating the interaction between extracellular environment and cell physiology. This is discussed in the section 1.5.

1.2.3 Scale up for mammalian cell culture processes

The growing demand for the commercial production of therapeutic and diagnostic biologics has motivated the development of more efficient and reliable mammalian cell culture production technology. The development of industrial cell culture processes is characterised by constraints which are related to issues such as costs, competitiveness, and the meeting of project timelines. As discussed in section 1, time allowed for proper process development is rather short and in such cases culture conditions and scale-up protocols have to be defined to maximize cell productivity and final titers and to minimise process length and overall costs. Rapid progress in the development and use of large-scale animal cell cultures has been seen in the last twenty years. Many of these processes are operated successfully at scales up to or greater than 10,000L, but there is not a single set of guidelines on how to design, operate and scale-up these bioprocesses.

Animal cells are sensitive to shear stress. Cell damage and non-lethal physiology changes are found in the range of shear stress of 1~10 N/m² (Ma et al., 2002). Dissolved oxygen concentration requirements are typically in the range 0.05~0.5 mmol oxygen.L⁻¹ hr⁻¹ for 10⁶ cells mL⁻¹ for human cells (Fleischaker and Sinskey, 1981) and 0.03~0.8 mmol oxygen.L⁻¹ hr⁻¹ (10⁶ cells mL⁻¹) for a range of industrially relevant cell lines.
(Aunins and Henzler, 1993) compared to 44–250 mmol oxygen. L⁻¹ hr⁻¹ for bacterial fermentation process (Perry and Green, 1998). Mammalian cells grow relatively slowly and the doubling times are about 12–100 hours or more compared to 12 minutes ~ 24 hours for bacterial fermentation (Arathoon and Birth, 1986). From a reaction engineering point of view, process development for animal cell culture is different to that for bacterial fermentation. The scale-up methods and related issues are discussed below.

Anchorage-dependent cells are usually cultivated in a solid supporting matrix. Packed-cell bioreactors such as hollow fiber and ceramic-matrix bioreactors, roller bottles, stirred tanks on suspended microcarriers have been used for large-scale production (Yoshida et al., 1997; Jackson et al., 1996; Mendonca et al., 1999). In large-scale microcarrier cultures it is important to avoid high shear conditions as the size of the microcarrier is in the same order of magnitude of Kolmogorov eddy size in a highly turbulent flow environment. But vigorous mixing is needed to suspend the solids and prevent aggregation of beads. However, suspension culture is currently regarded as the preferred scale up method for animal cell culture, and suspended cell cultures have been extensively used in stirred-tank and airlift bioreactors. The discussion in this thesis is focused on stirred tanks only. Production volume for freely suspended batch or fed batch stirred bioreactors of 8–10 m³ (Pullen et al., 1984; Phillips et al., 1985; Nelson, 1988a, b) and 15 m³ (Werner, 1994) have been reported. However, there has been little consideration of the important balance between mixing, mass transfer requirement, and shear effects in the large-scale cell culture system (Varley and Birch, 1999).

For the rational design of a reactor system and scale-up strategies, an understanding of the effect of shear stress on cellular viability, metabolism, and product formation is required. Mammalian cells in most bioreactor systems are subjected to two kinds of fluid mechanical forces: those due to turbulence within the liquid and those associated with the gas-liquid interface. But the latter effect is still controversial and no consensus of opinion has been reached. McQueen et al. (1987) showed that hybridoma cells were damaged by extremely intense flows, which produced eddy sizes comparable to the size of the animal cells. But in a cell culture stirred tank, the flows normally are not so intense. Kunas and Papoutsakis (1990) found that in a bench scale bioreactor, hybridoma cells could withstand impeller speeds up to 600 rpm as long as there was no air entrainment from the central vortex. Kioukia et al. (1992) found that the most
important region for cell damage was the bubble disengagement region and the more frequently cells passed through this region the greater the cell damage. However, when Chinese hamster overy (CHO) cells were subjected to 0.1 N m\(^{-2}\) shear generated in a flow chamber in protein-free media without protectants, recombinant protein production ceased with no change in cell morphology. After addition of shear protectants, the productivity of the cells was restored (Keane et al., 2003). They also found that on increasing the shear stress, the glucose uptake rate increased but lactate productivity decreased.

The interactions between cells and bubbles may also be responsible for cell damage, and there are three main regions where damage may occur: bubble generation region, bubble rising region; bubble disengagement region. Small bubbles (<2mm diameter) are more damaging to cells than large bubbles (~10mm diameter). Bubbles as large as 10–20 mm in diameter have been chosen for use in the commercial culture of several mouse hybridoma (Birch et al., 1987). The mechanisms of bubble-associated cell damage are still not fully identified. Estimates of typical flow stress based on bubble formation, growth and detachment from spargers in airlifts and stirred vessels have been proposed and shown to be insignificant (Hua et al. 1993; Cherry and Hulle, 1992). The energy dissipation rate in the wake carried behind a rising bubble is around 4–8 kW/m\(^3\) for the bubble diameter greater than 1mm, resulting in the micro-eddy size greater than ~20\(\mu\)m. In comparison with this, suspended cells, such as hybridomas, tend to be between 10–17 \(\mu\)m in diameter, which is smaller than the eddy size. No cell damage due to bubble rising is expected if the bubble diameter is greater than 1mm (Michaels et al., 1996; Chisti, 2000). The maximum energy dissipation rates of 10\(^7\)–10\(^8\) W/m\(^3\) was calculated for bubble rupture of small bubbles (less than 2mm) (Boulton-Stone and Blake, 1993; Garcia-Briones et al., 1994). The eddy size scale approaches the typical cell diameter (10–20 \(\mu\)m) and these bubbles are implicated in cell damage. But the maximum energy-dissipation rates decline with the increasing bubble diameter. Ma et al. (2002) designed a microfluidic device and found that four different cell lines were able to resist relatively intense energy dissipation rate up to 10\(^7\)–10\(^8\) W/m\(^3\), which is higher than the maximum energy dissipation rates generated by impeller bioreactors. Fig 1-3 summaries the reported energy dissipation rates at which cells are damaged.
### Lethal cell response

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Cell</th>
<th>Mode of growth</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>CHO-K1</td>
<td>Anchored</td>
<td>Gregoriades <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>b</td>
<td>Hybridoma</td>
<td>Suspended</td>
<td>Thomas <em>et al.</em> (1994); Zhang <em>et al.</em> (1993)</td>
</tr>
<tr>
<td>c</td>
<td>MCF-7</td>
<td>Suspended</td>
<td>Ma <em>et al.</em> (2002)</td>
</tr>
<tr>
<td>d</td>
<td>Mouse myeloma</td>
<td>Suspended</td>
<td>McQueen and Bailey (1989)</td>
</tr>
<tr>
<td>e</td>
<td>Hela S3, mouse L929</td>
<td>Suspended</td>
<td>Augenstein <em>et al.</em> (1971)</td>
</tr>
<tr>
<td>f</td>
<td>CHO-K1, SF-9, HB-24</td>
<td>Suspended</td>
<td>Ma <em>et al.</em> (2002)</td>
</tr>
</tbody>
</table>

### Hydrodynamic conditions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Process</th>
<th>Description of energy dissipation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agitation</td>
<td>Volume average in typical animal cell bioreactors</td>
<td>Varley and Birch (1999)</td>
</tr>
<tr>
<td>2</td>
<td>Agitation</td>
<td>Volume average in a 10L mixing vessel (RT, 700 rpm)</td>
<td>Zhou and Kresta (1996)</td>
</tr>
<tr>
<td>3</td>
<td>Agitation</td>
<td>Maximum in the 10L mixing vessel</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Agitation</td>
<td>Volume average in a 22,000L mixing vessel (RT, 240rpm)</td>
<td>Wernersson and Tragardh (1999)</td>
</tr>
<tr>
<td>5</td>
<td>Agitation</td>
<td>Maximum in the 22,000L mixing vessel</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bubble rupture</td>
<td>Pure water, bubble diameter: 6.32mm</td>
<td>Garcia-Briones <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>7</td>
<td>Bubble rupture</td>
<td>Pure water, bubble diameter: 1.7mm</td>
<td>Boulton-Stone and Blake (1993); Garcia-Briones <em>et al.</em> (1994)</td>
</tr>
</tbody>
</table>

![Diagram of Lethal Cell Response](image)

*Fig. 1-3 Energy dissipation rates for cell damage*
Most of the agitated tanks used in animal-cell culture operate at relatively low mechanical power inputs and impellers usually do not break up large gas bubbles throughout the tank. Michaels et al. (1996) found that cell-bubble interactions within the bulk fluid for Chinese hamster ovary cells do not appear to affect cells significantly even in the absence of protective additives and bubble coalescence and break-up within the bulk culture caused no significant damage either.

Although overwhelming reports are on the deleterious effect of shear stress on cell physiology, the nonlethal physiological responses of mammalian cells have been reported due to the shear stress in the reactor. Most reports are in the range of energy consumption of 1~100 kW/m\(^3\) (the shear stress of 1~10 N/m\(^2\)) (Ma et al., 2002). Shiragami and Unno (1994) studied the effect of shear stress on the activity of cellular enzyme of animal cells using a flow channel. They found that the metabolite production increased with exposure to moderate shear stress levels. They correlated this with the dissipation energy of the flowing medium and found that shear stress caused changes in the quantities of surface protein per cell. Fluid shear stress altered cell function (Chittur et al., 1988), enhanced acid metabolism (Nollert et al., 1991), activated the G-protein (Gudi et al., 1996) and induced the transcriptional activator c-fos in human and bovine cells, Hela and Chinese hamster ovary cells (Ranjan et al., 1996).

In agitated bioreactors for mammalian cells, marine and other low-shear impellers are usually used because of the relatively low oxygen uptake rate requirements. The agitation intensity is typically 100 times less than that in a microbial fermentation (Varley and Birch, 1999). Because the relatively low substrate consumption rate and low rate of change in pH and temperature, mixing and circulation times are less critical in animal cell culture and agitation rate need only to be sufficient to keep cells in suspension. Nienow et al. (1996) found that the specific power input Pg/V is around 3.5 W/m\(^3\) in the large scale aerated vessel (volume 8,000L, the diameter 2m and maximum liquid height 2.6m). The power input of 10 W/m\(^3\) is usually used for animal cell culture, and this value is an extremely low power consumption rate, compared to 1,000~2,000 W/m\(^3\) recommended for bacterial fermentation process (Aiba et al., 1965).

However, this low level of agitation may be insufficient for mixing and homogenization in many cases (Ozturk, 1996). It has become apparent that pH gradient, dispersion of
Introduction

nutrients and waste product, gas dispersion (oxygen and carbon dioxide) are of particular importance (Wayte et al., 1997; Nienow et al., 1996). Bliem and Katinger (1988) found that a number of industrially relevant cell lines used at large-scale are relatively insensitive to shear. These included BHK 21, Namalwa, Vero, and some CHO lines and a range of hybridoma cells. Successful cultivation of many animal cell lines under relatively intense agitation indicates that its impact of shear sensitivity has been overestimated (Nienow et al., 1996; Kunas and Papousakis, 1990).

As bioreactor volumes increase, mixing times for equivalent impeller tip speeds increase. The mixing times of 1–5s in 10–50 L fermentors may rise to 20–30s in 1m$^3$–2m$^3$ vessels and 2mins and beyond at the scale of 100 m$^3$ to 120 m$^3$. If oxygen uptake rate is sufficiently high and the gas oxygen solubility is low, extended mixing times mean that oxygen deprivation occurs in certain regions of the fermentor. Along with the potential for oxygen depletion, there may be equally serious problems in the control of pH and temperature. Cacciuttolo et al. (1998) measured glucose and lactate in a murine hybridoma culture and found a shift toward anaerobic metabolism at the 200L scale that was not observed at the 3L bioreactor, even by using constant impeller tip speed as a scale-up criterion.

Under low agitation rate, gas bubbles are not well dispersed throughout the bioreactor. The operational conditions in the large-scale mammalian cell culture fall into impeller flooding regime, where bubbles move along the impeller shaft and burst out at the top liquid surface. Tramper et al. (1995) calculated the oxygen concentration distribution in animal cell bioreactors (stirred tanks, bubble columns, and air-lift reactors) at a scale of 10m$^3$. They found that oxygen gradient in the bulk of the liquid phase and in the stagnant layer surrounding the air bubbles were often quite severe and these gradients were greater than gradients in stagnant layers surrounding particles (cells or microcarrier particles).

Gas liquid mass transfer is important in maintaining desired dissolved oxygen and dissolved carbon dioxide levels in the culture media. Traditionally the gas flow rate is kept very low and large gas bubbles are maintained in the reactor to ensure minimum shear effects. Most of the correlations available for $k_{La}$ have been derived in microbial fermentation at high agitation speeds and high aeration rates. They are not generally appropriate for animal cell system. There are limited data for application at large scale.
Nienow et al. (1996) provided the value of $k_{l,a}$ for cell medium in the range of 1–10 h$^{-1}$ corresponding to P/V of 10–100 W/m$^3$ (the gas flow rate of $6.3 \times 10^{-4}$ m$^3$ s$^{-1}$) in an 8000 L fermentor.

The partial pressure of metabolically produced carbon dioxide ($pCO_2$) and hence mass transfer of carbon dioxide from solution to the gas bubbles is important in animal cell culture. Rise in $pCO_2$ leads to a drop in pH because of the rise in $H_2CO_3$. $pCO_2$ must therefore be carefully monitored and controlled. Good gas dispersion can remove carbon dioxide from the solution. But under normally operating conditions in animal cell culture, carbon dioxide concentration can accumulate in the culture media to inhibitory levels unless adequate ventilation is provided (Kimura and Miller, 1996). Gray et al. (1996) found that the productivity with CHO cells was better when $pCO_2$ was between 30 and 76 mm Hg. They suggested that the bubble size be between 2–3 mm to ensure an adequate balance between oxygen transfer rate and carbon dioxide stripping.

Several scale-up criterion have been used for animal cell culture. Arathoon and Birth (1986) obtained very similar growth profile and viable CHO cells after 4 days in 10-, 100-, 1000-liter vessels while the maximum shear rates were kept constant or decreased with increasing scale. Backer et al. (1988) compared the performance of hybridoma cell lines for monoclonal antibody production in 150L and 1300L bioreactors. Maximum agitation rate used were 140 rpm for 150L and 75 rpm for 1300L vessel. Nelson (1988b) used constant Kolmogorov length scale as the basis of scale-up to minimise the shear effects. Chisti (1993) scaled up to 0.3 m$^3$ commercial plant using data from a 0.02 m$^3$ vessel for hybridoma suspension cultures based on the similar fluid turnover (i.e. impeller pumping rate per unit bioreactor volume) but the configurations (the ratios of impeller diameter to tank diameter, impeller clearance) of the large-scale unit were different from those of the small scale reactor. Zhou et al. (1996) balanced oxygen supply and carbon dioxide removal in 150L and 250L bioreactor. The cell growth obtained at both scales were comparable to that at 36L scale.

1.3 Micro-scale bioprocess operations

Historical approaches, based largely on bench and pilot-plant studies, are laborious and time-consuming and require large quantities of materials, which are often not available at the early stage of process development, especially in the case of high-value and low-
volume products such as the new generation of biologics. In many cases millilitre quantities may be all that is available for test purposes at an early stage. Additionally, in an increasingly competitive business environment, it is often the case that even a modest reduction in the “time-to-market” can make a significant difference between success and failure of a product. This demands new methods for the rapid assessment of processability of the new generation of medicines that can keep pace with the speed at which new drug discoveries are coming forward.

The automated shaken micro-well plates have been widely used in recent years. Its popularity stems from small quantities of materials, parallel sample preparation and reproducible sampling techniques. The microwell system has the potential to provide process information for scale-up and production yield. However the engineering environment in the microwell is not fully characterised to date and limits its capacity of process development. Another alternative is scaling down the conventional laboratory-scale bioreactors. The working volume can be decreased to millilitre scale and the reactor can be fully controlled by optical sensors. The section 1.3.1 will discuss the details of microwell and the section 1.3.2 will present some miniature bioreactors which have been developed and are developing.

1.3.1 Microtitre plates

Micro-well miniaturisation technology has emerged as a proven analytical tool for high-throughput screening (HTS) in drug discoveries. Microtitre plates have been widely used for high-throughput screening (HTS) (Aner, 2001; Lahana, 1999), and combinatorial chemistry (Hart, 2001; Nakayama, 2001), ELISA-test (Erdile et al., 2001). HTS uses automated liquid handling systems which greatly reduces the labour requirement and increases the operational and analytical accuracy. Several thousands of samples can be handled in the microtitre plates in parallel and the working efficiency can be greatly increased.

The multiwell systems have the potential for changing the ways laboratory fermentations are run. For example, the labour and time involved in separately reviving the stock cultures on agar plates and the subsequent inoculation of tubes or Erlenmyer flasks are major limiting factors in the progress of “screening” large microbial strain collections. However, in 96- or 384-well microtitre plates liquids can be manipulated and analysed automatically so that large number of microbial strains can be handled in
parallel rather than in sequence. This system can also provide process information which could be used for scaling up to large scale.

The microwell plates in which experiments are performed are based on a standard footprint of $86 \times 128 \text{ mm}^2$ and usually have a height of $14\text{ mm}$. This enables automation and integration with associated equipment such as spectrometric plate readers and high performance liquid chromatography (HPLC), which produces the means of handling small volumes and measuring outcomes from reactions in these microwell plates. Currently 96-, 48-, 24-, 12-, and 6-wells of variable geometry and volume are commercially available although 384, 1536 and now 3456-well plates are increasingly using in Ultra-High Throughput Screening (UHTS).

BiOLOG (Hayward, Calif.) has developed a culture identification system based entirely on growth of unknown microbes in 96-well plated containing a fixed array of carbon sources. In addition to clinical culture identification, BiOLOG system has been used to survey microbial communities (Fredrickson et al., 1991; Garland et al., 1991) and to screen for microorganisms from nature that are capable of degrading toxic chemicals (Gorden et al., 1993). Generic microwell plates have been used to evaluate the physiology of many strains in parallel (Collins et al., 1987). The microwell reactor has been used for screening and evaluating the aerobic and anaerobic microorganisms (Weiss et al., 2001b; Duetz et al., 2000; Duetz and Witholt, 2001), animal cells (Girard et al., 2001), and biocatalyst (Doig et al., 2001; Weiss et al., 2001a). Automated culture methods with increased throughput rates can be applied to screen for secondary metabolites (new drugs and antibiotics) and new biocatalyst and new enzymes. The methods can also be used for cultivation of clone libraries (expression studies, media development and optimisation) and strain development by random mutagenesis or generic engineering. These studies have explored the potential of microbioprocessing using instrumented shaken microwell systems. The process information obtained from the automated cell culture methods can be of great importance for biopharmaceutical industry to develop a whole process at very small scale, therefore reduce “time-to-market” and cost.

However, the use of microwell plates for the growth and maintenance of microbial strains has been mainly limited to clonal libraries in *E. Coli* (Giege et al., 1998; Hersh et al., 1996) and yeasts (Anand et al., 1990) because they can grow anaerobically and
small amount of biomass generally suffice as the use of high-copy-number vectors ensures high levels of the desired gene or gene product. The mixed aerobic-anaerobic growth can lead to undesirable variations in biomass from well to well, especially since oxygen diffusion rates for an individual well may be affected by its position in the system. Such well-to-well variations will affect the screening results. Therefore, there are two major problems in miniaturisation of such a microbial growth system: how to reach oxygen transfer rate and mixing over long periods similar to those achieved in regular growth system like stirred vessels or shaking flasks while preventing cross-contamination and excessive evaporation (evaporation can be alleviated if the growth system can be placed in a high humidity chamber); how to set up a reliable and reproducible system for all operations such as fermentation, biotransformation, precipitation and other unit operations.

Currently, the quantitative bioprocess design and development data regarding mixing and gas-liquid mass transfer are not available. The main reason is that at the small scale, the appropriate techniques to measure and characterise the typical engineering parameters are lacking. Conventional power-consumption techniques, such as torque measurement, or temperature rise, can not be applied to the microwell bioreactor. And the liquid volume varies from 50 μL-4000 μL, and the small volume results in a very shallow liquid depth. Such a liquid depth makes it difficult to measure the dissolved oxygen concentration because it is not possible to maintain the oxygen probe fully submerged in the liquid during operation.

Professor Govind Rao (www.howarddoc.edu/bcc/SET/abstract-microbioreactor.htm) proposed to construct a low cost instrumented microbioreactor, which is based on microwell. Fig.1-4 gives detailed information about the microreactor. Each well of a 96 well plate can be independently monitored for pH and DO by the sensors which are built in side-walls of the reactor. Eventually each well could be independently controlled for pH and DO. Bernhard et al. (2001) reported a 2~10μm thick pH-sensitive or O₂ sensitive sensing layer was immobilized inside each microwell (1~14 μm deep and 22 μm width) using a technically expedient, photo-initiated polymerization reaction. This technique may be applied to the 96-well or 24-well reactors. Usually sensors have to be built in the bottom of the well reactor so that at least the sensors are submerged in the liquid during measurements. John et al. (2003) reported an oxygen sensor layer with the thickness of 10 μm, placed in a 96-well round-bottom plate. The
“Cellscreen” system was reported to measure cell numbers in 96-well plates without affecting the culture process (Brinkmann et al., 2002).

An alternative is to design sensors for pH and DO separate from the well reactor. The set of the sensor may be manipulated by a robotic arm and inserted into each well for a short period to measure key parameters. This method requires knowledge of liquid movement to ensure that the sensors remain fully in contact with the liquid.
<table>
<thead>
<tr>
<th>Ref:</th>
<th>Research targets</th>
<th>System investigated</th>
<th>Microwell geometry and agitation</th>
<th>Parameters determined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermann et al. (2003, 2001)</td>
<td>Oxygen transfer rate</td>
<td>Sulfite oxidation in aqueous media</td>
<td>96-deep square wells, 0~1000rpm, e (shaking diameter) = 3 ~ 50 mm</td>
<td>OTR=16mmol l⁻¹ h⁻¹ (300rpm, 50mm), Critical shaking frequency, OTR dependency on surface material</td>
</tr>
<tr>
<td>Elmahdi et al. (2003)</td>
<td>pH control in fermentation process</td>
<td>A micro-pH probe and its use in growth of S. erythraea CA340</td>
<td>96-deep square wells, 600rpm, e=3mm</td>
<td>Implementation of pH control in microscale fermentation</td>
</tr>
<tr>
<td>Lye et al. (2002)</td>
<td>Microbial growth and enzyme induction</td>
<td>Enzymatic expression in E. coli</td>
<td>96-deep square wells, 300rpm, e=3mm</td>
<td>Biomass growth and flow visualization</td>
</tr>
<tr>
<td>Weiss et al. (2002)</td>
<td>Mixing and hydrodynamics</td>
<td>pH indicator dyes</td>
<td>96-deep (flat or round base), 240-900rpm, e=1-12mm</td>
<td>Mixing time (5-500 s), flow visualisation</td>
</tr>
<tr>
<td>Doig et al. (2002)</td>
<td>Quantification of whole-cell bioconversion kinetics</td>
<td>Enzymatic activity in a recombinant E. coli</td>
<td>96-round, 96-deep square and 24-well, 300-1300rpm, e=3mm</td>
<td>Enzymatic expression, substrate inhibition, and OTR=33mmol l⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Girard et al. (2001)</td>
<td>Mammalian cell culture</td>
<td>Growth of CHO and HEK 293 cell lines</td>
<td>12-wells, 180rpm, e=20mm</td>
<td>Process development</td>
</tr>
</tbody>
</table>
Table 1.2 summaries the recent research on the performance of different unit operations in the microwell bioreactor. The majority of the studies have been focused on the evaluation of micro-organism and mammalian cell growth in the microwell reactors. There are two papers which reported the scalability of the reactor (Girard et al., 2001; Doig et al., 2002). Girard et al. (2001) compared the growth of the HEK cells in a 3L bioreactor, 100ml spinner and 2ml shaken microwell reactor, and found that cell aggregate size and transfection efficiency were comparable at different scales. Doig et al. (2002) compared the effect of substrate concentration on the specific enzymatic activity in a 96-round well reactor and a 2L stirred reactor, and found that a microwell system reasonably replicated the overall trend observed in the stirred tank and concluded that it could be used for rapid, small-scale acquisition of initial quantitative kinetic data. Regarding the hydrodynamics and mixing in the reactor, Weiss et al. (2002) used soluble pH indicators and a fluorescence pH sensor and mixing patterns were observed by a video camera. The mixing time was estimated from a few seconds to several minutes. The discrepancies can be due to different addition methods of alkali. Similar results have been reported on the hydrodynamics of the 96-well reactor with
0.75mL of an aqueous solution of bromocresol blue (1%, w/v) (Duetz and Witholt, 2001). However, both studies are based on the experimental observations and underpinning physical laws have not reported. To characterise the gas-liquid mass transfer, Hermann et al. (2001, 2003) developed an optical method based on sulfite oxidation and measured the mass transfer in 96-well plates. The shaking intensity (shaking frequency and shaking diameter), and filling volume were reported to affect the mass transfer coefficient.

The microtiter plates have been widely used for mammalian cell culture. The cells are usually cultivated without shaking operations. Cell growth for mouse hybridoma cell lines in shallow static suspension were greater than that observed in a 1-liter stirred culture (Arathoon and Birch, 1986). But the cell growth rate and specific glucose consumption rate for hybridoma cells were found to be similar in both static six-well plates and controlled batch bioreactor (deZengotita et al., 1998).

1.3.2 Microreactors in biochemical process research

In recent years research and development of miniaturized chemical systems has grown dramatically, allowing the realization of the micro total analytical system (μ-TAS). The application of similar technology to that used for μ-TAS has also led to the development of so-called micro-reactors. Areas that have attracted most attention to date have focused on fast gas and liquid phase reaction covering heterogeneous and homogeneous catalysis, catalytic oxidation, heterocyclic synthesis, and photochemical reactions. The micro-scale processes have clearly indicated the value of using micro-reactor technology in chemical and biochemical discovery and development.

The development of micro-fabricated technology allows new miniature bioreactors for fermentation and cell culture process. The new microbioreactor incorporated with small sensors within the reactor may be rapidly used to provide information on the effect of engineering parameters (oxygen transfer rate, pH, temperature and power consumption). The microbioreactor may also be used to study physiological response of cells from medium composition, cell growth and product formation kinetics, and metabolic pathway.

A hollow-fiber micro-bioreactor was developed for screening factors affecting
mammalian cell culture (Gramer and Poeschl, 1998). Hollow fibers with a 10kDa molecular weight cutoff (0.02cm internal diameter, and 0.022cm external diameter) were placed in a silicone tube (working volume is 16 mL). The micro-bioreactor supported good growth of hybridoma cells including increased cell density, metabolic activity and antibody concentration over the 3-day period. Predictions from the micro-bioreactor that the initial growth phase of the cell line is dependent on the serum concentration in the medium reservoir were tested by production-scale hollow-fibre bioreactor. The results indicated that the micro-bioreactor may be used as an efficient screening tool to determine how well a new cell line will perform in a hollow-fibre system or how well an established cell line will perform under new operation conditions. But the significant limitation is the inability to mimic the process control schemes of large-scale hollow-fibre systems, including control of pH, continuous addition, and removal of media.

Kostov et al. (2001) have recently presented the design of a microreactor (Fig.1-5) with a working volume of 2mL and reported basic data on the responses of pH, dissolved oxygen and optical density probes using E. coli fermentation as a test bed. Initial results demonstrated that fermentation at the microwell-scale was feasible, however, achieving adequate oxygen transfer rate in the microreactor proved difficult and comparison of $K_La$ with data from a 1L conventional fermentor showed the difficulties in using magnetic-bar stirrer in the microreactor.
Fig. 1-5 Cuvette-based microbioreactor. At the left cuvette wall, blue and UV LED together with 530 nm photodetector are used to measure pH; at the right cuvette wall, blue LED, oxygen sensing patch, and 590 nm photodetector are used to measure dissolved oxygen; red LED and 600 nm photodetector are used to measure optical density through the front and back wall. The air supply inlet and outlet are positioned at the corners of the cuvette. LEDs are fired in succession to prevent crosstalk (for clarity, figure not to scale).

A multidisciplinary research program at MIT (http://www-mtl.mit.edu/mtlhome/mems/chembiomems.shtml) aims at developing new platforms for bioprocess discovery and development, specifically banks of miniaturized, automated fermentors, each with integrated bioanalytical devices, and all operating in parallel (see Fig. 1-6). Such system will address the continuing demand in bioprocess science and engineering for fast and accurate analytical information that can be used to rapidly evaluate the interactions between biological systems and bioprocess operations. And the microfermentors will provide the platforms for efficiently incorporating modern tools of biology to improve bioprocess screening and development. Applying microfabrication technology to bioprocess development will result in rapid screening of strains and metabolic pathways with dramatic productivity increases analogous to those experienced in drug discovery. Crucial issues, such as evaluating performance of the microfermentors, and design and fabrication strategies for the microfermentors are being carried out.
Recently a new miniature bioreactor with a diameter equal to that of a single well of a 24-well plate has been developed (Lamping et al., 2003). The configuration of the miniature bioreactor is based on the laboratory scale bioreactor. Mixing in the miniature bioreactor was provided by a set of three impellers mechanically driven via a microfabricated electric motor and aeration was achieved with a single tube sparger. Temperature, pH, oxygen dissolution concentration and biomass were controlled by a set of optical sensors. Detailed information of the miniature bioreactor is presented in Chapter 2.

1.4 New bioprocess development approach

Bioprocess development in the biopharmaceutical industry is facing great challenges as discussed in section 1.1 and conventional bioprocess development is not meeting these challenges (section 1.2). Microwell bioreactors and miniature bioreactors (Section 1.3) have the potential for accelerating the process development for new drugs. A new bioprocess development strategy presented in Fig.1-7 is a simultaneous approach that integrates drug research and process development.
The new strategy starts with the microwell bioreactors, in which drug targets are screened and culture conditions are established for expressing the proteins from target genes. However, in the microwell bioreactor surface aeration prevails at the gas-liquid interface and mixing is not very intense. Close to the interface, the concentration of dissolved oxygen is relatively high and growth of microorganisms is aerobic. However, immediately below the interface, the concentration of dissolved oxygen is very low and the growth of microorganisms becomes anaerobic. The mixed aerobic-anaerobic growth can lead to undesirable variations in biomass from well to well, especially since oxygen
diffusion rates for an individual well may be affected by its position in the system. These reasons mean that currently there is insufficient understanding of the factors that affect fermentation and cell growth in microtiter plates.

The miniature bioreactor may provide an alternative for drug discovery platform and give process information at the early stage of new drug development cycle. The engineering environment in the miniature bioreactor can be controlled, and the good mixing and oxygen transfer in the reactor improve cell growth and fermentation. The optimal process conditions obtained in the miniature bioreactor may be scaled up directly to laboratory scale bioreactors.

The shake flask is the starting point for the conventional bioprocess development. However, a full engineering study of its mixing and mass transfer has not been carried out to date. The shake flask is still widely used in the “screening” of optimum operation conditions and it is necessary to establish a link with the laboratory scale bioreactors. The reactor may also be used in the new development chain from the microwell reactor.

The present thesis focuses on establishing the engineering parameters in all these reactors and laboratory fermentors with the aim of providing a common basis for scale-up and scale-down between them.

1.5 CFD application to bioprocess development

The success of bioprocess development requires understanding the performance of cells in a bioreactor at different scales. The engineering flow environment in most items of bioprocess equipment has long been recognised as a key factor in determining the overall performance. The section 1.5.1 discussed the flow heterogeneity that exists in a bioreactor and the effect of the hydrodynamic environment on cell physiology. Minor changes in flow environment causes considerable variations in the bioreactor performance. Section 1.5.2 and 1.5.3 described the application of Computational Fluid Dynamics (CFD) to understanding of the flow environment in different systems and to help developing bioprocess development.

1.5.1 The effect of flow environment on cell physiology

The cells of bacteria, animals and plants are commonly cultured in a bioreactor, such as microwell, shake flask, stirred fermentor, airlift bioreactor, and bubble column.
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Engineering flow of these reactors is complicated because of the temporal and spatial variations of the flow environment around the cells. The hydrodynamic environment affects cell physiology and the interaction between cells and extracellular environment often deteriorate the overall production performance at pilot or production scale. And cell response sometimes is lagged in time, which means that cell response depends not only on the present micromixing conditions, but also on previous flow conditions in the reactor. Two typical problems related to flow environment are the mixing and power input.

The mixing does not reach a homogeneous state even in a well-mixed stirred tank. A large European project aimed at analysing the effect on scale-up of inadequate mixing revealed that gradients in the concentrations of various substrates and products are distributed around the whole tank (Larsson et al., 1996). These problems are very important for those fermentation processes in which nutrients are continuously introduced into the broth. Dynamically changing environmental conditions may result in drastic changes in cell metabolism and final outcome of the process. In order to address the issue of intracellular response to fast variations in the extracellular substrate concentrations, two different techniques for fast sampling devices to measure the changes in metabolite concentration within seconds or even milliseconds were developed (Theobald et al., 1997, 1993; Rizzi et al., 1996). It was reported that the levels of many metabolites in yeast *Saccharomyces cerevisiae* change within the first minute of the glucose pulse and metabolic regulation occurs on the time scale of seconds. The study of metabolic modelling should be based on the coupling of the fluid dynamics with the unstructured kinetic rate expression. The dynamic response of the concentration of phosphoenolpyruvate (PEP) and pyruvate (Pyr) after glucose incubation of E.coli growing in continuous culture was measured by the rapid stopped-flow sampling technique (Buziol et al., 2002). A rapid decreases of the PEP pool accompanied by a corresponding increase of the Pyr pool was observed after the stimulus of the high glucose concentration. In an impeller-driven fermentor, individual cells repeatedly pass through the non-homogeneous zones of substrate, dissolved oxygen, and energy dissipation, as a result, the intracellular responses (reactions) to different zones are expected to be different and the cells continuously try to adapt to a situation that lasts for only a few seconds. Analysis of acetate and formate concentration in a 22 m³ cultures indicated that acetate and other anaerobic products may be formed in
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The importance of mixing may be illustrated with an example from α-amylase production by filamentous fungi (Agger et al., 2001). It was reported that the specific rate of enzymatic formation in a continuous stirred bioreactor (chemo-stats) decreased significantly with increasing biomass concentration. But by deleting the creA gene, which is responsible for glucose repression, the specific productivity of the enzyme remained constant to a very high biomass concentration. The decrease of the productivity was caused by glucose repression while cell growth was not affected by the glucose concentration. The high glucose concentration in the feed solution yielded a higher biomass concentration, but this high concentration above the repression limit inhibited enzymatic production. Rapid dispersion of the substrate into the medium improved enzyme formation.

This contradiction of power input between improving mixing and keeping cells viable presents a big challenge in optimising the existing bioreactor operation conditions and scaling up from laboratory to production scale. Increasing power input can improve the efficiency of mixing in many stirred fermentors in which cells are grown. But diameter of the smallest turbulent eddy is about 50~300µm, compared with the diameter of the microorganisms which is typically 1-5µm. Consequently regardless of how well the reactor may be mixed on a macroscopic scale, the bacterial cell may be sitting in a stagnant pool whose nutrients have been rapidly depleted. There is no practical solution for the existing fermenters. For example, as the size of smallest eddy is proportional to the one quarter of power of the energy input \( \lambda \text{min} = 11.4\left(\frac{V}{\varepsilon}\right)^{0.25} \) (Tennekes and Lumley, 1973), it would take 16 times as much energy to halve the eddy size assuming the energy could actually be dissipated in the fermentor.

On the other hand, increasing power input results in high shear stresses and high energy dissipation rate in the bioreactor. These have complex effects on cell physiology and product formation. As the size of microorganisms like bacteria, yeast and mycelial microfungi is less than that of smallest turbulent eddy, the shear field may not damage cells. But it may still affect product formation. Increasing Rushton turbine tip speed reduced the specific protein production rate and a distinct lag phase was found at a
higher tip speed for a process utilising the filamentous microfungus *Neurospora sitophila* (Moo-Young *et al.*, 1992).

A reactor model that includes hydrodynamics and biokinetics is essential for evaluation the effect of non-homogeneous concentration, temperature, and energy dissipation on the performance and stability of the cells. The state-of-the-art computational fluid dynamics (CFD) techniques can provide a useful tool for understanding the interaction between the flow environment and living cells. In the present thesis, focus is put on the assessment of the engineering flow environment in different bioreactors (microwell reactor, shake flask, miniature and laboratory bioreactors).

### 1.5.2 Mass transfer in an impeller-driven reactor

The solubility of oxygen at normal pressure and temperature is low but the cell growth and the related metabolic activities are highly dependent on the dissolved oxygen in the fermentation broth. Efforts have been made to investigate the mass transfer performance in the bioreactor and various correlations have been reported for the calculation of volumetric mass transfer coefficient in single- and multiple-impeller systems. The $k_{l,a}$ values are usually correlated to the power consumption and superficial gas velocity. The general form for calculation of $k_{l,a}$ values is given below:

$$K_{l,a} = A \left( \frac{P_g}{V} \right)^\alpha \left( U_g \right)^\beta$$  \hspace{1cm} (Eqn. 1-1)

Eqn.1-1 does not take into account the effect of viscosity and physicochemical properties except through the calculation of $P_g$ (Kawase and Moo-Young, 1988; Cooke *et al.*, 1988). Demdoerfer and West (1960) suggested a modification of the basic $K_{l,a}$ correlation to include a viscosity term, i.e.,

$$K_{l,a} = A \left( \frac{P_g}{V} \right)^\alpha \left( U_g \right)^\beta \mu$$  \hspace{1cm} (Eqn. 1-2)

Correlations published in the literature are summerised in Table 1.3. There are large differences in the reported values of $A$, $\alpha$, $\beta$ from different reports. These differences can be attributed to the different impeller types and operation conditions, and different experimental techniques. Gogate *et al.* (1999) reported that different measurement techniques resulted in large deviation of the $k_{l,a}$ values. It has also been demonstrated that the assumed gas flow patterns has a dramatic influence on the value of $k_{l,a}$ derived.
from concentration measurements. But from the point of view of experimental measurement, an ideal gas flow pattern is normally implicitly assumed. Unfortunately the flow patterns is neither approximately plug-flow nor back-mixed. This can lead to different $k_{L,a}$ correlations even in the same reactor and with the same operational conditions (Moucha et al. 1995; Linek et al. 1996). A single correlation for calculation of $k_{L,a}$ is very difficult to establish. Van’Riet (1979) commended that deviation of ±40% is common in measurement of $k_{L,a}$.

In this study, the mass transfer performance will be based on the correlation of Van’Reit (1979) and the deviation of ±40% for the miniature bioreactor.

**Table 1. 3 Correlations for mass transfer coefficients from the literature**

<table>
<thead>
<tr>
<th>References</th>
<th>Coalescing medium</th>
<th>Non-Coalescing medium</th>
<th>Operational conditions</th>
<th>Impeller configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van’ Riet (1979)</td>
<td>$A=0.026$</td>
<td>$A=0.002$</td>
<td>$500&lt;Pg/V&lt;10,000$</td>
<td>Various impellers, various reactor sizes</td>
</tr>
<tr>
<td></td>
<td>$\alpha=0.4$</td>
<td>$\alpha=0.7$</td>
<td>$W/m^3$, various D/T ratios</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\beta=0.5$</td>
<td>$\beta=0.2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moo Young and Blanch (1981)</td>
<td>$A=0.0275$</td>
<td>$A=0.017$</td>
<td>$440&lt;Pg/V&lt;10,000$</td>
<td>Turbine stirrer, six-bladed,</td>
</tr>
<tr>
<td></td>
<td>$\alpha=0.42$</td>
<td>$\alpha=0.52$</td>
<td>$W/m^3$,</td>
<td>D=T/3, T=0.15m</td>
</tr>
<tr>
<td></td>
<td>$\beta=0.43$</td>
<td>$\beta=0.43$</td>
<td>$0.037&lt;U_g&lt;0.111m/s$</td>
<td></td>
</tr>
<tr>
<td>Moo Young and Blanch (1981)</td>
<td>-</td>
<td>-</td>
<td>$30&lt;Pg/V&lt;18,000$</td>
<td>Turbine stirrer, six-bladed,</td>
</tr>
<tr>
<td></td>
<td>$\alpha=0.4$</td>
<td>$\alpha=0.71$</td>
<td>$W/m^3$,</td>
<td>D=T/3, T=0.15m</td>
</tr>
<tr>
<td></td>
<td>$\beta=0.35$</td>
<td>$\beta=0.36$</td>
<td>$0.01U_g&lt;0.05m$</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>A (m)</td>
<td>α</td>
<td>P (m)</td>
<td>T (m)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>Linek et al. (1987)</td>
<td>0.005</td>
<td>0.593</td>
<td>0.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Hamby et al. (1992)</td>
<td>1.2</td>
<td>0.7</td>
<td>0.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Moucha et al. (1995)</td>
<td>0.0177</td>
<td>0.58</td>
<td>0.588</td>
<td>0.19</td>
</tr>
<tr>
<td>Linek et al. (1996)</td>
<td>0.00861</td>
<td>0.637</td>
<td>0.54</td>
<td>0.19</td>
</tr>
<tr>
<td>Humphrey (1998)</td>
<td>-</td>
<td>0.95 *</td>
<td>0.67 *</td>
<td>5L</td>
</tr>
<tr>
<td>Arjunwadkar et al. (1998)</td>
<td>0.002</td>
<td>0.68</td>
<td>0.58</td>
<td>0.18</td>
</tr>
<tr>
<td>Vasconcelos et al. (2000)</td>
<td>0.0083</td>
<td>0.62</td>
<td>0.49</td>
<td>0.392</td>
</tr>
</tbody>
</table>
1.5.3 CFD analysis of the engineering environment of cells

Development of computer hardware and numerical algorithms has allowed the solution of very complex flow problems that were previously impossible to solve. Computational Fluid Dynamics (CFD), a highly sophisticated integration of applied computer science, physics, chemistry and engineering science, has become a very useful engineering tool in chemical and pharmaceutical industry for research, development, design and manufacture. Computational results may be applied very quickly, saving millions of dollars of capital, reducing waste, and improving product quality, energy efficiency and process safety. A focus on CFD R&D was identified in Technology Vision 2020: The U.S. Chemical Industry as a high-priority for meeting the industry’s future goals.

CFD can provide a complete description of flow and mixing conditions in process equipment having complex geometry. The integration of CFD and modelling of the cell growth kinetics can elucidate the interactive relationship between the dynamic cell response and the engineering environment changing in time and space. While experimental investigation of the effects of engineering flow environment on mass transfer and cell growth is focused on overall “average” performance of the reactor, CFD analysis can provide temporal and spatial variations of flow in the reactor and allows one to capture the essence of the flow patterns and their effect on the various bioreactions. The residence time distribution of the microorganisms in different zones in the reactor combined with laboratory experiments that simulate the conditions in different zones, can be used to establish critical gradients that lead to microorganism death, mutations, or shifts in yield structure.

Simulations obtained using an integrated CFD and biokinetic model were performed by Larsson et al. (1996) and the pattern of rapid glucose fluctuations was shown to depend on the turbulence level at the location of the feed. The network of zones model of Vlaev et al. (2000) has been used to simulate a 3 m³ mechanically agitated pilot scale fermentor and the results indicated that significant O₂ and nutrient gradients were present even in this relatively small reactor.

The CFD techniques are employed in this thesis to analyze the flow environment in the miniature bioreactor, a 20L bioreactor, a shake flask and a 24-well reactor and 96-well
reactor. Correlations between the power consumption and mass transfer rate are established for different reactors. These correlations form the basis for the new bioprocess development approach.

1.5.4 CFD application to bioprocess development

The engineering flow environment of the reactor at different scales can be analysed by CFD. Krishna et al. (1998) provided a scale up philosophy for gas-solid fluidized beds and bubble column slurry reactors relying on hydrodynamics analogies between these two reactor types using CFD within Eulerian framework. Boychyn et al. (2001) applied CFD to map the flow field in the feed zone of an industrial multichamber-bowl centrifuge and established the profile of energy dissipation rate. Then a small high-speed rotating-disc device was designed with the capacity to reproduce the CFD-predicted energy dissipation rate in the feed zone. Boulding et al. (2002) used the same technique to provide information about the local energy dissipation rates in two filtering centrifuges with 0.5- and 30-L capacities to represent laboratory- and pilot-scale equipment. An ultra scale-down filtering centrifuge with a maximum working volume of 35mL was designed to mimic the operating conditions identified by the critical regime analysis for the laboratory and pilot-scale units. The activity of single-chain antibody fragments recovered from the three units were comparable at equal maximum energy dissipation rate. In a recent study, Neal et al. (2003) extended the approach to separation of precipitated polyclonal antibody using a (exposure) time-integrated fluid stress as a scaling factor. Therefore, by making the appropriate engineering connections with the aid of CFD, it is possible to establish from a few tens of millilitres what will happen to process materials during actual production.

1.6 Aims of the thesis

The discussions in the preceding sections cover the role of discovery for a new drug development and its need for a new bioprocess development methodology based on miniaturization. This thesis is focused on investigating the new approach from microwell bioreactors to laboratory scale 20L bioreactor. The aim of the study is to establish a methodology for comparing different micro-scale bioreactors from a mathematical basis. Small devices including a mechanically agitated bioreactor, shake flask and shaken micro-titre plate are used for test the methodology.
The miniature bioreactor was designed based on the overall dimensions of a single well of a 24-well plate. Computational fluid dynamics techniques are combined with model of mass transfer to provide a detailed description of flow and transport processes occurring in the miniature bioreactor. Power consumption and mass transfer predictions for the miniature bioreactor are compared a laboratory scale 20L bioreactor. Similar analysis is carried out for the shake flask and results compared with the miniature bioreactor. Finally, an analysis of flow and mass transfer is provided for the microtitre plate shaken under a range of operating conditions.
Chapter 2 Experimental materials, methods and Equipment

The present chapter provides information on materials, methods and bioreactors employed in this thesis. Section 2.1 and 2.2 describes the miniature bioreactor and online measurement method. Section 2.3 includes the shake flask and microwell bioreactors which are described in chapter 5 and chapter 6 respectively. The section 2.4 gives a brief introduction to the fermentation or cell culture method in the above bioreactors. The methods to measure the two important parameters, mass transfer coefficient and power consumption, in the above different bioreactors are presented.

2.1 Construction of the miniature bioreactor

The success of the design of an aerated agitated fermenter in 1947 opened a new area of biochemical engineering. This type of fermenter is still widely used in pharmaceutical and the new biotechnology industries. In the reactor, operational parameters, such as temperature, pH, substrate concentration and dissolved oxygen concentration, can be controlled. The miniature bioreactor was designed such that its geometry was similar to the large-scale fermenter, but the volume was scaled down to 6.5mL. Fig.2-1 shows the main elements of the miniature bioreactor together with some of its associated instrumentation and interconnections. The bioreactor was machined from square Plexiglas to allow visual inspection of mixing and gas bubbles.

The cylindrical chamber of the miniature bioreactor was 16 mm in diameter and 48 mm high (working volume of 6mL). The mixing of the contents of the bioreactor was achieved by means of three, 6-bladed open flat-turbine impellers, each having a diameter of 7.0 mm and width of 1.5 mm. The impellers were driven from the top of the bioreactor with a micro-fabricated electric motor (Smoovy, Switzerland) with a shaft diameter of 1 mm and an infinitely variable speed control (maximum speed and torque of 15,000 rpm and 2.2mNm, respectively). The bottom impeller was placed approximately 6mm from the base of the chamber, the distance between two impellers was 7.5 mm and the distance between the top impeller and the free liquid surface was 7.0 mm. The bioreactor was equipped with four baffles of width 1.8 mm and thickness 0.6 mm. The air from a compressed air supply was sparged through a perforated plastic cap placed at the discharge tip of a single tube of internal diameter 1.0 mm. The sparger
was placed directly beneath the hub of the bottom impeller (Fig.2-1).

The prototype of the second generation of the miniature bioreactor was built from stainless steel with parts that can be fully sterilised for cell culture experiments (Fig.2-2).

![Diagram of bioreactor components](image)

Fig.2-1 Main components of the miniature bioreactor. All measurements are in mm.
2.2 Online measurement in the miniature bioreactor

Air-water was used to establish the engineering performance of the miniature bioreactor for mass transfer operations and *E-coli* DH5α was chosen as a fermentation system because of its robustness and tolerance to contamination. Variables including air flow rate, dissolved oxygen concentration, pH, temperature and cell density were measured online in the miniature bioreactor.

2.2.1 Air flow rate

The air flow rate was measured using a standard laboratory rotameter with a flow rate in the range of 0.2 – 100 ml/min of air (Barnant Company, Illinois, USA). The gas supply had a separate connection via a two-way valve to a compressed nitrogen cylinder allowing mass transfer experiments to be carried out as described later in this section.
2.2.2 pH and temperature

Temperature was measured by means of a fine diameter copper-constantine thermocouple. pH of the solution or fermentation broth was measured by an optical probe using the method of Junker *et al.* (1988). The pH sensor consisted of: a fibre optic probe of diameter 1.5 mm (FCR-UV200F, Knight Optical Technologies Ltd, Surrey, UK); a xenon pulsed light source (XE-2000) and a blue LED light source (LED-475) coupled to a multiple channel spectrometer (MC2000). The fluorophor, 1-hydroxypyrene-3,6,8-trisulfonic acid trisodium salt (HPTS, Sigma-Aldrich, Dorset, England) with a pKa value of 7.3 was selected for the experiments described here. Two different excitation wavelengths of 405nm and 460nm were used. The fluorescence intensity was measured at a single emission wavelength of 520nm. A linear calibration curve was obtained in 20g/L of HPTS in water using the method of (Junker *et al.*, 1988) The spectrometer was used to monitor the change in fluorescence.

2.2.3 Dissolved oxygen concentration and \( k_l \),a measurement

On-line measurements of dissolved oxygen tension were performed by means of fibre optic probes. The oxygen sensor, the optrode (Fibre optic oxygen sensor, AVS-OXYKIT 1.5, Knight Optical Technologies Ltd, Surrey, UK) consisted of a 1 mm diameter silica glass optical fibre sealed in a 7 cm long stainless steel tube rod. The working tip of the probe was dip-coated with a ruthenium complex immobilised in a sol-gel matrix. An optical fibre carried light from a blue LED [470nm output peak] to the immobilised ruthenium /sol-gel coating layer at the working tip of the stainless steel tube. The 470 nm light excites the ruthenium complex to fluoresce. The level of this fluorescence is quenched in the presence of oxygen molecules, which diffuse through the sol gel matrix and interact with the trapped ruthenium. The reduction in the fluorescence signal is related to the concentration of oxygen through the Stern-Volmer equation (Wang *et al.*, 1999). The level of fluorescence signal was detected using a second fibre optic, part of the integrated optical probe and the fluorescence light was analysed by a sensitive CCD detector array grating spectrometer (Type: AVS- MC2000; Knight Optical Technologies Ltd; www.knightoptech.com). This has multiple spectrometer channels for other parallel spectral sensors, such as pH, turbidity, and other fluorescence investigations as described below. The primary channel for oxygen was used to monitor both LED light source excitation level and fluorescence emission simultaneously capturing full spectra in milliseconds.
KLa values were calculated from the dissolved oxygen concentration profiles obtained as a function of time by using the dynamic gassing out technique (Van’t Riet, 1979). Before each experiment, the optrode was calibrated at 100% and 0% air saturation by sparging air and nitrogen, respectively. A typical experiment started with fresh deionised water containing a known amount of sodium chloride salt. The oxygen concentration was continuously monitored as a function of time. In the miniature bioreactor, nitrogen was sparged till the level of oxygen had fallen to zero. At this point, the gas supply was switched rapidly to air set at a fixed flow rate of 1 vvm corresponding to a superficial velocity of 0.0005 ms$^{-1}$ and the rise in oxygen concentration was monitored.

2.2.4 Cell density

The measurement of cell density is achieved by combining the multiple optical fibre probes, as shown in Figure 1. Each fibre is fabricated from a single 600 micrometre active core diameter fibre. One fibre is used to deliver the light produced by a miniature tungsten light source to the bioreactor contents. The other two probes are used for light collection from the bioreactor, one for transmitted light (625 nm) and one for scattered light. This arrangement allows a range of measurements including turbidity, transmission/colour and nephelometry to be carried out by a multiple channel spectrometer (MC2000). In the present study the broth turbidity is sufficiently low to allow reliable cell density data to be obtained from the measurement of transmitted light (probes A and B).

2.3 Shake flask and microwell bioreactors

Specially manufactured Perspex wells were used to mimic one well of a 96 and 24 well plate (Whatman UniPlate®, Whatman Plc, Maidstone, Kent, U.K.). These wells allowed visual inspection and easy access for an optical oxygen probe. The Whatman plates are manufactured from polypropylene which is slightly more hydrophobic than Perspex. Polypropylene has a contact angle of 108° whereas Perspex has a contact angle of approximately 72°.

The chamber of the 24-well reactor had a square cross-section of dimensions 17.2 x 17.2 mm and a hemispherical base of radius 8.6 mm. The total depth of well was 40 mm giving a working volume of between 0.5 and 4 ml.
The shape of the 96-well reactor was a square cross section narrowing conically at the base. The upper section had dimensions of 8 x 8 mm and the total depth was 40 mm. The working volume of the vessel was between 0.2 and 0.5 mL.

The systems was shaken using an Eppendorf Thermomixer comfort (Eppendorf UK Limited, Cambridge, UK), which provided orbital shaking with a shaking diameter of 6 mm at between 300 and 1400 rpm.

The oxygen probe (as described in section 2.2.3) was positioned at the base of the Perspex well by with the active tip flush with the wall, which allowed to measure the area of lowest mass transfer.

2.4 Fermentation and cell culture

2.4.1 Fermentation in the miniature bioreactor and 20L bioreactor

All fermentations in the miniature bioreactor were carried out with *Escherichia coli*, DH5α in batch mode at 37°C, an air flow rate of 1vvm at impeller speeds 1300, 1500 and 1850 rpm. No attempt was made in these experiments to optimise the fermentation process, the aim was to demonstrate that fermentation was achievable in the miniature bioreactor and obtain basic engineering and fermentation parameters for comparison with conventional scale fermentation. Data for the latter were obtained in a 20 L (15 L working volume) LH20L03 HI-CAT series bioreactor (Adaptive Biosystems Ltd., Progress Business Park, Luton, UK). pH was fixed at the start of fermentation, but not controlled.

The cultures were grown on a semi-defined medium which consisted of, per litre, D-glucose (10 g), MgSO₄·7H₂O (1.2 g), (NH₄)₂SO₄ (4 g), KH₂PO₄ (13.3 g), citric acid (1.7 g), Na₂EDTA (8.4 mg), CoCl₂·6H₂O (2.5 mg), MnSO₄·4H₂O (15 mg), CuSO₄·2H₂O (1.5 mg), H₃BO₃ (3 mg), NaMoO₄·2H₂O (2.5 mg), ZnCl₂ (13 mg), Fe (III) citrate (100 mg), thiamine hydrochloride (4.5 mg; Sigma-Aldrich, Fancy Road, Poole, UK) and casamino acids (10 g/L; Oxoid). The pH of the medium was adjusted to 6.3 prior to sterilisation by addition of 4 M NaOH. All chemicals were obtained from BDH (Dorset, England) unless otherwise stated. Sterilisation of the miniature bioreactor was achieved by rinsing the equipment with 1M NaOH followed by sterile water. Seed culture was
prepared by inoculating 10ml of the medium in a McCartney bottle with a single colony from nutrient agar plates. The culture was allowed to grow overnight at 37°C and rotated at 200 rpm by a horizontal shaken platform in an incubator. 10ml of seed culture was used to inoculate 500ml of fresh medium, which was grown for 6 hours under the same conditions. The 500ml culture was used as the starting point for all experiments in the miniature bioreactor and the 20L fermenter. In the case of the miniature bioreactor, 0.6ml of the culture was used to inoculate 5.4ml of fresh medium in the bioreactor. A 10% inoculation was also used for the 20L fermentation. In the large fermenter foaming was controlled automatically by the addition of 100 % polypropylene glycol (PPG) pumped at a concentration of 0.1 mL/L. The 20L bioreactor was operated in the batch mode and the initial fermentation conditions were as follows: temperature, 37°C; air flow, 1 vvm (12 Lmin⁻¹); DO₂, 100%; agitator speed, 530 rpm; pH 6.3. The aeration and agitation were kept constant throughout the fermentation. Oxygen levels were monitored using an Ingold polarographic probe (Mettler-Toledo Ltd., Beaumont Leys, Leicester, UK). Online data were logged by Propack data logging and acquisition software (Acquisition Systems, Fleet, Hampshire, UK. Cultures were grown for 14 h.

2.4.2 Fermentation process at the 24-well reactor

The shaken fermentation was carried out in 24 well deep-square microwell plates (Whatman UniPlate®, Whatman Plc, Maidstone, Kent, U.K.). The bacterial strain *E. Coli* and the culture media were described in section 2.4.1. The shaking frequency was 1000 rpm and the shaking amplitude of 3mm. Parallel fermentations were conducted on the same plate to allow sampling at different time points.

2.5 Power consumption

A profile of energy dissipation rate was obtained from CFD analysis of the Navier-Stokes equation described the liquid velocity profile in the reactor. The power consumption in the miniature bioreactor and the 20L bioreactor was calculated as:

\[ P = \int r_a \rho_a e_a dV \]  
\( \text{(Eqn.2-1)} \)
The subscript of $\alpha$ referred to liquid phase, and $\tau$ was the liquid volume fraction. $\varepsilon$ was the energy dissipation rate, which was obtained from k-$\varepsilon$ turbulent model.

The power consumption in the shake flask and the microwell reactor was assumed to be equal to the viscous dissipation rate, obtained from the following relationship:

$$P = \int_{V} \mu \Phi_{\nu} dV$$  \hspace{1cm} (Eqn.2-2)

where $\mu$ was the liquid viscosity. In Eqn.2-2, the viscous dissipation function, $\Phi_{\nu}$, was expressed in terms of shear rates as follows:

$$\Phi_{\nu} = 2 \left[ \left( \frac{\partial u}{\partial x} \right)^2 + \left( \frac{\partial v}{\partial y} \right)^2 + \left( \frac{\partial w}{\partial z} \right)^2 \right] + \left( \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right)^2 + \left( \frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right)^2 + \left( \frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right)^2$$  \hspace{1cm} (Eqn.2-3)
Chapter 3 Theoretical analysis

3.1 Introduction

In this chapter, the general governing equations for the single phase and two-phase flow are presented. The turbulent flow model, two-fluid model, free-surface flow model and population balance model are described.

3.2 Turbulent single-phase flow

3.2.1 Governing equations

CFD provides solution of the fundamental governing equations of flow in complex geometries. The continuity, momentum and energy equations, which are also known as Navier-Stokes equations, are solved numerically. Briefly, the governing equations describing the instantaneous behaviour of turbulent liquid flow are three momentum transport equations for velocity components U, V, W respectively and a continuity equation. They are summarized below:

\[ \frac{\partial \rho}{\partial t} + \nabla \cdot (\rho U) = 0 \]  \hspace{1cm} (Eqn.3- 1)

and

\[ \frac{\partial \rho U}{\partial t} + \nabla \cdot (\rho U \otimes U) = -\nabla p' + \nabla \cdot \tau + B \]  \hspace{1cm} (Eqn.3- 2)

The velocities in Eq.3-1 and Eq.3-2 are defined as follows:

\[ U = \tilde{U} - U' \]  \hspace{1cm} (Eqn.3- 3)

Here, \( U \) is the velocity that is resolved in the numerical simulations, \( \tilde{U} \) is the instantaneous velocity and \( U' \) is the unresolved part of the numerical simulations. The interpretation of the terms \( U \) and \( U' \) depends on the method of derivation. If Eqn.3-1 and Eqn.3-2 are derived through the Reynolds ensemble averaging, \( U \) and \( U' \) represent the mean velocity and the fluctuating velocity. When Eqn.3-1 and Eqn.3-2 are obtained through a filtering operation, for example large eddy simulation, these terms are respectively the grid scale (GS) and the sub-grid scale (SGS) velocities.
The stress term is

\[
\tau = \mu_{\text{eff}} (\nabla U + (\nabla U)^\tau - \frac{2}{3} I(\nabla U)) \tag{Eqn.3-4}
\]

where \( \mu_{\text{eff}} \) is the effective viscosity of liquid, which is composed of two components: the molecular viscosity and the turbulent viscosity.

\[
\mu_{\text{eff}} = \mu + \mu_T \tag{Eqn.3-5}
\]

The modified pressure \( p' \) is:

\[
p' = p + \frac{2}{3} \rho k + \left( \frac{2}{3} \mu_{\text{eff}} - \zeta \right) \nabla \cdot U - \rho_g \mathbf{g} \cdot \mathbf{x} \tag{Eqn.3-6}
\]

3.2.2 k-\( \varepsilon \) model

The standard k-\( \varepsilon \) model, as presented by Launder and Spalding (1974), is by far the most widely used two-equation eddy viscosity model. This model has been widely used in stirred tank reactors (Brucato et al., 1998; Kresta and Wood, 1991; Ranade and Joshi, 1990). The instantaneous velocity components and the pressure can be replaced by the sum of a time-averaged mean component and a root-mean-square fluctuation component according to the model. The eddy viscosity model relates the individual Reynolds stresses to mean flow gradients:

\[
\rho u_i' u_j' = -\rho \mu_T \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) + \frac{2}{3} \rho \delta_{ij} k \tag{Eqn.3-7}
\]

The turbulent eddy viscosity \( \nu_t \) is calculated from:

\[
\mu_T = C_\mu \frac{k^2}{\varepsilon} \tag{Eqn.3-8}
\]

where \( C_\mu \) is a parameter which depends on the specific k-\( \varepsilon \) model.

The transport equations for the turbulence kinetic energy and turbulence dissipation rate \( \varepsilon \) are calculated from their conservation equations:

\[
\frac{\partial k}{\partial t} + \nabla \cdot (\rho U k) - \nabla \cdot \left( (\mu + \frac{\mu_T}{\sigma_k}) \nabla k \right) = P + G - \rho \varepsilon \tag{Eqn.3-9}
\]
\[ \frac{\partial \rho \varepsilon}{\partial t} + \nabla \cdot (\rho \mathbf{U} \varepsilon) - \nabla \cdot (\mu + \frac{\mu_t}{\sigma_z} \nabla \varepsilon) = C_1 \frac{\varepsilon}{k} (P + C_3 \max(G,0)) - C_2 \rho \frac{\varepsilon^2}{k} \]  
(Eqn.3-10)

respectively where \( P \) is the shear production defined by:

\[ P = \mu_{eff} \mathbf{VU} \cdot (\nu \mathbf{U} + (\nabla \mathbf{U})^T) - \frac{2}{3} \nabla \cdot \mu_{eff} \mathbf{VU} + pk \]  
(Eqn.3-11)

and \( G \) is production due to the body force defined by:

\[ G = G_{buoy} + G_{rot} + G_{res} \]  
(Eqn.3-12)

where \( G_{buoy}, \ G_{rot} \) and \( G_{res} \) are terms representing production due to buoyancy, rotation and resistance respectively. Assuming \( G = G_{buoy}, G \) is defined by:

\[ G = -\frac{\mu_{eff}}{\rho \sigma_p} \mathbf{R} \nabla \rho \]  
(Eqn.3-13)

3.2.3 Large eddy simulation

Large eddy simulation (LES) is becoming more popular in the simulation of turbulent flow. LES is based on spatial filtering of the equations of motion rather than time averaging used in traditional turbulence modelling like k-\( \varepsilon \) model. The space filtering of a function \( f(x_i,t) \) is defined as

\[ \overline{f(x_i,t)} = \int_{-\infty}^{\infty} G(x_i-x_i') f(x_i',t) dx_i' \]  
(Eqn.3-14)

where \( G \) is a filter function.

The model of LES proposed by Smagorinsky (1963) is employed. The turbulent viscosity or the SGS viscosity is formulated as follows:

\[ \mu_t = \rho (C_s \Delta)^2 |\mathbf{S}| \]  
(Eqn.3-15)

where \( C_s \) is a model constant with a value of 0.1 and \( \mathbf{S} \) is the characteristic filtered rate of strain. \( \Delta \) is the filter width and is defined by:

\[ \Delta = (\Delta_x \Delta_y \Delta_z)^{1/3} \]  
(Eqn.3-16)
3.3 Turbulent gas-liquid flow

3.3.1 Governing equations

The numerical model of the turbulent gas-liquid flow is based on two-fluid model, where transport equations governing conservation of mass, momentum are derived for each phase simultaneously. The transport equations for mass and momentum are formulated for each phase in volume-averaged form:

\[
\frac{\partial a \rho_a}{\partial t} + \nabla \cdot (a \rho_a U_a) = \nabla \cdot (\Gamma_a \nabla (a + r_a)) + \sum_{\beta=1}^{N_p} (m_{a\beta} U_\beta - \dot{m}_{a\beta} U_a)
\]  
Eqn. (3-17)

\[
\frac{\partial a \rho_a U_a}{\partial t} + \nabla \cdot (a (\rho_a U_a \otimes U_a - \mu_a (\nabla U_a + (\nabla U_a)^T)))
= r_a (B - \nabla \rho') + r_a S_a + \sum_{\beta=1}^{N_p} C_{a\beta}^{(a)} (U_\beta - U_a) + \sum_{\beta=1}^{N_p} (m_{a\beta} U_\beta - \dot{m}_{a\beta} U_a)
\]  
Eqn. (3-18)

The phase \(a\) denotes the continuous phase liquid phase and \(\beta\) denotes the dispersed gas phase.

The term \(m_{a\beta} U_\beta - \dot{m}_{a\beta} U_a\) in the Eqn. 3-17 and Eqn. 3-18 only arises if inter-phase mass transfer takes place. \(m_{a\beta}\) is the mass flow rate per unit volume into phase \(a\) from phase \(\beta\), which is assumed to be is zero in this study.

The volume fractions sum to unity:

\[
\sum_{\alpha=1}^{N_p} r_\alpha = 1
\]  
(Eqn. 3-19)

The general variable \(\phi\) can be expressed:

\[
\frac{\partial a \rho_a \phi_a}{\partial t} + \nabla \cdot (a \rho_a U_a \phi_a - \Gamma_a \nabla \phi_a) = r_a S_a + \sum_{\beta=1}^{N_p} C_{a\beta}^{(a)} (U_\beta - U_a) + \sum_{\beta=1}^{N_p} (m_{a\beta} U_\beta - \dot{m}_{a\beta} U_a)
\]  
(Eqn. 3-20)

The second term of right sides for the equation accounts for the inter-phase transfer of \(\phi\) between two phases, which is explained in detail in the following.
3.3.2 Interface model

A bubble moving in a liquid is subjected to several forces caused by the liquid flow, including drag force, acting in the opposite direction to the fluid motion relative to the bubble, and lift force normal to the liquid motion, virtual mass effects. Drag force is the most important of the forces. The gas and liquid momentum equations are then coupled by the interfacial force terms, which can be given in terms of the drag coefficient $C_d$,

$$
F_a = -F_b = 0.5 r_a \rho_a C_d A \left| U_\beta - U_\alpha \right| (U_\beta - U_\alpha)
$$

(Eqn.3-21)

where $A$ is the projected area of the bubbles per unit volume and $C_d$ is the drag coefficient. Assuming the spherical bubbles, the projected area is expressed by:

$$
A = n_b \frac{\pi d_b^2}{4}
$$

(Eqn.3-22)

And the number of bubbles per unit volume is defined by

$$
n_b = \frac{6 r_\beta}{\pi d_b^3}
$$

(Eqn.3-23)

Therefore, the interfacial force can be rewritten for the Eqn.3-21 as

$$
C_{\alpha\beta} = \frac{3}{4} \frac{C_d}{d} r_\beta \rho_\alpha \left| U_\beta - U_\alpha \right|
$$

(Eqn.3-24)

d is gas bubble diameter, which is determined from population balance model, $\left| U_\beta - U_\alpha \right|$ is slip velocity.

The drag coefficient $C_d$ is determined from the modified Reynolds number

$$
Re = \frac{\rho_\alpha d}{\mu_m} \left| U_\alpha - U_\beta \right|
$$

(Eqn.3-25)

where

$$
\frac{\mu_m}{\mu_\alpha} = \left(1 - \frac{r_\beta}{r_{fm}}\right)^{-2.5 r_{fm} \frac{\mu_\beta + 0.4 \mu_\alpha}{\mu_\beta + \mu_\alpha}}
$$

(Eqn.3-26)

$r_{fm}$ is the maximum attainable value for the volume fraction of the disperse phase, set to 1.0 for gas bubble (Kuo & Wallis, 1988).

For gas bubble diameters are greater than 1.8mm and the Newton and distorted particle
regimes, the Ishii and Zuber (1979) modification takes the form of a multiplying factor to the drag factor. The factor is given in terms of the volume fraction of the disperse phase by:

\[
E = \left( \frac{1+17.67 f(r_\beta)^7}{18.67 f(r_\beta)} \right)^2, \quad C_D = 0.44 \ E \tag{Eqn.3-27}
\]

where 

\[
f(r_\beta) = \frac{\mu_a}{\mu_m} (1-r_\beta)^{0.5} \tag{Eqn.3-28}
\]

For the spherical cap regime, the Ishii and Zuber modification also takes the form of a multiplying factor:

\[
E = (1-r_\beta)^2, \quad C_D = \frac{8}{3} \ E . \tag{Eqn.3-29}
\]

3.3.3 Population balance model

Mass transfers in a fermenter between gas and liquid are directly related to the interfacial area and the residence time of the gas phase. To predict the size distribution of the gas bubbles taking full account of the breakage and coalescence effects is therefore very valuable in optimising operation of existing reactors and evaluating engineering performance of the miniature bioreactor.

In many papers about CFD calculations of dispersed two-phase flows, the particles are assumed to have the same size and shape, i.e. the mono-disperse assumption. In reality, a wide spectrum of particle sizes and shapes exist at very point. Particularly, in gas-liquid flows gas bubbles with different sizes and shapes have different forces, which result in different velocities and volume fraction distributions for gas phase. The differences of volume fraction distribution and velocities have an effect on the gas bubble sizes and shapes too. It is important to take breakage and coalescence effects into account and predict the local gas bubble sizes distribution.

Population balance is a well-established method used to analyse the size distribution of the dispersed phase and accounting for the breakage and coalescence effect. A general form of the population balance equation is:
The theoretical model developed by Luo and Svendsen (1996) for break-up of bubbles in turbulent dispersion is employed. The model is based on the theories of isotropic turbulence and probability but significantly the model contains no unknown or adjustable parameters. Binary break-up is assumed. The break-up rate of bubbles of volume $V_j$ into bubbles of volume $V_i$ is expressed as:

$$g(V_j : V_i) = f_b 0.923(1-r_p)(\frac{\xi}{d_j})^3 \int_{\xi_{\text{min}}}^{\frac{11}{11}} (1+\xi)^2 e^{-\chi_c \xi} d\xi$$  \hspace{1cm} (Eqn.3-31)

where

$$\chi_c = \frac{12[f_{BV}^2 + (1-f_{BV})^2 -1]a}{\beta \rho_d \epsilon^2 d_j^5 \xi^3}$$  \hspace{1cm} (Eqn.3-32)

$\xi$ is the dimensionless size of eddies in the inertial subrange of isotropic turbulence and $\chi_c$ is the critical dimensionless energy for break-up. The coefficient $f_b$ is added for calibration of the model.

The birth rate of group-i particles due to break-up of large particles is:

$$B_b = \sum_{j=1}^{N} g(V_j : V_i) n_j$$  \hspace{1cm} (Eqn.3-33)

where $N$ is the total number of particle groups. The death rate of group-i particles due to break-up to smaller particles is:

$$D_b = g(V_i : V_j f_{BV}) n_i$$  \hspace{1cm} (Eqn.3-34)

The coalescence of two bubbles is often assumed to occur in three steps. First the bubbles collide trapping a small amount of liquid between them. This liquid film then drains until the liquid film separating the bubbles reaches a critical thickness. The film ruptures and the bubbles join together. The coalescence process is therefore modeled by a collision rate of two bubbles and a collision efficiency relating to the time require for
coalescence ($t_{\text{co}}$) and the contact time ($\tau_{\text{co}}$). Prince and Blanch (1990) considered the collisions resulting from three different mechanisms, turbulence ($\theta_{ij}^T$), buoyancy ($\theta_{ij}^B$) and laminar shear ($\theta_{ij}^{LS}$). The total coalescence rate is:

$$Q_{ij} = (\theta_{ij}^T + \theta_{ij}^B + \theta_{ij}^{LS}) e^{(-t_{ij}/\tau_{co})}$$  \hspace{1cm} (Eqn.3-35)

The birth rate of group-i particles due to coalescence of group-j and group-k particles is:

$$B_{ij} = \frac{1}{2} \sum_{j=1}^{i} \sum_{k=1}^{j} Q_{jk} n_j n_k$$  \hspace{1cm} (Eqn.3-36)

The death rate of group-i particles due to coalescence with other particles is:

$$D_{ij} = n_i \sum_{j=1}^{N} Q_{ij} n_j$$  \hspace{1cm} (Eqn.3-37)

In the miniature reactor, a simplified model is adopted to estimate the average bubble size from the following correlation (Wilkinson et al., 1993):

$$d_b = \left( \frac{We_c \sigma}{\rho_a} \right)^{0.6} e^{-0.4}$$  \hspace{1cm} (Eqn.3-38)

The critical Weber number, $We_c$, in Eqn.3-38 was assumed to have a value equal to 0.6 (Hinze, 1955). The energy dissipation term, $\varepsilon$, in the Eqn.3-38 was based on flow conditions around the impeller blades, and considering the small size of the miniature bioreactor, bubble coalescence was assumed to be negligible.

3.4 Gas-liquid free-surface flow

3.4.1 Governing equations

The presence of a free surface at the interface introduces specific mathematical complications which were resolved by the Volume of Fluid (VOF) method. The VOF method (Hirt and Nichols, 1981; Gueyffier et al., 1999; Scardovelli and Zaleski, 1999) calculated the transient motion of the gas and liquid phases using Navier-Stokes equations, and account for the topology changes of the gas-liquid interface. The VOF method defines a fractional volume or ‘color function’ $r(x,t)$ that indicates the fraction of the computational cell filled with liquid. The colour function varies between 0, if the cell is completed filled by gas, and 1, if the cell consists only of the liquid phase. The location of the gas-liquid interface is tracked in time by solving a balance equation for
this function:

\[
\frac{\partial r(x, t)}{\partial t} + \nabla \cdot (ur(x, t)) = 0
\]  
(Eqn.3-39)

The liquid and gas velocities are assumed to equilibrate over a very small distance and the mass and momentum conservation equations can be considered to be homogeneous.

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{U}) = 0
\]  
(Eqn.3-40)

\[
\frac{\partial \rho \mathbf{U}}{\partial t} + \nabla (\rho \mathbf{U} \cdot \mathbf{U}) = -\nabla p - \nabla \cdot \mathbf{\tau} + F_s
\]  
(Eqn.3-41)

where \( p \) is pressure, \( \mathbf{\tau} \) is the viscous stress tensor. The density and viscosity used in Eqn. (3-40) and (3-41) are defined as

\[
\rho = r_L \rho_L + r_G \rho_G , \quad \mu = r_L \mu_L + r_G \mu_G
\]  
(Eqn.3-42)

3.4.2 Continuum Surface Force Model

The continuum surface force (CSF) model, originally proposed by Brackbill et al. (1992), is employed to model the force due to surface tension acting on the gas-liquid interface. In this model, the surface tension is modeled as a body force \( F_s \):

\[
F_s = \sigma \kappa(x) r(x, t)
\]  
(Eqn.3-43)

Where \( \sigma \) is the surface tension coefficient, and \( \kappa(x) \) is the surface curvature defined by:

\[
\kappa = \frac{1}{|n|} \left( \left( \frac{n}{|n|} \cdot \nabla \right) |n| - \nabla \cdot n \right)
\]  
(Eqn.3-44)

where \( n \) is a vector normal to the surface given by:

\[
n = \nabla r(x, t)
\]  
(Eqn.3-45)
3.5 Numerical solution

3.5.1 Finite volume method

The above governing equations are solved by finite difference, finite element, and finite volume methods. The third one is probably the most popular numerical discretisation method used in CFD, especially in commercial software packages. The conservation of a general flow variable $\phi$ within a finite control volume can be expressed as a balance between the various processes tending to increase or decrease it, illustrated in below:

\[
\begin{bmatrix}
\text{Rate of change} \\
\text{\phi in the control volume with respect to time}
\end{bmatrix} =
\begin{bmatrix}
\text{Net flux of \phi due to convection into the control volume} \\
\text{Net flux of \phi due to diffusion into the control volume} \\
\text{Net rate of creation of \phi inside the control volume}
\end{bmatrix}
\]

(Eqn.3-46)

Eqn.3-46 is used as starting point for computational procedures in the finite volume method.

3.5.2 CFX software package

The CFD software used in my project is the CFX-4 developed by AEA Technology, Oxfordshire, United Kingdom. CFX4 is a commercial suite of CFD software with multi-phase capabilities. Computational grid is based on the unstructured set of blocks each containing structured grid. The structure grid within each block is generated using general curvilinear coordinates ensuring accurate representation of the flow boundaries. Discretization of the partial differential equations is based on the conservative finite volume method. Non-staggered grid is employed where all variables are evaluated at the geometrical centres of control volumes. Velocities required at the cell faces are calculated by applying an improved Rhie and Chow’s (1983) interpolation algorithm. Transport variables such as diffusion coefficients and effective viscosities are evaluated and stored at the cell faces. The differencing schemes used for the convective term in this project are the high order QUICK scheme or high hydrid scheme for velocities. The SIMPLEC algorithm was used for pressure correction in order to reduce the number of global interactions for convergence. The quadratic, fully implicit backward scheme has been used for time differencing. It provides also many subroutines for the users to accommodate the boundary conditions or other problems of concern.
Chapter 4 Engineering characterisation of the miniature bioreactor and scale-up to 20L bioreactor

4.1 Introduction

The engineering performance of bioreactors is affected by many variables, including impeller configuration, rotational speed, air sparging and the physical properties of the process fluid. Evaluation of the engineering performance may be made from mixing conditions, power consumption and mass transfer rate in the vessel. As the miniature reactor size is very small, conventional experimental techniques for power consumption and flow parameters are very difficult to use. In the present thesis, CFD analysis is employed to provide the power input information, which is coupled with a mass transfer model to map the mass transfer profile. The predictions are then compared with the experimental data. The experimental data are obtained from the dissolved oxygen concentration measured by micro-fabricated fibre optic probes (Junker et al., 1988; Wang et al., 1999; Lamping et al., 2003). The single phase flow in the miniature bioreactor is discussed in the appendix 1. The two-phase flow in a laboratory scale (20L) bioreactor is analyzed using the source-sink technique and two-fluid model presented in appendix 2. The CFD predictions are compared with the experimental observations and previous publications.

4.2 CFD characterisation of two-phase flow in the miniature bioreactor

The simulation is based on the governing equations described in chapter 3. The source-sink technique is employed to simulate the movement of the impellers and the stationary baffles. The k-ε model is used to capture the turbulent characteristics. As the reactor is very small, gas bubble breakage is assumed to prevail and coalescence is assumed to be negligible. A simplified equation (Eqn.3-39) is used to describe the bubble size in the impeller sweeping region while out of this region, the bubbles are assumed to keep the same size until bursting out at the top surface.

4.2.1 Computational domain and boundary conditions

The miniature reactor used has a diameter, T=16mm, and is equipped with three-impeller six-bladed paddle turbine and four baffles equally around the periphery. The shaft of impellers was concentric with the axis of the reactor. Other key dimensions are: impeller diameter $D_i = 7\text{mm}$; blade height, $0.875\text{mm}$; hub diameter, $1.6\text{mm}$; and the
baffle width, 1.6mm. The Height of the calculation domain, H = 32mm so that the working volume is 6.5 ml. Cylindrical coordinate system is used with the origin located at the centre of the impeller centre. To reduce the computational requirement, the flow domain in the miniature reactor is divided into two symmetric parts (each encompassing two baffles and three impeller blades. The extension of the approach to simulate the complete flow for the whole domain is straightforward). The grid resolution is chosen so that the impeller is described with three control cells in axial direction resulting in a grid of 57 × 55× 60 in axial, radial and tangential directions, respectively (Fig. 4-1).

The reactor walls and baffles are defined as impermeable walls with friction. The impeller blades are defined as stationary solids. For the gas phase, at the inlet of gas (at the orifices of gas sparger), the normal component of the velocity is specified for a given flow rate (1 vvm in this study). The other velocity components are assumed to be zero. Here no outflow of gas or liquid is allowed. At the top surface of the reactor as gas outlet, the velocities are determined from a mass balance based on the specified inlet conditions, but for the liquid, a free surface condition is assumed. Across an axis of symmetry, there are no convective or diffusive fluxes. Accordingly, the velocity normal to the boundary is set as zero for both phases. The gradients of all other variables are set equal to zero.

The computations are carried out for the impeller rotational speed of 1000, 2000, and 4000 rpm. The impeller tip speed is 0.37m/s, 0.733m/s, and 1.47m/s and the Reynolds number is 809, 1633, and 3267 respectively.

The air-water system is investigated for the report, the fluid properties are set as viscosity = 0.001 kg/(ms), density = 1000 kg/m$^3$ for water and viscosity = 1.0×10$^{-5}$ kg/(ms) and density = 1.29 kg/m$^3$ for air.
Fig. 4-1 The geometry, dimension, and simulation grids for the miniature bioreactor.
4.2.2 Gas volume fraction profile

CFD analysis of gas flow produces the gas volume fraction profile in the vertical plane between two baffles at different rotational speeds. It is well known that the gas flow patterns depend on the relative rates of gas input and stirring. When the rotational speed is low (1000 rpm and 1 vvm) (Fig. 4-3, a), the gas phase is not well dispersed and the flow is dominated by air flow up the stirred shaft. The lower impeller is in “flooding” condition, the middle and upper one are in the “ineffective dispersion” condition (Abardi et al., 1990). When the rotational speed is increased to 2000 rpm (Fig. 4-3, b), the bottom impeller is still in “flooding”, but part of the gas phase is dispersed by the middle and upper impeller although some of gas still flows along the shaft. When the rotational speed increases to 4000 rpm (Fig. 4-3, c), the gas phase is well dispersed by three impellers. A meandering of the gas fraction field is distributed in the bulk of fluid, and along the wall, some regions are free of gas phase because of the liquid secondary circulation loop. The region along the shaft is found free of gas phase as well. The evolution of gas volume fraction as a function of the rotational speed and the flow patterns in the vessel is shown in Fig. 4-2 (Nienow et al., 1978). The minimum agitator tip speed for dispersion of air bubbles has been estimated by Westerterp et al. (1963) to be approximately 1.5–2.5 m/s. At the rotational speed of 4000 rpm, the impeller tip speed is 1.47 m/s, which is very close to the above value.

![Diagram of gas flow in a stirred aerated bioreactor](image)

**Fig. 4-2** Gas flow pattern in a stirred aerated bioreactors as a function of impeller speed and gas flow rate (from Nienow et al., 1978)
Fig. 4-3 Gas volume fraction in the vertical midplane between two baffles under the aeration condition of 1 vvm at rotational speeds of (a) 1000 rpm (b) 2000 rpm (c) 4000 rpm
4.2.3 Liquid flow patterns

The effect of air sparging on liquid flow patterns can be seen in Fig. 4-4. At a rotational speed of 1000 rpm (a), the gas moves upward with no obvious dispersion by the impellers. Compared to single (liquid) phase flow (Fig. A1-9, page 191), the liquid motion is affected by the air flow. For 1000 rpm (a) and 2000 rpm (b) the ring vortices below the impellers become smaller than for the case of single phase and no vortex above the impellers is seen. The liquid above the middle and upper impeller moves upward rather than downward. As the rotational speed increases to 4000 rpm, the liquid flow patterns changes as well. Ring vortices below and above the impellers are observed and the liquid just above the impellers moves downward.

4.2.4 Liquid energy dissipation

The turbulent intensities are also affected by the gas flow patterns. At the rotational speed of 1000 rpm, the highest energy dissipation is distributed in the vicinity of the impeller tips and just above the middle and upper impeller. As the rotational speed increases to 4000 rpm, the energy dissipation increases and at the same time the highest energy dissipation is radially distributed. The average power input is obtained by integrating the local energy dissipation over the working liquid and it is compared with the unaerated conditions (see appendix 1) and published data. The simulation results give a ratio of gassed to ungassed power, $P_g/P$, of 0.8-0.9. The reported ratio of $P_g/P$ varies between 0.9 and 0.4 depending on the type of impeller and gas flow number (Smith et al., 1977).
Fig. 4-4 Liquid flow patterns in the vertical plane between two baffles under aeration rate of 1 vvm at rotation speeds of (a) 1000 rpm, (b) 2000 rpm, (c) 4000 rpm.
Fig. 4-5 Liquid energy dissipation profile in the vertical plane between two baffles under aeration conditions at rotational speeds of (a) 1000 rpm (b) 2000 rpm (c) 4000 rpm.
The energy dissipation rate around the impeller blades is higher than in the bulk zone, which agrees well with experimental measurements (Wernersson and Tragardh, 1999; Wu and Patterson, 1989). The highest energy dissipation rate is two orders of magnitude greater than the “whole” tank averaged energy dissipation, and in agreement with reported observations (Calabrese and Stoots, 1989; Wemnersson and Tragardh, 1999). This heterogeneity of energy dissipation rate leads to detrimental effects on cell growth. Cell damage is related to high rates of energy dissipation (Maguire et al., 2003). Aloi and Cherry (1996) argued that energy dissipation rates rather than shear rate or shear stress should be used to quantify cell damage in stirred tanks. They reported that in a 1L stirred bioreactor equipped with a Rushton impeller, Sf-9 insect cells exhibited a calcium response (an indicator of cell damage) beginning at the local energy dissipation rate greater than 10 m²/s³. The maximum energy in the miniature bioreactor is in the same order of magnitude for rotational speeds greater than 1000 rpm.

4.2.5 Mass transfer coefficient profile
The penetration model proposed by Higbie (1935) has been used previously to describe mass transfer in air-lifts (Ayazi Shamlo et al., 1995) and mechanically agitated mixing vessels (Kawase and Moo Young, 1990). According to Higbie's model, mass transfer at a gas-liquid interface is assumed to occur by a series of encounters between the liquid and the gas. Each encounter lasts for only a short time so that steady-state conditions are never established and any mass transfer that occurs is due to the unsteady molecular diffusion. The encounters are made by turbulent fluctuations in the bulk fluid. In the bulk fluid, there exists a whole range of eddy sizes between the largest eddies (in the same order of magnitude of the impeller diameter) and the smallest eddies (the scale is called the Kolmogorov length scale) and this is usually represented by an energy spectrum. The molecular diffusion takes place at the Kolmogorov scale. The length of the smallest eddies depends on the energy dissipation rate and is given by:

\[ l_k = \left( \frac{\nu^3}{\varepsilon} \right)^{\frac{1}{3}} \]  
(Eqn.4-1)

The velocity of the smallest eddies is assumed to be:

\[ u_s = 1.4 (\varepsilon \cdot l_k)^{\frac{1}{3}} \]  
(Eqn.4-2)

Therefore, combining the Eqn.4-1 and Eqn.4-2, the surface renewal time for each encounter at smallest size is:
Lamont and Scott (1970) analyzed the energy spectrum of eddies at different sizes and proposed the following equation for the local liquid phase mass transfer coefficient, $K_L$:

$$K_L(r, z, \theta) = 0.4 [\varepsilon (r, z, \theta) \nu]^{1/2} \left( \frac{\varepsilon}{\nu} \right)^{1/2}$$  

(Eqn. 4-4)

Where the local energy dissipation rate, $\varepsilon$, is obtained from the CFD simulation of flow in the vessel. The coefficient of 0.301 is recommended by Kawase and Moo Young (1990) for the mechanical stirring tank, which is also employed in the study.

The local specific surface area available for mass transfer is given by:

$$a(r, z, \theta) = \frac{6r_p(r, z, \theta)}{d_b}$$  

(Eqn. 4-5)

where $r_p$ is the local gas volume fraction. We used Eq 4-3 and 4-4 to predict the $K_La$ distributions in the miniature bioreactor. To compare these predictions with experimental data, the overall (average) volumetric mass transfer coefficient was calculated by integrating the local values over the entire working volume of the miniature bioreactor. Thus:

$$K_{La} = \frac{\int K_L(r, z, \theta) a(r, z, \theta) dv}{V}$$  

(Eqn. 4-6)

The local and overall $K_{La}$ are predicted for a range of impeller speeds at an operational airflow rate of 1 vvm used in our experiments. The local mass transfer coefficient profiles are presented in Fig. 4-6. At low rotational speed (1000 rpm), the mass transfer coefficient is determined by the local gas holdup rather than by the energy dissipation. The homogeneity of the mass transfer distribution is very poor and the majority of the reactor suffers from low dissolved oxygen concentration. But this poor distribution can be improved by increasing rotational speed. At a rotational speed of 4000 rpm, since the gas phase is well dispersed, the mass transfer in the reactor becomes more homogeneous. This indicates that above a critical rotational speed, a well-mixed model can be considered for measurement of dissolved oxygen concentration. However, it is still seen that there is poor mixing and mass transfer at the bottom part of the reactor.
Fig. 4-6 Mass transfer coefficient profile in the vertical plane between two baffles under aeration rate of 1 vvm at rotational speeds of (a) 1000 rpm, (b) 2000 rpm, (c) 4000 rpm
For distilled water an average value of $4 \times 10^4$ ms$^{-1}$ has been recommended for the liquid mass transfer coefficient, $K_L$, and CFD analysis gives the value of the same of order of magnitude. Solution ionic strength is known to increase the overall volumetric mass transfer coefficient, $K_{L,a}$, partly through its impact on interfacial area, $a$ (Van't Riet, 1979). The effect of ions on $K_{L,a}$ however is not easy to predict, according to experimental information it varies with the type of ions present in solution and is dependent on the prevailing fluid energy dissipation rates. Van't Riet's (1979) analysis of the published experimental data indicate that such uncertainties coupled with different measurement techniques mean that reported empirical correlations are likely to vary in their estimations of $K_{L,a}$ by up to ±40%.

The performance of the miniature bioreactor is assessed initially under defined flow conditions through a series of experiments carried out with air-water (Lamping, 2003). The results from these experiments also allow a basis for comparison with conventional bioreactors for which many correlations are available (Smith et al., 1977; Van’t Riet, 1979). The experimental $K_{L,a}$ values are based on the well-mixed model for both the gas and liquid phases, as suggested by Dunn and Einsele (1975). The well-mixed model is considered appropriate for this case as discussed above. According to this model, $K_{L,a}$ is obtained from the oxygen probe response data by the following expression:

$$K_{L,a} = \frac{1}{t} \cdot \ln \left( \frac{C^*}{C^* - C} \right) = \frac{1}{t} \cdot \ln \left( \frac{1}{C_L} \right)$$

(Eqn.4-7)

where $C_L$ is a normalized oxygen concentration defined by: $C_L = (C^* - C)/C^*$.

The optrode used in the present investigation has a response time, $\tau_p$ (the time needed to record 63% of a stepwise change), of 42s at 20°C, measured using a standard procedure described elsewhere (Dunn and Einsele, 1975). CFD predictions give an overall volumetric mass transfer coefficient, $K_{L,a}$, in the miniature bioreactor typically in the order of 100h$^{-1}$ (0.03 s$^{-1}$) indicating that the impact of the probe response time on $K_{L,a}$ was an important consideration. A first order response model recommended by Badino et al. (2001) was used to account for the fibre optic probe response time. Thus,

$$\frac{dC_p}{dt} = \frac{1}{\tau_p} \left( C_L - C_p \right)$$

(Eqn.4-8)
where $C_p$ is the normalized dissolved oxygen concentration measured by the probe. Substituting for $C_L$ using Eqn.4-7, integrating and rearranging gives the following expression for $K_{L,a}$:

$$C_p = \frac{1}{t_m - \tau_p} \left[ t_m \cdot \exp \left( - \frac{t}{t_m} \right) - \tau_p \cdot \exp \left( - \frac{t}{\tau_p} \right) \right]$$

(Eqn.4-9)

where $t_m = 1/K_{L,a}$. Eqn.4-9 is solved for $K_{L,a}$ using Microsoft Excel at each time measurement and the results averaged. $K_{L,a}$ data are obtained for air-water in the presence of sodium chloride for a range of impeller speeds.

\[
K_{L,a} = 0.002 (P/V)^{0.7} \nu_s^{0.2}
\]

Fig. 4-7 Overall volumetric mass transfer coefficient as a function of the impeller power input per unit volume. The plots are for the miniature bioreactor and refer to air-water system: (■) are experimental data and (●) are the values obtained from CFD simulations. The solid line is the relationship reported by Van't Riet (1977) based on a comprehensive review of $K_{L,a}$ data for Rushton turbine impellers. The dashed lines are the ± 40% deviation from Van't Riet’s equation due to experimental uncertainties and different measurement techniques.
Fig. 4-7 shows the results plotted as a function of the mean energy dissipation rate in the miniature bioreactor. For comparison, CFD simulated values are compared with the experimental data. The continuous line in Fig. 4-7 shows the predicted $K_{L_a}$ based on the correlation of Van't Riet (1979). The slope of the best line of fit (dashed-line) through the data points has a slope which agrees well with the 0.7, which is the exponent of $(P/V)$ in the equation reported by Van't Riet (1979). However, the absolute values of $K_{L_a}$ measured in our experiments are consistently lower by approximately 40% compared to those expected from Van't Riet's expression. The difference is thought to be due to the differences in the vessel-impeller configuration between the two systems. Van't Riet's equation is based on data obtained for standard a single Rushton turbine impeller, which is more efficient than the open flat turbine configuration used in the present study. It is notable that CFD-predicted $K_{L_a}$ values are in good agreement with experimental $K_{L_a}$ data, at least in the range of power per unit volume examined ($5 \times 10^2 < P/V < 5 \times 10^4$ W/m$^3$), which covers the range of interest to most fermentations.

Measurements of $K_{L_a}$ in the miniature bioreactor are made for two impeller speeds at the end of the fermentation period during the stationary phase. In these experiments care is taken to ensure that during the de-aeration stage in the gassing out method the oxygen concentration does not fall below 30% saturation (Badino et al., 2001). The results are plotted in Fig. 4-8 where the solid line shows the line of best fit obtained from the air-water experiments. The results indicate that volumetric mass transfer coefficients in the miniature fermenter fall within the range reported for conventional mechanically agitated bioreactors. It is notable that the concept of power input per unit volume as a scale-up parameter successfully links the performance of the miniature bioreactor to conventional fermentor. Kostov et al. (2001) have provided $K_{L_a}$ data for a magnetically stirred 2mL miniature bioreactor equipped with optical probes. Using *E. coli* fermentation, values of $K_{L_a}$ of 9.8 h$^{-1}$, 27.5h$^{-1}$ and 44.4h$^{-1}$ were reported for air flow rate of 1, 2 and 3 vvm. These $K_{L_a}$ values are similar to those reported recently by Duets et al. (2001) for air-water in a microtitre plate and are lower than values observed typically in shake flasks and conventional mechanically stirred systems. By comparison $K_{L_a}$ values measured during *E-coil* fermentation in our turbine-stirred miniature bioreactor at a fixed aeration rate of 1vvm were 68h$^{-1}$ and 128h$^{-1}$ at power input per unit volume of 413 Wm$^{-3}$ and 1190 Wm$^{-3}$ respectively (Lamping, 2003). These are well within the range of values observed in conventional mechanically agitated systems as shown in Figs 4, 5 and 7 reported in the literature for air-water (Van't Riet, 1979).
Fig. 4-8 Overall mass transfer coefficients measured during fermentation of E-coli in the miniature bioreactor (♦). The data compare very well with the $K_{L,a}$ obtained for air-water (•) and the values obtained from CFD simulations (●).

In a series of experiments $K_{L,a}$ was measured by challenging the miniature bioreactor in a way that more closely mimicked the conditions in a shaken microwell (Lamping, 2003). With air to the sparger turned off the oxygen transfer conditions in the miniature bioreactor occurs solely via surface aeration, similar to conditions in a shaken microwell plate. As shown in Fig. 4-9 this mode of operation resulted in significantly lower $K_{L,a}$ values, which falls in the range achieved in the shaken system of Duetz et al. (2000) and magnetically stirred system of Kostov et al. (2001).

The volumetric mass transfer coefficient at the free surface is also obtained from CFD simulation. The free surface is not changed using the free surface grid algorithm (CFX Solver) at a rotational speed of 1000–5000 rpm. The gas-liquid mass transfer area $a$ is obtained from the free surface area over the entire working volume. The liquid mass transfer coefficient $K_{L}$ is calculated only from the free surface basing on Eqn.4-6. The
prediction results are overall higher than the experimental results. Taniguchi et al. (2002) similarly obtained the overpredicted values in a gas-injected vessel. The main reason is that only part of the energy is consumed for mass transfer and some energy is consumed for keeping the free surface shape. It is also found that at the surface aeration condition, the volumetric mass transfer coefficient from the experimental measurement and theoretical analysis does not increase as expected as the power consumption increases.

The miniature bioreactor is also operated in a "bubble column" mode by operating the switching off the impeller motor and pumping air through the single tube sparger at a rate of 1 vvm (corresponding to a superficial gas velocity of 0.0005 m.s⁻¹). The overall measured $K_{l,a}$ value in that case is 67 h⁻¹, significantly higher than the $K_{l,a}$ values achieved via surface aeration alone. These results suggest that oxygen starvation may occur become limiting factor for rapidly growing (oxygen-demanding) microorganisms in agitated microwell systems (Lamping et al., 2003).

![Graph](image)

Fig. 4- 9 Overall mass transfer coefficients were measured (♦) and simulated (▲) under conditions of surface aeration only. Significantly lower $K_{l,a}$ data compared to values obtained under aeration via the sparger (solid line) indicate that in microwell scale fermentation in shaken systems involving oxygen demanding micro-organisms may be a potential problem.
4.3 Scalability of the miniature bioreactor

The engineering performance of the miniature bioreactor, especially the mass transfer and fermentation process, is evaluated from both CFD analysis and experimental measurement. The geometry of the miniature bioreactor is similar to a conventional laboratory-scale bioreactor, and the scalability of the miniature bioreactor to a 20L bioreactor is investigated in the section. As discussed in the chapter 1, there are many criteria that may be used as the basis to scale up from small-scale to large-scale. The method based on equal power input per unit volume (or mass) has met with success in many cases (Humphrey, 1964; Gaden, 1961). The method is chosen and discussed below.

4.3.1 Overall mass transfer coefficient

From the various correlations discussed in section 1, if the specific power consumption is maintained, changes in gas superficial velocity may affect mass transfer rate. In scaling up from laboratory scale to pilot-scale or production-scale, the volumetric air flow rate per liquid unit volume is usually kept constant. In this case, 1.0 vvm is applied to the miniature bioreactor and a 20L bioreactor. But this leads to two different gas superficial velocities: 0.0066 m/s for the 20L bioreactor and 0.000497 m/s for the miniature bioreactor. To compare the results for the two bioreactors therefore the overall volumetric mass transfer coefficient data for the each reactor are adjusted by assuming $K_La \propto v_s^{0.2}$, as recommended by Van't Riet (1979).

The results are shown in Fig. 4-10 where the solid line was obtained from Van't Riet's equation and the dashed-lines represent ± 40% deviations from it. The $K_La$ values for the miniature bioreactor consistently fall below those of the 20L fermentor, but the best lines of fit through the data points for the two scales have the same slope which very close to the exponent of P/V in Van't Riet's equation. It is also notable that while the $K_La$ data for the miniature bioreactor are lower than the 20L scale the difference are within the expected 40% deviation. Taken together the observations based on Fig. 4-7 and Fig. 4-10 support the view that the engineering performance of the miniature bioreactor is adequately described by current CFD techniques and that its performance, at least as far as mixing and oxygen transfer is concerned, matches those of laboratory bioreactors.
Fig. 4-10 Overall volumetric mass transfer coefficients (♦) (pink) for the miniature bioreactor compare well with (blue) (■) those obtained in a 20L (15L working volume) fermenter and (▲) are the values obtained from CFD simulations.

4.3.2 Biomass

Fig. 4-11a and Fig. 4-11b show respectively the variation of dissolved oxygen and dry cell weight (g/L) as a function of time for fermentation of E. coli, DH5α, in the miniature bioreactor (Lamping, 2003). The data refer to experiments carried out at a fixed impeller speed of 1500rpm corresponding to a simulated mean energy dissipation rate, $\varepsilon$, of 0.53 W/kg. In each case data are also shown from parallel experiments carried out in the 20L fermenter using standard probes and running under normal fermentation conditions described in the chapter 2. Fluorescence-based fibre optic probes, for monitoring of fermentation parameters including pH and dissolved oxygen, have been fully described and successfully tested previously (Junker et al., 1988). In the case of pH, both a pH indicator dye immobilised on the working tip of the fibre optic probe as well as the HPTS dye directly introduced into the culture medium were used. The former is preferable but in the experiments its response was found to be very slow and
erratic (data not shown). In contrast acceptable results were obtained from the probe responding to the dissolved HPTS dye. In the case of oxygen, the response of the commercial immobilised fluorophore probe was reliable and its calibration reproducible. No attempt was made in these experiments to run the fermentations in the two scales under comparable conditions. The agreement between the responses of the two probes demonstrated by the data in Fig. 4-11a and Fig. 4-11b therefore are encouraging and any differences between the probes are thought to be due to differences in the geometry and operational conditions of the two bioreactors.

4.4 Summary

The flow patterns, local and average power input, energy dissipation rate and bubble size are derived from an analysis of the multiphase flow in the miniature bioreactor and 20L conventional fermenter using computational fluid dynamics (CFD). Volumetric mass transfer coefficients are predicted using Higbie's penetration model with the contact time obtained from the CFD simulations of the turbulent flow in both bioreactors. Predicted volumetric mass transfer coefficients in both bioreactors agree with the experimental data. The liquid circulation and energy dissipation rate also matched the published experimental results. Predicted volumetric mass transfer coefficients in the miniature bioreactor are in the range 100 hr$^{-1}$ to 400 hr$^{-1}$, typical of those reported for large-scale fermentation although they were underpredicted compared with the experimental data.
Fig. 4- 11 The fibre optic response curves for the dissolved oxygen (6a) and biomass Concentration (6b) obtained in the miniature bioreactor are compared with profiles obtained in a 20L (15 L working volume) fermenter. The data were obtained during fermentation of E. coli. No attempt was made to run the fermentation in the miniature bioreactor under the optimum conditions. The differences in profiles for the two scales are thought to be due to differences in the geometrical configuration of the two systems.
4.5 Concluding Comments

K_{La} and gas volume fraction from CFD analysis in the miniature bioreactor are underpredicted compared to experimental data. However, the agreement with the experimental data in the 20L bioreactor is good. The main reason for the differences in the miniature bioreactor is thought to be due to the two-fluid model used in the simulations. The model is based on Eulerian approach, and allows for calculation of separate velocity and turbulence intensities for two phases. However, this model is only suitable for conditions of dilute dispersed gas phase, and in each grid the gas volume fraction is relatively low compared to liquid phase. In a 20L bioreactor, the grid size is significantly greater than bubble size (Fig. 4-12), the gas phase only takes up a small fraction of the volume of the grid cell. This allows a meaningful suater diameter to be obtained from the different sizes of bubbles, the drag force for the gas phase to be calculated, and applied to the gas momentum equations. But in the miniature bioreactor (Fig. 4-12), the grid size is smaller than the bubble size, and the grid cell may be completely filled with gas bubble, giving a gas volume fraction equal to 1.0. However, this violates the two-fluid model because it suggests that the liquid phase is not continuous. Additionally, according to the two-fluid model, the bubbles are always assumed to be located within the grid cells, otherwise the drag force becomes larger than the real value, which leads to accumulation of gas phase in the bottom part of the reactor. Although small modifications are made to simulations of the miniature bioreactor compared to the 20L bioreactor, the predicted gas volume fraction and mass transfer coefficient are still underpredicted.

The problem may be resolved by using an advanced numerical algorithm developed based on the Lagrangian approach, which can track every bubble within the reactor. This approach has been developed for bubble column (Krishna and van Baten, 1999) as the gas bubbles are only subjected to buoyant force and interfacial forces (drag, lift and virtual mass forces), and the number of gas bubbles is very small. For the miniature bioreactor, however, thousands of bubbles need to be tracked which will take huge computation resources and time. For this reason, this approach was not tested in the present study.
Miniature bioreactor and scale-up to 20L bioreactor

Fig. 4-12 Gas bubbles size and grid cell size for bioreactors at different scales

20L bioreactor

6.5mL bioreactor
Chapter 5 Shake flask

5.1 Introduction

The chapter is focused on a theoretical and experimental analysis of mixing and oxygen transfer in shake flasks, with the aim of comparing the process performance of shake flasks with conventional laboratory bioreactors. CFD techniques are used to obtain an estimate of the air-liquid interfacial area and power dissipated in the shaken liquid. These parameters are combined with models of mass transfer to predict the volumetric mass transfer coefficients and the predictions compared with available experimental observations reported previously. The mass transfer predictions are used to estimate the oxygen transfer rates (OTR) in the shake flask and the results are compared with experimental oxygen up-take rate (OUR) for a mammalian cell culture system. A comparison with laboratory bioreactors is also provided.

5.2 Simulation results

In a shake flask, the topological changes at the free surface are simulated by the Volume of Fluid (VOF) method, which is described in section 3.4.

5.2.1 Simulation domain and its grid

Simulations are carried out for different shaking frequencies and amplitudes using a standard orbital shaker with a 250mL shake flask of a geometry shown in Fig. 5- 1. The numbers of grid cells used in the simulations are 200,000 for the bottom section and 320,000 for the top section of the shake flask. Finer grids do not significantly affect the accuracy of predictions but greatly increase computational time. The fluid in the flask is assumed to have the physical properties of water and air at ambient temperature.
5.2.2 Gas-liquid interface changes

Oxygen transfer from the gas phase to the liquid phase is achieved at the gas-liquid interface. The interface is changed along with the circular movement of the shake flask. Fig. 5-2 shows typical simulations of the gas-liquid volume fraction profiles demonstrating the changes at the gas-liquid interface. The simulations shown are for two shaking frequencies (100 rpm, 200 rpm) and two amplitudes (e=40 mm, 60mm). At the starting point (t=0), the gas-liquid interface is flat (simulation not shown). The simulations are shown as a series of snapshots in time (the moving angle from 270° to 540°) as the shake flask moves through one and a half cycles (revolutions). The interface changes are very slow for the first a half of the cycle (0-180°), and hence the snapshots are not displayed. The flow simulations for subsequent cycles remain effectively the same.
Shake flask

(a) A rotational speed of 100rpm and a shaking diameter of 40mm, a working volume is 25mL.

(b) A rotational speed of 200rpm and a shaking diameter of 40mm, a working volume is 25mL.
A rotational speed of 200rpm and a shaking diameter of 60mm, a working volume of 25mL.

Fig. 5-2 Gas-liquid interface changes as the flask moves around the circle

A photograph snapshot of the liquid motion in shake flasks presented by Maier and Buchs (2001) at a rotational speed of 200rpm and a shaking diameter of 2.5mm supports these changes from CFD analysis shown in Fig. 5-2. Based on this information, they also proposed a model to calculate the mass transfer coefficient. Increasing shaking frequency from 100 rpm to 200 rpm causes the gas-liquid interface changes to become more sharply. In Fig. 5-2 (b) (100 rpm), most of the liquid stays at the bottom and the maximum liquid height is about 2.5cm. However, in Fig. 5-2 (a) (200rpm), the majority of the liquid moves from one side to the other and the maximum liquid height is about 5.0cm, which nearly doubles the height at 100 rpm. Shaking diameter also affects the gas-liquid interface changes. Fig. 5-2 (c) (e=60mm) shows that most of the liquid moves from one side to the other and the sharp changes allows the rapid exchange of oxygen between the gas and liquid phase. Fig. 5-3 presents the maximum liquid height for different shaking frequencies and different shaking diameters. Increasing the shaking frequency or the shaking diameter causes the liquid height to increase.
Fig. 5-3 Computed maximum liquid height at a working volume of 25mL.

(▲) e=40 mm, (■) e=60 mm

5.2.3 Velocity distribution

Fig. 5-4 shows the computational velocity vectors at the end of one complete cycle. The instantaneous velocity distribution is not symmetrical about the vertical axis and is complicated by the profiled base and the tapering side walls of the flask, as well as the centrifugal forces imparted on the liquid by the shaking action of the platform. For example, at the right-hand-side near the base of the flask, the flow is uniform, while on the opposite side, the liquid velocity is more randomly distributed. The corresponding profile of the energy dissipation shown in Fig. 5-5 reveals that most of the energy is dissipated in the regions close to the walls of the flask, as expected from the velocity field.
5.2.4 Energy dissipation

The first attempt at measuring the power consumption in a shake flask was based on temperature increase in heat-insulated flask (Sumino et al., 1972), but the temperature increased very slowly. Kato et al. (1995) proposed evaluation of the power input by recording the total electric power consumption of the whole shaking machine. However, this method was highly dependent on the accuracy of the registration of the electric power. A torque measurement device was designed by Buchs et al. (2000 a and b) and an empirical equation proposed to relate the power consumption to the shaking frequency, filling volume and shake flask geometry. However, the equation does not include the shaking diameter. The above methods only provide the average power consumption in the reactor at best. A profile of energy dissipation may be obtained from CFD analysis of flow in the flask.

The energy dissipation distribution is presented in Fig. 5- 5. Most of the energy is dissipated in the regions close to the walls of the flask and matches the velocity field profile (Fig.5- 4). It is clearly shown that the energy input through the vessel wall
during the rotational movement serves for mixing in the reactor as well as for mass transfer.

![Energy dissipation rates in the flask at a rotational speed of 200rpm and a shaking diameter of 40mm, a working volume of 25mL.](image)

**Fig. 5-5** Energy dissipation rates in the flask at a rotational speed of 200rpm and a shaking diameter of 40mm, a working volume of 25mL.

For a given liquid, the intensity of mixing in a shake flask is a function of the shaking frequency, amplitude as well as the volume of the liquid (fill volume). Flow simulations are therefore carried out to assess the impact of these parameters on the energy dissipation rate. The predictions are also compared to the experimental results from Buchs et al. (2000 a). The simulation results are shown in Fig. 5-6 (a) and (b). These plots show that power consumption increases with an increase in shaking frequency and a decrease in fill volume. For a shaking frequency of 200rpm and amplitude of 40 mm, the dependency of the energy dissipation rate on fill volume is found to be \( E \sim V^{-0.7} \), which agrees well with the experimental data of Buchs et al. (2000a).

The dependency of power input per unit volume, \( E \), on frequency is a non-linear function of the shaking amplitude. For example, for amplitudes of 60mm and 40 mm, energy dissipation rates and their dependency on shaking frequency agree well the experimental results of Buchs et al. (2000a) and Sumino et al. (1972). Thus

\[ E \propto N^{2.7} \]

(Eqn. 5-1)
(a) The impact of shaking frequency and fill volume at a shaking diameter of 40mm (■) \( V_L = 100 \) mL, (▲) \( V_L = 25 \) mL

(b) The impact of shaking diameter on power consumption at a working volume of 25mL

Fig. 5-6 Effects of shaking intensity on power consumption

However, the exponent of N in Eqn.5-1 is found to decrease as shaking amplitude decreased. For example, at a shaking amplitude of 20mm, the CFD predicted exponent of N is 1.6.
According to Buchs et al. (2000 a), the shaking amplitude has little effect on the power consumption. The correlation below was proposed to calculate the power input:

$$ Ne' = \frac{P}{\rho N^3 D^4 V_{L}^{\frac{1}{3}}} = \frac{70}{Re} + \frac{25}{Re^{0.6}} + \frac{1.5}{Re^{0.2}} $$

(Eqn.5- 2)

where $Ne'$ is the modified power number and $Re$ is the Reynolds number, which is defined as:

$$ Re = \frac{\rho N D^2}{\mu} $$

(Eqn.5- 3)

Using the Eqn. 5-2, the predictions for the working volume of 25ml in the 250mL shake flask are also displayed in Fig. 5- 6 (b). The predictions match well with the power consumption values from CFD analysis at the shaking amplitude of 60mm. According to the CFD predictions, the power consumption decreases as amplitudes (40mm or 20mm) decreases.

5.2.5 Volumetric liquid-phase mass transfer coefficient, $k_{L,a}$

The sharp changes at the gas-liquid interface and the short contact time between the two phases suggested that the liquid mass transfer coefficient, $k_{L}$, may be modelled using the Higbie's penetration model (Higbie, 1935) described by the equation Eqn.5-4.

$$ k_{L} = 2 \sqrt{\frac{\varepsilon}{\pi t_{cont}}} $$

(Eqn.5- 4)

The contact time in the equation $t_{cont}$ was calculated from the position of the interface and the local velocity of gas phase, using a modified approximation method based on the length of moving path and corresponding to the average velocity originally developed by Taniguichi et al. (2002). Interfacial area, $a$, was calculated from the gas-liquid interfacial area and wetted area along walls (Maier and Buchs, 2001).

The liquid film coefficient $k_{L}$ was calculated by the equation below (van Suijdam et al., 1978)
where $k_L$ is proportional to $N^{0.5}$. Maier and Buchs (2001) proposed calculation of the contact time based on the formation and dissolution of the liquid film on the flask wall. However this method requires an accurate description of the liquid film as the flask is moving rotationally. From the diagram to calculate the contact time, the index of the dependency of liquid film coefficient on shaking frequency is greater than 0.5, but no data for the index was presented in the paper. From CFD analysis, the index is around 0.54, very close to the value used by van Suijdam et al. (1978). The effect of shaking frequency on the liquid film coefficient is displayed in Fig. 5-7 (a).

An approximate estimate of the gas-liquid interfacial area may be obtained from the following simple expression (Henzler and Schedel, 1991; Gaden, 1962):

$$a = \frac{A}{V_L} = \frac{A}{V_s V_L} \propto \frac{1}{D} \frac{D^{3}}{V_L} = \frac{\pi D^3}{V_L}$$  \hspace{1cm} (Eqn.5-6)

According to Eqn.5-5, $a$ is approximately 211m$^2$/m$^3$ for the shake flask fluid used in this study. However, Eqn.5-5 suggests that interfacial area is independent of amplitude and frequency of shaking. CFD analysis (Fig. 5-7 b) shows that at the interfacial area is a function of the energy dissipation. The relationship between the two may be expressed by the following expression:

$$a \propto \varepsilon^{0.25}$$  \hspace{1cm} (Eqn.5-7)

For a fixed shaking diameter, the relationship between interfacial area, $a$, and shaking frequency is predicted to be as follows:

$$a \propto N^{0.6}$$  \hspace{1cm} (Eqn.5-8)

This agrees well with the experimental observations of van Suijdam et al. (1978).
(a) Liquid mass transfer coefficient as a function of shaking frequency

(b) Gas liquid interfacial area as a function of power consumption ($\Delta$) $e=40$mm, (■) $e=60$mm

Fig. 5-7 Effects of shaking intensity on liquid mass transfer $k_L$ and specific exchange area
Many researchers have presented $K_{La}$ data as a function of shaking frequency. The correlations for the mass transfer coefficient ($K_{La}$) proposed in the literature are summarised in Table 5-1. Two reported empirical equations are plotted in Fig. 5-8a which show the dependency of $K_{La}$ on shaking frequency for air-water systems. The good agreement between the CFD predictions and the empirical equations confirm the general applicability of the CFD technique and provides confidence in its predictions. Much less data is available on the influence of shaking amplitude on $K_{La}$. Interestingly, the CFD predictions shown in Fig. 5-8b suggest that $K_{La}$ is not sensitive to changes in shaking amplitude.

<table>
<thead>
<tr>
<th>References</th>
<th>Correlation proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Suijdam et al. (1978)</td>
<td>$K_{La} \sim N^{1.1} \sqrt{g}$</td>
</tr>
<tr>
<td>Henzler and Schedel (1991)</td>
<td>$K_{La} \sim \left(\frac{g}{V}\right)^{\frac{1}{3}} \left(\frac{D^3}{V_L}\right)^{\frac{8}{9}} \left(\frac{V}{D^3 g}\right)^{\frac{8}{27}} \left(\frac{N^2 \sqrt{eD}}{g}\right)^{\frac{1}{2}} \left(\frac{g}{V}\right)^{\frac{1}{2}}$ (Eqn.5-10)</td>
</tr>
<tr>
<td>Veljkovic et al. (1995)</td>
<td>$K_{La} \sim N \left(\frac{V}{V_L}\right)^{0.845}$          (Eqn.5-11)</td>
</tr>
<tr>
<td>Maier and Buchs (2001)</td>
<td>$K_{La}(OTR_{max}) \sim N^{0.84} V_L^{-0.84} e^{0.27} D^{-1.25}$ (Eqn.5-12)</td>
</tr>
</tbody>
</table>
(a) Impact of shaking frequency on the mass transfer rate at an amplitude of 40mm, filling volume of 25ml

\[ K_{a} (\text{s}^{-1}) \]

\[ \text{Shaking frequency (rpm)} \]

(b) Impact of shaking diameter on the mass transfer rate at a filling volume of 25ml

\[ K_{a} (\text{s}^{-1}) \]

\[ \text{Shaking frequency (rpm)} \]

(▲) e= 40mm (■) e=60mm

Fig. 5-8 Impact of shaking frequency and diameter on the mass transfer coefficients.
Currently, there is no reliable criterion for comparing gas-liquid mass transfer rates in shake flasks to those in laboratory bioreactors. In the latter case, the concept of energy dissipation rate has been used successfully for scale-up. In Fig. 5-9, $K_{L}a$ data are plotted against the mean energy dissipation rate obtained from CFD analysis for the shake flask. $K_{L}a$ data are also shown from experiments reported previously for a 20L laboratory and a mechanically stirred miniature reactor with an operating volume of 6mL (Lamping et al., 2003). It is notable that the power input to the liquid and the $K_{L}a$ in the shake flask are the lowest of the three systems. The 6mL miniature bioreactor and the 20L laboratory unit have similar geometrical configurations and in both cases aeration is achieved by sparging air at 1vvm. The differences in $K_{L}a$ for the two bioreactors is purely due to different superficial velocities prevailing in the two systems (0.0005ms$^{-1}$ and 0.0066ms$^{-1}$ for the miniature and the 20L bioreactor, respectively). By assuming the well-established relationship $K_{L}a \propto V^{0.2}$ for air-(salt)-water the mass transfer data are well correlated for the two systems. However, in the shake flask, gas-liquid contacting is achieved by surface aeration and therefore the concept of a superficial gas velocity does not apply. The data in Fig. 5-9 for the shake flask and the miniature bioreactor appear to be correlated. However, it should be noted that the range of liquid mass transfer coefficient, $K_L$, in the shake flask ($0.2 < K_L < 0.5$ mh$^{-1}$) is lower than the range observed in the stirred bioreactor ($1.0 < K_L < 3.5$ mh$^{-1}$). In contrast, the interfacial area in the shake flask is in the range ($300 < a < 800$ m$^2$m$^{-3}$) while for the stirred vessel the range is normally $100 < a < 300$ m$^2$m$^{-3}$. 
5.3 Analysis of cell growth and fermentation

The CFD analysis presented in the preceding section indicates that specific power input and gas-liquid mass transfer in the shake flask are low compared to laboratory and pilot scale bioreactors. Considering the practical range of operation of commercially available shakers, high $K_L a$ values on the order of 250-400 h$^{-1}$, typically reported for laboratory and pilot-scale bioreactors, are difficult to achieve in shake flask operations. Therefore, growth of cells that have high demand for oxygen may be impeded under shake flask operation. Recently, Katzer et al. (2001) provided experimental data for the production of several secondary metabolites using fungi and actinomycetes growing in a number of different 250ml (working volume 50 ml) shake-flasks with and without baffles for shaking frequencies of 180 rpm and 240 rpm and a fixed shaking amplitude of 50mm. Production of secondary metabolites was achieved in the shake flask but compared to growth in a 50 l (working volume) batch fermentation the rate of production in the shake flask was severely impeded after nearly 7 days. Initially, the $K_L a$ in the shake flask was in the range between 80 h$^{-1}$ to 100 h$^{-1}$, but as the fermentation progressed broth viscosity increased, which limited oxygen transfer, impeding biomass growth and secondary metabolite production.
The growth of *E. coli* in the shake flask (Fig. 6-13) shows that cells have a longer lag phase compared to the miniature bioreactor and the laboratory scale bioreactor. The existence of baffles in the shake flask improves the mass transfer rate, but the location and shape of the baffles affect the hydrodynamics in the reactor. Villatte *et al.* (2001) designed four different baffles, varying size, shape and angle between the baffle and the bottom wall. It was found that the dissolved oxygen concentration in the flask with baffles was higher, but the protein production and biomass concentration from the yeast *P. pastoris* varied in the flask with different baffles even though they had the same oxygen concentration levels. The factors influencing the performance of cells are still unknown. Possible reasons may be different flow hydrodynamics due to the baffles in the flask. Buchs (2001) provided additional examples of oxygen demanding fermentation operations.

In the case of mammalian cells, some preliminary cell growth experiments were carried out using Hybridoma cells (cell line VPM 8) obtained from the European Collection of Cell Cultures (ECACC) (Health Protection Agency, Salisbury, Wiltshire) (Zhang *et al.*, 2003). These cells were grown in cell culture medium RPMI 1640 plus 2mM glutamine plus 1mM sodium pyruvate plus 10% Foetal Bovine Serum (FBS) (Sigma). The Hybridoma cells were incubated at 37 °C in 5% CO₂. The cell culture experiments were performed in a purpose built 250mL shake flask fabricated from Perspex (Corning) with the dimensions shown in Fig. 5-1. Each set of experiments involved 25 ml of cell culture in six identical shake flasks, three of which were used as sacrificial runs and were discarded after four samples had been taken from each of them for analysis. Cell growth curves were obtained for shaking frequencies of 50, 100 and 200 rpm, at an amplitude of 30 mm. Experiments were also performed at a fixed frequency of 100 rpm and amplitudes of 20, 30 and 50mm.

Cells were diluted approximately mid-log growth phase to a concentration of $1 \times 10^5$ cells /ml and grown until the stationary phase. Samples of 0.75 ml were taken every 12 hrs for analysis. Cell counts were performed using a tryphan blue exclusion method. The remainder of each sample was centrifuged and the supernatant decanted and frozen for further analysis to determine the concentration of glucose and lactate with an YSI biochemistry analyser (Yellow Springs Incorporated, Ohio). All samples and tests were carried out in triplicate and the data averaged, unless otherwise stated.
Cell counts were carried out as function of time during cell growth experiments for three shaking frequencies and amplitudes. These results (data not shown) compare well with typical values reported for cell growth in mechanically stirred vessel (1.8x10^6 cells/ml) and shake flasks (0.9x10^6 cells/ml) and indicate that, within the range of parameters studied, cell growth is unaffected by operating conditions of the shake flask. Using the conditions at the maximum cell growth rate (0.0561h'''), maximum biomass concentration ~13.5x10^5 cells ml'' ( = 0.3192 gl''') and assuming a specific oxygen uptake rate Q_{O2} =0.5x10^{-10} mmol O_2 cell'' h''', the calculated oxygen uptake rate was found to be 0.73 mmol O_2 l'' h''. Assuming a typical concentration driving force of 10 ppm (= 10mg l'' = 0.312 mmol l''') the minimum K_{La} required for cell growth was calculated to be about 7x10^{-4} s'', which is significantly lower than the prevailing K_{La} in the shake flask during the growth period. These calculations demonstrate that while the volumetric mass transfer rates were lower in the shake flask compared to a mechanically stirred vessel, the prevailing oxygen transfer rates remained higher than the oxygen uptake rate of the cells throughout the growth so that growth was not oxygen limited for the mammalian cells examined (data not shown).

5.4 Summary

Parallel fermentation and cell culture experiments carried out under shake flask conditions are the method of choice in most laboratories in the early stages of process development. Despite their popularity, however, little is known about the basic transport processes of mass and momentum that occur in shake flasks during operation. Computational fluid dynamics was used to quantify the key engineering parameters in shake flasks including specific power input, interfacial area and gas-liquid volumetric mass transfer coefficient. These results show that gas-liquid interfacial area in shake flasks is in the range 300 m^2 m^{-3} to 800 m^2 m^{-3}, which is higher than the range for agitation in laboratory reactors (100 m^2 m^{-3} to 300 m^2 m^{-3}). However, the analysis also shows that the power dissipated in the liquid in shake flasks (40 Wm^{-3} to 600 Wm^{-3}) is an order of magnitude lower than those reported for mechanically stirred bioreactors. As a result, the calculated volumetric mass transfer coefficient K_{La} range in shake flasks (10 h^{-1} to 100 h^{-1}) is significantly lower than the reported range of values for mechanically stirred bioreactors (100 h^{-1} to 400 h^{-1}).
The consequences of these differences for scale-up of fermentation and cell culture may be significant and depend on cell requirement for oxygen. Using the analysis of the engineering flow, previously published data were assessed on cell growth and product formation in shake flask fermentation of bacterial and fungi systems. It is important to note that, where comparative data is available from experiments in mechanically stirred bioreactors, the results indicate that insufficient dissolved oxygen in shake flask operation may severely limit biomass growth and product formation. It has been argued that, for oxygen-demanding cells, the inability to mimic the engineering flow environment of mechanically stirred bioreactors renders shake flask operations inappropriate for process scale-up. Moreover, there is evidence in the literature to suggest that unless the flow environment between the two systems is matched, data obtained in media screening and strain selection from shake flask studies may not represent the optimum conditions under laboratory and pilot-scale operations (Clark et al., 1995).

Mammalian cells have a comparative low demand for oxygen and the specific power input requirement for biomass growth in mammalian cell cultures are nearly 100 times lower than those for bacterial and fungi systems. The preliminary results obtained under shake flask operations indicate that biomass growth of Hybridoma cells is essentially independent of the specific power input and is comparable to those reported for laboratory and pilot scale operations.
Chapter 6 Microwell Bioreactors

6.1 Introduction

In this chapter, the CFD analysis of flow in the microwell reactors (24 well and 96 well) is presented and the predictions are compared to the observations from experiments. Mass transfer coefficients and cell culture experiments are provided in 24 microwell bioreactors, and the experimental results are compared to those obtained in the miniature bioreactor and the shake flask.

6.2 Simulation results and discussions

The gas-liquid interface (free surface) changes are captured by the Volume of Fluid (VOF) method, which was reported by Zhang et al. (2003). The numerical method is described in section 3.4 of chapter 3.

6.2.1 Configuration of microwell reactors and the simulation grids

Simulations were carried out in two different microwell vessels: 24 deep well and 96 deep well. The dimensions and configurations are displayed in Fig. 6-1. The upper part of the two reactors has a square cross-section but the lower part is spherical. The non-uniform grids are used for both reactors. In the lower part, the grids are 40×40×20 for the 24 well and 40×40×40 for the 96 well so that the free surface between the gas and the liquid can be captured accurately. In the upper part, the density of the grids decreases as the height of the reactors increases. Overall, the grids used for the upper part are 40×40×60 for the 24 well and 40×40×40 for the 96 well. In the central region, the grids are 40×40×20 for both the 24 well and the 96 well.
(a) Dimension of the 24 well plate and the computational domain and part grids
Buchs et al. (2000b) defined a phase number (Ph) which was used to characterise the liquid phase movement in the shaking bioreactor. Ph is defined by the Eqn.6-1 as a function of the liquid film Reynolds number (Eqn.6-2) (See the Reynolds number defined in chapter 5 (Eqn.5-3). The calculated phase numbers and corresponding Reynolds and Froude numbers (Eqn.6-3) for typical shaking conditions are listed in table 6-1.

\[ Ph = \frac{e}{d} \left(1 + 3 \log \text{Re}_f\right) \]  

(Eqn.6-1)
Microwell Bioreactors

\[ \text{Re}_f = \frac{\rho (2\pi \lambda)}{\mu} \left( \frac{d^2}{4} \right) \left[ 1 - \frac{1 - \frac{\pi}{4} \left( \frac{V_f}{d} \right)^{2/3}}{1} \right] \]  
(Eqn. 6-2)

\[ Fr = \frac{(2\pi \lambda)^2 e}{2g} \]  
(Eqn. 6-3)

Table 6.1 Operational parameters and related dimensionless numbers

<table>
<thead>
<tr>
<th>Well type</th>
<th>Diameter of the well (m)</th>
<th>Filling volume (mL)</th>
<th>Shaking frequency (rpm)</th>
<th>Shaking amplitude (mm)</th>
<th>Ph</th>
<th>Re_f</th>
<th>Fr</th>
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<td>24</td>
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<td>500</td>
<td>3</td>
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<td>6.38</td>
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6.2.2 Liquid flow patterns

The mixing patterns are described by the liquid velocity from the CFD analysis. Fig. 6-2 shows the liquid velocity profile in the vertical Y (midplane) in the 24 and 96 well bioreactors. The curves represent the contour of liquid volume fraction, which defines the area below the curves as the liquid and the area above the curves as the gas phase. At a shaking frequency of 1000 rpm and a shaking diameter of 3mm (Fig. 6-2 a), a small part of the liquid at the bottom of the well remains stagnant during the reactor movement. The local liquid velocity at the gas-liquid interface changes dramatically. Small vortices can be seen at the interface or just below the interface. But the vortices are very small so that the liquid at the bottom is not affected. Increasing the shaking frequency to 1400 rpm or the shaking diameter to 6mm increases the liquid velocities at the bottom. At the gas-liquid interface small vortices can still be found (Fig. 6-2 b). However a different liquid velocity profile is found in a 24 well reactor at a shaking frequency of 500 rpm and a shaking diameter of 3mm (Fig. 6-2 c). The bulk of liquid moves nearly in the same direction. No clear vortices can be found in the well.
The liquid velocity in the 96 well reactor at a shaking diameter of 3mm and at a shaking frequency of 1000 rpm (Fig. 6-2 d) is very different from that in a 24 well reactor under the same shaking conditions. Overall, the liquid moves from left to right, and in the bottom part of the reactor, the liquid displays a similar velocity profile. At a shaking frequency of 1000 rpm and a shaking diameter of 6mm (Fig. 6-2 e), the overall liquid movement is very similar to that at the shaking diameter of 3mm, but at the bottom part of the reactor, the liquid velocity changes direction and a small vortex forms. This means that in addition to the rotational movement of the liquid, vertical movement can also be induced if the shaking diameter is increased. Duetz and Witholt (2001) observed that the higher oxygen transfer rate (OTR) at a shaking diameter of 50mm was not only due to the larger surface area but also due to a better degree of vertical mixing. Weiss et al. (2002) proposed different mixing mechanisms based on visual observations and fluorescence mixing studies for a 96 well reactor and a mechanism based on horizontal and vertical interchange of the liquid phase matched the experimental results. This can also be seen from the liquid patterns in Fig. 6-2 d or e.
(a) 1000 rpm and 3mm shaking diameter in the 24 well

(b) 1000 rpm and 6mm shaking diameter in the 24 well
(c) 500 rpm and 3mm shaking diameter in the 24 well

(d) 1000 rpm and 3mm shaking diameter in the 96 well
(c) 1000 rpm and 6mm shaking diameter in the 96 well

Fig. 6-2 Liquid velocity profiles of the vertical y midplane in a 24 and a 96 well bioreactor

6.2.3 Power consumption

Experimental measurement of power input for shaken liquids is difficult because of the lack of commercially available torque meters with sufficient sensitivity for this function. Because the absolute torque and power input for a shaken micro-well are low, indirect measurements based, for example, on the power input to the electric motor are not appropriate. If the specific power consumption of 2 ml of liquid for a single 24 well is 1.0 kW/m³ (or below), the total power input into the system is nearly 0.048 W or below. The smaller the well plates are, the more accurate the measurements need to be. Currently there are no experimental reports on power consumption on microtitre plate. However, accurate estimations of power input and energy dissipation rates in such systems are very important because of their impact on the rate at which transport processes occur in the liquid. Mixing and oxygen transfer rates in a fermenter, for example, are critically affected by energy dissipation rate. CFD analysis of flow can provide an estimation of the power input. The equations used for calculation of power
Typical energy dissipation profiles at different horizontal sections for the 24 well reactor and 96 well reactor are presented in Fig. 6-3. The pictures on the right-hand side are the liquid volume fraction profiles. Both are obtained from the same shaking conditions: shaking frequency of 1000 rpm and shaking diameter of 6mm. Similar to the energy dissipation in the shake flask (Fig.5-5), most of energy is dissipated around the reactor wall, and the momentum is transferred from the moving walls to the liquid in the reactor. Comparing both simulations, the energy dissipation in the 96 well reactor is higher than that in the 24 well reactor, which can also explain why the mixing in the 96 well reactor is better than in a 24 well reactor. At the bottom part of 96 well reactor, a region of high energy dissipation can be clearly seen in the centre of the reactor, not along the wall. This region does not appear in the 24 well reactor. These observations confirm the existence of vertical mixing in the 96 well reactor.

(a) 1000 rpm and 6mm shaking diameter in the 24 well reactor
Simulation results show that power input and energy dissipation rate in the shaken micro-wells are strongly affected by the size of the well and the volume of shaken liquid as well as the shaking frequency and amplitude. The power input in the 24 well reactor does not increase linearly as the shaking frequency is increased (Fig. 6-4 a). The power consumption obtained for shaking frequencies of 300 rpm to 800 rpm shows that power decreases as the shaking frequency increases. Above the shaking frequency of 800 rpm, the power consumption increases with the shaking frequency as expected. These observations agree with experimental data reported previously for the shake flask with baffles at low viscosity (Buchs et al., 2001). The power consumption is more sensitive to changes in shaking amplitude than to frequency (Fig. 6-4 b). These simulations also indicate that power input increases significantly as the volume of liquid in the well is decreased (Fig. 6-4 c).
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(a) Shaking diameter of 3mm and filling volume of 2mL

(b) Shaking frequency of 1000 rpm and filling volume of 2mL
(c) Shaking frequency of 1000 rpm and the shaking diameter of 3mm.

Fig. 6-4 Power consumption in a 24 well reactor is a function of the shaking frequency, shaking diameter and filling volume.

The predicted values of the energy dissipation rates for the shaken 96-well reactor are higher than those for the 24-well reactor under the same conditions of operations (Fig. 6-5). Increasing the shaking diameter from 3mm to 6mm, the power consumption increases to the range of 1,000–2,000 W/m³ which is recommended for bacterial fermentation process in large-scale fermentors (Aiba et al., 1965). The power consumption at both 3mm and 6mm increases linearly as the shaking frequency is increased, which confirms that the flow conditions in a 24-well reactor are unfavourable compared to 96-well reactors.

Fig. 6-5 Power consumption in the 96-well reactor at a filling volume of 0.2 mL.
6.2.4 Gas-liquid interface changes and mass transfer

Changes at the gas-liquid interface determine the mass transfer and mixing conditions in the well reactor. As interface changes sharply, mixing is improved. Fig. 6-6 and Fig. 6-7 display the liquid volume fraction in the vertical Y midplane. At the starting point (t=0), the interface is flat, then as the reactor moves along the orbital platform, the extent of interface change is different for different well types and the shaking conditions.

At low shaking frequency, the surface tension keeps the liquid surface nearly horizontal and no obvious interface changes are observed. Hermann et al. (2003) proposed an equation to calculate the critical shaking frequency at which the interface starts changing:

\[ N_{crit} = \sqrt{\frac{\alpha D}{4\pi V_L P_L e}} \]  

(Eqn. 6-4)

According to this equation, the critical shaking frequency for the 24 well reactor at the shaking diameter of 3 mm is 250 rpm, and for the 96 well reactor at the shaking diameter of 3 mm is around 520 rpm (based on the round well shape). Hermann et al. (2003) confirmed the equation by using the maximum liquid height, which was measured by an aqueous solution of bromothymol blue. For a 96 round well plate, at 600 rpm and a shaking diameter of 3 mm the gas liquid interface started to change. Similar results were observed by Doig et al. (2002) for the 96 round well plate (from 300 rpm to 600 rpm no increase of oxygen transfer rate was found). But for the 96 square well reactor, the critical shaking frequency was found to decrease to 400 rpm and for the 24 round well reactor, it was below 300 rpm.

All simulations were carried out beyond the critical shaking frequency for all shaking conditions and different well types. In the 96 well plate (Fig. 6-6), by increasing the shaking frequency from 500 rpm (a) to 1000 rpm (b), the interface changes from a flat profile to a more curved shape. The maximum liquid height increases as well. But the impact of the shaking frequency is not as strong as that of the shaking diameter. The interface becomes more vertical at a shaking diameter of 6 mm (c) and the maximum liquid height nearly doubles compared to results achieved at a shaking diameter of 3 mm (b).
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(a) 500 rpm
3 mm shaking diameter

Gas

Liquid

$\text{t}=0.09\text{s}$ $\text{t}=0.12\text{s}$ $\text{t}=0.15\text{s}$ $\text{t}=0.18\text{s}$

(b) 1000 rpm
3 mm shaking diameter

Gas

Liquid

$\text{t}=0.045\text{s}$ $\text{t}=0.06\text{s}$ $\text{t}=0.075\text{s}$ $\text{t}=0.09\text{s}$
Fig. 6-6 Interface changes as the 96-well reactor moves on a rotary platform

Hermann et al. (2003) obtained similar results using a 96 round well plate. Similar hydrodynamics behaviour was also observed by Duetz and Witholt (2001) using an aqueous solution of bromocresol blue in a 96-deep square well reactor with a digital camera. At a rotational speed of 300 rpm, the maximum liquid height at a shaking diameter of 50mm was much higher than that at a shaking diameter of 25mm. The oxygen transfer rate with a shaking diameter of 50mm was 24mmolO₂ l⁻¹ h⁻¹, three fold higher compared to a shaking diameter of 25mm (8mmolO₂ l⁻¹ h⁻¹). The biomass of *P. putida* reached 10g dry wt l⁻¹ during growth on a glucose mineral medium using a culture volume of 0.75ml with the shaking conditions of 300 rpm and 50mm.

In a 24 well plate (Fig. 6-7), the gas-liquid interface changes are more sensitive to variation in the shaking diameter than to the shaking frequency. At a shaking frequency of 1000rpm and a shaking diameter of 6mm, the liquid height reaches up to the edge of the upper part of the well. However, most of the liquid still remains in the bottom part, compared to the same shaking conditions in a 96 well reactor.
(a) In the 24 well at a shaking frequency of 1000 rpm and a shaking diameter of 3mm
(b) In the 24well at a shaking frequency of 1000 rpm and a shaking diameter of 6mm

Fig. 6-7 Interface changes as the 24-well reactor moves on a rotary platform
It can be seen (Fig. 6-6 and Fig. 6-7) that the imposed shaking conditions produce good mixing which is demonstrated by the sharp gas-liquid interface changes. These simulations indicate that most of the mass transfer is likely to occur at the interface between the two phases. It is noticeable that as the liquid moves cyclically from side to side, the sidewalls of the well become exposed to air, increasing the potential for mass transfer. Gas-liquid mass transfer on the wall however would require the presence of a liquid film at the wall as the bulk liquid moves away from the wall. The formation of such a liquid film is critically affected by the hydrophobicity and hydrophilicity of the wall material. Many plastic wells are likely to have non-wetting surface properties making it difficult for a liquid film to form on the wall. Glass wells on the other hand are expected to produce a liquid film and therefore better mass transfer conditions can be expected. These considerations make the prediction of mass transfer in shaken micro-well difficult and suggest that scale-up rules developed for such systems may be system specific. However, the sharp changes at the interface between the two phases and the short contact time between the gas and liquid phases suggest that the volumetric liquid mass transfer coefficient may be modelled according to Higbie’s equation (Higbie, 1935). The calculation of the mass transfer coefficient was discussed in the previous chapter.

The polystyrene microwell reactors examined in this work have a strong hydrophobic surface, therefore the gas-liquid interfacial area “a” is obtained only from the gas-liquid interfacial area, and the wetting area along walls of the reactors is not included. In the CFD analysis an artificial thickness for the gas-liquid interface is assumed, and the region having a liquid volume fraction of 0.5 is used to represent the gas-liquid interface in Fig. 6-8. The static surface area over the volume for a 24 well reactor is 128 m^2/m^3, and interestingly, when it moves around and reaches steady state, the interfacial area does not change significantly (Fig.6-9a). Hermann et al. (2003) found that in a 96 conventional round well plate, the interfacial area for a hydrophobic surface fluctuated around the static specific area, which agrees with the CFD analysis.
Fig. 6-8 Gas-liquid interfacial area calculated from CFD.

Fig. 6-9 Gas-liquid interfacial area and the liquid mass transfer coefficient in the 24 well reactor at a shaking diameter of 3mm.
There appears to be a step change in the simulated liquid mass transfer coefficient, $k_L$, plotted in (Fig. 6-9b) between 600 and 800 rpm. It is not clear what causes the change. The $k_L$ is independent of wall materials and $k_L$ has been reported to have a value of 0.2 m/hr in the range reported for surface-aerated bioreactors (Kawase and Moo-Young, 1990). In the 24 deep square well reactor, the predictions of $k_L$ are greater than 0.2 m/hr. In the 96 deep square well reactor, $k_L$ is found to be in the range of 0.19–0.40 m/hr at shaking frequencies of 300–1200 rpm and at a shaking diameter of 3mm (data not shown). Hermann et al. (2003) reported that using a deep square 96 well, the maximum $k_L$ value of 0.52 m/hr was obtained at a shaking frequency of 800 rpm and a shaking diameter of 25mm. This is comparable to the predictions in both well reactors.

At a filling volume of 2 mL, the mass transfer coefficient increases when the shaking frequency increases from 800 rpm to 1400 rpm. However the experimental values of $k_{L*a}$ increase more sharply than the simulation results (Fig. 6-10a)(Lamping, 2003). Decreasing the filling volume to 1mL in the 24 well reactor, the mass transfer coefficients from CFD analysis show a similar trend as observed from experimental measurements. As the shaking frequency increases, the mass transfer rate increases first, then it reaches an approximately constant value above 600 rpm(Fig. 6-10b). Increasing the liquid volume, whilst keeping the same shaking conditions, causes the mass transfer coefficient to decrease. This is expected because the mass transfer coefficient is a volume-based variable (Fig. 6-11 b). Multiplying the volumetric mass transfer coefficients with the filling volume yields constants for different filling volumes (Hermann et al., 2003; Duetz et al., 2000). The lower the filling volume is, the greater the mass transfer coefficient is expected. However, comparing the values of $k_{L*a}$ from experiments, $k_{L*a}$ at a filling volume of 1 mL is less than or nearly the same as that at a filling volume of 2 mL. Measuring the $k_{L*a}$ by sensors in this study was not suitable for a small filling volume (less than 1 mL) because the sensors may not be fully submerged into the liquid. Overall, the mass transfer coefficient is more sensitive to the shaking diameter, which is similar to the gas-liquid interface changes (Fig. 6-11 a). Duetz and Witholt (2001) and Hermann et al. (2003) reached the same conclusions from experimental measurement using a sulfite oxidation method.
Fig. 6-10 Volumetric mass transfer coefficient as a function of shaking frequency in a 24 well reactor at a shaking diameter of 3mm. (■) CFD predictions; (□) Experimental data.
Fig. 6-11 Volumetric mass transfer coefficient as a function of shaking diameter, and filling volumes in a 24 well reactor.

The simulated mass transfer coefficients are very high in the 96 deep-square well plate (Fig. 6-12). Compared to the measurements reported by Hermann et al. (2003) using a conventional 96-well reactor, high mass transfer rates are achievable in the square well reactor. The mass transfer is improved as the shaking diameter increases. The predicted values from CFD analysis are difficult to compare with experimental data in the literature because there is little work published and the conditions used are also different. Diog et al. (2002) reported that the mass transfer coefficient (k_{L\alpha}) at a shaking frequency of 1000 rpm and a shaking diameter of 3mm for the same well was around 0.052 s^{-1} corresponding to the maximum oxygen transfer rate of 33 mmol.l^{-1}.h^{-1}. This is comparable to the predicted value of 0.065 s^{-1} in this study. Lamping (2003) measured k_{L\alpha} in the same deep square well at a fixed shaking diameter of 3mm. K_{L\alpha} values were found to be 0.036 s^{-1} at a shaking frequency of 1000 rpm and 0.023 s^{-1} at 500 rpm,
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which are lower than the predictions. The measured \( k_{La} \) value of 0.033 s\(^{-1}\) corresponding to the maximum oxygen transfer rate of 21 mmol.l\(^{-1}\).h\(^{-1}\) was also reported in the round well reactor, which was very close to the value of 0.025 s\(^{-1}\) from Hermann et al. (2003) at the same shaking conditions. Therefore, the predicted values are acceptable and CFD analysis can be used to provide very useful information in this miniature system.

![Graph showing volumetric mass transfer coefficient in a 96-well reactor.](image)

**Fig. 6-12** Volumetric mass transfer coefficient in a 96-well reactor.

6.2.5 Discussion

The specific power consumption and the volumetric mass transfer coefficients are higher in the 96 well plate than in the 24 well plate under the same shaking conditions. The mixing and mass transfer in the 24-well reactor at a low shaking frequency is similar to or better than that at a high shaking frequency under the shaking diameter of 3mm. Buchs et al. (2001) reported similar observations in the baffled shake flasks and described the hitherto unknown phenomenon as “out-of-phase”. The phenomenon was reported to occur usually when using low filling volumes, large numbers and sizes of baffles, and small shaking diameters. The specific power consumption in a 2L round bottom shaking flask with three large baffles at a shaking diameter of 25mm was reported to decrease nearly down to zero after an initial increase as the shaking frequency was increased. The biomass concentration at lower shaking frequency was 60% higher compared to the higher shaking frequency. Katzer et al. (2001) found that product yields at 180 rpm were greater than at 240 rpm because of vertical baffles in
shake flasks for different microorganism strains. Villatte *et al.* (2001) reported that biomass concentration and product yield with vertical baffles were lower than those without any baffles.

The square shape of the well reactor functions as four baffles; therefore the corners of the square wells disturb the flow in a way similar to that of baffles in shake flasks or fermentors. Parallel studies at UCL indicate that "out-of-phase" also affects the biotransformation process in the 24 deep-square well (data not shown).

### 6.3 Cell culture in the 24 well reactor

The cell growth curve for *E. coli* in the 24-well reactor is very similar to that in the miniature bioreactor (Fig. 6-13) (Lamping, 2003). Cells in the 24-well reactor and the shake flask in the initial phase grow more slowly compared to cells in the miniature bioreactor and the 20L bioreactor. A similar growth curve was reported by Duetz *et al.* (2000) for *Pseudomonas putida* in a 96 deep-square well plate. At a shaking frequency of 1000 rpm and a shaking diameter of 3mm, cell growth in the 24-well reactor is faster than that in the 250 mL shake flask at a shaking frequency of 200 rpm and a shaking diameter of 25mm. Interestingly *Corynebacterium glutamicum* cultured in a 96 round-bottom well reactor (1000 rpm, and 1mm shaking diameter) had nearly the same growth profile as in a 250mL shake flask (John *et al.*, 2003). In this experiment, the maximum specific growth rate from the 24 well reactor and shake flask was found to be the same as that in a 20L conventional stirred tank fermentor. The slopes during the exponential growth phase from the four different reactors are nearly parallel, which means that the maximum growth rate is the same, which is an intrinsic parameter of the cell-substrate system. After 4 hours, the cells in the miniature bioreactor, the 24-well reactor and the shake flask reach stationary phase and the optical density of the cell broth is nearly the same for the above three systems.
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Related engineering parameters for the fermentations:

<table>
<thead>
<tr>
<th>Reactors</th>
<th>Operating conditions</th>
<th>$P/V$ (W/m$^3$)</th>
<th>$K_{La}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20L bioreactor</td>
<td>Rotational speed of 530 rpm, aeration rate of 1vvm</td>
<td>1231</td>
<td>0.106</td>
</tr>
<tr>
<td>6.5 mL miniature bioreactor</td>
<td>Rotational speed of 1500 rpm, aeration rate of 1vvm</td>
<td>508</td>
<td>0.025</td>
</tr>
<tr>
<td>24 Well microtiter plate</td>
<td>Shaking frequency of 1000 rpm, shaking diameter of 3mm</td>
<td>72</td>
<td>0.018</td>
</tr>
<tr>
<td>250 mL shake flask</td>
<td>Shaking frequency of 200 rpm, shaking diameter of 25mm</td>
<td>280</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Fig. 6-13 Growth curves of *E. coli* in four bioreactors.
6.4 Comparison of the miniature bioreactor, the shake flask and the microwell reactor

The comparison of engineering performance for the different reactors is summarised in table 6.2. The power consumption in the conventional fermentor and the miniature bioreactor is higher than shaking flasks and shaken microwell reactors. It is also higher than other reactors, such as air-lift or bubble column reactor (Moo-Young, 1981). Higher power consumption results in higher mass transfer coefficients (Table 6.2 and Fig. 6-14). The highest mass transfer coefficient of 0.14 s\(^{-1}\) is achieved in the stirred miniature bioreactor and the 20L bioreactor. But the mass transfer rate is also affected by the gas-liquid interfacial area. At the same aeration rate, the volumetric mass transfer coefficients in the miniature bioreactor are lower than that in the 20L bioreactor because of the low superficial gas velocity in the miniature bioreactor. Gas-liquid mass transfer in the stirred reactor occurs at the surface of the dispersed gas bubbles. Increasing the mixing intensity reduces the gas bubble size and the liquid film around the gas bubble decreases, resulting in higher mass transfer rates. The mass transfer between gas and liquid is dominated by surface aeration in the shake flask and the shaken microwell reactors. The gas-liquid interfacial area and the wetting area along the walls, if hydrophilic walls are used, contribute to the mass transfer. In a 96 deep square well reactor, mixing is more intense compared to a 24 well reactor and a shake flask. The average power input is also higher than other shaking systems. This makes the 96-square well plate suitable for highly oxygen-demanding bacterial fermentation. In a 24-square well reactor, the power input is low, and the mass transfer rate is similar to the shake flask, but one order of magnitude lower than that in a stirred reactor. The cell growth profile in the 24 well reactor and the shake flask is similar to that in the miniature bioreactor and the 20L bioreactor but the cells grow relatively slower. The 24-well reactor may be more appropriate for mammalian cell culture because of less intense agitation and low oxygen demand.
Table 6.2 Comparison of laboratory scale bioreactors and micro-titre plate scale bioreactors

<table>
<thead>
<tr>
<th></th>
<th>20L bioreactor</th>
<th>The miniature bioreactor</th>
<th>The shake flask (250mL)</th>
<th>24-well reactor</th>
<th>96-well reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Volume, ml</td>
<td>15,000</td>
<td>6.5</td>
<td>10–100</td>
<td>0.5–4</td>
<td>0.1–0.5</td>
</tr>
<tr>
<td>Rotating speed, rpm</td>
<td>200–800 (1vvm)</td>
<td>1000–7000 (1vvm)</td>
<td>50–300 (40mm)</td>
<td>500–1500 (3mm)</td>
<td>500–1500 (3mm)</td>
</tr>
<tr>
<td>Power input kW/m³</td>
<td>0.1–3.0</td>
<td>0.5–15.0</td>
<td>0.04–0.6</td>
<td>0.07–0.10</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>$K_L a$ s⁻¹</td>
<td>0.015–0.14</td>
<td>0.015–0.12</td>
<td>0.006–0.026</td>
<td>0.005–0.028</td>
<td>0.056–0.10</td>
</tr>
<tr>
<td>Sampling</td>
<td>online</td>
<td>online</td>
<td>offline</td>
<td>offline</td>
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</tr>
</tbody>
</table>

Fig. 6-14 The mass transfer coefficients via the power consumption in different reactor systems.
Chapter 7 Conclusions and Recommendations

7.1 Conclusions

Advances in geomics, proteomics, bioinformatics, and metabolomics, combined with high throughput screening techniques have greatly accelerated the drug discovery process. However, process development has remained essentially unchanged for the past five decades. A simultaneous approach to integrate both process development and drug discovery at the early stage of drug development cycle is required to reduce the development time and cost. But at the early stage, only small quantities of materials are available for evaluating the process performance. A new bioprocess development approach based on micro-titre scale bioprocess operations is proposed and studied in this thesis.

The new approach starts with experiments using the micro-titre plate, a miniature bioreactor, and the shake flask. The engineering performance of all these reactors are evaluated in the thesis and compared to a 20L bioreactor at laboratory scale. Engineering characterisation of three different types of reactors is made with the aid of CFD and the engineering performance in terms of mass transfer and cell growth are compared using an experimental and theoretical basis.

A new miniature bioreactor with a diameter equal to that of a single well of a 24-well plate is designed. Mixing in the miniature bioreactor is provided by a set of three impellers mechanically driven via a micro-fabricated electric motor and aeration is achieved with a single tube sparger. Parameter sensitive fluorophors are used with fibre optic probes for continuous monitoring of dissolved oxygen tension and an optical based method is employed to monitor cell biomass concentration during fermentation.

The flow patterns in the miniature bioreactor from CFD analysis of single-phase flow are typical to a multi-impeller stirred tank. In aerated conditions, at lower rotational speeds, gas bubbles move towards the shaft and the dispersion is very poor. By increasing the rotational speed, the dispersion can be improved. The evolution patterns are in good agreement with experimental observations. The energy dissipation rate is heterogeneously distributed in the reactor, and the highest energy dissipation rate occurs around the impeller regions. Volumetric mass transfer coefficients are predicted using Higbie's penetration model with the contact time obtained from the CFD simulations of
the turbulent flow in the bioreactor. Comparative data are provided from parallel experiments carried out in a 20L (15L working volume) conventional fermenter. Predicted and measured volumetric mass transfer coefficients are in good agreement in the miniature bioreactor and 20L bioreactor although the simulation data in the miniature bioreactor is underpredicted. The $k_La$ data from experiments and simulations in the miniature bioreactor are in the range of 100 hr$^{-1}$ to 400 hr$^{-1}$, typical of those reported for large-scale fermentation. The fermentation process is evaluated by cultivating *E. coli* under 1 vvm and different rotational speeds. The cell growth profile is nearly the same as that in a 20L bioreactor.

The flow in the shake flask is characterised by the free surface model of CFD. The gas-liquid interface changes are mapped as the reactor moves along the rotary platform. The flow patterns in the shake flask are found to be very complicated. The energy dissipation rate is non-homogeneous, and the highest energy dissipation rate occurs along the shaking wall. The power consumption is obtained by integrating the local energy dissipation over the entire working volume and compared to experimental observations by Buchs (2001). The energy consumption is found to be comparable to the experimental values. CFD predictions of power consumption are more sensitive to shaking amplitude than to shaking frequency. The CFD predicted correlation of power consumption as a function of shaking frequency is supported by experimental data. The volumetric mass transfer coefficients obtained from Higbie’s penetration model are in the range of experimental results. The correlations of liquid mass transfer coefficient $k_L$ and the specific gas-liquid interfacial area as a function of the shaking frequency are obtained and compared to those from experimental data. CFD predictions are in good agreement with experimental observations.

24-well and 96-well micro-titre plate reactors are investigated and different flow patterns are observed under different shaking conditions. “Out-of-phase” conditions are found in the 24-well reactor at lower shaking frequency. The liquid at the base of the 24 well reactor remained stationary, and the mixing performance is improved by increasing shaking frequency or shaking diameter. In a 96 well reactor good mixing is achieved at a shaking frequency of 1000 rpm and a shaking diameter of 6mm because of the existence of horizontal and vertical mixing.
The energy dissipation rates in the two reactors are found to be non-homogeneous. Most of the energy is dissipated along the reactor wall. But in the 96-well reactor, the energy dissipation rate is relatively high at the base of the reactor, which confirms good mixing in the reactor. The power consumption is strongly affected by the size of the well and the volume of shaken liquid as well as the shaking frequency and amplitude. The power consumption decreases in the 24-well reactor as the shaking frequency increases from 300 rpm to 800 rpm, and then increases as expected after the shaking frequency of 800 rpm. In the 96-well reactor, the power consumption increases monotonically as the shaking frequency increases. The power input is found to be more sensitive to shaking diameter than shaking frequency in both microtitre plate reactors. The volumetric mass transfer coefficients are higher in the 96-well reactor than in the 24-well reactor under the same shaking conditions. The predicted mass transfer coefficients are in the same order of magnitude as the experimental data. The mass transfer rate is more sensitive to changes in shaking diameter compared to shaking frequency.

High mass transfer rates in the miniature bioreactor are achieved and are comparable to a 20L bioreactor as the convective mass transfer prevails in both reactors. The power consumption in impeller-driving mixing tanks is higher than that in the shaking system. At the same shaking conditions, the mass transfer rate in a 96-well reactor is higher than that in a 24-well reactor; and the power consumption in a 96 well reactor is higher as well. The volumetric mass transfer coefficients in the shake flask are in the same order of magnitude of the 24-well reactor, but the power consumption is relatively higher. Overall, the power consumption can be correlated to the mass transfer performance in the different reactors.

Analysis of the available data on cell growth suggests that all three small-scale systems (microwell plate, shake flask and miniature bioreactors) have the capacity for fermentation and cell culture. However, the growth profiles show differences in the three systems. The miniature bioreactor produces growth profiles that are closest to those in the conventional bioreactor under conditions of equal energy dissipation rate allowing direct scale-up and/or scale down.
7.2 Recommendations

7.2.1 The miniature bioreactor

7.2.1.1 Experimental work
The miniature bioreactor has been evaluated as a fermentor using *E.coli* under controlled conditions. Different types of cells from bacteria, fungi, and mammalian system should be cultivated in the reactor and the performance of cell growth and product formation should be evaluated. These data should be combined with the information from theoretical analysis to study the interaction between the extracellular flow environment and cell physiology.

The miniature bioreactor has the advantage of a small working volume and of higher rotational speed availability. In a conventional reactor (20L), the maximum energy dissipation is around $1.0 \times 10^4$ W/m$^3$ corresponding to the rotational speed of 1100 rpm. This energy dissipation rate may be achieved in the miniature bioreactor with the impellers operating at 4,000 rpm. The average energy dissipation rate in the miniature reactor may be controlled easily through the adjustment of speed. In this way, it could be used to quantify rapidly the sensitivity of mammalian and bacterial cells to hydrodynamic forces.

The miniature bioreactor can be used for “screening” processes. Several miniature bioreactors can be integrated on an operational platform (Fig. 7.1). The control panel can be designed to control the operational conditions in individual reactors. This parallel operation could accelerate process development.

7.2.1.2 Theoretical analysis
The source-sink model employed in the study is an approximation for the simulation of the impellers and baffles. Sliding grid technique mimics the impeller movement, and could provide the main features of flow in the mixing tank. Currently the technique combined with k-ε model underpredicts the energy dissipation rate. An improvement in the technique could lead to a better understanding of the flow in the miniature bioreactor. The mixing conditions in the miniature bioreactor are highly turbulent. Large-eddy simulation should be considered for capturing the detailed turbulent structures. Because of advances in computer hardware and software, direct numerical simulation has become possible and should be applied to describe the flow in the miniature bioreactor.
Because the mesh size in the miniature bioreactor is smaller than the bubble size, results lead to incorrect gas-liquid interaction forces and therefore gas volume fraction distribution can be not predicted directly. In the present study, this problem was resolved through simplifying assumptions. A Lagrangian approach to simulate the motion of single gas bubbles in the liquid using volume-of-liquid technique should be studied to capture the formation of gas cavities behind the impeller blades.

Cell growth kinetics, substrate concentration, and product formation kinetics should be included in the hydrodynamic model.

The simulation results should be validated from experimental observations.

7.2.2 24-well and 96-well reactors

7.2.2.1 Theoretical analysis
The continuum surface model assumes that there is an artificial thickness between the gas and liquid phase. The artificial thickness cannot represent the real gas-liquid interface. The advanced numerical algorithm in free surface flow replaces the artificial thickness by the real interface. Future work should be directed towards thin area to allow improved predictions.

The mass transfer coefficients were calculated from Higbie’s penetration model based on some assumptions. However, they should be predicted directly from the dissolved oxygen concentration distribution which can be solved from CFD, and the mass transfer rate in the well reactors can be evaluated without any assumption in the model.

The simulation results should be validated from experimental observations.

7.2.2.2 Experimental work
The operating platform should be re-designed for the fermentation process in the microwell shaken system. Currently platforms allow the shaking frequency to vary, but the shaking diameter is fixed. The CFD predictions suggest that the mass transfer rate is more sensitive to changes in shaking diameter. The performance of a fermentation process in a microwell reactor, in which the shaking diameter is changed, should be studied and results compared to conventional bioreactors.
The sensors used for measuring key process parameters in the microwell reactor need to be submerged in the liquid. However, as the liquid in the well system is shallow and the bulk of the liquid moves along the rotary platform, the sensors may be exposed to the gas phase and give inaccurate information. New sensor systems should be studied which allow for rapid detection and accurate measurement.

Finally, for the cell culture system, contamination between single wells must be prevented and evaporation should be reduced to keep a constant working liquid in the reactor.

Fig.7-1 Prototype of parallel miniaturised bioreactor
## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Constant in Eqn. 1-1</td>
</tr>
<tr>
<td>A</td>
<td>Projected area of the bubbles per unit volume $m^2/m^3$</td>
</tr>
<tr>
<td>a</td>
<td>Specific gas-liquid interfacial area $m^2/m^3$</td>
</tr>
<tr>
<td>A</td>
<td>Constant in Eqn. A2-6 $1.22$ for DT</td>
</tr>
<tr>
<td>Ab</td>
<td>Baffle area $m^2$</td>
</tr>
<tr>
<td>A_r</td>
<td>Impeller blade area $m^2$</td>
</tr>
<tr>
<td>B</td>
<td>Birth rate due to breakage $1/(m^3s)$</td>
</tr>
<tr>
<td>B_c</td>
<td>Birth rate due to coalescence $1/(m^3s)$</td>
</tr>
<tr>
<td>C</td>
<td>Clearance of the impeller above the vessel base wall $m$</td>
</tr>
<tr>
<td>c</td>
<td>Constant in Eqn. 1-1</td>
</tr>
<tr>
<td>C_w</td>
<td>Constant $0.15$</td>
</tr>
<tr>
<td>C, C_1, C_2, C_3, C_\mu</td>
<td>Oxygen concentration mole/m$^3$</td>
</tr>
<tr>
<td>C_b</td>
<td>Drag constant for baffles in Eqn. A1-2</td>
</tr>
<tr>
<td>C_d</td>
<td>Drag coefficient</td>
</tr>
<tr>
<td>c_f</td>
<td>Drag constant for impellers in Eqn. A1-1</td>
</tr>
<tr>
<td>Cs</td>
<td>A constant in large eddy simulation $0.1$</td>
</tr>
<tr>
<td>D</td>
<td>Death rate of bubbles $1/(m^3s)$</td>
</tr>
<tr>
<td>D</td>
<td>The maximum diameter of a shake flask $m$</td>
</tr>
<tr>
<td>D_b</td>
<td>Death rate due to breakage $1/(m^3s)$</td>
</tr>
<tr>
<td>d_b, d</td>
<td>Bubble diameter $m$</td>
</tr>
<tr>
<td>D_c</td>
<td>Death rate due to coalescence $1/(m^3s)$</td>
</tr>
<tr>
<td>D_i</td>
<td>Diameter of the impeller $m$</td>
</tr>
<tr>
<td>D_i</td>
<td>Impeller diameter $m$</td>
</tr>
<tr>
<td>e</td>
<td>Shaking diameter of a shake flask or a microtitre plate $m$</td>
</tr>
<tr>
<td>E</td>
<td>Modifying factor for drag coefficient</td>
</tr>
<tr>
<td>F, C_\alpha \beta</td>
<td>interfacial force $N$</td>
</tr>
<tr>
<td>f_B</td>
<td>Coefficient for calibration of breakage model</td>
</tr>
<tr>
<td>Symbol</td>
<td>Meaning</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Fr</td>
<td>Froude number</td>
</tr>
<tr>
<td>Fs</td>
<td>Body force</td>
</tr>
<tr>
<td>G</td>
<td>Production due to body force</td>
</tr>
<tr>
<td>g</td>
<td>Gravitational acceleration</td>
</tr>
<tr>
<td>g(Vj;Vi)</td>
<td>Break-up rate of bubbles of volume Vj into bubbles of Vi</td>
</tr>
<tr>
<td>H</td>
<td>Liquid height</td>
</tr>
<tr>
<td>k</td>
<td>Turbulent kinetic energy</td>
</tr>
<tr>
<td>K_L</td>
<td>Liquid mass transfer coefficient</td>
</tr>
<tr>
<td>K_La</td>
<td>The volumetric mass transfer coefficient</td>
</tr>
<tr>
<td>l_k</td>
<td>The size of the smallest turbulent eddies</td>
</tr>
<tr>
<td>n</td>
<td>A normal to the free surface</td>
</tr>
<tr>
<td>N</td>
<td>Shaking frequency or impeller rotational speed</td>
</tr>
<tr>
<td>N₀</td>
<td>Minimum rational speed for dispersing gas bubbles</td>
</tr>
<tr>
<td>n_b</td>
<td>Number of baffles</td>
</tr>
<tr>
<td>n_b, n</td>
<td>Bubble population density</td>
</tr>
<tr>
<td>n_bmax</td>
<td>Number of bubbles corresponding to the maximum stable bubble size</td>
</tr>
<tr>
<td>N_crit</td>
<td>Critical shaking frequency for shaken microwell</td>
</tr>
<tr>
<td>N_e'</td>
<td>Modified power number</td>
</tr>
<tr>
<td>n_f</td>
<td>Number of impeller blades</td>
</tr>
<tr>
<td>N_p</td>
<td>Number of phases</td>
</tr>
<tr>
<td>N_p</td>
<td>Impeller power number</td>
</tr>
<tr>
<td>N_Q</td>
<td>Impeller pumping number</td>
</tr>
<tr>
<td>P</td>
<td>Shear production</td>
</tr>
<tr>
<td>p</td>
<td>Pressure</td>
</tr>
<tr>
<td>P</td>
<td>Power consumption</td>
</tr>
<tr>
<td>p'</td>
<td>Modified pressure</td>
</tr>
<tr>
<td>P_g</td>
<td>Power consumption under gassed condition</td>
</tr>
<tr>
<td>Ph</td>
<td>Phase number</td>
</tr>
<tr>
<td>Q</td>
<td>Coalescence rate</td>
</tr>
<tr>
<td>r</td>
<td>Volume fraction</td>
</tr>
<tr>
<td>r</td>
<td>radial coordinate</td>
</tr>
<tr>
<td>r_{pbm}</td>
<td>The maximum attainable value for the volume fraction</td>
</tr>
<tr>
<td>Re</td>
<td>Reynolds number</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Re_f</td>
<td>Liquid film Reynolds number</td>
</tr>
<tr>
<td>S_u, S</td>
<td>Source term for the corresponding equations</td>
</tr>
<tr>
<td>T</td>
<td>Diameter of the stirred reactor</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>τ</td>
<td>Surface renewal time</td>
</tr>
<tr>
<td>T</td>
<td>Tank diameter</td>
</tr>
<tr>
<td>t_ij</td>
<td>Time for coalescence between bubbles (size i) and bubbles (size j)</td>
</tr>
<tr>
<td>t_m</td>
<td>Mass transfer time</td>
</tr>
<tr>
<td>U</td>
<td>Mean velocity vector (velocity component)</td>
</tr>
<tr>
<td>U_0</td>
<td>Instantaneous velocity</td>
</tr>
<tr>
<td>U'</td>
<td>Unresolved velocity</td>
</tr>
<tr>
<td>U_g</td>
<td>Gas superficial velocity</td>
</tr>
<tr>
<td>u_t</td>
<td>Velocity of the smallest turbulent eddies</td>
</tr>
<tr>
<td>U_tip</td>
<td>Impeller tip speed</td>
</tr>
<tr>
<td>V</td>
<td>Working volume</td>
</tr>
<tr>
<td>V_L</td>
<td>Liquid filling volume</td>
</tr>
<tr>
<td>V_s</td>
<td>The volume of a shake flask</td>
</tr>
<tr>
<td>w</td>
<td>Impeller blade width</td>
</tr>
<tr>
<td>We</td>
<td>Dimensionless Weber number</td>
</tr>
<tr>
<td>We_c</td>
<td>Critical Weber number</td>
</tr>
<tr>
<td>x</td>
<td>Spatial vector (spatial component)</td>
</tr>
<tr>
<td>z</td>
<td>Axial co-ordinate</td>
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**Greek Letters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>𝜔</td>
<td>Gas bubble coalescence and break up frequency</td>
</tr>
<tr>
<td>𝜎</td>
<td>Constant in Eqn.1-1</td>
</tr>
<tr>
<td>𝜏</td>
<td>Gas (dispersed) phase</td>
</tr>
<tr>
<td>𝛼</td>
<td>Constant in Eqn.1-1</td>
</tr>
<tr>
<td>𝛼</td>
<td>Liquid (continuous) phase</td>
</tr>
<tr>
<td>Γ</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>𝜌</td>
<td>Density</td>
</tr>
<tr>
<td>𝜖</td>
<td>Energy dissipation rate</td>
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<table>
<thead>
<tr>
<th></th>
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<th>Eqn. 6-2</th>
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|   |   |   |
|---|---|---
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>Viscosity</td>
<td>kg/(ms)</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Surface curvature</td>
<td>1/m</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Surface tension</td>
<td>N/m</td>
</tr>
<tr>
<td>$\xi$</td>
<td>Liquid diffusivity</td>
<td>m$^2$/s</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Viscous stress tensor</td>
<td>N/m$^2$</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Kinematic viscosity</td>
<td>m$^2$/s</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Angular co-ordinate</td>
<td></td>
</tr>
<tr>
<td>$\pi$</td>
<td></td>
<td>3.1415926</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular velocity</td>
<td>m/s</td>
</tr>
<tr>
<td>$\phi$</td>
<td>General variable</td>
<td></td>
</tr>
<tr>
<td>$\xi$</td>
<td>Dimensionless size of eddies</td>
<td></td>
</tr>
<tr>
<td>$\Delta$</td>
<td>Filter width</td>
<td>m</td>
</tr>
<tr>
<td>$\chi_c$</td>
<td>The critical dimensionless energy for break-up</td>
<td></td>
</tr>
<tr>
<td>$\mu_{\text{eff}}$</td>
<td>Effective viscosity</td>
<td>kg/(ms)</td>
</tr>
<tr>
<td>$\tau_{ij}$</td>
<td>Contact time between bubbles (size i) and bubbles (size j)</td>
<td>s</td>
</tr>
<tr>
<td>$\theta_{ij}^B$</td>
<td>Coalescence rate due to buoyancy</td>
<td>m$^3$/s</td>
</tr>
<tr>
<td>$\theta_{ij}^{LS}$</td>
<td>Coalescence rate due to laminar shear</td>
<td>m$^3$/s</td>
</tr>
<tr>
<td>$\theta_{ij}^T$</td>
<td>Coalescence rate due to turbulence</td>
<td>m$^3$/s</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>Mixture viscosity</td>
<td>kg/(ms)</td>
</tr>
<tr>
<td>$\lambda_{\text{min}}$</td>
<td>The smallest eddy size</td>
<td>m</td>
</tr>
<tr>
<td>$\tau_p$</td>
<td>Probe response time</td>
<td>s</td>
</tr>
<tr>
<td>$\mu_T$</td>
<td>Turbulent viscosity</td>
<td>kg/(ms)</td>
</tr>
<tr>
<td>$\Phi_V$</td>
<td>Viscous dissipation term</td>
<td>m$^2$/s$^2$</td>
</tr>
</tbody>
</table>
Bibliography


Bibliography


Bibliography


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Bibliography


Appendix 1 Single phase flow in the miniature bioreactor

Continual motion of the impellers in a baffled vessel introduces complexity into the flow field. In the past decade, there have been several methods introduced to address this problem using different reference frame and fixed/moving grids. In this chapter, five different techniques: Source-Sink (SS), Snapshot (SP), Sliding Grids (SG), Multiple Reference Frame (MRF) and Inner-Outer (IO) were used and compared. Characteristic flow parameters in the miniature bioreactor are discussed.

A1.1 Introduction

Several approaches have been developed to simulate flow in a baffled stirred tank. These approaches include black box, momentum source-sink, inner-outer, computational snapshot, multiple reference frame, and sliding grid. A brief description of the development of these approaches are presented in this section.

The simplest approach is the imposed boundary conditions (IBC), or black box approach, modelling the impeller implicitly by substituting the actual impellers by time-averaged velocity components and turbulence quantities (Ranade and Joshi, 1990; Kresta and Wood, 1991; Jenne and Reuss, 1999; Joshi et al., 2001). An important prerequisite of this method is the availability of reliable velocity and turbulence data. This approach depends on the accuracy of the experimental data and can not present the flow characteristics within or near impeller region. Because currently no experimental data have been measured in the miniature bioreactor, this approach is not employed in the study.

The approach of momentum source-sink (SS) proposed by Pericleous and Patel (1987) introduces a quadratic source for the presence of the impellers and a sink term for the baffles in the tangential momentum equation. This simple method has been applied to simulate the single phase or two phases in the stirred tanks (Morud and Hjertager, 1996; Revstedt et al., 1998; Revstedt and Fuchs, 2001; Xu and Mcgrath, 1996; Lamping et al., 2003).

The computational snapshot method (SP), proposed by Ranade and Dommeti (1996), is
Single phase flow in the miniature bioreactor

similar to the source-sink method. Suitable mass sources (and sinks) are specified at the front and back side of the blades to simulate suction and ejection of fluid occurring at the back and front sides of impeller blade. It was reported that the trailing vortices were captured by the approach. This approach has been applied to a 45°-pitched impeller and two-phase system (Ranade, 1997; Ranade and Deshpande, 1999; Ranade et al., 2001; Zhang et al., 2002). The advantage of this method is that only a few snapshots of the flow are required to get a good description of the flow as steady-state simulations are carried out for a few impeller positions. However, the maximum tangential velocity is lower than the experimental data because of inadequate resolution of the trailing vortices, which causes the underestimation of radial velocity. The results are also sensitive to the relative position between the impeller blades and baffles.

Brucato et al. (1994) proposed the inner-outer (IO) approach to account for the complex flow in the stirred tank. The impeller region is first simulated using a rotating reference frame and the information of the flow near the impellers is extracted. This information is implemented in the reactor without impellers in a similar case to IBC. This approach has been extended to multi-impellers and two-phase system (Brucato et al. 1998; Micale et al., 1999).

Another approach of multiple reference frame (MFR) used by Luo et al. (1994) is a steady-state approximation in which individual cell zones move at different rotational speeds. The different frames are realized in the same calculation domain. But the interface between the different frames is carefully treated in this approach. Harvey and Lee (1995) applied this method to laminar flow and Wechsler et al. (1999) extended it to turbulent flow. This approach was also employed to computation of stirred vessel flows on parallel-vector computers (Bartels et al., 2002).

The explicit method to simulate the impeller movement is sliding grids (SG), which uses the moving grids to mimic the real movement of the impellers but keeps the grids for the outer walls and baffles stationary. SG developed by Luo et al. (1993) has now been developed into a standard option for the problem (Lee et al., 1996; Jaworski et al., 1997; Ng et al., 1998). This method is frequently used probably due to the fact that the approach is implemented in most commercial software packages, such as Fluent and CFX. SG is useful in accurately predicting the periodic flow patterns in a stirred reactor because the computational grid allows one to determine the boundary conditions easily.
Single phase flow in the miniature bioreactor

and precisely. By using appropriate grids the accuracy of the results can be controlled. The main disadvantage of this method is that one should explicitly add an acceleration term to the momentum equations to account for the moving grid system. Furthermore, mass flow continuity must be maintained at the grid interface. The interpolation procedure at the boundary implies reduced efficiency and a potential reduction in the accuracy of the numerical solution compared to a fixed grid approach. Another disadvantage is that the calculation time of this method can be an order of magnitude longer than that need for steady state calculations.

The first comparison of IBC, IO and SG was reported by Harris et al. (1996) and the turbulent kinetic energy profiles were found to exhibit severe underprediction for IO and SG methods. They suggested the extension of the IBC method to include multiphase, reacting flow and turbulence modeling. Tabor et al. (1996) compared the SG and MRF methods. They observed vortices trailing from the impeller blades and concluded that MRF performed better than SG in their case. Brucato et al. (1998) compared the IBC, IO and SG methods. They challenged the conclusions from Harris et al. (1996) and reported that the IBC method was very sensitive to the boundary conditions set at the impeller region. The predictions from IO were better than those from IBC. The best results were obtained with the SG method as far as mean flow velocities were concerned. Wechsler et al. (1999) compared the results of a turbulent simulation employing MFR with those from SG in a stirred tank with a four-45°-pitched-blade impeller. Encouraging agreement of the flow field between MRF and SG was found but MFR computation required only a fraction of the CPU time of the calculation with SG.

In this chapter, five different approaches (SS, SP, IO, MFR and SG) were systematically compared and an approach that is relatively precise and easily implemented into CFX source codes was chosen for the further analysis.

A1.2 Impeller simulation techniques

A1.2.1 Source-Sink approach

Pericleous and Patel (1987) introduced a quadratic source arising from the difference of the square of the impeller angular velocity and fluid velocities for the tangential
momentum equation:

\[ S_u = 0.5 \rho [(\omega r)^2 - W^2] C_f n_f A_f \]  
(Eqn. A1- 1)

The baffles was described in a similar way by defining a time-averaged sink term for the tangential momentum equation:

\[ S_u = -0.5 \rho W^2 C_g n_g A_b \]  
(Eqn. A1- 2)

A1.2.2 Snapshot approach

Suitable mass sources and sinks are specified at the front and back side of the blades to simulate suction and ejection of fluid occurring at the back and front sides of impeller blade. For all the computational cells adjacent to the backside of the impeller blades, the rotating impeller blades can be simulated by defining the mass sink \( S_u \) for the continuity and momentum equations,

\[ S_{u_a} = -r_a \rho_a A_{bc} W_{bc} \]  
(Eqn. A1- 3)

where \( A_{bc} \) is area of the surface of the computational cell which is adjacent to the impeller blade and \( W_{bc} \) is normal vector of the rotational velocity of the blade averaged for that computational cell surface respectively. The corresponding sink terms for the other variables \( \phi \) are defined as

\[ S_{\phi_a} = -r_a \rho_a A_{bc} W_{bc} \phi_a \]  
(Eqn. A1- 4)

where \( \phi_a \) is the value prevailing over the computational cell. For the computational cells adjacent to the front side of the impeller blades, a mass source for the continuity equation needs to be defined to simulate the displacement of fluid caused by the blade rotation. It is defined by the above equation with a positive sign to the right hand side. However, a source term is not added for other variables like momentum and turbulent kinetic energy.

A2.2.3 Inner-Outer Approach

In this approach, the whole vessel volume is subdivided into two partly overlapping zones: the inner and outer boundaries depicted in Fig. A1-1. First, a simulation of the flow in the inner domain is carried out in a reference frame rotating with the impeller. A first trial flow field is thus computed in the inner part including the distribution of velocity, turbulence energy and dissipation on the outer boundary surface. The outer boundary conditions are then imposed for the outer part in a laboratory reference frame which is similar to IBC method. The information on the whole vessel, including all
values on the inner boundary surface, is obtained. These values are used as boundary conditions for a second inner simulation and the procedure is repeated until the system attains a satisfactory numerical convergence.

**Fig. A1-1** Alternative subdivisions of the flow domain into zones for IO

### A2.2.4 Multiple Reference Frame

Fluid motion in a rotating subdomain is solved in a rotating frame, and the solution is matched at the interface between the rotating and stationary regions via velocity transformations from one frame to the other. However, the governing equations for the conservation of mass, turbulent kinetic energy (k) and energy dissipation rate ($\varepsilon$) remain unchanged. The momentum equations are changed because of the presence of centrifugal and coriolis forces in a rotating frame of reference which is accounted for by appropriate source terms. The velocity vector in the stationary frame of reference $U_i$ and the velocity vector in a rotating frame of reference $u_i$ are related to each other by:

$$U_i = u_i + \omega_{ij} \alpha_j x_k$$  \hspace{1cm} \text{(Eqn. A1-5)}$$

where $\omega_{ij}$ represents the angular velocity vector and $x_i$ the position relative to the rotation axis, $\varepsilon_{ijk}$ is the Levi-Civita tensor.

In contrast to SG approach, only one position of the impeller relative to the baffles is considered because of the assumption of an azimuthally constant flow field in the far-field of the impeller at some radial distance from the rotational axis of the impeller. In mixing tanks, since the impeller-baffle interactions are relatively weak, large-scale transient effects are not present in the bulk fluid and the MRF model can be used.
A2.2.5 Sliding grids

With the approach, the tank is divided into two regions: one region associated with the tank wall while the baffles remains stationary; the other region associated with the impeller rotates relative to the stationary mesh. The two grids slide past each other in a time-dependent manner, exchanging information at the cylindrical interface.

A1.3 Comparison of different impeller models and flow characterisation

As conventional instruments are difficult to use in the miniature bioreactor due to its small dimensions, no experimental data are available for this new bioreactor. There are also few papers concerning the flow driven by open flat turbine impellers. However, the basic configuration of the impellers is very similar to that of large-scale six-bladed Rushton impellers, so it is reasonable to borrow the experimental data from Rushton impellers for comparison of CFD analysis results in the miniature bioreactor.

A1.3.1 Simulation conditions and boundary conditions

The simulation was run at a rotational speed of the impeller of 4000 rpm, corresponding to a tip speed of 1.47 m/s and the Reynolds number of about 3267. The fluid in the study was assumed to be water, the density of 1000 kg/m$^3$ and the viscosity of 0.001 kg/m/s.

The boundary conditions of the simulation were as described in previous papers (Zhang et al., 2002; Lamping et al., 2003). The reactor walls and baffles were defined as impermeable walls with friction. The impeller blades were defined as stationary solids. At the top of the surface, free surface condition was set. The mathematical expressions were written as follows:

On the symmetry axis: \( \nu = w = 0 = \frac{\partial \phi}{\partial r}, \phi \neq \nu, \ w \)

At the impermeable walls and baffles: \( \frac{\partial \phi}{\partial r} = 0; \phi \neq \nu; \ v = 0 \)

At the top of the surface: \( \frac{\partial \phi}{\partial x} = 0; \phi \neq \nu; \ u = 0.0 \)

A1.3.2 Axial velocity

A similar trend from five approaches was observed for the axial velocity along the axial coordinate near the impeller edges and at the midplane of two baffles Fig.A1- 2 (a). Below the impeller, the flow is upward and reaches to its peak value. And above the impeller, the flow becomes downward. The axial velocity in the lower impeller region is
higher than in the middle and upper impeller. This can be explained by the flow pattern in the miniature bioreactor in the section of A1.3.5. Relatively under-predictions are obtained from SP and above the upper impeller region, IO has a higher axial velocity than others. The velocity from SG, SS and MRF has similar profiles. Experimental values from the literature show that the axial velocity has a sharp change across the impeller blades from around 0.15U_{tip} to −0.15U_{tip} (Wu and Patterson, 1989; Bakker, 1995), and similar simulation results were reported by Brucato et al. (1998) using IBC and IO techniques. Except for the lower impeller, the prediction values from SS, SG and MRF are in good agreement with the reported experimental data.

A large deviation for the radial profile of the axial velocity at the midplane of the middle impeller between baffles was seen from Fig.A1-2 (b) from different approaches. A small axial velocity along the radial coordinate is obtained from SG and SP. The IO technique, however, gives a sharp change of radial velocity. MRF and SS have the same profile after 1.5D_{i} but a big difference from two approaches exists near the impeller edge. Close to the middle impeller edge, an axial velocity of −0.1U_{tip} is computed from SS but −0.025U_{tip} from MRF. Experimental data reported from Bakker (1995) using a standard Rushton impeller showed that the axial velocity at the impeller edge was measured to be 0.1U_{tip} then it decreased from the impeller tip to 1.8D_{i}, along the radial distance. The discrepancy between the prediction and experimental data may be owing to different configurations of the impellers employed.
Fig. A1-2 Axial (at $2r/D_i = 1.07$ and $\theta = 90^\circ$) and radial profiles (at the midplane of the middle impeller and $\theta = 90^\circ$) of mean axial velocity.

A1.3.3 Radial velocity

The radial velocity profile along the axial coordinate is very similar from all approaches (Fig. A1-3a). The peak values are found to be located at the impeller center plane. A similar trend was reported from the simulation by Micale et al. (1999) in a dual-impeller stirred vessel when the flow pattern was a “merging” flow. Their study showed that the radial velocity was severely underpredicted close to impeller periphery. A value of $0.1 \sim 0.2U_{\text{tip}}$ was simulated from IO and SG techniques, but the experimental data was reported to be around $0.6U_{\text{tip}}$ (Rutherford et al., 1996). However, for a single Rushton impeller, simulation results from these techniques were in good agreement with the experimental data (Brucato et al., 1998; Ranade, 1997). Similar underpredictions are found from IO, SG, MRF, and SP for multi-impeller system in this study. SS approach produces a higher value of $0.35U_{\text{tip}} \sim 0.45U_{\text{tip}}$, which is still lower than the
experimental data for the dual Rushton impellers. The discrepancies may be owing to different radial positions for the value and different impeller geometries.

The radial velocity along the radial coordinate obtained from SS, SG and MRF agree well with experimental observations. But this velocity from IO and SP is severely underpredicted (Fig.A1- 3b). A maximum radial velocity 0.4Utip from SS, SG and MRF is found close to the impeller blade tip, but the radial positions from the three techniques are slightly different. The decay slope for the radial velocity along the impeller stream from SS technique is much steeper than from SG and MRF. The radial velocity in the impeller stream has been widely studied in a Rushton-impeller stirred tank and some correlations have been put forward to fit with their experimental results. For example, \( V=0.74(2r/D_i)^{0.99} U_{tip} \) from Lee and Yianneskis (1998) was used to fit well with their experimental measurements and the data from Dyster et al. (1993). The predictions in this study show that the maximum radial velocity for the flat-paddle (0.4Utip) is lower than that for the Rushton impeller (0.7Utip). The decay slope from SG and MRF matches the index of the above correlation.

Angle resolved flow characteristics in stirred tank help in the understanding of the complex trailing vortices behind the impeller blades. Several attempts have been made in recent years to measure angle resolved radial, tangential velocities and turbulent intensities (Lee and Yianneski, 1998). Fig.A1- 3c presents the azimuthal variation of mean radial velocity at the middle impeller centre-plane and a radial position of 1.2Di. 0° corresponds to the center of the leading blade, and 60° corresponds to the centre of the next successive impeller blade. The radial velocity is obviously angularly dependent on the impeller discharge stream from SG, MRF, SP and SS. As there is no impeller in the outer region for IO technique and this region is chosen to present the data, no azimuthal variation is obtained from the technique for the outer region (see section A1.2). SP technique displays a different radial velocity profile, which decreases behind the leading blade then increases to the peak value at the successive blade. The same result was found in the simulation for the Rushton impeller by Ranade et al. (2001). The simulation results from SS, SG and MRF, which increase behind the leading blade to the peak at around 10° then decrease away from the leading blade, agree well with the experimental measurements for a Rushton impeller by Lee and Yianneskis (1998).
Fig. A1-3 Axial (at 2r/D_i=1.07 and θ=90°), radial (at the midplane of the middle impeller and θ=90°) and tangential profiles (at the midplane of the middle impeller and 2r/D_i=1.2) of mean radial velocity.
A1.3.4 Tangential velocity

The predictions of the tangential velocity along the axial direction are presented in Fig. A1-4a. Close results are obtained from SS, MRF, IO and SP techniques. The maximum tangential velocity reaches up to 0.65-0.8U_{\text{tip}} which agree well with the reported experimental data (around 0.65-0.7U_{\text{tip}}) for a Rushton impeller (Mahouast et al., 1989; Wu and Patterson, 1989; Dyster et al., 1993). For a single Rushton impeller, Brucato et al. (1998) reported that the simulation results from SG technique were in good agreement with those experimental measurements and IO technique had a slight underprediction but the results were within accepted range. However, the prediction result from SG technique in this study is a little higher than that from other techniques and the maximum value is about 0.9-1.1U_{\text{tip}}.

Simulation results of the tangential velocity along the impeller stream are similar for all techniques but discrepancies among those techniques are observed to be around the impeller tip. The same maximum tangential velocity of 1.26U_{\text{tip}} is obtained from MRF and SG techniques at the tip of the middle impeller then the velocity decreases very steeply at around 1.2D_{i}. MRF worsens the deviation. Because SG and MRF have a moving grid or frame, in which the tangential velocity is proportional to the radial position, the radial position of the interface between the fixed grid (frame) and the moving grid (frame) affects the tangential velocity. Tangential velocity from SS increases to the peak close to the impeller tip then decreases very slowly to zero at the outer walls. The slow decrease of the tangential velocity was also found from experimental report by Ranade and Joshi (1990), and Cooper and Wolf (1968). SP technique gives a maximum tangential velocity of 1.0U_{\text{tip}} then decreases steeply similar to MRF and SG. The tangential value from IO is underpredicted in the impeller stream but overpredicted in reactor wall region.

Examination of azimuthally deviation of tangential velocity reveals that the tangential velocity increases to the peak just behind the leading blade then decreases and close to the successive blade the velocity increases again when MRF, SS and SG are used. Similar experimental observations were reported for the Rushton impeller by Ranade et al. (2001). The maximum value (0.5U_{\text{tip}}) in the study is observed to locate at 10-20° and the minimum value (0.2U_{\text{tip}}) at 40° behind the leading blade. However Stoots and Calabrese (1995) reported that the maximum value (0.8U_{\text{tip}}) is located at 25° and the
minimum value (0.5U_{tip}) is located at the successive blade.

Fig. A1 - 4 Axial (at 2r/D_i=1.07 and \( \theta=90^\circ \)), radial (at the midplane of the middle impeller and \( \theta=90^\circ \)) and tangential profiles (at the midplane of the middle impeller and 2r/D_i=1.2) of mean tangential velocity.
A1.3.5 Turbulent intensities

The turbulent kinetic energy is an important parameter which presents the turbulent intensities in the reactor. The axial and radial profiles of the turbulent kinetic energy $k$ scaled by $U_{\text{tip}}^2$ are shown in (Fig.A1-5). It can be seen that all techniques have a similar trend but $k/U_{\text{tip}}^2$ from SS are higher than other techniques. $k/U_{\text{tip}}^2$ along axial direction are higher just above or below the impeller blades than at the central of impeller stream for the middle and upper impellers (Fig.A1-5a). But this is not the same in the lower impeller region. The maximum $k/U_{\text{tip}}^2$ in the lower impeller region is also lower than that in the middle or upper impeller regions. The range of $0.1\sim0.15\ U_{\text{tip}}^2$ turbulent kinetic energy is obtained from SS. Radial profile of $k$ shows that $k$ is very high in the vicinity of impeller blade then decreases with the radial distance away from the impeller blade edge (Fig.A1-5b). The turbulent kinetic energy decreases more quickly from SS technique than SG and MRF, especially in the region of $2r=1.8\sim2.2Di$. But in the region of $1.5\sim1.8Di$, SS, SG and MRF have nearly the same value of $k/U_{\text{tip}}^2$. Severe underprediction of turbulent kinetic energy was reported from Harris et al. (1996) and Brucato et al. (1998) using IO and SG techniques for a single Rushton impeller. This underprection is also consistent with the report from Lee et al. (1996) and Tabor et al. (1996) using SG technique. Michale et al. (1999) reported the same underprediction of turbulent kinetic energy for a dual impeller system using IO and SG techniques. MRF is very similar to SG based on the technical feature, therefore underprediction is also found from MRF.
For the Rushton impeller, at the impeller center plane, the turbulent kinetic energy of $0.1-0.15U_{tip}^2$ was reported from Mohouast et al. (1987) and Wu and Patterson (1989). Calabrese and Scoots (1989) reported that the maximum value of $0.36U_{tip}^2$ was found in the impeller region. A close examination of the turbulent kinetic energy aims to present the trailing vortex behind the impeller blade. Understanding fluid dynamics characteristics of trailing vortices and capacities to computationally simulate these vortices is essential for reliable design and scale-up of the miniature bioreactor, especially for the aerated fermentor. Trailing vortices behind rotating impeller blades control gas accumulation and therefore pumping and power capacities of the impeller. The contour of turbulent kinetic energy from SS is presented in Fig.A1- 6a. The maximum value of turbulent kinetic energy is observed to be $0.17 U_{tip}^2$. Close examination of the contour reveals that behind each blade (the rotation is anticlockwise)
there is one small vortex. These vorticities are also be seen from the z-vorticity contour in Fig.A1- 6b.

Fig.A1- 6 Contour plots of turbulent kinetic energy (a) turbulent kinetic energy $k$ from SS; (b)z-vorticity from SS; (c) turbulent kinetic energy $k$ from SG in the middle impeller midplane. Values are in percent of $U_{tip}^2$. The impeller rotates anticlockwise.
The energy dissipation rate $\varepsilon$ is of paramount importance for many mixing applications as it controls the flow at the microscale. Knowledge of the magnitude and deviation of $\varepsilon$ across the whole vessel is essential. But it is very difficult to directly measure the distribution of the energy dissipation. A very sophisticated attempt, based on 3-D laser-induced fluorescence, was undertaken by Dahm et al. (1991). Indirect ways of measuring $\varepsilon$ include turbulence intensities and length scales through LDA or chemical reaction method (Schaer et al., 1999). But all of the methods are based on approximations for macro length scale or micromixing time, which may not be appropriate in stirred tank flow.

Similarly to the turbulent kinetic energy, the energy dissipation rate from SS is higher than from other techniques (Fig.A1-7). The maximum of $\varepsilon/N^3D^2$ is found to be about 16 from SS but it is about 5 from the other techniques. Ng and Yianneskis (2000) using SG technique and $k$-$\varepsilon$ model for a standard Rushton impeller found the predicted maximum $\varepsilon/N^3D^2$ to be around 7.5-8.0 which was very close to the values in the study. The peaks of the energy dissipation along the axial profile are located at all impeller midplanes (a) and in the impeller streams but very close to impeller tips (b). The energy dissipation profiles on the centre line of the middle impeller stream (b) from all techniques are very similar to the experimental data in a fermentor (MBI lecture notes). The energy close to the edge of the impeller blade is very high and decay rapidly with the distance from the blade to walls. After $2r/D_i=1.5$, the predicted valued from all different techniques are nearly the same except SP approach.

The indicted estimates reported by Lee and Yianneskis (1998) based on LDA data showed that the LDA studies yielded the maximum $\varepsilon/N^3D^2$ values of 22 near the blade tip and decreased the values comparable with those of Wu and Patterson (1989) at $2r/D_i=1.5$. The predictions from SS are very close to the experimental values.

The energy dissipation rate distribution throughout the reactor is very inhomogeneous (Fig.A1-8). Energy dissipation is concentrated in the impeller swept region. Dissipation rates in the tank cover a range of more than three orders of magnitude. Interestingly the energy dissipation in the lower impeller region is different from that in the middle and upper impeller region. Similar turbulent kinetic energy distribution at the lower impeller region was reported by Michale et al. (1999) for a “diverging” flow. In the impeller swept area, the highest energy dissipation is found at the front side of impeller blades.
tips and decreases along the swept direction. In the horizontal plane between the middle and lower impellers, most of energy is dissipated around shaft, baffles and outer walls, but only a very small amount of energy is dissipated in the bulk of fluid.

Fig.A1 - 7 Axial (at 2r/Di=1.07 and $\theta=90^\circ$) and radial (at the midplane of the middle impeller $\theta=90^\circ$) profiles of energy dissipation rates.
A detailed knowledge of the flow patterns existing in the miniature bioreactor, especially in the vicinity of the impellers, is necessary. The flow patterns are a strong function of the reactor configuration, such as the geometry of the impellers, and presence of the baffles. Also they are affected by the rheological properties of fermentation broth and conditions of gas-sparging.

It was reported (Rutherford et al. 1996; Mishra and Joshi, 1994) that for disc-turbine and disc-turbine impeller system, if the impeller spacing is greater than the impeller diameter, each impeller gives its characteristic upper and lower ring vortices independently, which is called “parallel flow”. If the spacing is equal to the impeller diameter, the flow patterns merge midway between the spacing and form two large ring

Fig.A1- 8 Energy dissipation rates at different locations
Single phase flow in the miniature bioreactor

vortices, which is called “merging flow”. But if the spacing between the lower impeller and the bottom wall is less than the impeller diameter, the flow is called “diverging flow”.

Fig.A1- 9 presents the streamline and flow patterns in the miniature bioreactor. In the upper impeller region, a large ring vortex formed in the top of the reactor. Between the upper and middle impeller, the flow patterns are the same as the “merging flow” as the impeller spacing is nearly equal to impeller diameter. But between the middle and lower impeller, the flow patterns are very complicated. The flow pumped out by the middle impeller is severely affected by the flow from the lower impeller. And no vortex is formed below the middle impeller. However, the vortices formed by the lower impeller are located above the lower impeller. The flow patterns are also presented by the liquid axial and radial velocity profiles in Fig.A1- 10.
Single phase flow in the miniature bioreactor
Fig. A1- 9 Streamline and flow patterns in the miniature bioreactor at a rotational speed of 4000 rpm.
Fig.A1- 10 Liquid axial velocity and radial velocity profiles in the midplane between two baffles
A1.3.7 Pumping number

A comparison of predicted values of gross parameters, such as pumping number and power number of the impellers, within the literature can provide useful indication about the validity of simulation. The predicted value of the pumping number is calculated as:

\[ N_q = \frac{\int_0^\infty \int_0^r Vrd\theta \, dz}{2 \pi ND_i^3} \]  

(Eqn. A1-6)

\( R_i \) is the impeller radius and \( D_i \) is the impeller diameter. \( V \) is the radial velocity along the impeller stream, and \( w \) is the width of the impeller blades.

The cumulative pumping numbers for three impellers at the outer edge of blades are obtained from the above different approaches (Fig. A1-11). SS, IO and SP techniques give a very similar pumping number of 0.5, but MRF and SG techniques yield a higher pumping number of 1.5. Stoots and Calabrese (1995) and Cooper and Wolf (1968) reported that pumping number for a disc turbine at the outer blade edge \((r=Di/2)\) was 0.78 and 0.8 respectively. For a dual disc-turbine combination at the outer blade edge, Mishra and Joshi (1994) found that the pumping number was small for the small clearance between the lower impeller and the vessel bottom, and a cumulative pumping number was increased for a small the impeller clearance. The cumulative number for two impellers was found to be equal to the pumping number for a single impeller if the impeller clearance was smaller than the impeller diameter. In their study with a similar configuration of the miniature bioreactor (similar impeller clearance and bottom clearance), the cumulative pumping number was measured to be 0.60-0.65 for a double
Single phase flow in the miniature bioreactor

A turbine, which is very close to the predicted value from SS, SP and IO. But MRF and SG over-predicted the number.

A1.3.8 Power number

The power number is calculated by integrating the simulated local energy dissipation rates over the entire volume of the vessel and dividing it by the group $\rho_1 N^2 D_i^5$.

$$N_p = \frac{\int r_a \rho_a \varepsilon dV}{\rho_a N^2 D_i^5}$$  (Eqn. A1-7)

where $r_a$ is the local liquid fraction which is taken to be equal to 1.0 for the case of single (liquid) phase, and the local fluid density $\rho_a$ is the liquid density, $\rho_1$. Different predicted values for the three impellers are obtained as in Fig.A1-12. Compared to experimental values for a large-scale unit with similar configuration (Uhl and Gray, 1966), the power number is approximately 4.0–5.0 for the micro-impeller in turbulent flow regime. There are three blades, assuming that the power number of the three impellers is not affected by the impeller spacing, the predicted value is roughly 12. MRF, SG, IO and SP give an underprediction of the value, but SS technique provides a reasonable power number in the miniature bioreactor. This is consistent with the discussion from the energy dissipation section.

Fig.A1-12 Predicted power number from different approaches.

Given different rotational speeds, a power curve is obtained based on the equation 4-2 (Fig.A1-13). The power curve is very similar to the typical power curve from flat blade turbine system. It is agreed that in the turbulent regime, the power number is found to be constant and is specific to the type of impeller and the geometry of the impeller. But for a multi-impeller system, the power number is also a function of impeller spacing.
Hudcova et al. (1989) found that for an unaerated dual impeller system comprising two disc turbines, the flow patterns generated by the two impellers affected each other for the impeller spacing in the range between 0.5D and 1.5D, resulting in the power number increasing by 50% compared relative to the single impeller system. But if the impeller spacing was above 2D, the power number was the addition of the power number of each impeller. In the miniature reactor, the impeller spacing is nearly the same as the impeller diameter. The predicted value of power number is about 10. Assuming the power number in this three-impeller system doubling the power number for the single impeller, the power number for the single impeller is about 5, which agrees well with the experimental data.

![Power Curve](image)

Fig.A1- 13 Power curve for the impellers of the miniature bioreactor.

A1.4 Conclusions

Five different approaches: SnaPshot, Source-Sink, Inner-Outer, Multiple Reference Frames, and Sliding Grids are employed to simulate the mixing in the miniature bioreactor. Results for three velocity components and turbulent intensity obtained from those approaches show that the predictions from SS, MRF and SG agree with the experimental data. The predicted gross numbers such as power number and pumping number from SS are more close to reported experimental data than MRF and SG.
Appendix 2 Two-phase flow in a 20L bioreactor

A2.1 Introduction

Multidimensional two-phase flow models are divided into Lagrangian or Eulerian type of models. The continuity and momentum equations are solved for the continuum while the dispersed phase follows a Langrangian approach. This approach is currently limited to low concentration of the dispersed phase. A Lagrangian attempt to simulate the motion of single gas bubbles in the liquid using volume-of-liquid (VOF) technique was made by Krishna and van Baten (1999). The rise trajectories of bubbles in the size range 4-20mm were captured. The simulation technique determined the rise velocity in a bubble column and reproduced precisely the experimental observations. However such a simulation may require a grid with millions of grid cells and the use of a massively parallel computer.

Eulerian models are divided into algebraic slip and two-fluid models. Algebraic slip model considers the two phases as a mixture and conservation equations are solved using mixture properties. Only gravitation, pressure, and drag forces are taken into account in the momentum equation for the gas phase, and the slip velocities of the gas phase are calculated from algebraic equations. The slip velocities relative to the mean velocity are used in the convection terms in the equations for gas volume fractions. The disadvantage of this simple approach is the fact that additional interface forces are neglected. Two-fluid model considers the two phases as interspersed continua and a set of conservation equations are solved for each phase. The interfacial force acting on the two phases is required. The results of the flow simulation are used as an input to calculate the distribution of the gas fraction and other parameters.

An overview of CFD simulations of gas-liquid phase in a stirred tank is given in Table A2.1. Only the Eulerian approach has been considered for aerated stirred tank reactors to date. Different approaches have been employed for the simulation of impellers (See appendix 1). Trägardh (1988) reported two-dimensional simulations with the algebraic slip model for a stirred vessel equipped with two Rushton impellers. This algebraic slip model was also employed by Bakker & Van den Akker (1994). Good agreement of the predicted gas holdup and overall mass transfer coefficient with experimental data was observed. But the hydrodynamics were not compared with experimental data.
Morud & Hjertager (1996) employed two-fluid model and source-sink for impellers in two dimension and compared numerical simulations with LDA measurement data of the gas-phase velocity. The radial gas velocities in the impeller region were well predicted, but the axial gas velocities were mostly overpredicted. Friberg and Hjertager (1999) extended the simulations of Morud & Hjertager (1996) from two to three dimension and good agreement of the velocities in all directions was obtained. Gas cavities situated behind the impeller blades were also captured numerically by Friberg and Hjertager (1999). The simulation by Jenne and Reuss (1997) was similar to that by Morud & Hjertager (1996). Despite no quantitative comparison with experimental data, the overall gas hold-up seemed to be well predicted. Ranade & Deshpande (1999) and Ranade et al. (2001) used two-fluid model and snapshot method for impellers and trailing vortices and accumulation of gas behind the impeller blades were observed. The predicted velocities agreed well with experimental PIV data. The turbulent kinetic energy, however, was underpredicted. The Euler-Lagrange method by Wu et al. (2001), in combination with the IBC method for impellers and large eddy simulation for turbulence, was used to describe the gas-liquid flow in a stirred tank, equipped with two Rushton impellers. Although there is no quantitative comparison with experimental data, the results indicate that this technique is promising.

Mass transfer in a bioreactor between gas and liquid is directly related to the interfacial area and the residence time of the gas phase. To predict the size distribution of the gas bubbles taking full account of the breakage and coalescence effects is therefore very valuable in optimising operation of existing reactors and studying scale-up and scale-down between these bioreactors. In many CFD calculations of dispersed two-phase flows, the particles are assumed to have the same size and shape, i.e. the mono-disperse assumption (Trägardh, 1988; Morud & Hjertager, 1996; Ranade et al., 1999, 2001). Simple calculation of bubble size can be made by suggesting that break-up rates and coalescence rates of bubbles are equal by Friberg (1998):

\[
d_b = 1.4 \frac{\sigma_{0.6}}{\rho_l} \frac{1}{\epsilon_{0.4}}\]  
\text{(Eqn. A2-1)}

Another approach is to calculate the number density using first order conservation equation:

\[
\frac{\partial r_{\alpha} \rho_{\alpha} n_b (V_b, t)}{\partial t} + \nabla \cdot (r_{\alpha} \rho_{\alpha} n_b (V_b, t) U_{\alpha}) = B - D\]  
\text{(Eqn. A2-2)}
### Table A2.1 Parameters in numerical studies of aerated stirred tanks.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Impeller</th>
<th>2D/3D</th>
<th>Two-phase model</th>
<th>Bubble size</th>
<th>Reactor parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane et al. (2002)</td>
<td>MRF</td>
<td>3D</td>
<td>Two-fluid</td>
<td>Eqn.A2-2</td>
<td>A Rushton impeller (C/T=1/4)</td>
</tr>
<tr>
<td>Zhang et al. (2002)</td>
<td>SP</td>
<td>3D</td>
<td>Two-fluid</td>
<td>Eqn.A2-2</td>
<td>Three open flat impellers</td>
</tr>
<tr>
<td>Venneker et al. (2002)</td>
<td>SG</td>
<td>2D</td>
<td>Algebraic Slip</td>
<td>Eqn.3-31</td>
<td>A Rushton impeller (C/T=1/2)</td>
</tr>
<tr>
<td>Djebbar et al. (2002)</td>
<td>IBC</td>
<td>2D</td>
<td>Drift-flux</td>
<td>Eqn.A2-2</td>
<td>Different impellers</td>
</tr>
<tr>
<td>Wu et al. (2001)</td>
<td>IBC</td>
<td>3D</td>
<td>Euler-Lagrange</td>
<td>Constant size</td>
<td>Two Rushton impellers</td>
</tr>
<tr>
<td>Lo (2001)</td>
<td>SG</td>
<td>3D</td>
<td>Two-fluid</td>
<td>Eqn.3-31</td>
<td>A Rushton impeller (C/T=1/3)</td>
</tr>
<tr>
<td>Ranade et al. (1999, 2001)</td>
<td>SP</td>
<td>3D</td>
<td>Two-fluid</td>
<td>2.0mm</td>
<td>A Rushton impeller (C/T=1/3)</td>
</tr>
<tr>
<td>Friberg and Hjertager (1999)</td>
<td>SG</td>
<td>3D</td>
<td>Two-fluid</td>
<td>Eqn.A2-1</td>
<td>A Rushton impeller (C/T=1/2)</td>
</tr>
<tr>
<td>Jenne and Reuss (1997)</td>
<td>IBC</td>
<td>2D</td>
<td>Two-fluid</td>
<td>Constant size</td>
<td>A Rushton impeller (C/T=1/3)</td>
</tr>
<tr>
<td>Morud and Hjertager (1996)</td>
<td>SS</td>
<td>2D</td>
<td>Two-fluid</td>
<td>Constant size</td>
<td>A Rushton impeller (C/T=1/2)</td>
</tr>
<tr>
<td>Bakker and Van den Akker (1994)</td>
<td>IBC</td>
<td>3D</td>
<td>Drift-flux</td>
<td>Eqn.A2-2</td>
<td>A Rushton impeller (C/T=1/3)</td>
</tr>
<tr>
<td>Trägardh (1988)</td>
<td>SS</td>
<td>2D</td>
<td>Algebraic Slip</td>
<td>Constant Size</td>
<td>Two Rushton impellers (C/T=1/2)</td>
</tr>
</tbody>
</table>

\(n_b(V_b,t)\) in the left side of equation represents the number of bubbles of the volume \(V_b\) at a given time. \(D\) and \(B\) represent death and birth rate of bubbles respectively. The death term consists of breakage to small bubbles and coalescence to larger one. The birth term consists of larger bubbles breaking into the size range and smaller bubbles coalescing into the required size range.
The complete calculation of different gas bubble size groups due to breakage and coalescence was implemented in the CFX-solver. The MUtiple-SIze-Group (MUSIG) model (See chapter 3 for details) provides a frame-work in which the population balance method together with the break-up and coalescence models were incorporated into three dimensional CFD simulations (Lo, 2001). The simulation results were not compared with the experimental data. Venneker et al. (2002) solved single phase flow then employed the modified algebraic slip model to calculate the gas volume fraction and gas bubble sizes using the population balance equations similar to MUSIG model. The simulation displayed the different bubble size distribution in the reactor in two dimension, but the simulation did not present the gas accumulation behind the impeller blades.

No report to date includes the two-fluid and population balance equations for multiple impellers in three dimension. A test simulation in a 20L bioreactor with three Rushton impellers for gas-liquid phase was run in the chapter. Source-sink approach (see Appendix 1) was employed to account for the interaction between the continually moving impellers and stationary baffles and walls. The momentum transport equations in cylindrical coordinates for the gas and liquid phase were solved along with the standard k-ε model of turbulence. The interfacial force was calculated according to the Eqn. 3.22-3.30.

**A2.2 Numerical results and discussions**

**A2.2.1 20L bioreactor configuration**

20L bioreactor is a conventional fermenter, with a working volume of 15L. The vessel is D=220mm in diameter and H=345mm in height (working domain). There are three standard six-bladed Rushton impellers along the shaft, which are located at the centre of the reactor. The impeller diameter is 70mm. There are four baffles equally spaced along the periphery and the baffle width is B=D/10. The height of each blade is 12mm and the clearing space between two impellers is 96mm. The clearance between the lowest impeller disc and the bottom of the vessel is 85mm. The gas sparger ring is located below the lower impeller. The flow domain was divided into two symmetric parts (each encompassing two baffles and three impeller blades). The grid resolution was chosen so that the impeller is described with five control cells in axial direction resulting in a grid
Two-phase flow in a 20L bioreactor of 57 * 45 * 60 in axial, radial and tangential directions, respectively. The detailed dimension and numerical grid are presented in Fig. A2-1.

A source term at the gas sparger for gas volume fraction and momentum equations was employed and a degassing boundary condition at the top surface was used to account for the gas removal from the reactor. Other boundary conditions were the same as the single phase in the miniature bioreactor (see Appendix 1). The rotational speed for the reactor was 400rpm, which resulted in its tip speed of 1.47m/s and the Reynolds number of
about 33,000. The airflow rate in the 20L bioreactor was set at 1 vvm, equivalent to 0.007ms⁻¹ (compared to 0.0005ms⁻¹ in the miniature bioreactor).

**A2.2.2 Aerated liquid flow pattern**

The flow patterns in the aerated fermentor are functions of the geometry of the impellers, clearance from the bottom, clearance above the upper impeller, impeller spacing and sparging conditions. In the 20L bioreactor, two large ring vortices are formed between the top impeller and the lower impeller (Fig. A2-2 a). A combination of three impellers acts as a single-impeller and produces only one radial outflow. The function of lower and upper impellers is impaired and only the middle impeller functions normally. Under aerated conditions, Bouaifi *et al.* (2001) mounted disc-turbine in the lower part and A-315 in the upper part, and the impeller spacing is three times the impeller diameter. Only one loop between the two impellers was observed in the whole reactor, which agrees well with CFD analysis.

Gas sparging affects the flow pattern in the reactor by comparing Fig. A2-2 a and b. Compared to the unaerated condition (Fig. A2-2 b), a ring vortex is found above the top impeller and below the lower impeller respectively. The flow pattern between the top impeller and the middle impeller is very similar to that between the middle impeller and the lower impeller. The downward flow out of the top impeller meets the upward flow out of the middle impeller at the nearly halfway axial location between two impellers and small vortices are formed below the top impeller and above the middle impeller. However, they are smaller than that at the top of the reactor. Rutherford *et al.* (1996) reported that the clearance between two impellers are greater than 0.385T, the Rushton impellers operate independently and each impeller produces its own characteristic upper and lower ring vortex. The discrepancies between the simulation results and the experimental report can be explained by the fact that the impeller clearance in the reactor is nearly the same as the critical value 0.385T, and the interactions between the two impellers affects the independence of each impeller.
Two-phase flow in a 20L bioreactor

(a) Gas-liquid Flow 400rpm

(b) Single phase Liquid Flow 400rpm
The flow patterns under the aerated conditions at a rotational speed of 200rpm are presented in Fig. A2-2 c. Only one large ring vortex is formed between the middle and lower impeller. The top impeller functions very similarly to the unaerated condition but the ring vortex just above the impeller become small and the secondary liquid circulation is observed at the top surface. The liquid flow is severely affected by gas sparging under the aerated and agitation conditions.
A2.2.3 Liquid and gas speed profile

The liquid flow patterns are also presented by the liquid speed \( \sqrt{U^2 + V^2 + W^2} \) profile in the vertical plane between two baffles (Fig. A2-3a). The pumping capacity of the top and bottom impellers is impaired compared to the middle impeller. At the bottom of the reactor, the liquid velocity is very small because the downward flow pumped by the bottom impeller is affected by the large vortex. The similar profile is found at the top of the impeller.

Gas speed profile (Fig. A2-3b) indicates that the gas bubbles are not well dispersed. The gas is sparged into the reactor but gas bubbles are not dispersed by the first impeller. According to the equation for the minimum rotational speed for dispersing gas bubbles:

\[
\frac{N_g D_l}{D_i} = A + B \left( \frac{D_r}{D_i} \right)^4 \quad A=1.22 \text{ and } B=1.25 \text{ for a disc impeller} \quad (\text{Eqn. A2-3})
\]

The minimum rotational speed for the reactor is around 720rpm. Therefore the high speed of gas bubbles along the shaft is expected. Between the first and second impellers, the speed of the gas phase is nearly zero along the walls of the reactor. However, above the second impeller, as the impeller pumps out of the liquid along with the gas phase, the gas moves radially toward the wall and a meandering movement is taken to the upper part the reactor. A small stagnant loop can be clearly seen near the wall in the top of the tank. This effect can also be seen in the drawing of the bulk flow patterns in an aerated stirred vessel of Nienow et al. (1978) (Fig.4-2).
Two-phase flow in a 20L bioreactor

A2.2.4 Energy dissipation rate

The energy dissipation rate profile (Fig. A2-4 a) in the vertical plane between the baffles again shows that the function of the radial pumping is performed normally by only the middle impeller. The energy dissipation around the top and the bottom impellers decreases rapidly along radial direction while around the middle impeller the energy dissipation is very high along the impeller pumping jet to the reactor wall. Without the interaction between impellers, the energy dissipation profile around the top
Two-phase flow in a 20L bioreactor

and the bottom impeller is the same as that around the middle impeller. The distribution of the energy dissipation in the tank is a combination of the “parallel flow” and “merging flow” reported by Rutherford et al. (1996). A high energy dissipation rate zone shaped like pie are observed from the horizontal plane just below the middle impeller (Fig. A2-4b).

The unaerated power consumption is calculated from the Eqn. A1-7 and a power curve can be obtained (Fig. A2-5). The power number is independent of Reynolds number if the flow is highly turbulent. After the rotational speed of 400rpm, corresponding to the Reynolds number 33,000, power number keeps a constant value of 17.4. The power number for a single standard Rushton impeller is 6.0, and if the impellers act independently of each other, the power number is tripled to 18.0. The interaction between impellers leads to decrease in the power number in the reactor.

Aerated power consumption is always lower than that in an unaerated system. In the case of the rotational speed of 400rpm and the gas flow rate of 1vvm, the power number is reduced to 14.2. Compared to the unaerated condition, 20% reduction of power consumption is obtained from the simulation. Many correlations have been proposed to calculate the gassed power consumption. For example, the reduction of power consumption can be 60% for 18 Rushton blades (MBI lecture notes). But those correlations are based on the assumptions that the gas is well dispersed in the reactor, and especially that the gas is effectively pumped and dispersed by the lower impeller.
Two-phase flow in a 20L bioreactor

Fig. A2-4 Energy dissipation rate distribution (a) in the vertical midplane between the baffles (b) in the horizontal plane below the second impeller (x=0.16m)
A2.2.5 Gas volume fraction

The gas volume fraction profile in the vertical plane between the baffles (Fig. A2-6 a) is very similar to the gas speed profile (Fig. A2-6 b). It is clearly shown that the gas phase is dispersed by the middle impeller, rather than by the lower impeller. As the lower impeller pumping is not sufficient to circulate gas bubbles at the bottom of the reactor, there is a large zone with a low gas holdup in the lower part of the reactor, which is also observed experimentally. High gas volume fraction regions are found around the shaft. This has been reported from the experimental observations on the flow patterns of the poorly dispersed system (Nienow et al., 1978). The gas bubbles are dispersed by the middle impeller, and a meandering of the gas fraction field is found in the upper part of the reactor. A region free of gas is observed just above the middle impeller and similarly the same region is found near the wall at the top of the reactor, which was also reported from the experiment (Venneker et al., 2002). Fig. A2-6 (b) shows that gas phase accumulates in the low pressure region on the trailing side of the impeller blade before being dispersed by the trailing vortices. Accumulation of gas phase is also found around the baffles.
Fig. A2.6 Gas volume fraction distribution (a) in the vertical midplane between the baffles (b) in the horizontal midplane of the second impeller

A2.2.6 Gas bubble size distribution
Prediction of local bubble-size offers the possibility of investigating the internal gas-liquid structure and the understanding of gas-liquid flow patterns can be used to improve the mass transfer process. The two-fluid model coupled with population balance equations produces the profile of the gas bubble size distribution in the reactor (Fig. A2.7). It can be clearly seen that the gas bubble size distribution is non-
homogeneous. In the numerical simulation, the bubble size determines the drag force, which affects the rise velocity of the gas bubbles. Constant bubble size distribution does not map the gas phase flow.

The spatial bubble size profile is a complex interaction of the convective bubble transport, bubble breakage by the impeller and the local turbulent intensity. A high gas holdup leads to large coalescence rates because of the large number of bubble collisions. A high turbulence intensity, especially the kinetic energy contained in the eddies of a wave-length of the order of the bubble diameter, leads to an increase in bubble breakage. The vertical profile of gas bubble sizes in Fig. A2-7 (a) shows that the smallest bubbles are found in the upward flow of the lower impeller, radial flow of the middle impeller and a small region of the downward flow at the top impeller due to break-up in the highly turbulent discharge stream. In the liquid circulation where the turbulent intensity is low, bubbles become larger due to coalescence. Relatively large bubbles are expected at the bottom of the reactor, as coalescence prevails in the region. The largest bubbles are found in the gas sparger region and along the shaft. In the region of the shaft, the turbulent intensity is relatively high (Fig. A2-7), but a high gas volume fraction is also found (Fig. A2-5). Breakage and coalescence coexist but coalescence prevails in the region.

The heterogeneous distribution of gas bubble size is also presented in the horizontal plane of the middle impeller (Fig. A2-7 b). The largest bubbles form behind the impeller blades as gas accumulates in the low-pressure region. After they are pumped out of the blade, they are broken up into small bubbles. Those small bubbles coalesce into large bubbles again in the region far away from impeller blade tip. Large bubbles are also found around the baffles.
Two-phase flow in a 20L bioreactor

Fig. A2-7 Gas bubble size distribution (a) in the vertical midplane between the baffles (b) in the horizontal midplane of the second impeller
A2.2.7 Mass transfer coefficient profile

The volumetric mass transfer coefficient $k_{L,a}$ can be calculated from the Eqn. 4-4-6 and the predictions from CFD analysis are presented in Fig. A2-8. The mass transfer is far from uniform throughout the reactor. The local $K_{L,a}$ value is determined by both the local values of the interfacial area "a" and the liquid mass transfer coefficient $k_L$. The local interfacial area is determined by the local gas holdup and the local gas bubble size. The liquid mass transfer coefficient $k_L$ is dependent on the main resistance of the liquid side, which is affected by the local turbulent intensity. High turbulent intensity reduces the surface renewal time in the stagnant liquid film around the bubble and results in high mass transfer coefficient. Therefore, high $k_{L,a}$ values are expected in the region where high gas holdup and turbulent intensity. These regions include the region around the shaft, in the outflow of the middle impeller and around the impeller blades. In reverse, poor mass transfer is found at the bottom of the reactor, along the wall in the upper part of the reactor and just above the middle impeller. Baffles play an important role in improving mass transfer rate in the reactor (Fig. A2-8 b). The mass transfer rate between two baffles is higher than that in the bulk of fluid.

Experimental measurement of $k_{L,a}$ for air-water system with different impeller rotational speeds is made for comparison with the simulated $k_{L,a}$ value. Fig. A2-9 shows the measured and predicted overall mass transfer coefficient plotted as a function of the mean energy dissipation rate. The continuous line is the well-established correlation of Van't Riet (1979). The slope of the best line of fit through the data points has a slope which agrees well with the value of 0.7, which is the exponent of $(P/V)$ in the correlation reported by Van’t Riet (1979). CFD predictions are in good agreement with the measured values.

A2.3 Conclusions

A simulation with two-fluid model coupled with population balance equations (MUSIG model) was run in three dimension for a multi-impeller bioreactor. Flow patterns, bubble properties and turbulent intensity were captured from CFD analysis. A non-homogeneous distribution of gas bubble size, gas holdup, and turbulent energy dissipation in the reactor were found in the reactor. Mass transfer coefficient was calculated based on Higbie’s penetration model and overall mass transfer coefficient from prediction agreed well with from the experimental measurements.
Fig. A2-8 Mass transfer coefficient distribution (a) in the vertical midplane between the baffles (b) in the horizontal midplane of the second impeller
Fig. A2-9 Comparison of predicted and measured mass transfer for air-water system: (♦) are experimental data and (●) are the values obtained from CFD simulations. The solid line is the relationship reported by Van't Riet (1977) based on a comprehensive review of $K_{L}a$ data for Rushton turbine impellers. The dashed lines are the ± 40% deviation from Van't Riet's equation due to experimental uncertainties and different measurement techniques.
Appendix 3 Publications by the author


* The publications and two posters attached as follows are related to this thesis.
Design of a prototype miniature bioreactor for high throughput automated bioprocessing

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Introduction

The availability of the complete gene sequences for a growing number of organisms is expected to lead to a significant increase in the number of genetically engineered strains that can be used as cellular producers of new biological materials e.g. drug candidates. There is consequently a pressing need to develop new fermentation methods that will allow for the selection of suitable strains and operation conditions faster and at lower costs. We are seeking to address this need through significant reduction in size while maintaining the scalability of the process.

The design of a miniature prototype bioreactor is presented with a detailed analysis of the fluid flow and related hydrodynamic parameters including the energy dissipation rate ($\varepsilon$) and mass transfer coefficient ($K_a$). These parameters will form the engineering basis for comparison with data from large scale bioreactors and shaken micro-litre systems.

The Bioreactor: essential characteristics

- diameter equal to that of a single well of a 24-well plate
- scalable
- has the potential for automation
- see Figure 1 (opposite) for details.

Results

The local power input, energy dissipation rate and gas volume fraction are derived from an analysis of the multiphase flow in the miniature bioreactor using computational fluid dynamics (CFD). Volumetric mass transfer coefficients are predicted using Higbie's penetration model with the contact time obtained from the CFD simulations of the turbulent flow in the bioreactor. Comparative data are provided from parallel experiments carried out in a 20L (15L working volume) conventional fermenter.

Experimental data were obtained to compare the performance of the miniature bioreactor with that of a standard 20L bioreactor and the CFD simulations.

Conclusion

Predicted and measured volumetric mass transfer coefficients in the miniature bioreactor are in the range 100hr⁻¹ to 400 hr⁻¹, typical of those reported for large-scale fermentation. The miniature bioreactor may be used to predict the performance of a full scale bioreactor.

Acknowledgements

S. Lamping's PhD research programme is supported by the Science and Engineering Research Council and Eli Lilly.

H. Zhang's research is funded by KC Wong Foundation and ORS at UCL.
Major advances in numerical techniques and the development of high speed computers in the past decade have revolutionized process design and development. Computational Fluid Dynamics (CFD) techniques provide a powerful means of obtaining process information for complex operations including homogenization, centrifugal separation, and multiphase operations [1,2,3]. Design and process optimisation can also be evaluated using these techniques, eliminating the cost, time and production delay associated with physical trials. In this poster this is demonstrated through the design of a new miniaturized bioreactor.

The publication of the human genome combined with advances in high throughput screening is leading to the discovery of a very large number of potential drug target molecules. What is needed now are novel process engineering methods that allow the biotechnology industry to decide early which few, amongst the thousands of target molecules that are screened, stand the highest chance of progressing into the process development and manufacturing phase. This demands the capability to make major decisions about the processability of new molecules when only millilitre quantities of test materials are available. In fermentation and animal cell culture current practice based on shake flask technology and laboratory bioreactors is slow and labour intensive and has not changed significantly for nearly 50 years. Here is described the design of a new mechanically agitated miniature bioreactor with a working volume of 6 ml, based on the dimensions of a single well of a 24-well plate. The miniature bioreactor offers a 'one step' approach to scalable product development, an alternative to conventional inoculation and seed train cell expansion and a significant reduction in time-to-market.

**Results**

The local power input, energy dissipation rate and gas volume fraction are derived from an analysis of the multiphase flow in the miniature bioreactor using computational fluid dynamics (CFD). Volumetric mass transfer coefficients are predicted using Higbie’s penetration model with the contact time obtained from the CFD simulation of the turbulent flow in the bioreactor. Comparative data are provided from parallel analysis carried out in a 20L (15L working volume) conventional fermenter.

**Application of Advanced Computational Techniques in the Design of a Novel Miniature Bioreactor for Process Development**

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**Design of a Novel Miniature Bioreactor for Process Development**

**The Bioreactor: essential characteristics**

- diameter equal to that of a single well of a 24-well plate
- scalable
- has the potential for automation

**CFD analysis of engineering flow in the miniature bioreactor and the 20L fermenter at equal average energy input of 576 W/M^2**

**Conclusion**

CFD is an important engineering tool in the design of the miniature bioreactor, prediction of its performance and assessment of its scalability. Key engineering parameters: energy dissipation, gas holdup and gas bubble size were obtained from CFD techniques. Predicted volumetric mass transfer coefficients in the miniature bioreactor and 20L fermenter are in good agreements with the measured values.

The relationship between the average power input per unit volume and the mass transfer coefficient is similar both in the miniature bioreactor and in 20L fermenter. The miniature bioreactor may be used to predict the performance of a full scale bioreactor.

**Publications**


**Acknowledgements**

H. Zhang’s research is funded by Henry Lester Trust, KC Wong Foundation and the ORS at UCL.
Numerical Simulation of Mixing in a Micro-well Scale Bioreactor by Computational Fluid Dynamics

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Received Nov. 25, 2001

The introduction of the multi-well plate miniaturisation technology with its associated automated dispensers, readers and integrated systems coupled with advances in life sciences has a compelling effect on the rate at which new potential drug molecules are discovered. The translation of these discoveries to real outcome now demands parallel approaches which allow large numbers of process options to be rapidly assessed. The engineering challenges in achieving this provide the motivation for the proposed work. In this study we used computational fluid dynamics (CFD) analysis to study flow conditions in a gas-liquid contactor which has the potential to be used as a fermenter on a multi-well format. The bioreactor had a working volume of 6.5 ml, with the major dimensions equal to those of a single well of a 24-well plate. The 6.5 ml bioreactor was mechanically agitated and aerated by a single sparger placed beneath the bottom impeller. Detailed numerical procedure for solving the governing flow equations is given. The CFD results are combined with population balance equations to establish the size of the bubbles and their distribution in the bioreactor. Power curves with and without aeration are provided based on the simulated results.

Keywords (T T): Numerical simulation, Fermenter, Snapshot method, Two-fluid, Population balance

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Introduction

Shake flasks are used routinely in the pharmaceutical, biochemical and medical fields for cell growth culture, media screening and for cell expansion. Shake flask fermentation requires relatively small amounts of material, typically between 50 and 500 ml, and relies on a horizontal platform, which is continuously shaken in a horizontal circle to induce bulk mixing of the broth and to promote oxygen dissolution by surface aeration. Oxygen transfer, cell growth and product yield in the shake flask are determined by bulk mixing. Shake flask fermentation practice, however, has remained essentially unchanged for nearly 50 years. Shake-flask methods are laborious and while "instrumented" shake flasks are now available, monitoring of cell growth and product yields are not possible and the conditions of nutrient and oxygen transfer are poorly defined. Most importantly, shake flask fermentation provides little information about full-scale process operation and scale-up. Such information is currently obtained through additional experiments used, laboratory scale fermenters, typically 2 to 10 litre in volume, and geometrically similar pilot-scale fermenters. Manpower and facility space requirements are high and progress is slow and the "time-to-market" is delayed because of the large number of such fermentation runs that are needed.

Robotic multi-well systems are now under intensive development for drug discovery. They have the potential for changing the ways laboratory fermentations are run but currently they are not geared to the study of unit operations. In the case of fermentation, the requirements of adequate mixing and oxygen transfer rate over long periods demand solutions to a number of fundamental issues. These provide the motivation for our research.

In recent years, CFD has emerged as an engineering tool to provide a complete description of flow and mixing conditions in a process equipment having a complex geometry. Here we use CFD simulation of flow in a micro-well scale bioreactor, based on Reynolds averaged Navier-Stokes equations and standard turbulent k-ε model the overall aim of the study is to assess scale dependency of mixing parameters at micro-well scale of operation. From a CFD perspective the time-dependent interaction between the im
Pellers blades and the baffles creates a major difficulty. Several approaches including the black box approach, inner core approach, multiple reference frame approach, computational snapshot approach and sliding mesh approach have been proposed in the past to deal with this problem. Ranade has discussed the advantages and disadvantages of each approach[1]. In the present study the snapshot method was used as the best compromise between computational time required to obtain acceptable results and accuracy. The gas phase was treated by a Eulerian approach, based on a two-fluid model. Gas bubble coalescence and break-up models were used to calculate the bubble population and bubble size.

Mathematical Model

The multidimensional two phase flow may be divided into Lagrangian or Eulerian type of models. Lagrangian models are generally limited to a low concentration of the dispersed phase, and therefore in the present study the governing equations and the solution procedure are based on the latter models. The numerical model of turbulent gas-liquid flow is based on a two-fluid model, where transport equations governing conservation of mass and momentum are solved for each phase simultaneously. The transport equations for mass and momentum are formulated for each phase in volume-averaged form[2]. Standard turbulent k-ε model is adopted. Turbulent kinetic energy k and its energy dissipation ε are obtained from the homogeneous model, where the transported quantities for the process are the same for all phases[3].

A bubble moving in a liquid is subjected to several forces caused by the liquid flow, including drag force, lift force and a force due to virtual mass effects. For the purpose of this study, the drag force is the most important force acting on a bubble. The gas and liquid momentum equations are coupled by the interfacial force terms due to the drag force by the following[2].

\[ F_x = - F_d = 0.75 \sigma_{\text{sg}} \frac{C_d}{d_b} \left| U_b - U_s \right| (U_b - U_s) \]  

(1)

The drag coefficient \( C_d \) is determined from the modified Reynolds number and making use of the correlation proposed by Ishii and Zuber[4] for the correction of the drag force in a bubble swarm, the interaction between bubbles is also taken into account.

The snapshot approach proposed by Ranade et al.[4] is adopted to simulate the motion of the impellers. Suitable mass sources and sinks are specified at the front and back sides of the blades to simulate suction and ejection of fluid occurring at the back and front sides of impeller blade. For all the computational cells adjacent to the backside of the impeller blades, the rotating impeller blades were simulated by defining the mass sink \( S_m \) for the continuity and momentum equations.

\[ S_m = -r_{\text{sg}} A_b W_{ib} \]  

(2)

where \( A_b \) is the area of the surface of the computational cell which is adjacent to the impeller blade and \( W_{ib} \) is the normal vector of the rotational velocity of the blade averaged for that computational cell surface respectively. The corresponding sink terms for the other variables \( \phi \) can be defined as

\[ S_m = -r_{\text{sg}} A_b W_{ib} \phi \]  

(3)

where \( \phi \) is the value prevailing over the computational cell.

For the computational cells adjacent to the front side of the impeller blades, a mass source for the continuity equation needs to be defined to simulate the displacement of fluid caused by the blade rotation. This is defined by equation (2) with a positive sign to the right hand side. However, Any source terms will not be added for other variables including momentum and kinetic energy.

The number of bubbles per unit volume is calculated from the first order conservation equation for the bubble number density[5].

\[ \frac{\partial n_b(V_b,t)}{\partial t} + \nabla \cdot \left[ \rho_b n_b(V_b,t) U_b \right] = B - D \]  

(4)

\( n_b(V_b,t) \) on the left side of equation (4) represents the number of bubbles of volume \( V_b \) at a given time. \( D \) and \( B \) represent the death and birth rates of bubbles, respectively. These terms account for the dynamic processes of coalescence and breakage of bubbles throughout the entire volume of the vessel. For the agitated vessels Bakker et al.[6] give the statistical equilibrium term \( B - D \) as equivalent to

\[ B - D = \omega (n_{b,\text{max}} - n_b) \]  

(5)

where \( \omega \) denotes an average effective coalescence and break-up frequency.

\[ \omega = c_w \frac{3}{2} \sqrt{2} \frac{F_b}{d_b} \frac{1}{2} k \]  

(6)

and \( c_w \) is a constant with a value of 0.15, \( n_{b,\text{max}} \) is the number of bubbles corresponding to the maximum stable bubble size estimated by the correlation:

\[ d_{b,\text{max}} = \left( \frac{W_{ib} \sigma}{\rho_b} \right)^{\frac{3}{4}} \epsilon_{\text{B}}^{-\frac{1}{4}} \]  

(7)

This correlation was proposed by Wilkinson et al.[7].
and $W_0$ is equal to $6.6^{[6]}$.

**Boundary Conditions**

For both phases, no-slip conditions are applied except that the radial gradient of the gas fraction is assumed to be zero along the bottom and side walls. The gas sparger is simulated as a mass source term for the above equations. The top surface is assumed to be flat, where the normal velocity of the liquid phase, the normal fluxes, $k$ and $\epsilon$ are zero on the surface. However, the top surface is simulated as a mass sink term for the gas phase. A baffle is defined as a thin surface because of its small width compared to its length.

**Simulation Results**

The micro-well scale vessel used has a diameter, $T = 16$ mm and is equipped with three-impeller six-bladed paddle turbine and four baffles equally spaced. Other key dimensions are: impeller diameter $D_i = 7$ mm; blade height, 0.875 mm; hub diameter, 1.6 mm; and the baffle width, 1.6 mm. The height of the calculation domain, $H = 52$ mm so that the working volume is 6.5 mL. Cylindrical coordinate system is used with the origin located at the centre of the vessel bottom. Half of the reactor is considered as a solution domain. In the simulated case, the stirred speed is 2000 r/min and gas flow rate is 1.0vvm. The grid resolution is chosen so that the impeller is described with three control volume in axial direction resulting in a grid of $57 \times 55 \times 60$ in axial, radial and tangential directions, respectively.

The power number is calculated from the simulated results using

$$N_p = 2 \int \frac{\rho \omega dV}{\rho \omega N^2 D_i^3}$$

where $\omega$ denotes the liquid phase for both single phase and gas-liquid phase. The simulated power curve for single phase is shown in Fig. 1. The impact of the gas phase on power consumption is shown in Fig. 2.

**CFD Predicted gas phase velocities for typical r-z planes are shown in Fig. 3. The vector plot for liquid phase shows the flow pattern generated by the paddle turbine. The gas is seen to rise up to the impeller and then move radially towards the wall. Large circulation zones are formed just above the impellers, while small circulation zones are formed just below it.**

**Fig. 2 Predicted influence of gas flow rate on impeller power curve.**

**Fig. 3 Gas velocity contour in the r-z plane.**

Most of the turbulent energy is dissipated in the impeller stream region as shown in Fig. 4. Gas volume fraction distribution shown in Fig. 5 indicates the accumulation of gas around impeller blades. The impeller pumping is not sufficient at the simulated speed of 2000 r/min to circulate gas bubbles in the lower part of the vessel.

**Fig. 4 Energy dissipation rate in the r-z plane.**

The gas bubbles in the lower part of the vessel
Conclusions

The flow patterns and associated hydrodynamic parameters in a micro-well scale bioreactor have been successfully predicted with the aid of CFD. A two-fluid model, a population balance model for bubble coalescence and break-up and a k-ε turbulence model were used in the CFD approach. The simulated results describe the flow patterns and gas hold up, bubble size distribution and power input. The detailed analysis of comparison of predicted results with experimental data and the use of this method to predict the local R_e, dissolved oxygen, substrate concentration and cell growth form the basis of our further investigation.

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<tr>
<td>D_i</td>
<td>impeller diameter</td>
</tr>
<tr>
<td>k</td>
<td>turbulent kinetic energy</td>
</tr>
<tr>
<td>N</td>
<td>rotational speed</td>
</tr>
<tr>
<td>N_p</td>
<td>power number</td>
</tr>
<tr>
<td>n_b</td>
<td>bubble population density</td>
</tr>
</tbody>
</table>

Greek letters

a: liquid phase
β: gas phase
e: turbulent energy dissipation / m^2/s^3
μ: viscosity / kg/(m*s)
σ: surface tension / kg/(m^2*s)
ρ: density / kg/m^3
n_w: gas bubble coalescence and break up frequency / s^-1

References

Design of a prototype miniature bioreactor for high throughput automated bioprocessing

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Abstract

A new miniature bioreactor with a diameter equal to that of a single well of a 24-well plate is described and its engineering performance as a fermenter assessed. Mixing in the miniature bioreactor is provided by a set of three impellers mechanically driven via a microfabricated electric motor and aeration is achieved with a single tube sparger. Parameter sensitive fluorophors are used with fibre optic probes for continuous monitoring of dissolved oxygen tension and an optical based method is employed to monitor cell biomass concentration during fermentation. Experimental measurements are provided on volumetric mass transfer coefficient for air–water and bacterial fermentation data are presented for \textit{Escherichia coli}.

The local and average power input, energy dissipation rate and bubble size are derived from an analysis of the multiphase flow in the miniature bioreactor using computational fluid dynamics (CFD). Volumetric mass transfer coefficients are predicted using Higbie’s penetration model with the contact time obtained from the CFD simulations of the turbulent flow in the bioreactor. Comparative data are provided from parallel experiments carried out in a 20 l (15 l working volume) conventional fermenter. Predicted and measured volumetric mass transfer coefficients in the miniature bioreactor are in the range 100–400 h\textsuperscript{−1}, typical of those reported for large-scale fermentation.

1. Introduction

The realization that there are approximately 30,000 genes in the human genome has shifted drug discovery research significantly towards proteomics and away from genomics. Currently, the available drugs on the market target nearly 500 of the estimated hundreds of thousands of human proteins with the expectation that this number will increase by a factor of 10–100 in the next few years. A major challenge for drug discovery now is to elucidate the relationship between proteins produced by each gene and disease. In this respect, advances in proteomics and automated high-throughput screening based on the shaken microwell plate system have provided the technology platform for a significant increase in the number of potential drug candidates that are likely to come forward. A related challenge that is yet to be addressed is the need to define the conditions for the translation of results from the microwell system to conventional laboratory scale. For example, in the case of \textit{Escherichia coli} fermentation, while a number of discovery companies now routinely run fermentation in a 24-well plate to produce small quantities of proteins for crystallographic studies, scale-up to laboratory fermentation, typically up to 10 l, is proving difficult to achieve. The ability to scale-up such information and the capacity to generate process data from unit operations carried out at the microwell scale have become important issues in the development pathway of a new drug. This has provided the motivation for a few recent studies on microwell scale bioprocessing, including the present investigation the aim of which is to describe the engineering design of a miniature bioreactor and assess its use as a fermenter.

Shaken flask fermentation, with working volumes between 50 and 500 ml, has been used with very little change for over five decades for cell growth culture, media screening and cell expansion (Kato, Hiraoka, Tada, Lee, & Koh, 1999; Buchs, 2001; Rhodes & Gaden, 1957; Maier & Buchs, 2001). “Instrumented” shake flasks have been used recently to establish bulk mixing and oxygen transfer in these systems and work is in progress to establish the relationships
between these parameters and cell growth and product yields (Anderlei & Buchs, 2001; Weuster-Botz, Altenbach-Rehem, & Arnold, 2001).

The automated shaken microcell system has been used in a few of recent publications to obtain process information on biological materials. These include studies on bacterial fermentation (Weiss, John, Klimant, & Heinze, 2001b; Duetz et al., 2000; Duetz & Witholt, 2001), animal cell cultures (Girard, Jordan, Tsao, & Wurm, 2001) and biotransformation (Doig, Pickering, Lye, & Woodley, 2001; Weiss, John, Klimant, & Heinze, 2001a). As far as oxygen transfer is concerned, the limited data available indicate that the volumetric mass transfer coefficient in a shaken microcell (Weiss, John, Klimant, & Heinze, 2001b) is likely to be lower, by at least a factor of 10, compared to a conventional scale fermenter. Walther et al. (1994) have described a miniature bioreactor with a working volume of 3 ml for cell culture in a space laboratory and Kostov, Harins, Randers-Eichhorn, and Rao (2001) have recently presented the design of a microreactor with a working volume of 2 ml and reported basic data on the responses of pH, dissolved oxygen and optical density probes using E. coli fermentation as a test bed. Mixing was achieved by the action of a magnetic stirrer placed at the bottom of the well. Initial results demonstrated that fermentation at the microwell-scale was feasible, but achieving adequate overall oxygen transfer rate in the microreactor proved difficult and comparison of $K_{L}a$ with data from a 1 l conventional fermenter showed the difficulties in using magnetic-bar stirrer in the microreactor.

In the present study, we report engineering data to demonstrate the operation of a new miniature bioreactor having a diameter equal to that of a single well of a standard 24-well plate. A microfabricated three-bladed turbine impeller was used to mix the contents of the miniature bioreactor and a single sparger placed underneath the bottom impeller provided the means for aeration. Microfabricated fibre optic probes (Junker, Wang, & Hatton, 1988; Wang, Shahriari, & Musris, 1999) were used for in situ measurement of process parameters including dissolved oxygen, pH and cell density. Volumetric oxygen transfer data were obtained for air-water and E. coli fermentations for different operational conditions. The results were compared with data obtained from parallel experiments using a 2 l mechanically agitated fermenter with a working volume of 15 l and with predictions from theory based on CFD simulations of multiphase flow in the miniature bioreactor and the Higbie's penetration model for mass transfer.

2. Materials and methods

The miniature bioreactor: Fig. 1 shows the main elements of the miniature bioreactor together with some of its associated instrumentation and interconnections. The bioreactor was machined from Plexiglas to allow visual inspection of mixing and gas bubbles. Air-water was used to establish the engineering performance of the miniature bioreactor for mass transfer operations and E. coli DH5a was chosen as a fermentation system because of its robustness and tolerance to contamination. Subsequent miniature bioreactors in our laboratory (not described here) are built from stainless steel with parts that can be fully sterilised for cell culture experiments.

The cylindrical chamber of the miniature bioreactor was 16 mm in diameter and 48 mm high (working volume of 6 ml). The mixing of the contents of the bioreactor was achieved by means of three, 6-bladed open flat-turbine impellers, each having a diameter of 7.0 mm and width of 1.5 mm. The impellers were driven from the top of the bioreactor with a microfabricated electric motor (Smooovy, Switzerland) with a shaft diameter of 1 mm and an infinitely variable speed control (maximum speed and torque of 15,000 rpm and 2.2 m Nm, respectively). The bottom impeller was placed approximately 6 mm from the base of the chamber, the distance between two impellers was 7.5 mm and the distance between the top impeller and the free liquid surface was 7.0 mm. The bioreactor was equipped with four baffles of width 1.8 mm and thickness 0.6 mm. The air from a compressed air supply was sparged through a perforated plastic cap placed at the discharge tip of a single tube of internal diameter 1.0 mm. The sparger was placed directly beneath the hub of the bottom impeller (Fig. 1). The air flow rate was measured using a standard laboratory rotameter with a flow rate in the range of 0.2–100 ml min$^{-1}$ of air (Barnant Company, IL, USA). The gas supply had a separate connection via a two-way valve to a compressed nitrogen cylinder allowing mass transfer experiments to be carried out as described later in this section.

On-line measurements of dissolved oxygen tension, pH, and cell density were performed by means of fibre optic probes; temperature was measured by means of a fine diameter copper-constantine thermocouple. The oxygen sensor, the optrode (fibre optic oxygen sensor, AVS-OXYKIT 1.5, Knight Optical Technologies Ltd., Surrey, UK) consisted of a 1 mm diameter silica glass optical fibre sealed in a 7 cm long stainless steel tube rod. The working tip of the probe was dip-coated with a ruthenium complex immobilised in a sol-gel matrix. An optical fibre carried light from a blue LED (470 nm output peak) to the immobilised ruthenium/sol-gel coating layer at the working tip of the stainless-steel tube. The 470 nm light excites the ruthenium complex to fluoresce. The level of this fluorescence is quenched in the presence of oxygen molecules, which diffuse through the sol-gel matrix and interact with the trapped ruthenium. The reduction in the fluorescence signal is related to the concentration of oxygen through the Stern–Volmer equation (Wang et al., 1999). The level of fluorescence signal was detected using a second fibre optic, part of the integrated optical probe and the fluorescence light was analysed by a sensitive CCD detector array grating spectrometer (Type: AVS-MC2000; Knight Optical Technologies Ltd.; www.knightopttech.com). This
has multiple spectrometer channels for other parallel spectral sensors, such as pH, turbidity, and other fluorescence investigations as described below. The primary channel for oxygen was used to monitor both LED light source excitation level and fluorescence emission simultaneously capturing full spectra in milliseconds.

In the present study, solution pH was measured by an optical probe using the method of Junker et al. (1988). The pH sensor consisted of: a fibre optic probe of diameter 1.5 mm (FCR-UV200F, Knight Optical Technologies Ltd., Surrey, UK); a xenon pulsed light source (XE-2000) and a blue LED light source (LED-475) coupled to a multiple channel spectrometer (MC2000). The fluorophor, 1-hydroxypyrene-3,6,8-trisulfonic acid trisodium salt (HPTS, Sigma-Aldrich, Dorset, England) with a $pK_a$ value of 7.3 was selected for the experiments described here. Two different excitation wavelengths of 405 and 460 nm were used. The fluorescence intensity was measured at a single emission wavelength of 520 nm. A linear calibration curve was obtained in 20 g l$^{-1}$ of HPTS in water using the method of (Junker et al., 1988). The spectrometer was used to monitor the change in fluorescence.

The measurement of cell density was achieved by combining three optical fibre probes, as shown in Fig. 1. Each fibre was fabricated from a single 600 µm active core diameter fibre. One fibre was used to deliver the light produced by a miniature tungsten light source to the bioreactor contents. The other two probes were used for light collection from the bioreactor, one for transmitted light (625 nm) and one for scattered light. This arrangement allowed a range of measurements including turbidity, transmission/colour and nephelometry to be carried out by a multiple channel spectrometer (MC2000). In the present study the broth turbidity was sufficiently low to allow reliable cell density data to be obtained from the measurement of transmitted light (probes A and B).

**Air-water experiments:** The performance of the miniature bioreactor was assessed initially under defined flow conditions through a series of experiments carried out with air-water. The results from these experiments also allowed a basis for comparison with conventional bioreactors for which many correlations are available (Smith, Van’t Riet, & Middleton, 1977; Van’t Riet, 1979; Ni, Gao, Cumming, & Pritchard, 1995). $K_La$ values were calculated from the dissolved oxygen concentration profiles obtained as a function of time by using the dynamic gassing out technique (Van’t Riet, 1979). Before each experiment, the optode was calibrated at 100% and 0% air saturation by sparging air and nitrogen, respectively. A typical experiment started with fresh de-ionised water containing a known amount of sodium chloride salt and with the impeller speed set at a predetermined value. The oxygen concentration was continuously monitored as a function of time. Nitrogen was sparged till the level of oxygen had fallen to zero. At this point, the gas supply was switched rapidly to air set at a fixed flow rate of 1 vvm corresponding to a superficial
velocity of 0.0005 m s\(^{-1}\) and the rise in oxygen concentration was monitored.

**Fermentation:** All fermentations in the miniature bioreactor were carried out with *E. coli*, DH5\(\alpha\) in batch mode at 37°C, an air flow rate of 1 vvm at impeller speeds 1300, 1500 and 1850 rpm. No attempt was made in these experiments to optimise the fermentation process, the aim was to demonstrate that fermentation was achievable in the miniature bioreactor and obtain basic engineering and fermentation parameters for comparison with conventional scale fermentation. Data for the latter were obtained in a 20 l (15 l working volume) LH20L03 HI-CAT series bioreactor (Adaptive Biosystems Ltd., Progress Business Park, Luton, UK). pH was fixed at the start of fermentation, but not controlled.

The cultures were grown on a semi-defined medium which consisted of, per litre, D-glucose (10 g), MgSO\(_4\) \(\cdot\) 7H\(\text{2} \text{O}\) (1.2 g), (NH\(\text{4}\))\(_2\)SO\(_4\) (4 g), KH\(_2\)PO\(_4\) (13.3 g), citric acid (1.7 g), Na\(_2\)EDTA (8.4 mg), CoCl\(_2\) \(\cdot\) 6H\(\text{2} \text{O}\) (2.5 mg), MnSO\(_4\) \(\cdot\) 4H\(\text{2} \text{O}\) (15 mg), CuSO\(_4\) \(\cdot\) 2H\(\text{2} \text{O}\) (1.5 mg), H\(_2\)BO\(_3\) (3 mg), Na\(_2\)MoO\(_4\) \(\cdot\) 2H\(\text{2} \text{O}\) (2.5 mg), ZnCl\(_2\) (13 mg), Fe (III) citrate (100 mg), thiamine hydrochloride (4.5 mg; Sigma-Aldrich, Fancy Road, Poole, UK) and casamino acids (10 g l\(^{-1}\); Oxoid). The pH of the medium was adjusted to 6.3 prior to sterilisation by addition of 4 M NaOH. All chemicals were obtained from BDH (Dorset, England) unless otherwise stated. Sterilisation of the miniature bioreactor was achieved by rinsing the equipment with 1 M NaOH followed by sterile water. Seed culture was prepared by inoculating 10 ml of the medium in a McCartney bottle with a single colony from nutrient agar plates. The culture was allowed to grow overnight at 37°C and rotated at 200 rpm by a horizontal shaken platform in an incubator. 10 ml of seed culture was used to inoculate 500 ml of fresh medium, which was grown for 6 h under the same conditions. The 500 ml culture was used as the starting point for all experiments in the miniature bioreactor and the 20 l fermenter. In the case of the miniature bioreactor, 0.6 ml of the culture was used to inoculate 5.4 ml of fresh medium in the bioreactor. A 10% inoculation was also used for the 20 l fermentation. In the large fermenter foaming was controlled automatically by the addition of 100% polyethylene glycol (PPG) pumped at a concentration of 0.1 ml l\(^{-1}\). The 20 l bioreactor was operated in the batch mode and the initial fermentation conditions were as follows: temperature, 37°C; air flow, 1 vvm; agitation speed, 530 rpm; pH 6.3. The aeration and agitation levels were monitored using an Ingold polarographic probe (Mettler-Toledo Ltd., Beaumont Leys, Leicester, UK). Online data were logged by Propack data logging and acquisition software (Acquisition Systems, Fleet, Hampshire, UK). Cultures were grown for 14 h.

**Simulation of flow in the miniature bioreactor:** Energy dissipation rate gas volume fraction and air-liquid interfacial area are important engineering flow parameters used in correlating experimental \(K_\text{o}a\) data. In the present study the power input, and hence energy dissipation rate, to the microimpellers could not be measured with confidence because of the very low values of torque involved. The power input was predicted as part of an analysis of the multiphase flow and mixing in the miniature bioreactor by using computational fluid dynamics, CFD (CFX 4.1, AEA Technology, UK). The standard Reynolds-Averaged-Navier-Stokes (RANS) model was used to solve the three-dimensional two-phase (gas-liquid) turbulent flow in the miniature bioreactor and the closure of the problem was achieved using the k-\(\varepsilon\) model. Turbulent kinetic energy, \(k\), and energy dissipation, \(\varepsilon\), were obtained from the homogeneous model, where \(k\) and \(\varepsilon\) were assumed to be the same for both phases throughout the vessel (Soon, Harbridge, Titchener-Hooker, & Ayazi Shamlou, 2000; Boysen et al., 2001). The motion of the gas bubbles and their interaction with the flowing liquid were described in terms of the drag force acting on the bubbles, the impact of lift force and the virtual mass effects were ignored. The gas and liquid momentum equations were coupled by the interfacial force terms due to the drag force by the following equation:

\[
F_a = -F_g = 0.75 \rho_a \frac{C_d}{d_b}(U_g - U_a)(U_g - U_a).
\]

The drag coefficient \(C_d\) was determined from the modified Reynolds number (Kuo & Wallis, 1988) and the interaction between bubbles were taken into account by making use of the correlation proposed by Ishii and Zuber (1979) for the drag force in a bubble swarm. The impeller was described by the inclusion of additional source terms in the momentum equations using the method of Pericleous and Patel (1987). Each baffle was treated as a thin surface and described by defining an appropriate time-averaged sink term in the momentum equations as recommended previously (Morud & Hjertager, 1996; Revstedt, Fuchs, & Tragardh, 1998; Xu & McGrath, 1996).

The average bubble size was estimated from the following correlation (Wilkinson, Vanschayk, Spronken, Laurent, & Van Dierendonck, 1993):

\[
d = \left( \frac{W_{e_0} \sigma}{\rho_a} \right)^{-0.6}(\varepsilon^{-0.4}).
\]

The critical Weber number, \(W_{e_0}\), in Eq. (2) was assumed to have a value equal to 0.6 (Hinze, 1955). The energy dissipation term, \(\varepsilon\), in Eq. (2) was based on flow conditions around the impeller blades, and considering the small size of the miniature bioreactor, bubble coalescence was assumed to be negligible. For both phases, no-slip boundary conditions were applied. Free-slip conditions were used along the bottom and side walls (Morud & Hjertager, 1996). The gas sparger was simulated as a solid body. The gas inlet was set on top plane of the solid body and the normal component of gas was specified according to the gas flow rate (1 vvm). At
gas outlet (top of the vessel), the velocity was determined from a mass balance based on inlet conditions. For a fixed impeller speed and gas flow rate, the CFD simulations provided the distribution of the relevant engineering parameters in the miniature bioreactor including the velocity field, bubble size, gas hold-up, and the energy dissipation rates. The local values of the energy dissipation rates were integrated over the vessel volume to obtain an average value over the entire contents. The average power input was obtained directly from the integrated energy dissipation rate and used for comparison with published data. The simulated parameters were also used in a mass transfer model to predict both the profile and the integrated overall mass transfer coefficients, $K_{ia}$, as described later on in section under results and discussion.

3. Results and discussions

Mixing and flow in the miniature bioreactor: The CFD simulations of speed and energy dissipation rate for a single phase liquid with the properties of water are shown in Fig. 2 for a speed of 2500 rpm. The single-phase power number, $N_p$, was obtained by integrating the simulated local energy dissipation rates over the entire volume of the vessel and dividing it by the group $\rho_l N^3 D^5$. Thus:

$$N_p = \frac{2 \int r_0 \rho_d \tau dV}{\rho_u N^3 D^5}, \quad (3)$$

where $r_0$ is the local liquid fraction which was taken to be equal to 1.0 for the case of single (liquid) phase, and the local fluid density $\rho_u$ was assumed to be liquid density, $\rho_l$. The simulated impeller power curve was obtained by assuming equal distribution of power input between the three impellers. These simulations predicted a power number of approximately 4.0 for the microimpeller in turbulent flow regime, comparable to published experimental values for a large-scale unit with similar configuration (Uhl & Gray, 1966). Eq. (3) was also used to predict the local and average power input for the two-phase, gas–liquid system, in the miniature bioreactor (simulations not shown). The results gave a ratio of gassed to ungassed power, $P_g/P$, of 0.8–0.9. The reported ratio of $P_g/P$ varies between 0.9 and 0.4 depending on the type of impeller and gas flow number (Smith et al., 1977).

Flow simulations were also performed for the 20 l standard fermenter equipped with a set of three Rushton turbines. The CFD simulated single-phase power curve predicted a power number 6.0 for turbulent flow which compared favourably with published values for the Rushton turbine impeller (Uhl & Gray, 1966).

For distilled water an average value of $4 \times 10^{-4} \text{ m s}^{-1}$ has been recommended for liquid mass transfer coefficient, $K_L$, and solution ionic strength is known to increase the overall volumetric mass transfer coefficient, $K_{L,a}$, partly through its impact on interfacial area, $a$ (Van’t Riet, 1979). The effect of ions on $K_{L,a}$ however is not easy to predict, but according to experimental information it varies with the type of ions present in solution and is dependent on the prevailing fluid energy dissipation rates. Van’t Riet’s (1979) analysis of the published experimental data indicate that such uncertainties coupled with different measurement techniques mean that reported empirical correlations are likely to vary in their estimations of $K_{L,a}$ by up to ±40%. Higbie’s (1935) penetration model of mass transfer at a gas–liquid interface has been used previously to describe mass transfer in air-lifts (Ayazi Shamloo, Pollard, & Ison, 1995) and mechanically agitated mixing vessels (Kawase & Moo-Young, 1990). According to Higbie’s model, mass transfer at a gas–liquid interface is assumed to occur by a series of encounters between the liquid and the gas. Each encounter lasts for only a short time so that steady-state conditions are never established and any mass transfer that occurs is due to the unsteady molecular diffusion. The relationship between the mass transfer coefficient, $K_L$, liquid-phase diffusivity, $\delta$, and exposure time, $t$, is given by

$$K_L = \frac{2}{\pi^{1/2}} \left( \frac{\delta}{t} \right)^{1/2}. \quad (4)$$

In a mechanically agitated vessel, Kolmogoroff’s theory of homogeneous and isotropic turbulence has been used to obtain the following equation for the surface renewal time, $t$, as follows:

$$t = \left( \frac{\gamma}{\Delta} \right)^{1/2}, \quad (5)$$

where the local energy dissipation rate, $\epsilon$, is obtained from the CFD simulation of flow in the vessel.

Combining Eqs. (4) and (5) and rearranging it leads to the following equation for the local liquid phase mass transfer coefficient, $K_{L,a}$:

$$K_{L,a}(r, z, \theta) = \frac{2}{\pi^{1/2}} \left[ \delta(r, z, \theta) N \right]^{1/4} \left( \frac{\delta}{\gamma} \right)^{-1/2}. \quad (6)$$

The local specific surface area available for mass transfer is given by:

$$a(r, z, \theta) = \frac{6 \rho _g (r, z, \theta)}{d_s}, \quad (7)$$

where $\rho _g$ is the local gas volume fraction. We used Eqs. (5) and (6) to predict the $K_{L,a}$ distributions in the miniature bioreactor. Fig. 3 shows the distribution of gas volume fraction and $K_{L,a}$ in the vessel for air–water. The profiles shown in Fig. 3 are for a speed of 2500 rpm and an air flow rate of 1 vvm. To compare these predictions with experimental data, the overall (average) volumetric mass transfer coefficient was calculated by integrating the local values over the entire working volume of the miniature bioreactor.
Thus

\[ K_{La} = \frac{\int K_L(r, z, \theta) a(r, z, \theta) \, dv}{V} \]  

(8)

The overall \( K_{La} \) were predicted for a range of impeller speeds and the operational airflow rate of 1 vvm used in our experiments. The results are presented and discussed below.

Air-water \( K_{La} \) results: The experimental \( K_{La} \) values were based on the well-mixed model for both the gas and liquid phases, as suggested by Dunn and Einsel (1975). The well-mixed model is considered appropriate for this case because of the small size of the miniature bioreactor. According to this model, \( K_{La} \) is obtained from the oxygen probe response data by the following expression:

\[ K_{La} = \frac{1}{\tau_p} \ln \left( \frac{C_{\infty} - C}{C_{\infty} - C} \right) = \frac{1}{\tau_p} \ln \left( \frac{1}{C_{\infty}} \right) \]  

(9)

where \( C_{\infty} \) is a normalized dissolved oxygen concentration defined by:

\[ C_{\infty} = \frac{C}{C_{\infty} - C} \]

The electrode used in the present investigation had a response time, \( \tau_p \) (the time needed to record 63% of a stepwise change), of 42 s at 20°C, measured using a standard procedure described elsewhere (Dunn & Einsel, 1975). CFD predictions gave an overall volumetric mass transfer coefficient, \( K_{La} \), in the miniature bioreactor typically in the order of 100 h\(^{-1}\) (0.03 s\(^{-1}\)) indicating that the impact of the probe response time on \( K_{La} \) was an important consideration. We used a first-order response model recommended by Badino, Facciotto, & Schmidell (2001) in our calculations to account for the fibre optic probe response time.

Thus,

\[ \frac{dC_p}{dt} = \frac{1}{\tau_p} (C_{L} - C_p), \]  

(10)

where \( C_p \) is the normalized dissolved oxygen concentration measured by the probe. Substituting for \( C_L \), integrating and rearranging gives the following expression for \( K_{La} \):

\[ C_p = \frac{1}{\tau_m - \tau_p} \left[ \tau_m \exp \left( -\frac{t}{\tau_m} \right) - \tau_p \exp \left( -\frac{t}{\tau_p} \right) \right] \]  

(11)

where \( \tau_m = 1/K_{La} \). Eq. (11) was solved for \( K_{La} \) using Microsoft Excel at each time measurement and the results averaged. \( K_{La} \) data were obtained for air-water in the presence of sodium chloride for a range of impeller speeds. Fig. 4 shows the results plotted as a function of the mean energy dissipation rate in the miniature bioreactor. For comparison, selected CFD simulated values of \( K_{La} \) are also shown. The continuous line in Fig. 4 shows the predicted \( K_{La} \) based on the correlation of Van’t Riet (1979). The slope of the best line of fit through the data points has a slope which agrees well with the 0.7, which is the exponent of \( P^{0.7} \) in the equation reported by Van’t Riet (1979). However, the absolute values of \( K_{La} \) measured in our experiments are consistently lower by approximately 40% compared to those expected from Van’t Riet’s expression. The difference is thought to be due to the differences in the vessel-impeller configuration between the two systems. Van’t Riet’s equation is based on data obtained for standard a Rushton turbine impeller, which is more efficient than the open flat turbine configuration used in the present study. It is
notable that CFD-predicted \( K_{l,a} \) values are in good agreement with experimental \( K_{l,a} \) data, at least in the range of power per unit volume examined \( (5 \times 10^2 < P/V < 5 \times 10^4 \text{ W m}^{-3}) \), which covers the range of interest to most fermentations.

We carried out additional experiments using the standard 20 l (working volume, 15 l) bioreactor in which we measured \( K_{l,a} \) for air–water as a function of impeller speed. The airflow rate in the 20 l bioreactor was set at 1vvm, equivalent to 0.007 m s\(^{-1}\) (compared to 0.0005 m s\(^{-1}\) in the miniature bioreactor). To compare the results for the two bioreactors therefore the overall volumetric mass transfer coefficient data for the each reactor were adjusted by assuming \( K_{l,a} \propto v^2 \), as recommended by Van’t Riet (1979). The results are shown in Fig. 5 where the solid line was obtained from Van’t Riet’s equation and the dashed-lines represent ±40% deviations from it. The \( K_{l,a} \) values for the miniature bioreactor consistently fall below those of the 20 l fermenter, but the best lines of fit through the data points for the two scales have the same slope which very close to the exponent of \( P/V \) in Van’t Riet’s equation. It is also notable that while the \( K_{l,a} \) data for the miniaturized bioreactor are lower than the 20 l scale the difference are within the expected 40% deviation. Taken together the observations based on Figs. 4 and 5 support the view that the engineering performance of the miniature bioreactor is adequately described by current CFD techniques and that its performance, at least as far as mixing and oxygen transfer is concerned, matches those of laboratory bioreactors.

E. coli \( K_{l,a} \) and fermentation results: Figs. 6a and b show respectively the variation of dissolved oxygen and dry cell weight (g l\(^{-1}\)) as a function of time for fermentation of E. coli, DH5\(\alpha\), in the miniature bioreactor. The profiles shown in Figs. 6a and b are used routinely to monitor the progress of fermentation and provide data for calculation of other important fermentation parameters including oxygen uptake rate. These aspects are beyond the scope of this investigation, the aim of which is to compare the performance of the miniature bioreactor with a large fermenter. The data in Figs. 6a and b refer to experiments carried out at a fixed impeller speed of 1500 rpm corresponding to a simulated mean energy dissipation rate, \( \epsilon \), of 0.53 W kg\(^{-1}\). In each case data are also shown from parallel experiments carried out in the 20 l fermenter using standard probes and running under normal fermentation conditions described in Section 2. Fluorescence-based fibre optic probes, for monitoring of fermentation parameters including pH and dissolved oxygen, have been fully described and successfully tested previously (Junker et al., 1988). In the case of pH, we used both a pH indicator dye immobilised on the working tip of the fibre optic probe, as well as the HPTS dye directly introduced into the culture medium. The former is preferable but in our experiments we found its response to be very slow and erratic (data not shown). In contrast acceptable results were obtained from the probe responding to the dissolved HPTS dye. In the case of oxygen, the response of the commercial immobilised fluorophore probe was reliable and its calibration reproducible. No attempt was made in these experiments to run the fermentations in the two scales...
Fig. 4. Overall volumetric mass transfer coefficient as a function of the impeller power input per unit volume. The plots are for the miniature bioreactor and refer to air-water system: (♦) are experimental data and (•) are the values obtained from CFD simulations. The solid line is the relationship reported by Van't Riet (1979) based on a comprehensive review of $K_{ia}$ data for Rushton turbine impellers. The dashed lines are the ±40% deviation from Van't Riet's equation due to experimental uncertainties and different measurement techniques.

Fig. 5. Overall volumetric mass transfer coefficients (♦) for the miniature bioreactor compare well with those obtained in a 20 l (15 l working volume) fermenter. Data refer to air-water. The two scales were operated at different superficial gas velocities and the data for the two scales were brought together by assuming $K_{ia} \propto \left(v_g\right)^{0.2}$ as recommended by Van't Riet (1979). The solid line and the dashed lines are explained in caption for Fig. 4.
under comparable conditions. The agreement between the responses of the two probes demonstrated by the data in Figs. 6a and b therefore are encouraging and any differences between the probes are thought to be due to differences in the geometrical configuration of the two systems.

Measurements of $K_{La}$ in the miniature bioreactor were made for two impeller speeds at the end of the fermentation period during the stationary phase. In these experiments care was taken to ensure that during the de-aeration stage in the gassing out method the oxygen concentration did not fall below 30% saturation (Badino et al., 2001). The results are plotted in Fig. 7 where the solid line shows the line of best fit obtained from the air–water experiments. The results indicate that volumetric mass transfer coefficients in the miniature fermenter fall within the range reported for conventional mechanically agitated bioreactors. It is notable that the concept of power input per unit volume as a scale-up parameter successfully links the performance of the miniature bioreactor to conventional fermenter. Kostov et al. (2001) have provided $K_{La}$ data for a magnetically stirred 2 ml miniature bioreactor equipped with optical probes. Using *E. coli* fermentation, values of $K_{La}$ of 9.8, 27.5 and 44.4 h^{-1} were reported for air flow rate of 1, 2 and 3 vvm. These $K_{La}$ values are similar to those reported recently by Duetz et al. (2000) for air–water and are lower than values observed typically in shake flasks and conventional mechanically stirred systems. By comparison $K_{La}$ values measured during *E. coli* fermentation in our turbine-stirred miniature bioreactor at a fixed aeration rate of 1 vvm were 68 and 128 h^{-1} at power input per unit volume of 413 and 1190 Wm^{-3}, respectively. These are well within the range of values observed in conventional mechanically agitated systems as shown in Figs. 4, 5 and 7 reported in the literature for air–water (Van’t Riet, 1979).

One practical problem with our current oxygen sensor is its relatively long response time. The Perspex bioreactor used in the present study necessitated the elimination of the effect of external light on the response of the probes during measurements. To achieve this the whole bioreactor was wrapped in a layer of thin aluminium foil during the experiments and the immobilised tip of the oxygen sensor was covered with an extra coating of silicon. The presence of this extra coating on the working tip of probe caused the observed increase in its response time. The new miniature bioreactors in our laboratory are now fabricated from stainless steel such that their contents are fully isolated from external lighting. Another consideration is the need to control the temperature of the broth in the miniature bioreactor during fermentation. In the present study, this was achieved by carrying out the experiments in an incubator. Having established the “proof of concept” in the present study, in the new design, broth temperature will be controlled by the insertion of a single miniature concentric cylinder tubular heat exchanger fabricated from stainless steel.

**Challenging the miniature bioreactor**: Having established the engineering parameters that define the performance of the miniature bioreactor, we used it to explore new process situations. Our future plan of work includes a detailed comparison of the performance of shaken microcell systems for gas–liquid contacting operations measured against the performance of the miniature bioreactor. In shaken microcell systems, oxygen transfer is normally achieved by surface aeration. We challenged the miniature bioreactor by running an experiment in which we measured the volumetric oxygen transfer rate, $K_{La}$, in water with the supply of air to the sparger turned off so that any oxygen transfer occurred entirely via surface aeration. The results are shown in Fig. 8 for three values of power input per unit volume. The solid line shows the best line of fit for oxygen transfer by aeration through the single tube sparger (see Fig. 4). The relatively low values of $K_{La}$ observed under the condition of surface aeration suggest that most current shaken microcell systems for fermentation are likely to run under conditions of severe oxygen limitation. We also challenged the miniature bioreactor by running it in a “bubble column” mode, i.e. under conditions where the only form of agitation was provided by the flow of air (zero impeller speed) into the vessel. We measured the overall mass transfer coefficient for an air flow rate of 1 vvm corresponding

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**Fig. 6**: The fibre optic response curves for the dissolved oxygen (a) and biomass Concentration (b) obtained in the miniature bioreactor are compared with profiles obtained in a 20 l (15 l working volume) fermenter. The data were obtained during fermentation of *E. coli*. No attempt was made to run the fermentation in the miniature bioreactor under the optimum conditions. The difference in profile of the two scales are thought to be due to differences in the geometrical configuration of the two systems.**
$\frac{K_l a}{s} = 0.002 (P/V)^{0.7} \psi_a^{0.2}$

**Fig. 7.** Overall mass transfer coefficients measured during fermentation of *E. coli* in the miniature bioreactor (▲). The data compare very well with the $K_{L,a}$ obtained for air–water (♦).

**Fig. 8.** Overall mass transfer coefficients were measured (x) under conditions of surface aeration only. Significantly lower $K_{L,a}$ data compared to values obtained under aeration via the sparger (solid line) indicate that in microwell scale fermentation in shaken systems involving oxygen demanding microorganisms may be a potential problem.

to the value used in our standard air–water and *E. coli* fermentation experiments. The calculated power input per unit volume for this system was based on the method of Ayazi Shamlou et al. (1995). The overall mass transfer coefficient obtained for this system was $67 \text{ h}^{-1}$ comparable to typical values obtained under low agitation ($P/V < 100$), but significantly higher than what was achievable by surface aeration.
4. Conclusions

In this paper we describe a new miniature gas–liquid bioreactor and use experimental and theoretical analysis to establish its performance as a fermenter. The bioreactor was designed to have the same diameter as that of a single well of a 24-well plate but was mechanically agitated and aerated such that its operation mimicked the flow conditions in a conventional mechanically stirred reactor. The miniature bioreactor was instrumented with microprobes for measurement of fermentation parameters including dissolved oxygen, pH, temperature and optical density. The overall mass transfer coefficient was predicted theoretically from theory and measured in the miniature bioreactor using air–water and E. coli fermentation. The results were compared well with data obtained from a 20 l (15 l) working volume. The miniature bioreactor was challenged by running it under conditions similar to those in a shaken microwell system. Significantly lower mass transfer rates were observed highlighting the potential of oxygen starvation during fermentation in most current microwell systems.

Notation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$A$</td>
<td>gas–liquid interfacial area, $m^2 m^{-3}$</td>
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<tr>
<td>$C_d$</td>
<td>drag coefficient</td>
</tr>
<tr>
<td>$C, C_L, C^*, C_\rho$</td>
<td>oxygen concentration, mol $m^{-3}$</td>
</tr>
<tr>
<td>$d_b$</td>
<td>bubble diameter, $m$</td>
</tr>
<tr>
<td>$D_i$</td>
<td>impeller diameter, $m$</td>
</tr>
<tr>
<td>$F$</td>
<td>drag force, $N$</td>
</tr>
<tr>
<td>$k$</td>
<td>turbulent kinetic, $m^2 s^{-2}$</td>
</tr>
<tr>
<td>$K_L$</td>
<td>liquid phase mass transfer coefficient, $m s^{-1}$</td>
</tr>
<tr>
<td>$n_b$</td>
<td>bubble population density, $m^3$</td>
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<tr>
<td>$N$</td>
<td>rotational speed, $s$</td>
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<tr>
<td>$N_p$</td>
<td>impeller power number, $W$</td>
</tr>
<tr>
<td>$r$</td>
<td>volume fraction, radial co-ordinate</td>
</tr>
<tr>
<td>$t$</td>
<td>surface renewal time, $s$</td>
</tr>
<tr>
<td>$t_m$</td>
<td>mass transfer time, $s$</td>
</tr>
<tr>
<td>$T$</td>
<td>tank diameter, $m$</td>
</tr>
<tr>
<td>$U$</td>
<td>velocity, $m s^{-1}$</td>
</tr>
<tr>
<td>$v_i$</td>
<td>superficial gas velocity, $m s^{-1}$</td>
</tr>
<tr>
<td>$V$</td>
<td>volume, $m^3$</td>
</tr>
<tr>
<td>$W_e$</td>
<td>dimensionless Weber number</td>
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<tr>
<td>$z$</td>
<td>axial co-ordinate</td>
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Greek letters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>$\alpha$</td>
<td>liquid phase</td>
</tr>
<tr>
<td>$\beta$</td>
<td>gas phase</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>turbulent energy dissipation, $m^2 s^{-3}$</td>
</tr>
<tr>
<td>$\theta$</td>
<td>angular co-ordinate</td>
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$\mu$ viscosity, kg (m s)$^{-1}$
$\nu$ kinematic viscosity, $m^2 s^{-1}$
$\zeta$ liquid diffusivity, $m^2 s^{-1}$
$\rho$ density, kg $m^{-3}$
$\sigma$ surface tension, kg (m$^{-2}$ s)$^{-1}$
$\tau_p$ probe response time, $s$

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References


CFD simulation of the engineering flow environment in shaken micro-well plates

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High throughput screening (HTS) platform technology based on the use of shaken microtitre plates has the potential to provide process information early in the development pathway of new drugs. The impact of such information may be significant in reducing the process development cycle-time. In this article we used computational fluid dynamics (CFD) techniques to simulate, for the first time, the complex flow patterns in shaken micro-well plates. The results provide new insight into the mixing environment in shaken systems and a rational basis for their scale-up.

1. Introduction
Major scientific advances in genetic engineering and analytical techniques combined with high throughput screening over the past decade have produced a significant increase in new potential drug target molecules. In contrast methods used for "screening" of process options for large scale production of therapeutics have changed little in the past century and now present one of the biggest challenges to process engineers. Historical approaches, based largely on pilot-plant studies, are laborious, requiring large quantities of materials, which are often not available at the developmental stage, especially in the case of high-value and low-volume products such as the new generation of biologics. In many cases only millilitre quantities may be available for test purposes at an early stage. Additionally, in an increasingly competitive business environment, it is often the case that even a modest reduction in the "time-to-market" can make a significant difference between success and failure of a product. This demands new methods for the rapid assessment of processability of the new generation of medicines that can keep pace with the speed at which new drug discoveries are coming forward.

We have hypothesised that the HTS technology has the potential to provide process information for scale-up and production yield. However, this requires knowledge of the engineering flow conditions in the micro-wells, which is currently lacking. This article provides a theoretical basis of assessing the mixing conditions in micro-wells by using computational fluid dynamics (CFD) techniques. The results provide a rational basis for establishing process scale-up rules based on the HTS technology.

The automated shaken micro-well plates have been used in recent years in process situations involving biological materials. These include studies on bacterial
fermentation (Weiss et al., 2001b; Duetz et al., 2000; Duetz and Witholt, 2001), animal cell cultures (Girard et al., 2001) and biotransformation (Doig et al., 2002; Weiss et al., 2001a). As far as mixing and oxygen transfer are concerned, the limited data available indicate that the volumetric mass transfer coefficient in a shaken micro-well (Weiss et al., 2001b) is lower, by at least a factor of 10, compared to a conventional scale fermenter. Gas-liquid mass transfer has been discussed for other small-scale culture systems used for screening purposes, such as shaking flasks (Van Suijdam et al. 1978, Marier and Buchs 2001). Duetz et al. (2000) characterised oxygen transfer in deep well microtitre plates with a square-shaped cross section area at different shaking frequencies, shaking diameters, filling volumes and tilting angles. Hermann et al. (2003) studied the maximum oxygen transfer capacity, the mass transfer coefficient and the specific mass transfer area at different shaking conditions, different well shapes and interfacial tensions in a single well of a 96-well micro-well plates. What makes comparison of data between the shaken micro-well and laboratory, pilot and full-scale operations difficult is a lack of information on the hydrodynamic parameters for the shaken system. Consequently, scale-up rules from micro-well scale to large-scale operation have yet to be achieved.

Recently a new miniature bioreactor with a diameter equal to that of a single well of a 24-well plate was developed and its performance tested by the authors (Lamping et al., 2003). Mixing in the miniature bioreactor was provided by a set of three impellers mechanically driven via a micro-fabricated electric motor and aeration was achieved with a single tube sparger. The hydrodynamic parameters in the miniature bioreactor were obtained from CFD simulations of flow and the results used to predict process information including local and overall volumetric mass transfer coefficients. Good comparison of predictions and measured mass transfer coefficients under different mixing conditions were obtained giving confidence in the ability of the CFD methodology to simulate flow in miniaturised flow devices.

2. CFD model of mixing in the shaken micro-well

The flow conditions in the shaken micro-well was obtained by solving the following flow equation (Versteeg, 1996):}

\[
\int_{\Delta t} \frac{\partial}{\partial t} \left( \int_{CV} \rho \phi dV \right) dt + \int_{\Delta t} \int_{A} \mathbf{n} \cdot \rho \phi \mathbf{u} dA dt = \int_{\Delta t} \int_{A} \mathbf{n} \cdot (\Gamma_{\phi} \nabla \phi) dA dt + \int_{\Delta t} \int_{CV} \mathbf{S}_f dV dt
\]

(1)

The flow in the micro-well was assumed to be laminar and the CFX 4.3 (AEA Technology, Oxfordshire, UK) finite volume code was used to solve the integrated form of Eq. (1). The presence of a free surface at the interface introduced specific mathematical complications which were resolved by the method of continuum surface
force (CSF) (Brackbill et al., 1992). Briefly, the CSF method leads to an extra body force, \( F_s \), in the Navier-Stokes momentum equation given by:

\[
F_s = \sigma \kappa V_r
\]  

(2)

Where \( \sigma \) is the surface tension coefficient, \( r \) is the volume fraction of the first phase, and \( \kappa \) is the surface curvature defined by:

\[
\kappa = \frac{1}{|n|} \left( \frac{n \cdot V}{|n|} |n - \nabla \cdot n| \right)
\]

(3)

for a normal to the surface given by:

\[
n = V_r
\]

(4)

The gas and liquid phase are treated as a homogeneous model, which they have the same velocity and pressure, but their volume fractions are different. As the CSF model is a direct simulation method, no turbulence model is needed.

The total power consumption is calculated from the general relationship for the laminar flow:

\[
\varepsilon = \frac{P}{V} = \frac{\int \mu \Phi_v dV}{V}
\]

(5)

\( \mu \) is the liquid viscosity and \( \Phi_v \), the viscous dissipation function, can be expressed in terms of shear rates:

\[
\Phi_v = 2 \left[ \left( \frac{\partial u}{\partial x} \right)^2 + \left( \frac{\partial v}{\partial y} \right)^2 \right] + \left( \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right)^2 + \left( \frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right)^2 + \left( \frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right)^2
\]

(6)

3. Results and discussion

Simulations were carried out for different shaking frequencies and amplitudes using a standard orbital shaker for a 96-well deep well with a circular cross section (diameter 8mm, height 40mm) and a 24-deep well with a square cross section (diameter 17mm, height 40mm). Figure 1 gives typical simulations of the gas-liquid volume fractions for the 96-well plate format for a shaking frequency of 1000 r.p.m. and an amplitude of 3mm. The fluid in the well was assumed to have the physical properties of water at ambient temperature. At the starting point \( (t=0) \), the interface was flat (simulation not
shown), the gas-liquid volume factions are presented as a series of snapshots in time as the well moves through one complete cycle.

Figure 1. CFD simulation of gas-liquid volume fraction in a 96-well plate

The flow simulations for subsequent cycles remained effectively the same. It may be seen that the imposed shaking conditions produce good mixing which is demonstrated by the sharp gas-liquid interface changes. These simulations indicate that much of the mass transfer is likely to occur at the interface between the two phases. It is noticeable that as the liquid moves cyclically from side to side, the side-walls of the well become exposed to the air increasing the potential for mass transfer. Gas-liquid mass transfer at the wall however would require the presence of a liquid film at the wall as the bulk liquid moves away from the wall. The formation of such a liquid film is critically affected by the hydrophobicity and hydrophilicity of the wall material. Many plastic wells are likely to have non-wetting surface properties making it difficult for a liquid film at the wall. Glass wells on the other hand are expected to produce liquid film and therefore better mass transfer conditions may be expected. These considerations make prediction of mass transfer in shaken micro-well difficult and indicate suggest that scale-up rules developed for such systems may be system specific. However, the sharp changes at the interface between the two phases and the short contact time between the gas and liquid phases suggest that the volumetric liquid mass transfer coefficient may be modelled according to Higbie’s equation (Higbie, 1935). The prediction of mass transfer for the shaken system will be reported in a separate publication. A key
parameter that determines the rate of mass transfer is the power input to the shaken liquid and the associated energy dissipation rate. These are discussed briefly below.

Simulation results showed that flow patterns, power input and energy dissipation rate in the shaken micro-wells were strongly affected by the size of the well and the volume of shaken liquid as well as the shaking frequency and amplitude. These simulations indicated that power input was more sensitive to changes in shaking amplitude compared to frequency (Figure 2) and that for otherwise similar conditions power input increased significantly as the volume of liquid in the well decreased (data not shown). Experimental measurement of power input to shaken liquids is made difficult because of the lack of commercially available torque meters with sufficient sensitivity for this duty. Because the absolute torque and power input in a shaken micro-well are low, indirect measurements based, for example, on the power input to the electric motor are not appropriate. Accurate estimations of power input and energy dissipation rates in such systems therefore are very important because of their impact on the rate at which transport processes occur in the liquid. Mixing and oxygen transfer rates in a fermenter, for example, are critically affected by energy dissipation rate. The predicted values of the energy dissipation rates (Figure 2) for the shaken micro-well system under normal conditions of operations (typically 3mm amplitude, 1000 r.p.m.) are lower than values reported for mechanically agitated bioreactors. This may explain the reported low volumetric mass transfer coefficient in shaken micro-wells compared to mechanically agitated systems. It is however vital that comparison of transport
processes (e.g. $K^a$) between the shaken micro-well and mechanically agitated systems are carried out at equal values of the energy dissipation rates. This forms the basis of our future work.

4. Symbols

- CV: Control volume
- $F_s$: Body force (N)
- $K_l$: Liquid mass transfer coefficient (m/s)
- $n$: A normal to the free surface
- $P$: Power consumption (W)
- $r$: Volume fraction
- $R$: Radius (m)
- $t$: time (s)
- $\Gamma$: Diffusion coefficient (kg/(ms))
- $\rho$: Density (kg/m$^3$)
- $\phi$: General variable for a particular property ($\phi = u, v, w$ and $r$)
- $\varepsilon$: Energy dissipation (W/m$^3$)
- $\mu$: Viscosity (kg/(ms))
- $\kappa$: Surface curvature
- $\psi$: Viscous dissipation term (m$^2$/s$^3$)
- $\sigma$: Surface tension
- $\xi$: Diffusivity (m$^2$/s)
- $V$: Liquid volume (m$^3$)

5. References

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