On-line monitoring of oxygen in Tubespin, a novel, small-scale disposable bioreactor

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Abstract A novel, optical sensor was fixed in a new type of disposable bioreactor, Tubespin, for the on-line (real-time) monitoring of dissolved oxygen concentrations during cell culture. The cell density, viability and volumetric mass transfer coefficient were also determined to further characterize the bioreactors. The k_La value of the Tubespin at standard conditions was 24.3 h^{-1} , while that of a spinner flask was only 2.7 h^{-1} . The maximum cell density in the Tubespin bioreactor reached $6 \times 10^6 \text{ cells mL}^{-1}$, which was two times higher than the cell density in a spinner flask. Furthermore, the dynamic dissolved oxygen level was maintained above 90% air-saturation in the Tubespin,

while the value was only 1.9% in a spinner flask. These results demonstrate the competitive advantage of using the Tubespin system over spinner flasks for process optimization and scale-down studies of oxygen transfer and cell growth.

Keywords Tubespin · Spinner flask · Dissolved oxygen · k_La

Introduction

The limited availability and high cost for conventional bioreactors, such as spinner flasks, limits the development of high-throughput cell culture to a considerable extent. In the last few years, however, there has been a trend towards the use of disposable seed bioreactors as well as production bioreactors (Eibl et al. 2010). Tubespin, a disposable scale-down bioreactor for mammalian cell culture based on orbital shaking technology, is mostly used for rapid identification of optimal process conditions and protein expression on a small scale (De Jesus et al. 2004; Sang et al. 2010; Stettler et al. 2006; Xie et al. 2011). Using 50 mL centrifugation tubes as culture vessels mounted on a rotational shaker in an incubator, the Tubespin bioreactor is a high throughput and reliable system which can afford cell density as high as $10^7 \text{ cells mL}^{-1}$, with high gas transfer rates assumed to be an important reason for its efficiency. Using a small-scale system such as the Tubespin

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bioreactor, hundreds of culture conditions can be screened in parallel, allowing for fast process development, medium optimization and small scale production of proteins.

Dissolved oxygen (DO) concentration plays an important role in the growth and metabolism of mammalian cells (Zupke et al. 1995). It not only affects the growth rate and protein synthesis (Miller et al. 1987) but also regulates glucose and glutamine consumption and lactate and ammonia generation (Vallejos et al. 2010). A DO concentration between 20 and 50% air-saturation is usually maintained in bioreactors for better cell growth and recombinant protein expression. The growth profile of animal cells cultured in Tubespin has been described previously, but until now the dynamic profile of dissolved oxygen in this disposable bioreactor has not been investigated. The real-time behavior of dissolved oxygen concentration in culture medium is still not very well understood; however, a suggestion from the Food and Drug Administration (FDA) in 2004 recommended that it should be investigated as early as possible in the process development.

In this study, a precision 4-channel oxygen meter, utilizing a new oxygen sensing technology based on the quenching of emissions, was used to quantify the amount of dissolved oxygen in Tubespin bioreactors. The real-time oxygen concentration, volumetric mass transfer coefficient and oxygen uptake rates of suspended CHO DG44 cells were investigated with this non-invasive method. These data were compared to those from cultures in spinner flasks to evaluate the use of Tubespin bioreactors for small-scale cell culture.

Materials and methods

Cell Culture

Suspension-adapted dihydrofolate reductase-deficient CHO cells (DG44) were cultured in serum-free proCHO5 medium (Lonza, Verviers, Belgium) as described previously (De Jesus et al. 2004). The cultures were incubated in 50 mL disposable bioreactors (Tubespin®, TPP Trasadingen, Switzerland) in an ISF-4-W incubator with CO₂ and controlled humidity. DG44 cells were also cultured in 125 mL spinner flasks (Wheaton, NJ, USA) with stirring at 60 rpm in a 5% CO₂ incubator (the standard condition for cell culture in spinner flasks) (Deshpande and Heinzle 2009;

Suh-Chin Wu 1998). The cells were sub-cultured at a seeding density of $4\text{--}5 \times 10^5$ cells mL⁻¹.

Cell viability assays

Viable and dead cells were directly counted with a hemacytometer using the dye exclusion method (0.4% Trypan blue in phosphate buffered saline) (Kim et al. 2005).

Dissolved oxygen and measurement of k_La

The dissolved oxygen (DO) concentrations in spinner flasks and Tubespin bioreactors were measured by non-invasive and pre-calibrated DO sensors (PreSens, Regensburg, Germany) (Deshpande and Heinzle 2009; Tolosa et al. 2002). Autoclaved oxygen patches were placed on the inside of the spinner flask and Tubespin bioreactor with sterile forceps.

With surface aeration, the k_La and oxygen uptake rate (OUR) can be determined from a stationary, liquid-phase O₂ balance:

$$\frac{dC}{dt} = k_La \cdot (C^* - C) - Q_{O_2} \cdot X$$

where C and C^* are the dissolved oxygen concentrations in the liquid phase and in equilibrium with the gas phase, respectively, and k_La is the volumetric, liquid-phase mass transfer coefficient. Q_{O_2} is the specific oxygen uptake rate, and X is the viable cell density.

To measure Q_{O_2} , the shaker or spinner was stopped for a few minutes (which does not affect cell growth) and the following equation was used:

$$\frac{dC}{dt} = OUR = Q_{O_2} \cdot X$$

Then, the shaker or spinner was restored to shaking or stirring, respectively, and the dissolved oxygen concentration was raised again so that k_La could be calculated.

Results and discussion

On-line monitoring of cell growth and DO in spinner flask and Tubespin bioreactor

On-line monitoring of oxygen in spinner flasks has been reported previously (Deshpande and Heinzle

2009); however, there are no reports about the dynamic dissolved oxygen concentration in Tubespín bioreactors and its effect on cell growth. In this study, CHO DG44 cells were cultured in Tubespín bioreactors at standard operating conditions (agitation at 160 rpm with 10 mL working volume or 180 rpm with 10 or 20 mL working volume) to understand the effect of such small-scale bioreactors on cell growth. Figure 1 shows the cell density and dissolved oxygen profile of the cells cultured in Tubespín. Cells in Tubespín at 160 rpm with 10 mL working volume reached the highest density of 4.4×10^6 cells mL⁻¹ at 72 h post-inoculation (Fig. 1a). Interestingly, under these conditions, cell-aggregation appeared when the cell density reached 4×10^6 cells mL⁻¹. It could be that a shaking speed of 160 rpm is not enough to maintain a suspension of cells at such high densities. Cells were also cultured in Tubespín bioreactors at 180 rpm with either 10 or 20 mL working volume. Under both conditions, the maximum cell density was 6×10^6 cells mL⁻¹, and the lowest dissolved oxygen concentrations were still above 90 and 74% air-saturation, respectively (Fig. 1b, c), suggesting that Tubespín bioreactors can maintain a high oxygen transfer capacity even when cell density reaches 6×10^6 cells mL⁻¹ in a simple batch process. This means that cells cultured using the Tubespín system would not suffer from oxygen limitations.

Cells were inoculated in spinner flasks with an initial density of 5×10^5 cells mL⁻¹, and for the first 72 h, the cell density increased quickly. Concurrently, the DO concentration decreased sharply after 72 h to a steady-state value of approximately 1.9% air-saturation, after which the oxygen consumption and supply were balanced in a dynamic equilibrium until 120 h. Because the cells experienced oxygen limitation at this phase, the cell density remained unchanged at approximately 3×10^6 cells mL⁻¹. After 120 h, the DO concentration increased again with a corresponding slight increase in cell density. This second growth phase might be explained by the decrease in the oxygen uptake rate during the steady-state phase which allowed the DO level to recover, thus allowing cells to proliferate again (Fig. 2).

According to the DO concentration and cell density data, Tubespín was a better bioreactor than the traditional spinner flask, and a shaker speed of 180 rpm with a 10–20 mL working volume is the most appropriate culture condition. Cells cultured in

Tubespín bioreactors were free of any oxygen limitations and grew to a higher density. In addition, cells cultured with too small of a working volume or too high of a shaking speed resulted in premature cell death due to the stronger shear forces. In contrast, cells cultured with too large of a working volume or too low of a shaking speed resulted in cell aggregation due to inadequate shaking (data not shown).

Calculation of volumetric mass transfer coefficient (K_La) and oxygen uptake rate (OUR) of CHO DG44 from the time profile of DO

The k_La describes the nature of the mass transfer within the reactor and serves as an important parameter for reactor design (Zhang et al. 2008). Additionally, it is often used to compare the efficiency of different bioreactors in terms of oxygen transfer for both microbial and animal cell culture (Maier and Buchs 2001). The k_La value of the Tubespín bioreactors was calculated at three different shaking speeds (160, 180 and 200 rpm) with three different volumes (10, 20 and 35 mL), while the k_La of spinner flasks was calculated at a stirring speed of 60 rpm with 100, 125 and 150 mL volumes (Table 1). As expected, the k_La increased for higher shaking speeds and decreased at higher volumes. Notably, even the lowest k_La value found with the Tubespín bioreactor was much higher than that of the spinner flask. The k_La of the Tubespín at the standard operating condition (180 rpm with a 10 mL volume) was 21.5 h⁻¹, while that of the spinner flask at its standard operating condition (60 rpm with a 100 mL volume) was only 2.7 h⁻¹. With such high k_La values, Tubespín bioreactors can easily support the oxygen consumption requirements of high density cultures.

Oxygen is utilized by the cell for the oxidation of FADH₂ and NADH₂, which reflects cell growth and metabolism, so the oxygen uptake rate per hour of cultured cells, OUR, is the most useful metabolic parameter for cell culture. Surprisingly, even though it is a novel system for small-scale process development, there are no data on the oxygen uptake rate of cells cultured in Tubespín bioreactors, so we measured the oxygen uptake rate of CHO DG44 cells at different culture times (Fig. 3). In the lag phase, the OUR was only 0.14×10^{-12} mol cell⁻¹ h⁻¹, but it increased in the log phase to 0.27×10^{-12} mol·cell⁻¹ h⁻¹ until it

Fig. 1 On-line monitoring of dissolved oxygen (DO) concentration along with cell density and viability measurements in Tubespín bioreactors: **a** CHO DG44 cells cultured in 50 mL disposable bioreactors (Tubespín) with a 10 mL working volume at 160 rpm and an initial cell density of 0.4×10^6 cells mL⁻¹; **b** CHO DG44 cells cultured in 50 mL disposable bioreactors (Tubespín) with a 10 mL working volume at 180 rpm and an initial cell density of 0.6×10^6 cells mL⁻¹; **c** CHO DG44 cells cultured in 50 mL disposable bioreactors (Tubespín) with a 20 mL working volume at 180 rpm and an initial cell density of 0.5×10^6 cells mL⁻¹

finally fell back down to 0.10×10^{-12} mol cell⁻¹ h⁻¹ when the cells entered the stationary phase.

Previous studies have found that an estimated k_{La} of 1 h⁻¹ can result in a maximum O₂ transfer rate of only 0.23 mM h⁻¹ (Deshpande and Heinzle 2004, 2009). Using this value and the OUR and k_{La} value we measured, it is reasonable to conclude that a spinner flask with a k_{La} of less than 3 h⁻¹ could only sustain a maximum cell density of approximately 3×10^6 cells mL⁻¹, while Tubespín, with a much

higher k_{La} value, could theoretically support the growth of cells up to 2×10^7 cells mL⁻¹.

Conclusion

In this study, on-line dissolved oxygen concentrations, volumetric mass transfer coefficient and oxygen uptake rate were measured in Tubespín, a novel disposable bioreactor, and the same parameters were investigated

Fig. 2 On-line monitoring of dissolved oxygen (DO) concentration along with cell density and viability measurements in spinner flasks. CHO DG44 cells were cultured in spinner flasks with a 100 mL working volume at 60 rpm. The culture was inoculated at a cell density of 0.5×10^6 cells mL⁻¹

Table 1 Values of k_La in the Tubespin bioreactor and spinner flask at different working volumes and agitation speeds

Shaking (stirring) speed	Tubespin			Spinner flask		
	10 mL	20 mL	35 mL	100 mL	125 mL	150 mL
160 rpm	19.6	12	7.6	N	N	N
180 rpm	24.3	21.5	14.2	N	N	N
200 rpm	37.5	28.9	16.2	N	N	N
60 rpm	N	N	N	2.7	2.7	1.9

The working volume and agitation speed conditions used here are the most acceptable ones for cultivation of DG44 cells in these two types of bioreactors. K_La was calculated by dynamic method using gas–liquid mass transfer equation. All of the experiments were repeated three times, and the data listed above were from one representative experiment

Fig. 3 Relationship between oxygen uptake rate (OUR), cell density and cell viability when CHO DG44 cells were cultured in the small-scale disposable Tubespin bioreactor

in a conventional spinner flask. Dissolved oxygen concentration and k_La were confirmed as the main limiting factors for small-scale bioreactors as well as large-scale bioreactors. Under normal growth conditions, with a high volumetric mass transfer coefficient, the cells cultured in Tubespin bioreactors were free of oxygen limitation and the cell density reached 6×10^6 cells mL⁻¹. However, cells cultured in spinner flasks were oxygen limited and the maximum cell density was only 3.0×10^6 cells mL⁻¹ (Table 2). These results

indicate that Tubespin is a more effective and efficient small-scale bioreactor system for cell culture and that a working volume of 10 or 20 mL with a shake speed of 180 rpm is the optimal condition. Additionally, oxygen uptake rates of CHO DG44 cells cultured in Tubespin bioreactors at different times were determined for the first time, and these data will be very helpful for further use of this disposable bioreactor. Based on our studies, Tubespin is an ideal system for process optimization and scale-down studies.

Table 2 Comparison between the disposable Tubespin bioreactor and spinner flask

	Tubespin	Spinner flask
$k_L a$	24.3	2.7
Minimum DO	90%	1.9%
Maximum cell density	6×10^6 cells mL ⁻¹	3×10^6 cells mL ⁻¹

Cells were cultured at a 10 mL working volume and 180 rpm for the Tubespin bioreactor and a 100 mL working volume and 60 rpm for the spinner flask. These are the standard operating conditions for the two types of bioreactors

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Conflict of interest The authors have no conflicts of interest.

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