ARTICLE

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Quantification of Power Consumption and Oxygen Transfer Characteristics of a Stirred Miniature Bioreactor for Predictive Fermentation Scale-Up

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ABSTRACT: Miniature parallel bioreactors are becoming increasingly important as tools to facilitate rapid bioprocess design. Once the most promising strain and culture conditions have been identified a suitable scale-up basis needs to be established in order that the cell growth rates and product yields achieved in small scale optimization studies are maintained at larger scales. Recently we have reported on the design of a miniature stirred bioreactor system capable of parallel operation [Gill et al. (2008); Biochem Eng J 39:164–176]. In order to enable the predictive scale-up of miniature bioreactor results the current study describes a more detailed investigation of the bioreactor mixing and oxygen mass transfer characteristics and the creation of predictive engineering correlations useful for scale-up studies. A Power number of 3.5 for the miniature turbine impeller was first established based on experimental ungassed power consumption measurements. The variation of the measured gassed to ungassed power ratio, P_g/P_{ug}, was then shown to be adequately predicted by existing correlations proposed by Cui et al. [Cui et al. (1996); Chem Eng Sci 51:2631–2636] and Mockel et al. [Mockel et al. (1990); Acta Biotechnol 10:215–224]. A correlation relating the measured oxygen mass transfer coefficient, k_La, to the gassed power per unit volume and superficial gas velocity was also established for the miniature bioreactor. Based on these correlations a series of scale-up studies at matched k_La (0.06–0.11 s^{-1}) and P_g/V (657–2,960 W m^{-3}) were performed for the batch growth of *Escherichia coli* TOP10 pQR239 using glycerol as a carbon source. Constant k_La was shown to be the most reliable basis for predictive scale-up of miniature bioreactor results to conventional laboratory scale. This gave good agreement in both cell growth and oxygen utilization kinetics over the range of k_La values investigated. The work described here thus gives further insight into the performance of the miniature bioreactor design and will aid its use as a tool for rapid fermentation process development.

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KEYWORDS: miniature bioreactor; predictive scale-up; oxygen transfer; power consumption; high-throughput operation

Introduction

In recent years the need to decrease the time scales involved in bioprocess development has led to the design and evaluation of several small scale bioreactor systems. These have tended to focus on stirred tank designs because of their geometrical similarity with the large scale bioreactors most likely to be used for industrial manufacture (Betts et al., 2006; Gill et al., 2008; Kostov et al., 2001; Puskeiler et al., 2005). These miniature bioreactor systems are generally capable of parallel and automated operation, enabling several experiments to be carried out simultaneously. These features are particularly valuable when considering the evaluation of libraries of recombinant biocatalysts or therapeutic proteins (Lye et al., 2003). The initial screening of these candidates and the subsequent optimization of the fermentation conditions for the chosen strains involves evaluation of a large number of variables. These include medium composition, nutrient feeding regimes and physical variables such as temperature, pH and dissolved oxygen levels. In facilitating parallel operation small scale bioreactors enable increases in the rate at which the necessary experiments are performed thus reducing fermentation development times and costs (Lye et al., 2003; Micheletti and Lye, 2006).

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Once the most promising strain and associated fermentation conditions are identified, scale-up studies are necessary to ensure that the optimal physiological conditions identified in the small scale studies are maintained at larger scale (Diaz and Acevedo, 1999). Despite the extensive literature that is available there is no common, generally applicable scale-up strategy. A viable scale-up strategy consists of comprehensive and detailed process characterization to identify key stress factors and parameters influencing cell growth and product yield and quality the most (Schmidt, 2005). The problem is further complicated by the fact that absolute geometric similarity is not possible across all scales (Aiba et al., 1973). Several criteria have been proposed for scale up (Hosobuchi and Yoshikawa, 1999; Ju and Chase, 1992; Oosterhuis and Kossen, 1985). These require that key parameters are maintained the same across the scales such as the volumetric mass transfer coefficient, k_La (Flores et al., 1997; Shin et al., 1996), power per unit volume, P_g/V (Pérez et al., 2006), oxygen transfer rate, impeller tip speed, mixing time (Junker, 2004) and volumetric gas flow rate per unit volume of liquid.

In the case of aerobic fermentations such as that of *Escherichia coli*, oxygen availability, gas–liquid mass transfer and mixing efficiency are the most critical considerations and usually dictate the criteria for scale-up. Oxygen is only sparing soluble in culture broth and as a result oxygen supply usually becomes rate-limiting as scale increases (Shin et al., 1996). This is especially true when dealing with high cell density cultures of fast growing cells such as *E. coli* or when the rheological properties of the fermentation broth offer high resistance to mass transfer (Diaz and Acevedo, 1999). Conditions involving even temporary depletion of dissolved oxygen during aerobic fermentations could result in irreversible cell damage (Ju and Chase, 1992) and a significant reduction in product yield (Hosobuchi and Yoshikawa, 1999). Consequently, the most utilized criteria for scale translation are based on empirical relationships which correlate P_g/V and k_La (Vilaca et al., 2000). This criteria account for the relevant parameters influencing gas–liquid mass transport, such as agitation (via power input) and aeration (superficial gas velocity) (Yawalkar et al., 2002).

Recently we have described the design, instrumentation and characterization of a novel miniature stirred bioreactor system (Gill et al., 2008), demonstrating parallel operation and reproducibility of fermentation results. In the present study we aim to further characterize the miniature bioreactor in terms of oxygen transfer and power consumption of the impeller in order to establish and verify a reliable basis for scale-up to conventional laboratory bioreactor scales.

Materials and Methods

Chemicals and Microorganisms

The chemicals used in this work were obtained from BDH (Dorset, UK) unless otherwise stated and were of the highest purity available. RO water was used for all experiments. *E. coli* TOP10 pQR239, which expresses cyclohexanone monooxygenase (CHMO) under the control of an L-arabinose promotor (Doig et al., 2001) was a kind gift from Prof. John Ward (Department of Biology and Molecular Biology, UCL). Cells were maintained as 40% (v/v) glycerol stock solutions at -80°C as previously described (Gill et al., 2008). For the *E. coli* TOP10 pQR239 fermentations the growth media consisted of 10 g L^{-1} each of tryptone, yeast extract, glycerol, NaCl (Sigma–Aldrich, Poole, UK) and 50 mg L^{-1} ampicillin (Sigma–Aldrich).

Characterization of Bioreactor Power Input

The ungassed power requirement of the novel miniature impeller design was measured using a small scale air bearing dynamometer; the setup is similar to that described by Nienow and Miles (1969). The glass vessel from the miniature bioreactor ($d_T = 60$ mm) fitted with four equidistant baffles containing 100 mL of RO water, was mounted onto the top plate of the air bearing (96 mm Ø). The miniature bioreactor impeller ($d_i = 20$ mm) was then located vertically in the vessel. In order to facilitate power measurements this was driven by an overhead electric motor, and positioned such that there was clearance of 20 mm from the base of the vessel (refer to Fig. 1 for more detailed dimensions). The torque generated was measured using a pre-calibrated force sensor (FS Series, Honeywell, Lanarkshire, UK). From the torque measurements the power draw can be calculated using:

$$P = FR\omega \tag{1}$$

where P is the power requirement, F the force applied, R the length of the arm pressing against the force sensor and ω is the angular velocity, given by

$$\omega = 2\pi N \tag{2}$$

where N is the stirrer speed. The gassed power input of the impeller was measured in exactly the same way as described above but, with a sintered sparger fitted alongside the stirrer in the vessel as in the actual bioreactor configuration (Gill et al., 2008). Aeration rates were varied between 1 and 2 vvm. All power measurements were performed in triplicate with a maximum coefficient of variance of 3.5%.

Miniature and Laboratory Scale Fermentations at Matched P_g/V and Constant k_La Values

Miniature Bioreactor (100 mL) Fermentations

The design characteristics of the parallel miniature bioreactors used for the scale-up investigations have previously been described in detail (Gill et al., 2008). Each bioreactor stand holds up to four bioreactors capable of independent

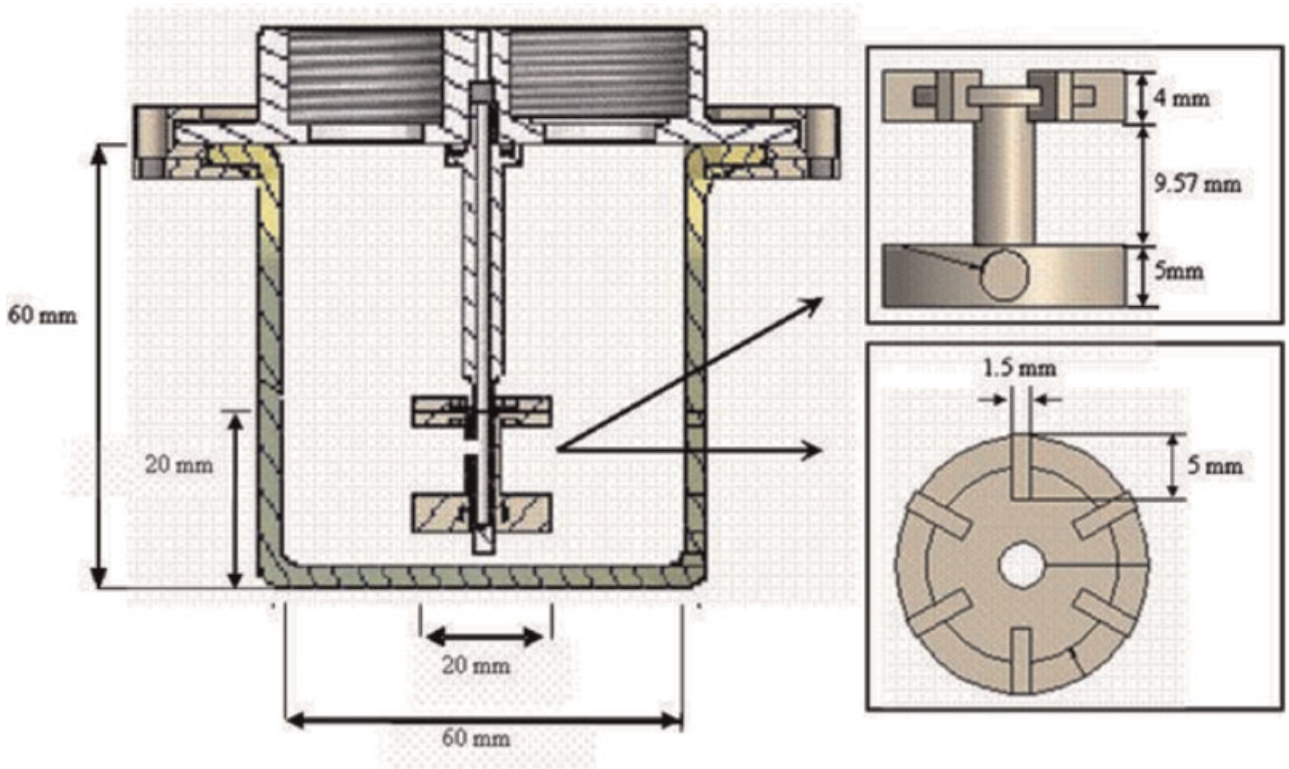

Figure 1. Mechanical drawing showing key design features and dimensions of a single miniature bioreactor and magnetically driven miniature turbine impeller. Further details of the parallel bioreactor system are given in Gill et al. (in press). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

operation. pH was measured by miniature pH probes (Easyferm 9-6-52, Hamilton, Bonaduz, Switzerland) and controlled at pH 7 (±0.1) by the metered addition of 3 M NaOH and 3M H_3PO_4. The dissolved oxygen was monitored using a miniature steam sterilisable polargraphic oxygen electrode (Oxyferm 9-12-52, Hamilton Bonaduz AG). The inlet and exhaust gas lines were filtered through 0.2 μm filters (Fisher Scientific, Loughborough, UK). Each miniature bioreactor was sterilized as a complete unit (121°C for 20 min) with all media components (apart from ampicillin) and 0.2 mL L^{-1} of added antifoam (polypropylene glycol 2000). After cooling the temperature was maintained at 37°C (±0.2) via an electrical disc heater positioned beneath the glass vessel. Filter sterilized (0.2 μm) ampicillin was added immediately prior to inoculation. Each bioreactor was inoculated with 2 mL (2% (v/v) inoculum) of broth from a shake flask culture (100 mL in a 1 L shaken flask) grown for 14 h on the same medium at 37°C and 200 rpm on a horizontal shaken platform (New Brunswick Scientific, Edison, NJ).

Three different P_g/V and k_La values were investigated, 657, 1,487, and 2,960 W m^3 and 0.06, 0.08, and 0.11 h^{-1} respectively. The agitation rates in the miniature bioreactors were varied between 1,000 and 2,000 rpm and aeration rates varied between 1 and 2 vvm (100–200 mL min^{-1}). Operating conditions for each P_g/V and k_La value are given in Tables III and IV respectively. A gas blending device was integrated into the four pot miniature bioreactor system providing aeration using oxygen enriched air and allowing the DOT to be accurately controlled at any required values. Both air and oxygen were fed into the gas blender and mixed according to the DOT set point requirement.

Laboratory Scale Bioreactor (2 L) Fermentations

The fermentations carried out in the miniature bioreactors were also performed in a 2 L (1.5 L working volume) LH 210 series laboratory scale bioreactor (Bioprocess Engineering Services, Charing, Kent, UK) at matched P_g/V and k_La values. The bioreactor ($d_T = 125$ mm) was fitted with two top driven six blade Rushton turbines ($d_i/d_T = 1/3$) and four equally spaced baffles. pH was measured by a steam sterilisable Ingold pH probe (Ingold Messtechnik, Urdorf, Switzerland) and controlled as described for the miniature bioreactors. Dissolved oxygen was measured and controlled using a polargraphic oxygen electrode (Ingold Messtechnik). Growth media and antifoam were sterilised as described for the miniature bioreactors. Ampicillin was added via a 0.2 μm filter (Fisher Scientific) prior to inoculation. Thirty milliliter (2% (v/v) inoculum) of inoculum was prepared and transferred to the 2 L bioreactor in the same way, and grown under the same conditions as described in Miniature Bioreactor (100 mL) Fermentations Section. The agitation

and aeration rates were adjusted to give the required P_g/V and k_La values as described in Tables III and IV, respectively.

Biomass Quantification

Biomass concentration was determined in the same way as described in Gill et al. (2008), by taking broth samples of up to 1 mL at regular intervals and measuring the OD_{600} off-line (Ultraspec 4000 spectrophotometer, Pharmacia Biotech, Piscataway, NJ). All biomass concentrations reported here are dry cell weight (DCW) concentrations and were determined from calibration curves of known DCW and the corresponding optical density.

Results and Discussion

Power Characteristics of the Miniature Turbine Impeller

For novel impeller designs such as that shown in Figure 1, it is important to understand their mixing and energy dissipation characteristics. The impeller power consumption in the case of aerated systems is always lower than that in unaerated systems since the transfer of power from the impeller to the fluid is greatly influenced by aeration. This power reduction is due to the formation of cavities behind the impeller blades and the different density of the fluid under gassed and ungassed conditions (van't Riet and Smith, 1973). The effect of aeration has been extensively studied by Nienow et al. (1977), Oosterhuis and Kossen (1981), Warmoeskerken and Smith (1981) and Yawalkar et al. (2002) for a single impeller system as studied here. It has been shown that the gassed power input is usually 30–40% of the ungassed power input depending on the type of impeller and aeration rates used (Oosterhuis and Kossen, 1985).

The results in Figure 2 illustrate how the measured power consumption of the miniature turbine impeller, for both gassed and ungassed conditions, increases exponentially with agitation rate. There is also a clear distinction that the power consumption under ungassed conditions is on average 25% higher than that of the gassed power consumption. The maximum power consumption determined was 0.41 W at an agitation rate of 2,000 rpm and the measured power consumption under gassed conditions was found to decrease with increasing aeration rate. The maximum values measures at an agitation rate of 2,000 rpm and aeration rates of 1, 1.5, and 2 vvm were 0.30, 0.29, and 0.26 W respectively. The difference between ungassed and gassed power inputs is more significant at higher stirrer speeds. As the stirrer speed decreases to <1,000 rpm the gassed power consumption approaches the ungassed power consumption with a maximum difference of 35%. Visual observations suggested that this was a result of poor gas dispersion at lower stirrer speeds.

For the ungassed power input data Figure 3 shows the relationship between the calculated Power number, N_p, of

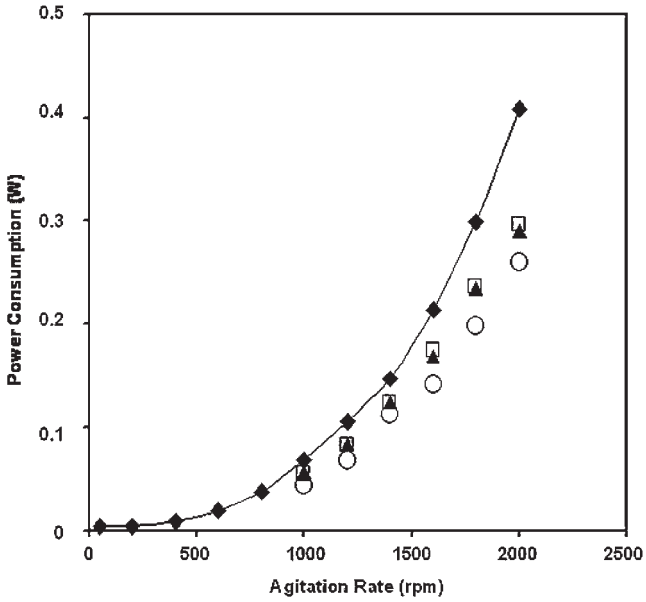

Figure 2. Ungassed and gassed power requirements of the miniature turbine impeller over a range of agitation rates: (◆) ungassed power; gassed power with aeration at (□) 1 vvm, (▲) 1.5 vvm, and (○) 2 vvm.

the miniature turbine impeller and the impeller Reynolds number, *Re*. N_p values can be calculated from the data in Figure 2 according to

$$P_{ug} = N_p \rho N^3 d_i^5 \tag{3}$$

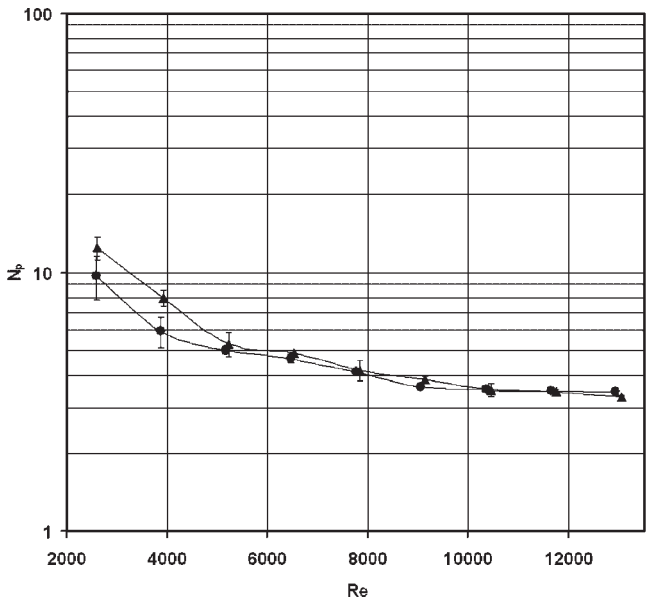

Figure 3. Variation of the measured Power number with Reynolds number for the miniature turbine impeller: (●) in water; (▲) in clarified fermentation broth. Error bars represent one standard deviation about the mean.

where P_{ug} is the measured ungassed power input, ρ the density of the liquid, N the agitation rate and d_i the impeller diameter. Results are shown for power measurements made in both water and clarified fermentation broth. The variation of N_p with Re is very similar for both process fluids and as Re increases, a constant value of N_p of 3.5 is achieved in both cases. Visual observation of the vessel suggested that fully turbulent flow had developed by the point where $Re > 8{,}000$.

This measured N_p value of 3.5 is lower than that typically reported in literature, $N_p = 6$, for a six blade Rushton turbine in the turbulent region (Nienow et al., 1994). However, Bujalski et al. (1987) have reported that geometric parameters such as the thickness of the disk and the impeller blades can have an appreciable effect on the Power number. Rutherford et al. (1996) also noted that N_p reduced as the ratio of impeller blade thickness to impeller diameter was increased from 0.008 to 0.033. The N_p correlation derived by Rutherford et al. (1996) is shown below

$$N_p = 6.57 - 54.771\left(\frac{b_t}{d_i}\right) \tag{4}$$

which relates N_p to b_t the impeller blade thickness and d_i the impeller diameter. This computes a Power number of 3.8 for the miniature turbine impeller design used (Fig. 1). This predicted value is in good agreement with the experimentally determined value of 3.5. In relation to other miniature impeller designs this measured N_p is in the same range as those reported for a magnetically driven gas inducing impeller ($d_T = 20$ mm, $d_i = 14.5$ mm) by Puskeiler et al. (2005), who estimated a value of 3.7 based on CFD analysis, and Betts et al. (2006) who predicted a Power number of 3 for a triple impeller system ($d_T = 0.023$ m, $d_i = 8.5$ mm) by direct measurements of the electrical energy input of the agitator motor.

Variation of P_g/P_{ug} Ratio With Flow Number

In aerated systems the ratio of P_g/P_{ug} will determine the actual power input during fermentation operation and can also give insight into the gas dispersion characteristics of the impeller. The extent of power decrease between gassed and ungassed operation, P_g/P_{ug}, ranges from 0.3 to 1, depending on the type of impeller and aeration rate (Aiba et al., 1973). The nature of the gas–liquid flow within the vessel may be described by the Flow number which is defined as

$$Fl = \frac{Q_g}{Nd_i^3} \tag{5}$$

where Q_g is the volumetric gas flow rate, N the agitation rate and d_i the impeller diameter. Figure 4 shows the measured P_g/P_{ug} ratio (solid symbols) as a function of the Flow number. For comparison, data from Hall et al. (2005) has also been included (open symbols). Their system consisted of a 0.045 m diameter glass vessel with a mechanically driven, eccentrically fitted 6-blade up pumping pitched blade turbine ($d_i = 0.024$ m) and is the only compatible data available in the literature at this scale at the present time. In the case of Hall et al. (2005) gas was introduced at a single flow rate of 0.5 vvm via a sintered glass sparger mounted at the base of the vessel. Two different configurations were used for the position of the sparger in the vessel: the first had the active area of the sparger located directly below the eccentrically positioned impeller, whilst the second had the active part of the sparger located opposite the impeller on the other side of the vessel. From Figure 4 it can be seen that the P_g/P_{ug} ratio is of the same order of magnitude for both systems and in the range of 0.58–0.85, suggesting similar gas dispersion characteristics. However to obtain similar values of P_g/P_{ug} the miniature bioreactor studied here needs to operate at significantly higher Flow numbers. This is probably due to the thicker blades of the miniature impeller design used here which are thought to be less efficient at gas bubble break-up and distribution due to decreased fluid velocity and turbulence in the impeller region (Rutherford et al., 1996).

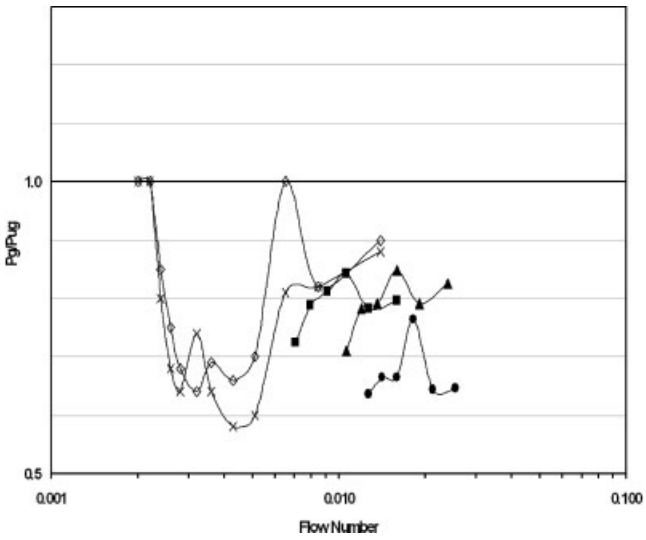

Figure 4. Comparison of the measured variation of P_g/P_{ug} with Flow number for the miniature bioreactor and the results reported by Hall et al. (2005) for a miniature reactor ($d_T = 0.045$ m). Miniature bioreactor aeration rates: (◆) 1 vvm, (■) 1.5 vvm, and (▲) 2 vvm. Hall et al. (2005) miniature reactor aeration rate of 0.5 vvm: (×) sparger positioned beneath the impeller axis; (◇) sparger positioned away from the impeller.

To ensure economical gas–liquid dispersion in a stirred tank reactor and to better understand the various flow patterns that occur, some knowledge of the minimum agitation rate (N_{CD}) for complete dispersion of the sparged gas is necessary. Nienow et al. (1977) proposed the following correlation for vessels with d_T of up to 1.8 m:

$$N_{CD} = \frac{4Q_g^{0.5}d_T^{0.25}}{d_i^2} \tag{6}$$

where N_{CD} is the minimum agitation rate for complete dispersion of the sparger gas (rev s^{-1}), Q_g the volumetric gas flow rate, d_T the vessel diameter and d_i is the impeller diameter. At agitation rates below N_{CD}, gas dispersion becomes inefficient resulting in little or no gas in the region below the impeller and potential oxygen starvation in the case of aerobic fermentations. In Figure 4 N_{CD} is represented by the minimum P_g/P_{ug} values in each data set. The flow pattern at this operating condition is characterized by a fully dispersed bubbled regime throughout the fluid without any flooding of the impeller (Yawalkar et al., 2002). At this point however the gas hold up is not sufficient to promote gross recirculation of bubbles throughout the dispersion. The point at which gross recirculation sets in is normally taken as the left hand maximum of the curves (Hall et al., 2005).

In the case of the miniature bioreactor the data in Figure 4 suggests that complete dispersion of gas occurred at flow numbers of 0.0135, 0.0194, and 0.028 for aeration rates of 1, 1.5, and 2 vvm, respectively. These flow numbers are achieved at significantly higher agitation rates, compared to those calculated by Equation (6) which predicts flow numbers of 0.033, 0.040, and 0.047 for achieving complete dispersion. These differences are again most likely a result of the thicker impeller blades as described earlier.

Correlation of Miniature Bioreactor Energy Dissipation Rates

For the purpose of predictive process design and scale-up it is useful to correlate the measured power input data shown in Figure 2 with the bioreactor geometry and operating conditions. The ungassed power requirement, P_{ug}, of an impeller in a stirred bioreactor can be easily calculated from the Power number (Eq. 3). However since the transfer of power from the impeller to the fluid is influenced very much by aeration, the power requirement for the gassed situation is lower than that for the ungassed situation. Considerable research has been done to correlate P_g/P_{ug} values to bioreactor design and operating conditions. Table I lists some of the most commonly used correlations derived by various authors with a particular emphasis on smaller vessels with single impellers ($d_T = 0.16$–1.83 m).

Figure 5 shows a parity plot comparing the measured P_g/V values here with those predicted from the correlations listed in Table I. The dual correlation proposed by Cui et al. (1996) provides the closest predictions to the experimental miniature bioreactor data. In their work the correlations were based on 526 data sets for single impeller systems with errors below 6%. Cui et al. (1996) concluded that gassed power reductions can vary considerably at the same Flow number, which means that the Flow number alone is not sufficient to describe the value of P_g/P_{ug}. Consequently they derived two separate criteria for predicting the P_g/P_{ug} depending of the value of the Flow number.

In terms of the other correlations, Mockel et al. (1990) established an empirical correlation which gave good agreement with measured and literature data. This correlation is unique in that it only takes into account the superficial gas velocity and impeller diameter together with a single constant, Z, which varies depending on the number of impellers. Although the correlation does not appear to be as widely used as some, it does show reasonably good agreement with the measured P_g/V data here, but with a tendency to over predict the experimental values. The correlation presented by Hughmark (1980) for a six-bladed disc turbine has been applied over a wide range of system configurations and operating parameters. The correlation was based on 391 data sets with a standard deviation of 0.1117 and is considered to be very reliable (Hughmark, 1980; van't Riet and Tramper, 1991; Yawalkar et al., 2002). Although Koloini et al. (1989) reported that Hughmark's correlation predicted higher values for power input compared to their experimental data, in the case of the miniature bioreactor this correlation also slightly under estimates the power input compared to the measured values, especially at higher stirrer speeds. Finally the Luong and

Table I. Summary of the most commonly reported gassed to un-gassed power consumption correlations (P_g/P_{ug}) for smaller scale stirred vessels (0.22–10 m^3).

References	Vessel diameter (m)	Impeller characteristics			Proposed correlation
		Type	Number	d_i/d_T	
Cui et al. (1996)	0.23–1.83	Flat blade turbine	1–2	0.33–0.5	$\frac{QN^{0.25}}{d_i^2} \le 0.055 : 1 - \frac{P_g}{P_{ug}} = 9.9\left(\frac{QN^{0.25}}{d_i^2}\right)$, $\frac{QN^{0.25}}{d_i^2} > 0.055 : 1 - \frac{P_g}{P_{ug}} = 0.52 + 0.62\left(\frac{QN^{0.25}}{d_i^2}\right)$
Mockel et al. (1990)	0.4–7	Flat blade turbine	1 ($Z = 750$) 2 ($Z = 490$) 3 ($Z = 375$)	0.25–0.4	$\frac{P_g}{P_{ug}} = \frac{1}{\sqrt{1 + Z\frac{u_s}{\sqrt{gd_T}}}}$
Hughmark (1980)	0.16–1	Flat blade turbine	1	0.33	$\frac{P_g}{P_{ug}} = 0.1\left[\frac{Q}{NV}\right]^{-0.25}\left[\frac{N^2 d_i^4}{gW_i V^{\frac{2}{3}}}\right]^{-0.2}$
Luong and Volesky (1979)	0.22	Flat 6 blade turbine	1	0.33	$\frac{P_g}{P_{ug}} = 0.497\left(\frac{Q}{Nd_i^3}\right)^{-0.38}\left(\frac{N^2 d_i^3 \rho}{\sigma}\right)^{-0.18}$

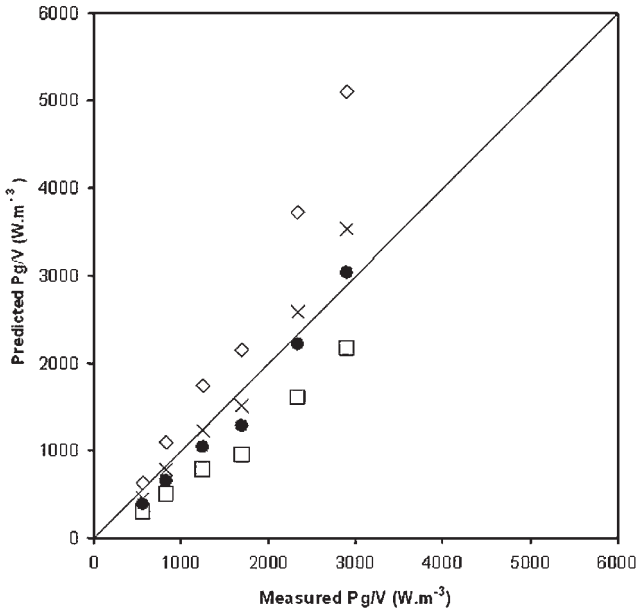

Figure 5. Parity plot of measured P_g/V values at an aeration rate of 1.5 vvm, with those predicted by the most commonly reported literature correlations: (□) Hughmark (1980); (×) Cui et al. (1996); (◇) Luong and Volesky (1979); (●) Mockel et al. (1990). Experimental measurements as shown in Figure 2. Correlations as described in Table I.

Volesky (1979) correlation based on the use of dimensionless groups and Newtonian fluids, is seen to greatly over predict the measured values and is unsuitable.

Correlation of Miniature Bioreactor k_La Values

Given the previously established importance of the k_La value on cell growth kinetics in the miniature bioreactor (Gill et al., 2008), correlations for the prediction of k_La values were also examined. These correlations usually relate k_La, power consumption and superficial gas velocity, and take the form:

$$k_La = C\left(\frac{P_g}{V}\right)^{\alpha} u_s^{\beta} \tag{7}$$

where k_La is the volumetric mass transfer coefficient, P_g/V the impeller gassed power per unit volume, u_s the superficial gas velocity, C is a constant and α and β are exponents. Various authors have predicted values for the constant, C, and exponents (α and β) for a variety of different vessels and these are listed in Table II. Figure 6 shows the k_La values for a range of different agitation rates that have been predicted according to the correlations listed in Table II. The P_g/V values substituted into these correlations were those measured previously (Fig. 2).

The correlations derived by Linek et al. (2004), Smith et al. (1977) and Zhu et al. (2001) all under estimate the k_La values for the miniature bioreactor. It can be seen in Figure 6, however, that they have very similar gradients to the measured k_La values. The van't Riet (1979) and Vilaca et al. (2000) correlation predicted k_La values that are in the same order of magnitude as the measured k_La, but grossly over and under estimated over a wide range of P_g/V values. All of the existing k_La correlations thus appear unsuitable for predicting accurate k_La values for the miniature bioreactor. This is most probably due to the fact that they have been developed for much larger scale vessels. As a result an empirical relationship was derived here specifically for the miniature bioreactor based on k_La values measured at different operating conditions (Gill et al., 2008) and the corresponding gassed power measurements (Fig. 2). The values determined for the constants C, α, and β from Equation (7) were 0.22, 0.35, and 0.52 respectively as shown in Table II.

Table II. Summary of the most commonly reported k_La correlations for stirred vessels (0.002–2.6 m^3).

		Impeller characteristics		
References	Vessel diameter (m)	Type	d_i/d_T	Proposed correlation and type of fluid
This work	0.06	Rushton turbine	0.33	Air–water with ions: $k_La = 0.224\left(\frac{P_g}{V}\right)^{0.35} u_s^{0.52}$
van't Riet (1979)	Various	Various	Various	Air–water with ions: $k_La = 2\times10^{-3}\left(\frac{P_g}{V}\right)^{0.7} u_s^{0.2}$
Vilaca et al. (2000)	0.21	Rushton turbine	0.4	Air–water–sulfite solution $k_La = 6.76\times10^{-3}\left(\frac{P_g}{V}\right)^{0.94} u_s^{0.65}$
Linek et al. (2004)	0.29	Rushton turbine	0.33	Air–water: $k_La = 0.01\left(\frac{P_g}{V}\right)^{0.699} u_s^{0.581}$
Smith et al. (1977)	0.61–1.83	Disc turbine	0.5–0.33	Air–water: $k_La = 0.01\left(\frac{P_g}{V}\right)^{0.475} u_s^{0.4}$
Zhu et al. (2001)	0.39	Disc turbine	0.33	Air–water: $k_La = 0.031\left(\frac{P_g}{V}\right)^{0.4} u_s^{0.5}$

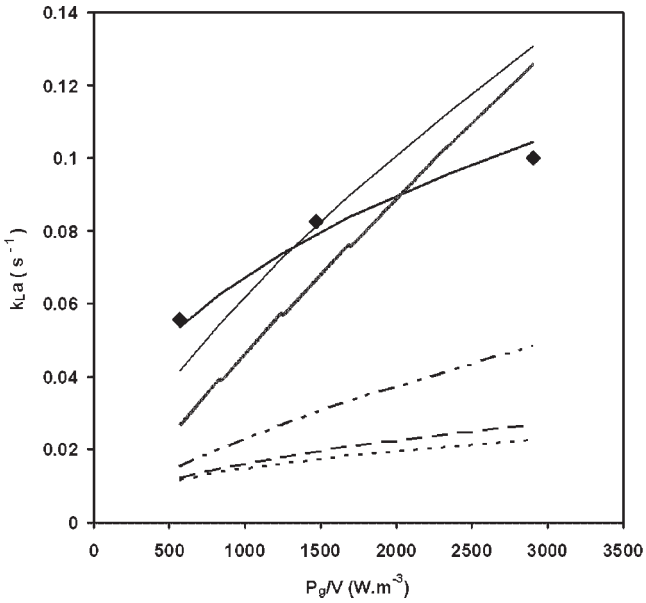

Figure 6. Comparison of experimentally determined k_La values at an aeration rate of 1.5 vvm, with those predicted from the miniature bioreactor correlation and various literature correlations: (◆) measured values; (▬▬) miniature bioreactor correlation; (—) van't Riet (1979); (▬▬) Vilaca et al. (2000); (— - - —) Linek et al. (2004); (— —) Smith et al. (1977); (- - - -) Zhu et al. (2001). Experimental k_La values taken from Gill et al. (in press). Correlations as described in Table II.

The correlations proposed by van't Riet (1979) and Vilaca et al. (2000) have much larger values for α than β, implying that the stirrer speed has a more significant influence on k_La. In the case of the miniature bioreactor correlation β is greater than α, suggesting that in fact there is much less dependence on P_g/V. As the superficial gas velocity is a more significant parameter here than in larger scale bioreactors, it might imply that the miniature bioreactor has certain bubble column characteristics. Finally, it can be seen from Figure 6 that the correlations of Linek et al. (2004), Smith et al. (1977) and Zhu et al. (2001) show a similar gradient to the miniature bioreactor correlation. The difference between α and β for these correlations is also less than for the van't Riet (1979) and Vilaca et al. (2000) correlations.

Fermentation Scale Translation at Matched P_g/V and k_La Values

Scale-Up Based on Constant P_g/N

In order to explore the ability of the miniature bioreactor to provide data representative of conventional laboratory scale bioreactors a series of fermentations were performed at matched P_g/V and matched k_La values. These were initially operated without DOT control so that the time at which cultures became oxygen limited (DOT ≤ 0) could be easily determined for different operating conditions.

The 2 L bioreactor used in this work was fitted with two six blade Rushton turbine impellers. In the case of matched P_g/V values the operating conditions required in the 2 L bioreactor were determined using the Hughmark correlation (1980). Although this correlation was primarily derived for single impeller systems, the few multiple impeller correlations available are either very specific to the vessel geometry (Cui et al., 1996) or based on larger impeller spacing. In the latter case the clearance between the two impellers must be sufficiently large to consider them as fully separate (Paglianti et al., 2001), that is, when the impeller spacing is $>2d_i$ (Gogate et al., 2000) which is not the case for the 2 L vessel used here. Taking into account the importance of impeller spacing, a dual Power number of 10.5 was used to compute the various operating condition required for the 2 L. This was estimated based on the ratio of impeller spacing to impeller diameter (Hudcova et al., 1989). Table III summaries the key parameters and operating conditions required for each bioreactor over a range of matched P_g/V values.

Figure 7 compares the performance of the two bioreactor scales at each matched P_g/V value. The fermentation kinetic parameters are summarized in Table III. At the lowest P_g/V value of 657 W m^{-3}, the 2 L bioreactor significantly underperforms compared to the miniature bioreactor, achieving a final biomass concentration of almost 3 g L^{-1} less. This is likely to be the result of operating at a reduced agitation rate, and the poor gas–liquid dispersion that was observed at the 2 L scale under these operating conditions. Given the poor oxygen transfer at the 2 L scale, the DOT reached zero much earlier, and cell growth is clearly seen to become oxygen limited. The performance of the 2 L

Table III. Summary of batch *E. coli* TOP10 pQR239 fermentation conditions carried out in miniature (0.1 L) and conventional (2 L) laboratory stirred bioreactors using constant P_g/V as a basis for scale translation.

P_g/V (W m^{-3})	657		1,487		2,960	
Bioreactor	0.1 L miniature bioreactor	2 L bioreactor	0.1 L miniature bioreactor	2 L bioreactor	0.1 L miniature bioreactor	2 L bioreactor
Agitation rate (rpm)	1,000	650	1,500	900	2,000	1,100
Aeration rate (vvm)	1.5	0.67	1	0.67	1	0.67
μ_{max} (h^{-1})	0.75	0.72	0.84	0.82	0.94	0.91
X_{final} (g L^{-1})	5.6	2.8	5.1	5.1	5.3	5.8

Kinetic parameters derived from Figure 7.

Table IV. Summary of batch *E. coli* TOP10 pQR239 fermentation conditions carried out in miniature (0.1 L) and conventional (2 L) laboratory stirred bioreactors using constant k_La as a basis for scale translation.

k_La (s^{-1})	0.06		0.08		0.11	
Bioreactor	0.1 L miniature bioreactor	2 L bioreactor	0.1 L miniature bioreactor	2 L bioreactor	0.1 L miniature bioreactor	2 L bioreactor
Agitation rate (rpm)	1,000	900	2,000	1,000	2,000	1,100
Aeration rate (vvm)	2	1.33	1	0.67	2	1.33
μ_{max} (h^{-1})	0.79	0.78	0.94	0.90	0.99	0.98
X_{final} ($g\,L^{-1}$)	5.6	6.2	5.3	5.5	6.3	6.6

Kinetic parameters derived from Figure 8.

bioreactor is improved at higher P_g/V values (>1,000 $W\,m^{-3}$), with very similar μ_{max} and X_{final} values obtained at both scales. The trends for oxygen depletion during the exponential growth phase as well as the time taken for both systems to become oxygen limited are similar and reproducible at these two higher P_g/V values. However, in both cases oxygen limitation occurs slightly earlier in the 2 L vessel than in the miniature bioreactor.

Scale-Up Based on Constant k_La

For experiments at matched k_La, values of 0.06, 0.08, and 0.11 s^{-1} were used and the cultures were again initially operated without DOT control. At both scales k_La values were experimentally determined using the dynamic gassing out technique as described by Gill et al. (2008). Figure 8 compares the performance of the two bioreactor scales at these matched k_La values. The operating conditions in each case, together with the derived growth kinetic parameters are given in Table IV. Compared to the earlier experiments at matched P_g/V values (Fig. 7) there appears to be better agreement in terms of cell growth and DOT profiles across the range of k_La values studied. There is particularly good agreement at the two higher k_La values. The trends for oxygen depletion during the growth phase as well as the time taken for both systems to become oxygen limited are similar and reproducible in both cases. Based on the results presented in Figures 7 and 8, for aerobic fermentations, and bioconversions where oxygen supply is critical, it would appear that k_La provides the best basis for scale translation over a range of operating conditions.

Scale Translation With DOT Control

Final scale-up experiments were performed using operating conditions that gave matched k_La values ($k_La = 0.08\ s^{-1}$) during the initial phase of the cultures but where DOT was also controlled at 30% to prevent the cultures becoming oxygen limited during the exponential growth phase. The operating conditions for the two bioreactor scales, at a k_La of 0.08 s^{-1} are given in Table IV. The results in Figure 9 show good agreement in terms of cell growth kinetics and DOT

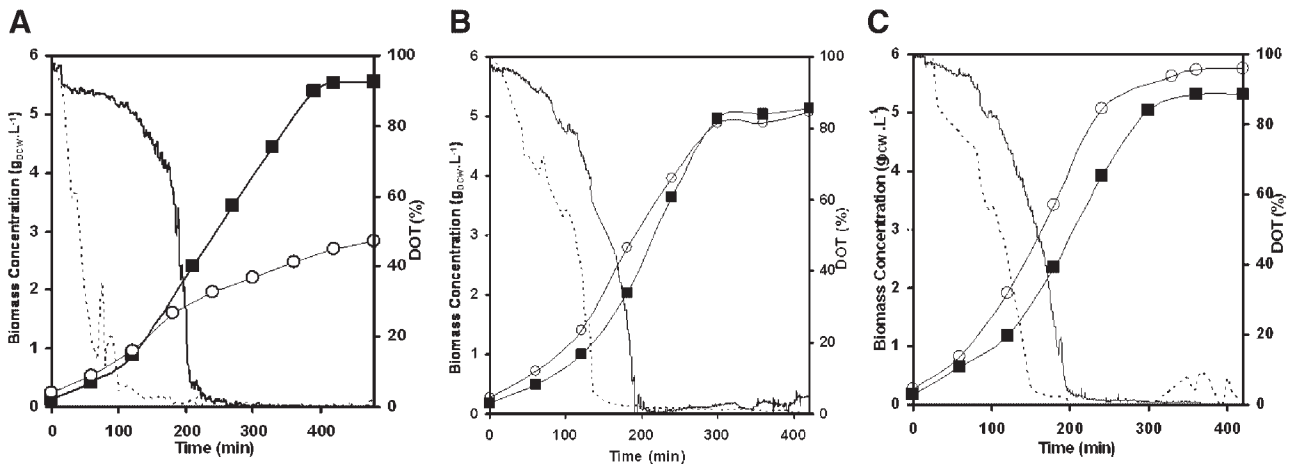

Figure 7. Comparison of growth and DOT profiles for batch *E. coli* TOP10 pQR239 fermentations carried out at matched P_g/V at miniature (0.1 L) and conventional laboratory bioreactor (2 L) scales: (**A**) $P_g/V = 657\ W\,m^{-3}$; (**B**) $P_g/V = 1{,}487\ W\,m^{-3}$; (**C**) $P_g/V = 2{,}960\ W\,m^{-3}$. Miniature bioreactor: (■) cell density, (—) DOT; laboratory bioreactor: (○) cell density, (- - -) DOT. Matched P_g/V operating conditions as described in Table III.

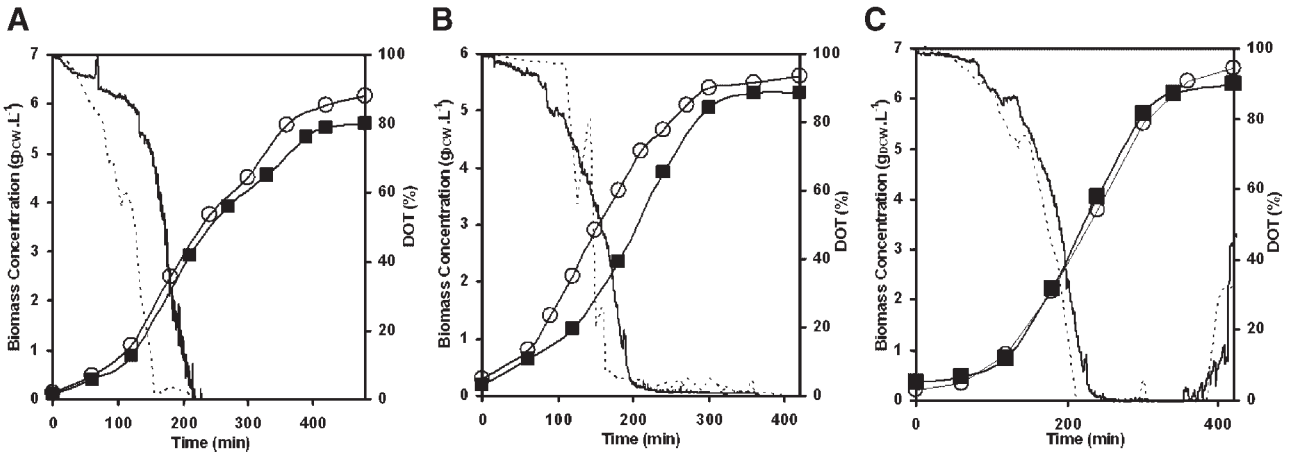

Figure 8. Comparison of growth and DOT profiles for batch *E. coli* TOP10 pQR239 fermentations carried out at matched k_La at miniature (0.1 L) and conventional laboratory bioreactor (2 L) scales: k_La (**A**) 0.06 s^{-1}; (**B**) 0.08 s^{-1}; (**C**) 0.11 s^{-1}. Miniature bioreactor: (■) cell density, (—) DOT; laboratory bioreactor: (○) cell density, (- - -) DOT. Matched k_La conditions as described in Table IV.

profiles at both scales. In the absence of any oxygen limitations growth rates as high as 0.87 h^{-1} were achieved, and the final biomass concentrations significantly increased in both the miniature bioreactor and the 2 L vessel with practically identical values of 7.6 and 7.7 g L^{-1}, respectively. It is clear at both scales that gas blending is required during the later stages of fermentation if higher cell growth rates and yields are to be attained.

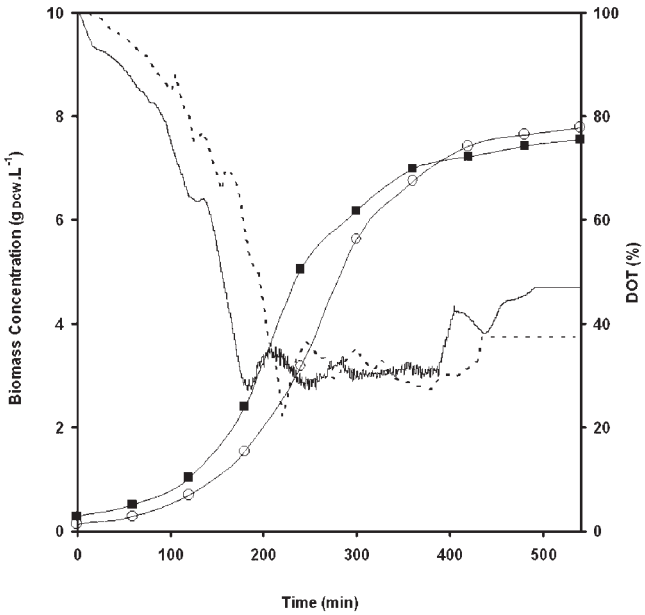

Figure 9. Comparison of growth and DOT profiles for batch *E. coli* TOP10 pQR239 fermentations carried out at matched k_La (0.08 s^{-1}) and DOT controlled at 30% in miniature (0.1 L) and conventional laboratory bioreactor (2 L) scales. Miniature bioreactor: (■) cell density, (—) DOT; laboratory bioreactor: (○) cell density, (- - -) DOT. Matched k_La conditions as described in Table IV.

Conclusions

In this work the engineering characteristics of the miniature bioreactor have been further examined in terms of power consumption and oxygen transfer in order to establish a reliable criterion for the predictive scale-up of fermentation results. Initially the power consumption of the miniature turbine impeller under both ungassed and gassed conditions was measured as a function of agitation and aeration rates. The ungassed power measurement yielded a Power number of 3.5 for the miniature turbine impeller. Investigation of the gas dispersion characteristics of the impeller showed that complete dispersion of the sparger gas occurred at lower Flow numbers than proposed by Nienow et al. (1977) predicts complete dispersion of gas to be achieved at lower agitation rates and hence higher Flow numbers. The main reason for these differences is most probably due to the thicker impeller blades of the miniature turbine impeller used in this work.

Suitable correlations for predicting operational power consumption and k_La values have been established. Various literature correlations for predicting P_g/P_{ug} for the miniature bioreactor were first evaluated. Those proposed by Cui et al. (1996) and Mockel et al. (1990) provided reasonable predictions of the experimental data. Similarly several literature correlations were evaluated for the estimation of the miniature bioreactor k_La values. However all of these correlations proved to be unsuitable and so an empirical correlation specific to this bioreactor was derived as follows:

$$k_La = 0.224\left(\frac{P_g}{V}\right)^{0.35} u_s^{0.52} \quad (8)$$

Finally a series of miniature and conventional scale *E. coli* TOP10 pQR239 fermentations were performed at matched

P_g/V and k_La values to define a suitable basis for scale-up of fermentation results. In the case of scale-up based on constant P_g/V, comparable results at both scales are only achieved at P_g/V values greater than 1,000 W m^{-3}. Scale-up at matched k_La values shows good agreement of fermentation results at both scales over the whole range of k_La values studied. It would thus appear that k_La is the most suitable criterion for scale-up of miniature bioreactor results. The results and correlations presented here will aid the use of the miniature bioreactor design as a tool for rapid fermentation process development.

Nomenclature

b_t	impeller blade thickness (m)
C	constant
d_B	width of baffle (mm)
d_i	diameter of impeller (mm)
d_T	diameter of vessel (mm)
DOT	dissolved oxygen tension (%)
F	force (N)
Fl	flow number (Q_g/ND^3)
g	acceleration of gravity (m s^{-2})
k_La	volumetric oxygen mass transfer coefficient (s^{-1})
N	impeller speed (s^{-1})
N_P	Power number
N_{CD}	minimum agitation rate for complete dispersion (s^{-1})
OD_{600}	optical density at 600 nm
P	power input (W)
P_g	gassed power requirement (W)
P_{ug}	ungassed power requirement (W)
Q_g	volumetric gas flow rate aeration rate (m^3 s^{-1})
R	length of the arm pressing against the force sensor (m)
Re	Reynolds number ($\frac{ND^2\rho}{\mu}$)
u_s	superficial gas velocity (m s^{-1})
V	volume (L)
W	impeller blade width (m)
X_{final}	final biomass concentration (g_{DCW} L^{-1})
Z	constant, depends on the number of stirrers for Mockel et al. (1990)

Greek Letters

α, β	exponents
ρ	density (kg m^{-3})
μ_{max}	maximum specific growth rate (h^{-1})
ω	angular velocity (s^{-1})
σ	air–liquid surface tension (N m^{-1})

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