

Article

Helical-Track Bioreactors for Bacterial, Mammalian and Insect Cell Cultures

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Received: 15 December 2012; in revised form: 17 February 2013 / Accepted: 15 March 2013 / Published: 26 March 2013

Abstract: We investigated the cultivation of bacterial, mammalian and insect cells in an orbitally-shaken 250-mL disposable tube that incorporates a helical track (HT) on its inside wall. The mass transfer coefficient of oxygen (k_{La}) was 200%–400% higher in the HT tube than in a shake flask. Bacterial growth and plasmid production were 30% higher in the HT tube than in a 1-L Erlenmeyer flask. Mammalian cell cultures achieved a 25% higher cell density in the HT tube as compared to a 250-mL square-shaped bottle while insect cells grew as well in HT tubes as in 250-mL shake flasks. Because of their performance, disposability, and compact size, we conclude that 250-mL HT tubes are a useful alternative to other shaken containers for the cultivation of bacterial, mammalian and insect cells.

Keywords: shake flasks; orbital shaking; bacteria; gas transfer; insect cells; mammalian cells

Nomenclature

c^*_G , interfacial oxygen concentration in the gas phase [M]; c_G , oxygen concentration in the atmospheric air [M]; c_L , dissolved concentration of oxygen [M]; c^*_L , dissolved oxygen concentration at saturation [M]; k_{GA} , oxygen mass transfer coefficient in the gas phase [h^{-1}]; k_{La} , oxygen mass

transfer coefficient in the liquid phase [h^{-1}]; OTR , oxygen transfer rate through the gas-liquid interface [$\text{M}\cdot\text{h}^{-1}$]; OTR_{plug} , oxygen transfer rate through the sterile closure [$\text{M}\cdot\text{h}^{-1}$]; t , time [h].

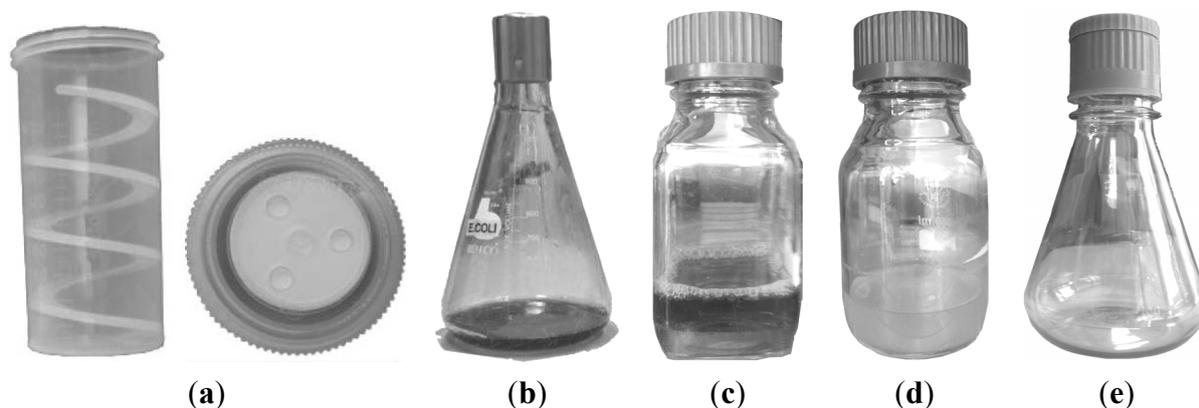
1. Introduction

The suspension cultivation of microbes and animal cells is often performed in shaken flasks and bottles because of their ease of use and cost effectiveness. In most cases, however, shaken containers are neither monitored nor controlled for the dissolved oxygen concentration (c_L) or pH. Consequently, the culture may become oxygen or pH limited, leading to reduced biomass accumulation [1,2]. In shaken flasks, mass transfer limitations can occur across the sterile closure or at the gas-liquid interface. Mass transfer resistance studies have shown that the closure can, in fact, be the limiting factor in passively aerated flasks [3,4]. In this study we tested a new orbitally shaken container for the cultivation of microbes and animal cells. The cylindrical 250-mL helical-track (HT) tube was developed to improve gas transfer by increasing the gas-liquid interface area. In addition, the tube has a ventilated cap with a membrane filter to improve gas transfer across the sterile closure. Cylindrical HT bioreactors ranging in working volume from 50 mL to 55 L, but not including the 250-mL HT tube described here, have been used for the cultivation of mammalian cells in suspension [5]. Here we determined the volumetric mass transfer coefficient of oxygen (k_La) in a disposable 250-mL HT tube. We also compared the growth of bacterial, mammalian and insect cells in the HT tube to that in conventional shaken flasks and bottles.

2. Experimental Section

2.1. The 250-mL HT Tube and Other Standard Vessels

Figure 1. Images of culture containers used in this study; (a) a 250-mL helical track (HT) tube (left) and its vented cap (right); (b) a 1-L Erlenmeyer flask with a stainless steel closure; (c) a 250-mL square-shaped bottle; (d) a cylindrical glass bottle; (e) a 250-mL flat-base Erlenmeyer flask with a vented cap.



The 250-mL polypropylene HT tube (kindly provided by Sartorius AG, Göttingen, Germany) was equipped with vented caps having a 0.22- μm polytetrafluoroethylene filter as a sterile barrier (Thermo Fisher Scientific, Waltham, MA, USA). The 0.8 cm-wide track started at the bottom of the tube and reached a height of 10.6 cm (Figure 1a). The outside diameter of the tube was 5 cm. The other standard

vessels used here included 1-L Erlenmeyer flasks (Bellco Glass, Vineland, NJ, USA) with a stainless steel closure for bacterial cultures (Figure 1b); 250-mL square-shaped (Figure 1c) and cylindrical glass bottles with a screw cap (Figure 1d) (SciLabware, Stone, UK) for mammalian cell cultures; and 250-mL flat-base Erlenmeyer flasks equipped with vented caps (BD Falcon, Franklin Lakes, NJ, USA) for insect cells cultures (Figure 1e).

2.2. Gas Transfer

The volumetric mass transfer coefficient (k_La) was measured at 37 °C using a dynamic method as described [6]. Non-invasive O₂ sensors (PreSens, Regensburg, Germany) positioned below the liquid level measured the dissolved oxygen concentration (c_L) in a 250-mL HT tube containing 100 mL of deionized water. The tube, without a closure, was agitated at various speeds on an orbital shaker. Nitrogen was flushed into the water until the c_L reached about 0% air saturation (air sat). Air was then flushed into the gas phase of the vessel to replace nitrogen, and the c_L was recorded over time as the vessel was agitated. The k_La was calculated from Equation (1) based on the change of c_L with time. As the vessels were not equipped with closures, the c_L^* was assumed to be constant and equal to 0.2 mM [2].

$$OTR = \frac{dc_L}{dt} = k_La(c_L^* - c_L) \quad (1)$$

The k_Ga represents the rate at which oxygen is transferred from the surroundings into the gas phase of the vessel [7]. Thus, the k_Ga is related to the mass transfer resistance of the closure. The HT tube with 100 mL deionized water was agitated at 200 rpm at 37 °C. It was flushed with nitrogen until the c_L reached 0% air sat as measured by non-invasive O₂ sensors positioned in the gas phase. The HT tube was then fitted with its closure, and the c_G was recorded over time as the container was agitated. Assuming that the gas phase was well mixed, Equation (2) was used to calculate the k_Ga . The maximal OTR through the sterile closure (OTR_{plug}) was calculated from Equation (2) [6,8].

$$OTR_{plug} = \frac{dc_G}{dt} = k_Ga(c_G^* - c_G) \quad (2)$$

2.3. Bacterial Cultivation and Plasmid Production

The dual expression vector pXLG^{CHO}-A2 carrying the human anti-Rhesus D IgG1 heavy and light chain cDNAs was constructed as described for pXLG^{CHO}-A3 except that the expression cassettes for the light and heavy chain genes were in a head-to-tail rather than a head-to-head orientation [9]. *E. coli* DH5 α were transformed with pXLG^{CHO}-A2 and grown overnight at 37 °C in a 1-L Erlenmeyer flask in 100 mL of Terrific Broth (TB) (Difco Laboratories, Franklin Lakes, NJ, USA) with 100 mg ampicillin/L (AppliChem, Darmstadt, Germany) with agitation at 200 rpm on an ES-X shaker (Kühner, Birsfelden, Switzerland). The culture was diluted 10 times in TB containing 100 mg ampicillin/L, and aliquots of 100 mL were transferred into 250-mL HT tubes and 1-L Erlenmeyer flasks with a metal closure (Figure 1a,b). The cultures were grown at 37 °C for 7 h. Turbidity was assessed by optical density at 600 nm (OD₆₀₀) using a spectrophotometer (Eppendorf, Hamburg,

Germany). The pH was measured off-line, and the dissolved oxygen concentration c_L was measured on-line with non-invasive O₂ sensors (PreSens) positioned below the liquid level.

For plasmid production, duplicate cultures of 100 mL were grown overnight in 250-mL HT tubes and 1-L Erlenmeyer flasks. Plasmid was recovered from each culture using a Nucleobond PC 500 maxiprep kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. The DNA was resuspended in 500 µL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4), and its concentration was determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

2.4. Mammalian Cell Cultivation

The DG44 strain of Chinese hamster ovary (CHO-DG44) cells was routinely cultivated in ProCHO5 medium (Lonza, Verviers, Belgium) supplemented with 13.6 mg hypoxanthine/L, 3.9 mg thymidine/L, and 4 mM glutamine. The cells were incubated at 37 °C with 5% CO₂ in cylindrical glass bottles of various sizes with agitation at 110 rpm in an ISF4-W incubator (Kühner) [10]. For cell growth studies, 100 mL of culture was transferred at a density of 0.3×10^6 cells/mL into 250-mL cylindrical and square-shaped glass bottles and 250-mL HT tubes. The bottles were agitated at 110 rpm as previously described [10] and the HT tubes at 160 rpm. The cultures were incubated at 37 °C in the presence of 5% CO₂ in an ISF4-W incubator with a shaking diameter of 5 cm. Cell density and viability were determined by the Trypan blue exclusion method. The biomass was determined by the packed cell volume (PCV) method using PCV tubes (TPP, Trasadingen, Switzerland) as described [11].

2.5. Insect Cell Cultivation

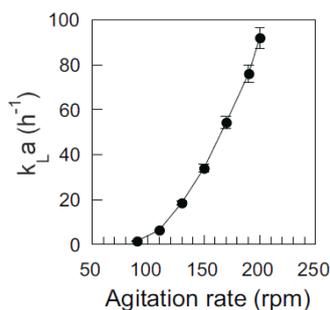
Spodoptera frugiperda Sf-9 insect cells were routinely cultivated in 5 mL of Sf900 II SFM medium (Invitrogen, Carlsbad, CA, USA) at 28 °C in TubeSpin[®] bioreactor 50 tubes (TubeSpins) (TPP) with agitation at 180 rpm in an ISF4-W incubator as described [12]. For cell growth studies, the cells were inoculated at a density of 0.6×10^6 cells/mL in 250-mL flat-base Erlenmeyer flasks equipped with vented caps and 250-mL HT tubes at working volumes of 50 and 100 mL, respectively. The Erlenmeyer flasks were agitated at 130 rpm with a shaking diameter of 2.5 cm, and the HT tubes at 140 rpm with a shaking diameter of 5 cm. The cultures were incubated at 28 °C in an ISF4-W incubator. The cell density, viability and PCV were assessed as described for mammalian cells.

3. Results and Discussion

3.1. k_La in 250-mL HT Tubes

The k_La was measured in 100 mL of deionized water in 250-mL HT tubes without a cap at agitation rates from 90 to 200 rpm. Over this range, the k_La increased with the agitation rate (Figure 2). At agitation rates used for insect (140 rpm) and mammalian cells (160 rpm), the k_La reached 25 and 50 h⁻¹, respectively (Figure 2). At the agitation rate used for *E. coli* cultures (200 rpm), the k_La was 90 h⁻¹ (Figure 2).

Figure 2. Determination of k_{La} in 250-mL HT tubes. The HT tubes were filled with 100-mL deionized water, and measurements were made at 37 °C using a dynamic method ($n = 4$, error bars represent SD).



3.2. Bacterial Cultivation and Plasmid Production

E. coli were cultivated in 1-L Erlenmeyer flasks and 250-mL HT tubes at 200 rpm. During the first 4 h of cultivation, the OD_{600} values were nearly identical in the two containers (Figure 3a). From 5–7 h of cultivation, slightly higher OD_{600} values were observed in the HT tubes than in the flasks (Figure 3a). Over time, the pH decreased from 7.2 to 6.4 (Figure 3b) and the c_L reached about 0% air sat in both cultivation systems (Figure 3c). Within 7 h of cultivation, the c_G decreased to 60% air sat in the flask and 90% air sat in the HT tube (Figure 3c). After 4 h of cultivation, the OTR reached a plateau in both vessels (Figure 3d). Plasmid DNA was purified from overnight cultures. The amount of plasmid DNA and the OD_{600} were 40% higher for the cultures grown in HT tubes as compared to those grown in flasks (Table 1).

Figure 3. *E. coli* cultivation in different containers. The 100-mL cultures in 1-L Erlenmeyer flasks (open symbols) and 250-mL HT tubes (closed symbols) were incubated at 37 °C with agitation at 200 rpm. At the times indicated, the (a) OD_{600} , (b) pH, and (c) c_G (squares) and c_L (circles) were measured. The c_G and c_L were reported as % air sat. (d) The OTR was calculated from Equation (1). This experiment was performed in duplicate; error bars represent SD.

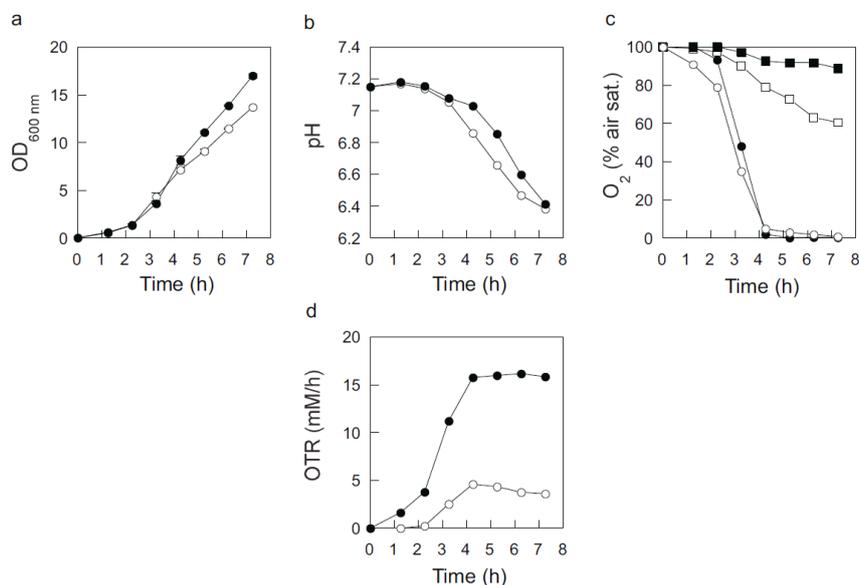


Table 1. Plasmid DNA production in *E. coli*.

Yield	1-L Erlenmeyer flasks	250-mL HT tubes
^a Total amount of plasmid	1.9 ± 0.1 mg	2.7 ± 0.3 mg
^b OD ₆₀₀	21 ± 0.5	29 ± 0.7

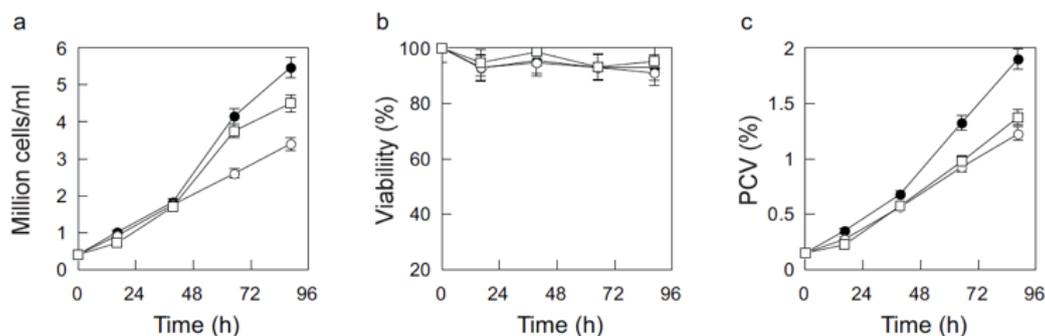
^a Recovered from a 100-mL overnight culture using Nucleobond PC 500 maxiprep kits; ^b Measured in overnight cultures before cell harvest.

It is known that bacterial growth is affected by the gas transfer in the culture vessel, and in the case of aerobic growth of *E. coli* in shaken cultures, the *OTR* may become limiting [13]. Here, there was not a difference between cultures in the HT tubes and 1-L Erlenmeyer flasks in terms of the dissolved oxygen concentration at saturation (c_L) (Figure 3c). However, the c_G and *OTR* values were higher in the HT tubes than in the flasks (Figure 3c,d). At the agitation rates used for the cell growth, the k_La values were 33 and 90 h⁻¹ in the 1-L Erlenmeyer and in the HT tubes, respectively. These differences translated into greater biomass and higher plasmid production in HT tubes (Figure 3e).

3.3. Cultivation of Mammalian Cells

CHO-DG44 cells were cultivated in 250-mL cylindrical and square-shaped bottles and in 250-mL HT tubes. At the agitation rates used for cell growth, the k_La values were 7, 8, and 50 h⁻¹ in the three vessels, respectively. The cells cultivated in the 250-mL HT tubes achieved the highest density (Figure 4a), but there were not any noticeable differences in cell viability among the three cultures (Figure 4b). The biomass was also the highest in the HT tube (Figure 4c). The pH decreased from 7.1 to 6.5 (cylindrical bottle), 6.6 (square-shaped bottle), and 6.7 (HT tube). While the c_L reached 0% air sat in 2 days in the cylindrical bottles, by the end of the other cultures it had only decreased to 10% air sat in the square-shaped bottles and 50% in the HT tubes.

Figure 4. CHO-DG44 cell cultivation in different containers. The 100-mL cultures were inoculated in cylindrical (open circles) and square-shaped (open squares) 250-mL bottles and 250-mL HT tubes (closed circles) at a density of 0.3×10^6 cells/mL. The cultures were incubated at 37 °C with agitation at 110 rpm (bottles) or 160 rpm (HT tubes). At the times indicated, the viable cell density (a), cell viability (b), and biomass (c) were measured. The experiment was performed in duplicate. Error bars represent SD.



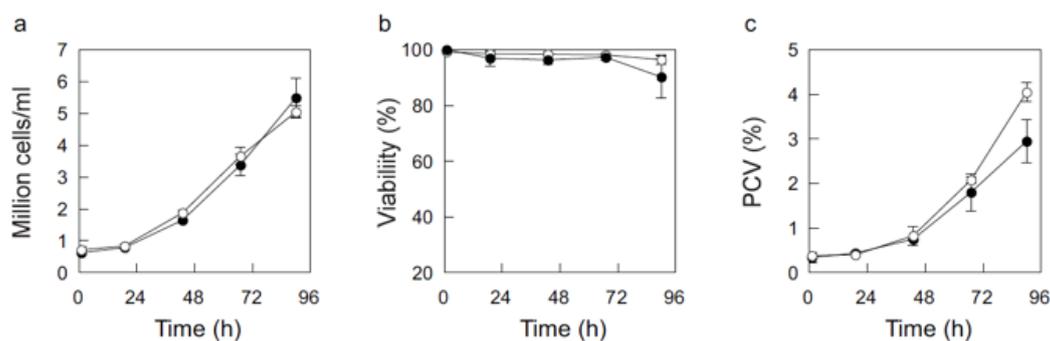
The maximal mammalian cell density was 25% higher in the HT tubes than in the cylindrical and square-shaped bottles (Figure 4a). The *OTR* was limiting in cylindrical and square-shaped bottles as

judged by the c_L values mentioned above. Moreover, higher dissolved CO₂ concentrations (data not shown) and lower pH values were observed in the bottles as compared to the HT tubes. Altogether, these results showed that the better gas transfer provided in the HT tubes was beneficial for CHO-DG44 cell growth in comparison with orbitally shaken glass bottles.

3.4. Cultivation of Insect Cells

Sf9 cells were cultivated in 250-mL Erlenmeyer flasks and HT tubes at 130 rpm and 140 rpm, respectively. At these shaking speeds the k_{La} values were 10 and 20 h⁻¹ for the flasks and HT tubes, respectively. Over the course of the cultures, the cell density (Figure 5a) and viability (Figure 5b) were about the same, but the biomass was higher in the Erlenmeyer flasks than in the HT tubes (Figure 5c). In both containers the c_L decreased after 2 days of culture from 100% air sat to about 80% air sat, and the pH varied between 6.0 and 6.3 over the course of the culture (data not shown). The dissolved CO₂ remained about 1% in both vessels (data not shown). Since the gas transfer did not seem limiting in either the Erlenmeyer flasks or HT tubes, no difference in cell growth in these two containers was expected. However, in the HT tube the working volume was double that in the flask. Thus, for the HT tube relative to the flasks, more and larger cultures can be maintained in an incubator.

Figure 5. Sf9 cell cultivation in different containers. The cells were inoculated into 250-mL Erlenmeyer flasks (open circles) and 250-mL HT tubes (closed circles) at a density of 0.6×10^6 cells/mL and incubated at 28 °C with agitation at 130 rpm for the Erlenmeyer flasks and 140 rpm for the HT tubes. The working volumes were 50 mL in the Erlenmeyer flasks and 100 mL in the HT tubes. At the times indicated, the (a) viable cell density, (b) viability, and (c) biomass were measured. The experiment was performed in duplicate. Error bars represent SD.



4. Conclusions

The versatility of 250-mL HT tubes may be of interest for the cultivation of bacteria and suspension-adapted animal cells for recombinant protein production [14]. The HT tube showed a higher gas transfer efficiency than did shaken flasks and bottles, confirming previous results using other cylindrical orbitally shaken containers with an internal helical track [15,16]. This property was clearly beneficial for bacterial and mammalian cell cultures but not necessarily for those of insect cells. The efficiency of gas transfer in the HT tube is probably due to the greater liquid free-surface area generated by the movement of liquid up the track. The 250-mL HT tube can be used with a working

volume of at least 100 mL, it has a small footprint on the shaker, and is disposable. For these reasons, it is an attractive option for the cultivation of microbes and animal cells.

Acknowledgments

The authors thank Divor Kiseljak and Yashas Rajendra for providing pXLG^{CHO}-A2. We gratefully acknowledge the very considerable support for equipment and consumables by Adolf Kühner AG and Sartorius AG. The work presented here has been partially supported by the KTI Program of the Swiss Economic Ministry and by a grant from the Academy of Finland (decision no. 135820).

Conflict of Interest

The authors declare no conflict of interest.

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