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Disposable 600-mL orbitally shaken bioreactor for mammalian cell cultivation in suspension

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ABSTRACT

Orbitally shaken bioreactors (OSRs) support the efficient cultivation of mammalian cells in suspension at various scales of operation. Here we introduce a disposable culture vessel with a ventilated cap having a nominal volume of 600 mL (TubeSpin® bioreactor 600) for medium-scale cell cultivation. It has a conical bottom designed to fit into a standard swinging bucket rotor to facilitate cell/liquid separation by centrifugation. At shaking frequencies suitable for mammalian cell cultivation (140–220 rpm), the volumetric mass transfer coefficient (k_La) ranged from 15 to 40 h^{-1}, the mixing time was less than 20 s, and the specific power consumption ranged from 0.03 to 0.5 kW/m^3. To define the working conditions for the TubeSpin® bioreactor 600, we measured cell growth and environmental culture conditions such as pH and dissolved O_2 and CO_2 concentrations over a range of shaking frequencies and working volumes using a stable CHO-derived cell line expressing a human IgG antibody. The optimal cultivation conditions were observed at working volumes of 300–500 mL and shaking frequencies of 180–220 rpm. In 300-mL batch cultures of the same cell line in a TubeSpin® bioreactor 600, 1-L cylindrical glass bottle, and 1-L Erlenmeyer shake flask, the maximum cell densities and recombinant antibody yields were similar. This study demonstrated the suitability of the TubeSpin® bioreactor 600 for cell culture applications with suspension-adapted CHO cells at culture volumes up to 500 mL.

1. Introduction

The use of disposable bioreactors is becoming increasingly important for cell culture and recombinant protein production within the biopharmaceutical industry due to their flexibility, small footprint, and cost-effectiveness. Disposable bioreactors with working volumes up to 2000 L are now available [1–3]. Among these, disposable orbitally shaken bioreactors (OSRs) with nominal volumes up to 2000 L have proven to be feasible for the cultivation of animal cells in suspension [4–9]. The disposable TubeSpin® bioreactor 50 with a ventilated cap and a working volume of 5–20 mL is widely used for high-throughput cell cultivation and protein production with both mammalian and insect cells [10–13]. Given the advantages of this vessel, a larger model, the TubeSpin® bioreactor 600, was introduced. It has a nominal volume of 600 mL with a comparable size and shape to a 500-mL centrifuge tube.

To determine if a bioreactor is suitable for cell cultivation, several engineering principles such as the volumetric power consumption, mixing time, mixing homogeneity, and gas transfer rate need to be defined. These experimentally determined values are commonly expressed in the form of coefficients that are used to identify optimal conditions for cell cultivation and to compare the characteristics of different bioreactors [14–16]. The aim of this study was to assess the suitability of the TubeSpin® bioreactor 600 for the cultivation of mammalian cells through a characterization of engineering principles and cell cultivation conditions using a stable Chinese hamster ovary (CHO) cell line since this is the most widely used mammalian host for recombinant protein production [17–19].

2. Materials and methods

2.1. Determination of the volumetric mass transfer coefficient

The volumetric mass transfer coefficient (k_La) [h^{-1}] was determined in ultra-high purity (UHP) deionized water at 37 °C using a static gassing-out method [20,21]. The cell cultivation vessels included TubeSpin® bioreactor 600 polypropylene tubes (TPP, Trasadingen, Switzerland), 1-L cylindrical glass bottles (Duran

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Schott Glass, Mainz, Germany) equipped with a vented cap having a 1.5 cm polyether sulfones membrane, and 1-L polycarbonate Erlenmeyer shake flasks with a vented cap (Corning, Corning, NY, USA). Each vessel was filled to the appropriate volume with UHP deionized water, and nitrogen gas was sparged into the liquid phase until the percent air saturation reached about zero. With the vessel cap removed, the headspace was quickly sparged with air to replace the nitrogen. Each vessel was then orbitally shaken on an ES-X shaker (Kühner AG, Birsfelden, Switzerland) at a shaking diameter of 50 mm. The percent air saturation in the liquid phase was measured using a non-invasive optical sensor patch (PreSens, Regensburg, Germany) that was attached to the inside wall of the vessel with a silicone gel. Each measurement was completed in the absence of the vessel cap. The k_La was calculated from the following mass balance equation (1):

$$\frac{dC_L}{dt} = k_La(C^* - C_L) \quad (1)$$

where C_L is the concentration of dissolved oxygen (DO) in the liquid phase [mol/L], C^* is the concentration of DO at saturation [mol/L], and t is the time [h]. The k_La value is derived from two variables, the liquid-phase mass transfer coefficient (k_L) [m/h] and the specific interfacial area (a) [m^{-1}], as defined in Eq. (2):

$$a = \frac{SA}{V_L} = \frac{\text{surface area}}{\text{liquid volume}} \quad (2)$$

Thus, the overall volumetric oxygen mass transfer coefficient is proportional to the ratio of the liquid surface area to the liquid volume.

2.2. Mixing time analysis

The mixing time was measured using the Dual Indicator System for Mixing Time (DISMT) [22,23]. Each vessel was filled with 300 mL of UHP deionized water at room temperature. The two pH indicators, Methyl Red (Fisher Scientific, Pittsburgh, PA) and Thymol Blue (AppliChem GmbH, Darmstadt, Germany), were each added at a final concentration of 1.3 mg/L. The water was acidified with the addition of 10 μL of 1 M HCl. The pH was changed by rapid addition, close to the vessel wall, of 10 μL of 1 M NaOH as the vessel was orbitally shaken at various shaking frequencies. The color change was recorded with a video camera at 30 frames per second (Nikon Coolpix S210, Nikon AG, Egg, Switzerland). The mixing time was determined by analyzing the recorded data using in-house imaging software as previously described [23,24]. The mixing time was represented in a mixing map format depicting the mixing time of each pixel with a color code. Measurements for each experimental condition were performed in duplicate.

2.3. Specific power consumption analysis

The specific power consumption can be defined as the electric power required to maintain the motion of liquid in a vessel [25,26]. The voltage required for the agitation of both an open vessel with a moving liquid and a closed vessel with a liquid that is not allowed to form a wave was measured using a digital multimeter (DT830B, Zhangzhou Lihao Electronic Co., Ltd., Fujian, China). The specific power consumption was calculated from the following equation (3):

$$\frac{P}{V_L} = \frac{I(V_O - V_C)}{zV_L} \quad (3)$$

where P/V_L is the specific power consumption [kW/m^3], I is the electric current [A], V_O is the electric potential or voltage of the open vessel with a moving liquid [V], V_C is the electric potential or voltage of the closed vessel [V], V_L is the liquid volume [m^3], and z is the number of vessels.

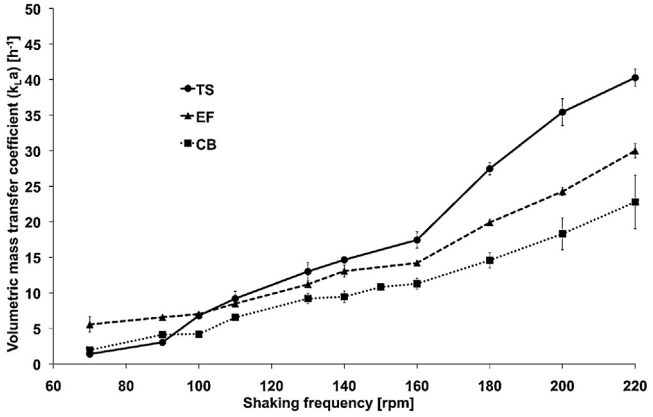

Fig. 1. Comparison of k_La as a function of shaking frequency in OSRs. The k_La was measured with a static method. The TubeSpin® bioreactor 600 (TS), 1-L Erlenmeyer shake flask (EF), and 1-L cylindrical glass bottle (CB) were filled with 300 mL of UHP deionized water at 37 °C and orbitally shaken at various shaking frequencies. Each experiment was performed in duplicate.

2.4. Cell culture

A recombinant CHO-DG44 cell line, designated CHO-IgG (courtesy of Dr. Mattia Matasci), that expresses a human IgG antibody was routinely cultured in suspension in 200 mL of ProCHO5 medium (Lonza, Verviers, Belgium) in TubeSpin® bioreactor 600 tubes orbitally shaken at 180 rpm. The cells were subcultivated by dilution to a seeding cell density of 5×10^5 cells/mL. All cell cultivations were performed in an ISF1-XC incubator (Kühner AG) at 37 °C with 5% CO_2 saturation and 85% humidity. The shaking diameter was set at 50 mm for all experiments. The cell density and cell viability were determined by the Trypan Blue exclusion method using a hemocytometer. The percent air saturation, percent CO_2 saturation, and pH were analyzed within 1 min of sampling using an off-line NOVA BioProfile pHOx analyzer (NOVA Biomedical, Waltham, MA). The percent air and CO_2 saturation refer to the measured level of each gas in water as compared to the level of the gas in water at saturation. The recombinant antibody in conditioned medium was quantified by sandwich ELISA as described [27].

3. Results

3.1. Measurement of k_La

The k_La of the TubeSpin® bioreactor 600 was determined at a working volume of 300 mL over a range of shaking frequencies from 70 to 220 rpm. For comparison, the k_La was determined in a 1-L shake flask and a 1-L cylindrical glass bottle at the same filling volume. For all three vessels, the k_La increased with the shaking frequency (Fig. 1). Above a shaking frequency of 100 rpm, a higher k_La value was recorded in the TubeSpin® bioreactor 600 than in either of the other two vessels, possibly indicating a more efficient usage of the liquid surface area to volume ratio as the shaking frequency increased.

3.2. Mixing time analysis

The homogeneity of liquid mixing in the three vessels was determined using the DISMT method at a working volume of 300 mL over a range of shaking frequencies. At shaking frequencies of 140 rpm

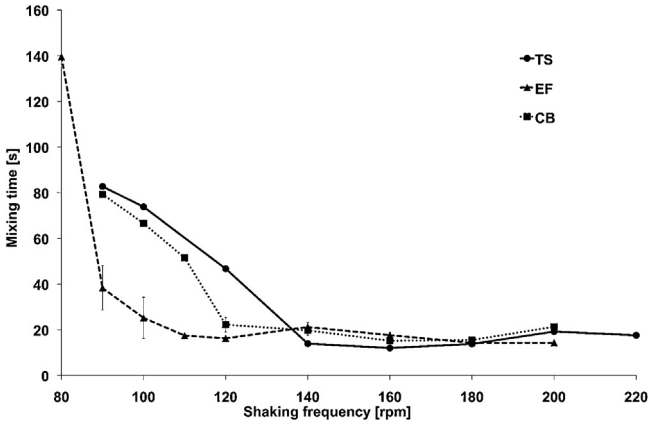

Fig. 2. Comparison of mixing time as a function of shaking frequency in OSRs. The mixing time values were calculated using DISMT. TubeSpin® bioreactor 600 (TS), 1-L Erlenmeyer shake flask (EF), and 1-L cylindrical glass bottle (CB) were filled with 300 mL of UHP deionized water at room temperature and orbitally shaken at various shaking frequencies. Each experiment was performed in duplicate.

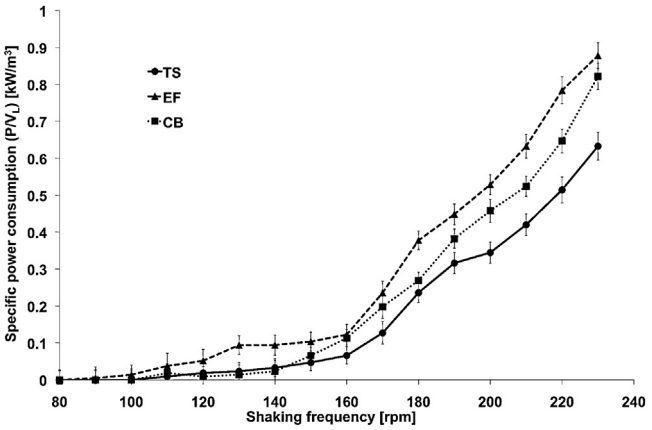

Fig. 4. Comparison of specific power consumption as a function of shaking frequency in OSRs. The TubeSpin® bioreactor 600 (TS), 1-L Erlenmeyer shake flask (EF), and 1-L cylindrical glass bottle (CB) were filled with 300 mL of UHP deionized water at room temperature and orbitally shaken at various shaking frequencies. Each value represents the average of six vessels.

or more, a mixing time of less than 22 s was observed with all three vessels. At shaking frequencies less than 140 rpm, the geometry of the vessels appeared to play an important role in mixing (Fig. 2).

For mammalian cell cultivation we routinely agitate the 1-L shake flask, 1-L cylindrical bottle, and TubeSpin® bioreactor 600 at 120 rpm, 110 rpm, and 180 rpm, respectively. Under these conditions, the mixing times were 17, 52 and 15 s, respectively (Fig. 2). A mixing map for each vessel was generated from video images recorded at these working conditions. Notably, a poorly mixed zone was observed in the center of the liquid in the cylindrical bottle but not in the TubeSpin® bioreactor 600 or the shake flask (Fig. 3). This may have been due to the vessel geometry and to the low shaking frequency. Furthermore, the surface area of the liquid was greater in the shake flask and TubeSpin® bioreactor 600 than in the cylindrical bottle under the conditions used to generate the mixing maps.

3.3. Specific power consumption

The specific power consumption was determined for the three different vessels at a working volume of 300 mL over a range of shaking frequencies. The specific power consumption was under 0.1 kW/m^3 at shaking frequencies up to 160 rpm for all three vessels (Fig. 4). For both the shake flask and the TubeSpin® bioreactor 600 there was a dramatic increase in the specific power consumption beginning at a shaking frequency of 160 rpm. Among the three vessels, the TubeSpin® bioreactor 600 required the lowest amount of power at shaking frequencies of 160 rpm and more (Fig. 4). With agitation conditions we routinely used for cell culture, the specific power consumption values for the TubeSpin® bioreactor 600 (180 rpm), shake flask (120 rpm), and cylindrical bottle (110 rpm) were 0.24, 0.05, and 0.02 kW/m^3, respectively.

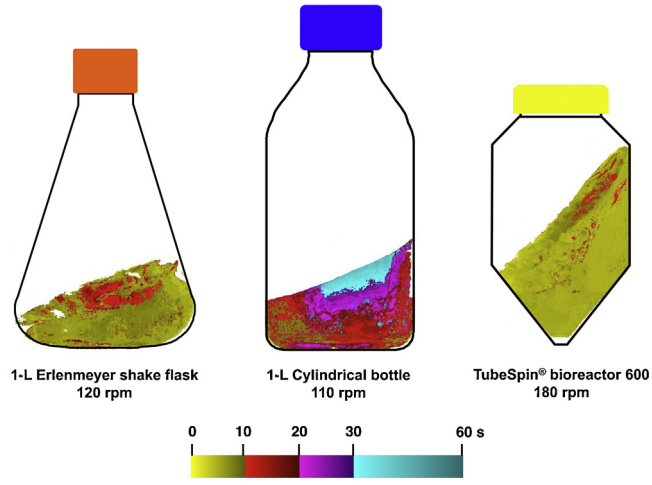

Fig. 3. Comparison of mixing time maps in OSRs. The mixing time maps were generated using DISMT and in-house software [22,23]. Each vessel was filled with 300 mL of UHP deionized water at room temperature, and the vessels were agitated at the shaking frequencies indicated. The color scale represents the mixing time in seconds for each pixel. Each experiment was performed in duplicate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Effect of working volume on cell cultivation

The effect of working volume on the growth of CHO-IgG cells was determined in the TubeSpin® bioreactor 600 at a shaking frequency of 180 rpm. For all working volumes tested, a maximum cell density of 7×10^6 cells/mL was achieved at 120 h post-inoculation (Fig. 5). However, in cultures with working volumes of 100 mL and 200 mL the cell density was lower at 168 h post-inoculation. For all working volumes tested, the viability remained about 100% to 96 h post-inoculation and then declined (Fig. 5). For a working volume

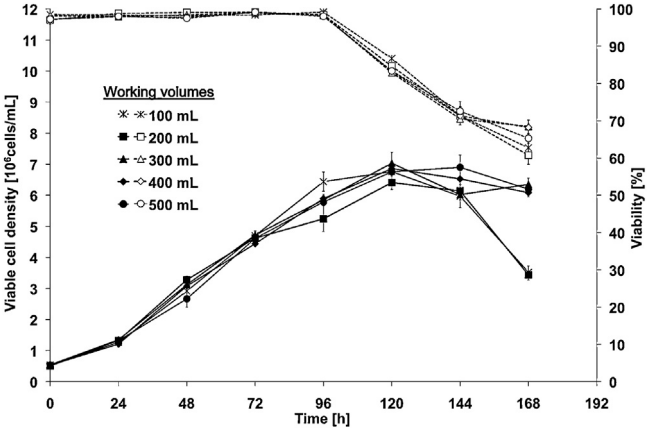

Fig. 5. Batch culture of CHO-IgG cells in a TubeSpin® bioreactor 600. The cultures were inoculated at 5×10^5 cells/mL and grown at 37 °C with a shaking speed of 180 rpm at a range of different working volumes as indicated. The viable cell density (closed solid lines) and the percent cell viability (open dashed lines) were determined with the Trypan Blue exclusion method at the times indicated. Each experiment was performed in triplicate.

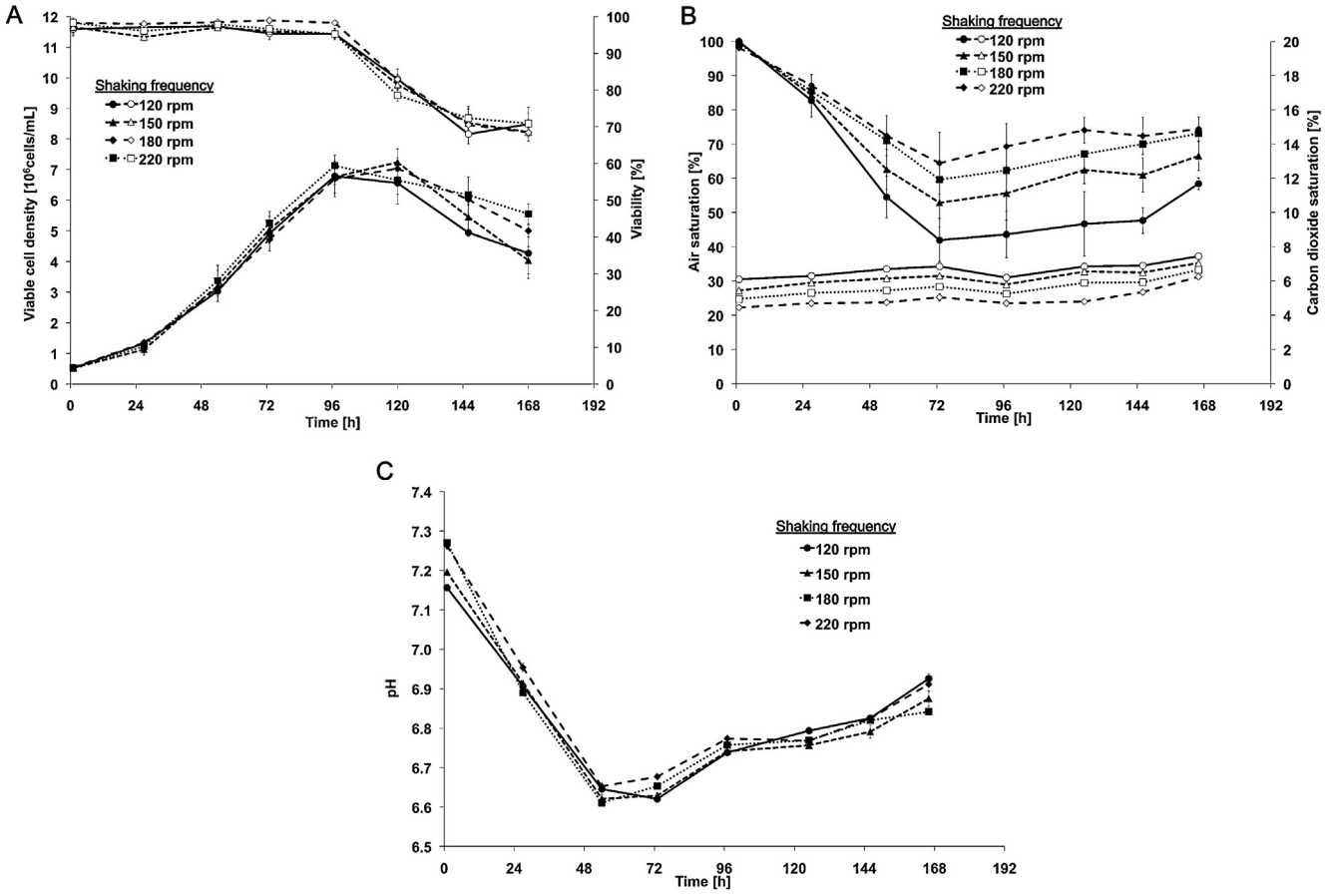

Fig. 6. Cell growth in a TubeSpin® bioreactor 600. The cultures were inoculated with 5×10^5 CHO-IgG cells/mL and grown at 37 °C at a working volume of 300 mL at different shaking speeds as indicated. The viable cell density (A, closed symbols) and the percent cell viability (A, open symbols) were determined with the Trypan Blue exclusion method at the times indicated. The air saturation (B, closed symbols), carbon dioxide saturation (B, open symbols), and pH (C) were determined at the times indicated with a NOVA BiopHox analyzer. Each experiment was performed in duplicate.

of 300 mL, the DO remained above 60% air saturation throughout the culture, but for working volumes of 400 and 500 mL, the DO declined to 40% air saturation by 96 h post-inoculation (data not shown). In contrast, no differences were observed in CO_2 saturation for the various cultures (data not shown). Taking these results into consideration, further studies in this vessel were completed at a working volume of 300 mL.

3.5. Effect of shaking frequency on cell cultivation

The effect of shaking frequency on the growth of CHO-IgG cells in the TubeSpin® bioreactor 600 was determined at a working volume of 300 mL. For all shaking frequencies tested, a maximum cell density of 7×10^6 cells/mL was observed, but this was achieved at different times post-inoculation depending on the shaking frequency (Fig. 6A). No significant differences in cell viability were seen in the various cultures (Fig. 6A). As predicted from the k_La values shown in Fig. 1, the air saturation levels in the cultures were directly correlated with the shaking frequency while the CO_2 saturation levels were inversely correlated with the shaking frequency (Fig. 6B). The latter was probably due to a decrease in CO_2 stripping from the culture as the shaking frequency decreased. At shaking frequencies of 180 rpm and above, efficient removal of CO_2 from the medium was achieved, and the CO_2 saturation level remained close to its level in the incubator (5%) (Fig. 6B). Despite the differences in CO_2 saturation levels, the pH profiles for the various cultures did not vary significantly with the shaking frequency (Fig. 6C). A shaking frequency of 180–220 rpm is therefore recommended for the cultivation of CHO cells in the TubeSpin® bioreactor 600 at a working volume of 300 mL since sufficient gas transfer and mixing are expected. These conditions were also found to be optimal for the cultivation of human embryonic kidney 293 (HEK-293) and insect cells in the TubeSpin® bioreactor 600 (data not shown).

3.6. Comparison of cell cultivation in different orbitally shaken vessels

Cell growth and recombinant protein production were compared in the three different OSRs. Prior to the experiment, the CHO-IgG cells were maintained in shake flasks at a working volume of 200 mL with agitation at 120 rpm for at least 3 passages. The conditioned cells were used to inoculate the three OSRs, and the cultures were agitated at 110 rpm (1-L cylindrical bottle), 120 rpm (1-L shake flask), and 180 rpm (TubeSpin® bioreactor 600). During the exponential growth phase, the cell density and viability in each vessel were similar, and the maximum cell density for each culture was about 7×10^6 cells/mL (Fig. 7A). A more rapid decline of the cell density and viability was observed in the cylindrical bottle than in the other two vessels after reaching the maximum cell density (Fig. 7A). The k_La values for the cylindrical bottle, shake flask, and TubeSpin® bioreactor 600 at the working conditions used in this experiment were 6, 8, and 27 h^{-1}, respectively (cf. Fig. 1). Predictably, the decrease in air saturation over time was more pronounced in both the cylindrical bottle and shake flask than in the TubeSpin® bioreactor 600 (Fig. 7B). In each vessel, the lowest air saturation level was observed at 72 h post-inoculation, and then

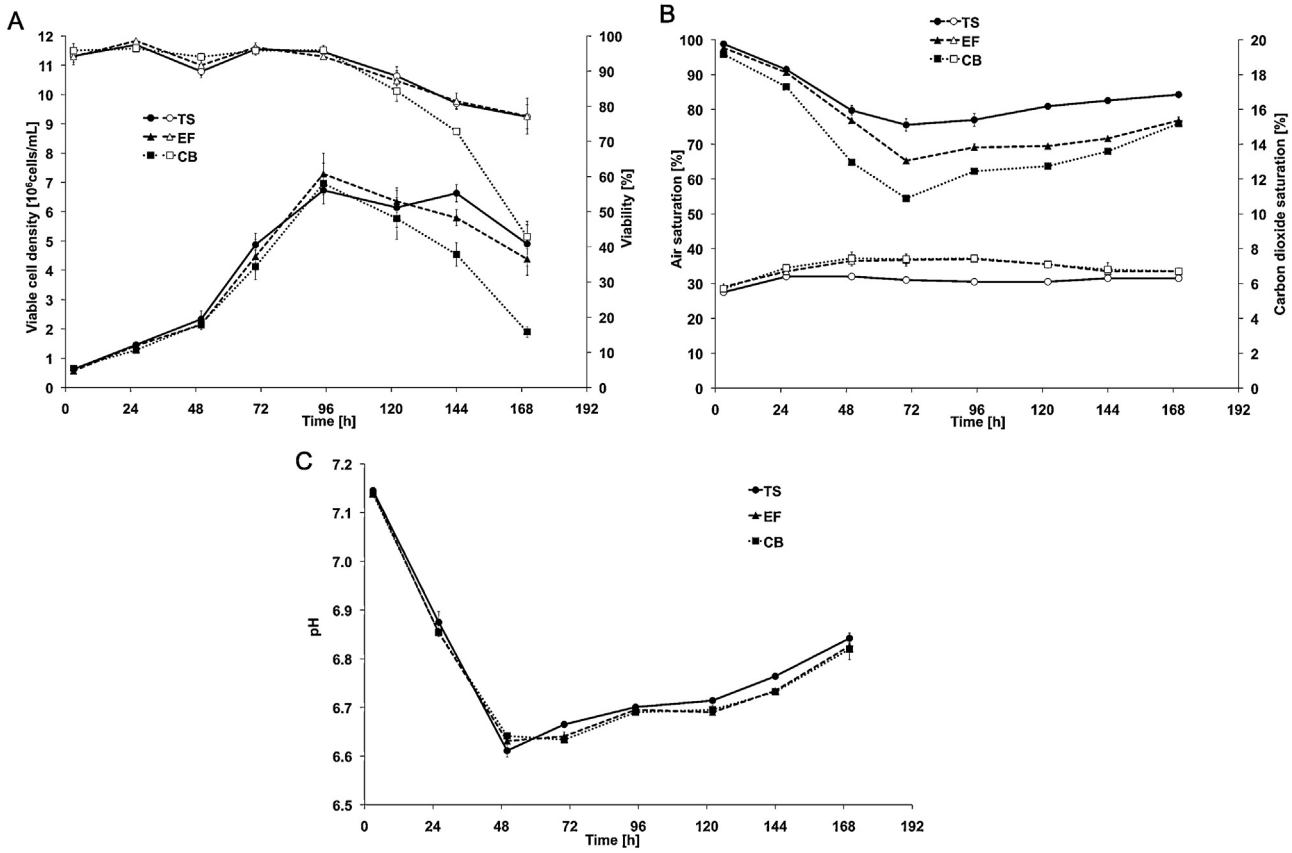

Fig. 7. Comparison of cell growth in different OSRs. The cultures were inoculated with 5×10^5 CHO-IgG cells/mL and grown at 37 °C at a working volume of 300 mL. The viable cell density (A, closed symbols) and percentage cell viability (A, open symbols) were analyzed using the Trypan Blue exclusion method. The air saturation (B, closed symbols), carbon dioxide saturation (B, open symbols), and pH (C) were determined with a NOVA BiopHox analyzer at the times indicated. Cells were cultivated in a TubeSpin® bioreactor 600 (TS), 1-L Erlenmeyer shake flask (EF), and 1-L cylindrical glass bottle (CB) at shaking speeds of 180, 120, and 110 rpm, respectively. Each experiment was performed in duplicate.

the level rose as the cells died (Fig. 7B). Likewise, the CO_2 saturation level was higher in the cylindrical bottle and shake flask than in the TubeSpin® bioreactor 600 (Fig. 7B). The pH profile in each OSR was similar (Fig. 7C). The maximum recombinant antibody yields for the cultures in the TubeSpin® bioreactor 600, shake flask, and cylindrical bottle were 155 ± 13 mg/L, 118 ± 9 mg/L, and 111 ± 16 mg/L, respectively.

4. Discussion

In the first part of this study, the engineering characteristics of the TubeSpin® bioreactor 600 were compared with two other cell cultivation vessels, a 1-L shake flask and a 1-L cylindrical glass bottle. The k_La in the TubeSpin® bioreactor 600 ranged from 15 to 40 h^{-1} at shaking frequencies from 140 to 220 rpm. Compared to the other two vessels, the TubeSpin® bioreactor 600 had a superior k_La at shaking frequencies of 110 rpm or more and a lower specific power consumption at shaking frequencies of 150 rpm or more. All three vessels had a minimal mixing time of about 20 s.

The results of these studies demonstrated that vessel geometry plays an important role in mixing, gas transfer, and specific power consumption. Of the three vessels, the shake flask has the largest vessel diameter (at its base) and its walls are inclined. The diameters of the TubeSpin® bioreactor 600 and the 1-L cylindrical bottle are similar to each other, but the former has a conical bottom while the latter has a flat bottom. Only the cylindrical bottle does not have inclined walls. Due to the different geometries, the minimal mixing time was achieved at different shaking frequencies for the three different vessels: 110 rpm for the shake flask, 120 rpm for the cylindrical bottle, and 140 rpm for the TubeSpin® bioreactor 600. At low shaking frequencies (90–100 rpm), the larger liquid surface area and lower liquid height in the shake flask resulted in higher k_La values and more rapid mixing times relative to the other vessels. At high shaking frequencies (>140 rpm), higher k_La values were recorded in the TubeSpin® bioreactor 600 than in the other two vessels. Assuming a constant k_L and the same liquid volume, a more efficient usage of the liquid surface area to volume ratio allowed better gas transfer to be achieved in the TubeSpin® bioreactor 600 relative to the other two vessels. As a consequence of the differences in vessel geometry, different cell culture conditions (i.e. shaking frequencies) were chosen for each vessel.

When measuring the specific power consumption, the electric efficiency factor of the shaker motor must be taken into consideration. This efficiency factor is defined as the mechanical output divided by the electrical input. The transformation of electric power to mechanical movement does result in a loss of power. However, this loss was assumed to be minimal for the ES-X shaker used here due to its mechanical design and to the conservation of angular momentum. This assumption was shown to be valid because the specific power consumption values measured here were comparable with the values obtained by other methods [28,29]. Although the specific power consumption of the cylindrical bottle increased exponentially with the shaking frequency, this was not true for the other two vessels. For the shake flask and the TubeSpin® bioreactor 600 the slope of the specific power consumption curve changed dramatically at a specific shaking frequency. For the shake flask this occurred at 160 rpm and was likely due to the liquid retracting from the center of the vessel, leaving a dry bottom. For the TubeSpin®

bioreactor 600 the slope of the curve changed at 190 rpm. This was probably due to the wave crest contacting the upper inclined wall of the tube (cf. Fig. 3). It is assumed that the change of the wave geometry required a greater amount of power.

In the second part of the study, the conditions for mammalian cell cultivation in the TubeSpin® bioreactor 600 were investigated. It was possible to achieve a maximum cell density of 7×10^6 cells/mL for culture volumes from 100 to 500 mL at a shaking frequency of 180 rpm. This agitation condition was chosen because the liquid contacted the top of the vessel when shaking frequencies greater than 180 rpm were used at a working volume of 500 mL. At 180 rpm, the specific power consumption was 0.23 kW/m^3 and the k_La was 27.5 h^{-1}. At this shaking frequency, sufficient power was provided to support rapid mixing and allowed a more efficient stripping of CO_2 than in the other two OSRs.

In the comparative cell growth study, cell cultivation in either the TubeSpin® bioreactor 600 or the 1-L shake flask was superior to that in the 1-L cylindrical bottle. For the 1-L shake flask, the k_La was 8 h^{-1} at a shaking frequency of 120 rpm. These conditions allowed a sufficient oxygen supply to be maintained for CHO cell cultivation, explaining the similar cell growth in the shake flask and the TubeSpin® bioreactor 600. However, for insect cells and other cell types having a greater demand for oxygen than do CHO cells, better cell growth is expected in the TubeSpin® bioreactor 600 than in the shake flask due to the difference in the k_La values (27 h^{-1} for the TubeSpin® bioreactor 600 and 8 h^{-1} for the shake flask) at the shaking frequencies used for cell cultivation. Furthermore, for cultures with a high oxygen demand, a high production of CO_2 is expected. The TubeSpin® bioreactor 600 has demonstrated a more efficient stripping of CO_2 as compared to the shake flask.

The TubeSpin® bioreactor 600 has several advantages to shake flasks and cylindrical bottles. It has a comparable size and shape to a 500-mL centrifuge tube and can be directly transferred to a swinging bucket rotor for centrifugation of the culture. This decreases the contamination risk associated with the transfer of the culture from one container to another prior to centrifugation. The TubeSpin® bioreactor 600 has a smaller footprint than a shake flask and more of them can be placed on a shaker platform. Finally, the working volumes for shake flasks and cylindrical bottles are about 20–40% of the nominal volume due to the geometry of the vessels. With a working volume range of 100–500 mL, the TubeSpin® bioreactor 600 provides a greater flexibility than the other two vessels. The Tubespin® bioreactor 600 has been used for transient protein production with CHO cells [30], and it is also suitable as an intermediate vessel for culture scale-up from the TubeSpin® bioreactor 50 to containers with a minimal working volume of about 5 L.

5. Conclusions

We have demonstrated that the TubeSpin® bioreactor 600 is a suitable single-use alternative to 1-L Erlenmeyer shake flasks and 1-L cylindrical glass bottles for CHO cell cultivation. The TubeSpin® bioreactor 600 exhibited high gas transfer, rapid mixing to homogeneity, and low specific power consumption. These properties enabled the vessel to support cell densities up to 7×10^6 cells/mL in batch cultures at working volumes of 100–500 mL. The best operating conditions were observed at shaking frequencies of 180–220 rpm at a shaking diameter of 50 mm. The TubeSpin® bioreactor 600 can be used for routine cell cultivation, medium scale (100–500 mL) protein production, and volumetric scale-up with CHO and other animal cells.

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