

Orbitally Shaken Single-Use Bioreactors

Wolf Klöckner, Sylvia Diederichs and Jochen Büchs

Abstract Orbitally shaken single-use reactors are promising reactors for upstream processing, because they fulfill three general requirements for single-use equipment. First, the design of the disposable parts is inherently simple and cost-efficient, because no complex built-in elements such as baffles or rotating stirrers are required. Second, the liquid distribution induced by orbital shaking is well-defined and accurately predictable. Third, the scale-up from small-scale systems, where shaken bioreactors are commonly applied, is simple and has been successfully proven up to the cubic meter scale. However, orbitally shaken single-use reactors are only suitable for certain applications such as cultivating animal or plant cells with low oxygen demand. Thus, detailed knowledge about the performance of such systems on different scales is essential to exploit their full potential. This article presents an overview about opportunities and limitations of shaken single-use reactors.

Keywords Animal cell culture · Hydromechanical stress · Orbitally shaken · Out-of-phase · Oxygen transfer · Power input · Scale-up

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1 Introduction

Orbitally shaken bioreactors are widely used for small-scale screening and process optimization. Complex mechanical and electronic parts such as the shaker drive, the power train, and the control unit are integrated in the shaker and, therefore, separated from the reactor vessel. This allows a simple and cost-efficient reactor design, important not only for many parallel experiments on a small scale but also crucial for single-use applications in general. Unlike shaken systems, stirred single-use reactors require a complex sealing of the stirrer shaft or a magnetic clutch for energy transmission that is disposed of with the bag-reactor after each cultivation [1].

The simple design and handling of shaken bioreactors has led to their wide acceptance for screening and process optimization. After the detection of optimized conditions and suitable strains on a small scale, the cultivation conditions have to be transferred to a larger production scale. However, the scale-up from a shaken bioreactor to a bubble-aerated stirred tank reactor requires detailed knowledge about the basic engineering characteristics of both systems. Consequently, extensive research has been conducted to determine suitable methods for the transfer of culture conditions from shaken to stirred tank reactors [2–5]. Nonetheless, problems may still occur due to differences in oxygen transfer, hydromechanical stress, aeration, mixing, power input and temperature control between both reactor types. These problems are partly avoided by using orbitally shaken large-scale bioreactors. It is obvious that a transfer of culture conditions is highly simplified when the same basic principle for mixing, aeration, and power input is applied during scale-up. But even if the same working principle is applied, changes in culture conditions resulting from an increased reactor scale need to be carefully considered. In particular, the maximum oxygen transfer capacity is reduced with increasing reactor size due to a reduced volumetric oxygen transfer area. The basic engineering parameters for utilizing orbitally shaken disposable bioreactors with volumes ranging from 50 mL up to 1,000 L are described in the following chapter.

2 Types and Scales of Orbitally Shaken Single-Use Reactor Systems

Different types of orbitally shaken single-use reactors for different scales are currently available on the market. Figure 1 illustrates the various sizes and shapes of some commonly used small-scale reactors. The TubeSpin® system (Techno Plastic Products AG), available for reactor volumes of 15, 50, and 600 mL, has been developed for optimizing cell culture processes. Small tubes with a volume of 15 or 50 mL allow more parallel experiments to be performed on one shaker compared to single-use Erlenmeyer flasks. In addition, the conical tube can be directly used for centrifugation during sample preparation. A comparison between

results obtained with CHO cells cultivated in 50 mL TubeSpin® reactors and conventional glass reactors was first described by Jesus et al. [6]. A comparable growth and antibody production was reported with both reactor types.

Single-use Erlenmeyer flasks made out of polycarbonate or polypropylene have a similar geometry to that of conventional glass flasks (see Fig. 1). However, the material properties of single-use flasks differ from the properties of borosilicate glass, commonly used for conventional flasks. The impact of material properties on the maximum oxygen transfer capacity is discussed in Sect. 3.2.

Cylindrical orbitally shaken vessels consisting of polycarbonate or polypropylene and with volumes of 5 to 50 L have been used to cultivate mammalian, plant and insect cells. These vessels usually have tube connectors on top for active aeration. Figure 2 depicts various rigid vessels and bag reactors that are used in scales from 10 to 200 L, whereby the recommended shaking parameters depend on culture requirements as described in Sect. 3.2.

Bag-reactors for orbitally shaken platforms are so far available with nominal volumes of 50 and 200 L (Sartorius Stedim Biotech). As Fig. 3 shows, shakers with special bag holders are required to operate them. The OrbShake SB 200-X bioreactor system was developed by the company Kühner, Birsfelden, Switzerland, in cooperation with Lausanne. Engineering parameters for the application of orbitally shaken bag-reactors are discussed in Sect. 3.

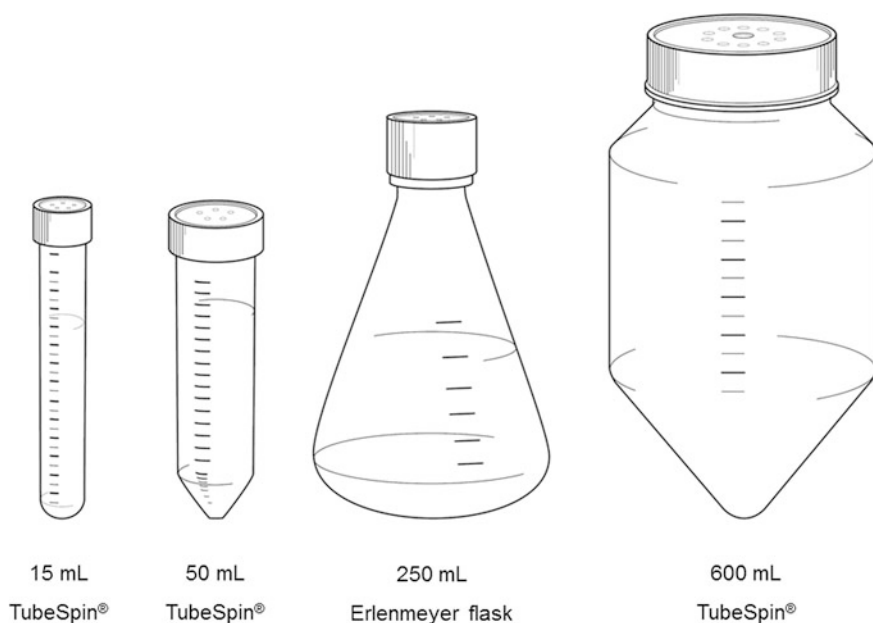


Fig. 1 Examples for orbitally shaken single-use reactor systems with volumes from 15 to 600 mL

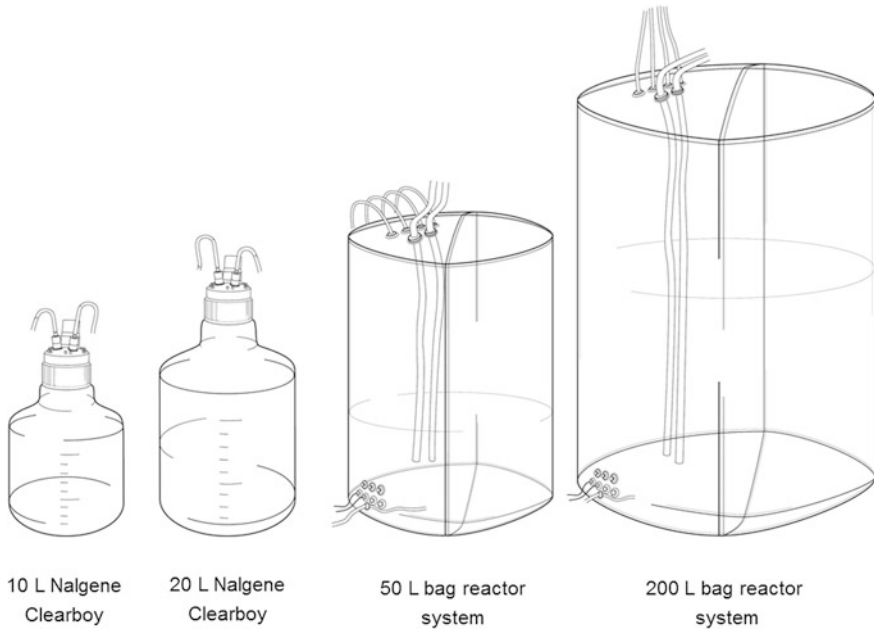


Fig. 2 Examples for orbitally shaken single-use reactor systems with volumes from 10 to 200 L

3 Engineering Parameters of Orbitally Shaken Single-Use Reactors

To choose the right cultivation conditions, it is important to know the fundamental engineering parameters of a bioreactor such as power input, hydromechanical stress, oxygen transfer and mixing performance. The following sections discuss the parameters for correctly applying orbitally shaken reactors.

3.1 Liquid Distribution, Power Input, and Hydromechanical Stress

The well-defined and homogeneous liquid distribution during shaking is a key benefit of orbitally shaken single-use reactors. Reproducible flow conditions are required for a detailed characterization and application of the system. A circulating liquid flow is induced during the shaking process, whereby the liquid follows the direction of the centrifugal force during one in-phase rotation. A balance between centrifugal force and gravitational force leads to a liquid distribution with the shape of a rotational paraboloid as previously described for shake flasks [7]. The typical liquid distribution for water-like viscosities in a cylindrical shaken reactor is shown in Fig. 4.



Fig. 3 Commercially available OrbShake SB 200-X and SB 50-X bioreactors (with kind permission of Kühner AG, Birsfelden)

By assuming that frictional forces are negligible for liquids with water-like viscosities, it follows that the pressure in the liquid is isotropic. This implies that the hydrostatic pressure p_{hydr} in the liquid is equal to the pressure induced by centrifugal acceleration p_{cent} :

$$p_{\text{hydr}} = \int_{h_0}^h g \cdot dz = p_{\text{cent}} = \int_0^r r \cdot (2 \cdot \pi \cdot n)^2 \cdot dr. \quad (1)$$

The gravitational acceleration in Eq. (1) is denoted by g and the shaking frequency by n . Geometric variables in Eq. (1) are defined as shown in Fig. 4. The liquid height in a cylindrical shaken bioreactor [7] follows from Eq. (1):

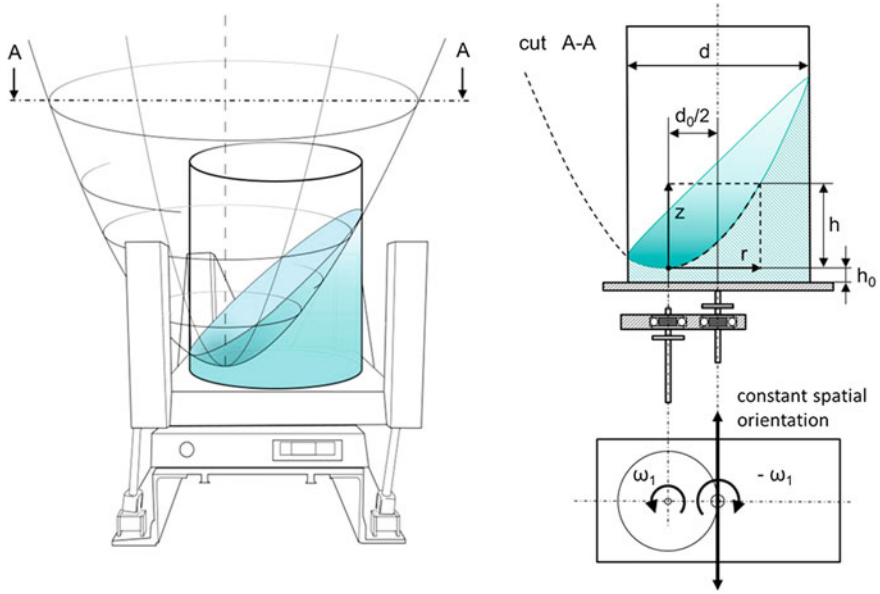


Fig. 4 Liquid distribution of a liquid with water-like viscosity during orbital shaking in a cylindrical single-use reactor

$$h = \frac{r^2 \cdot (2 \cdot \pi \cdot n)^2}{2 \cdot g} + h_0 \quad (2)$$

An accurate calculation of the power transfer and gas transfer areas during shaking can be realized with a mathematical model on the basis of Eq. (2) (manuscript in preparation).

Power is transferred during shaking due to friction between the rotating liquid bulk and the cylindrical reactor wall. Different measurement systems for determining the average power input ($P/V_\emptyset$) in cylindrical bioreactors have been described. A simple and effective determination of $P/V_\emptyset$ can be realized with a torque sensor integrated in the shaker drive [8, 9]. This method also allows online monitoring of $P/V_\emptyset$ during biological cultivations. However, integrating a torque sensor in the shaker drive is complex and requires a redesign of the shaker drive. This can be avoided by using a temperature method for determining $P/V_\emptyset$ in large-scale bioreactors. Moreover, this method only requires online monitoring of the liquid and surrounding air temperature during a cooling-down process [10]. A comparison between values measured with a torque sensor and values calculated with the temperature method showed comparable results for both techniques [11]. An extension of the temperature method allows one to consider the influence of viscosity changes on heat losses over the reactor wall [12]. Values for $P/V_\emptyset$ in cylindrical shaken single-use reactors range from 50 W/m³ to 2 kW/m³ depending on the filling volume, shaking frequency, and liquid viscosity. Figure 5 presents

the measurement values for P/V_ϕ in a 10 and 20 L vessel. In addition, a scale- and volume-independent correlation for P/V_ϕ in orbitally shaken reactors was recently developed for reactor volumes of up to 2,000 L [9].

Furthermore, hydromechanical stress in shaken bioreactors was investigated in several research studies using Erlenmeyer flasks [13, 14]. Here, maximum stable drop-size measurements were conducted in coalescence inhibited liquid–liquid two-phase systems in order to determine the ratio of maximum local energy dissipation ($\varepsilon_{\max}$) to volumetric energy dissipation (ε_ϕ). Values for $\varepsilon_{\max}/\varepsilon_\phi$ ratios in Erlenmeyer flasks were between one and seven and therefore about ten times lower compared to the ratios determined in stirred tank reactors [13, 14]. The evenly distributed energy dissipation in orbitally shaken bioreactors leads to lower levels of hydromechanical stress compared to that of stirred tank reactors at the same level of volumetric power input. The evenly distributed energy dissipation is attributed to the fact that the size of the reactor wall (that acts as a power introducing element in orbitally shaken reactors) is much larger than the size of a stirrer in conventional bioreactors relative to the reactor liquid volume [13]. However, differences between the conical glass wall in Erlenmeyer flasks and the cylindrical plastic wall in single-use bags might lead to differences in hydromechanical stress between both systems. This influence has not been described in the literature up to now.

3.2 Aeration and Maximum Oxygen Transfer Capacity

The applicability of orbitally shaken single-use reactors for aerobic bioprocesses highly depends on their potential to deliver a sufficient amount of oxygen to the

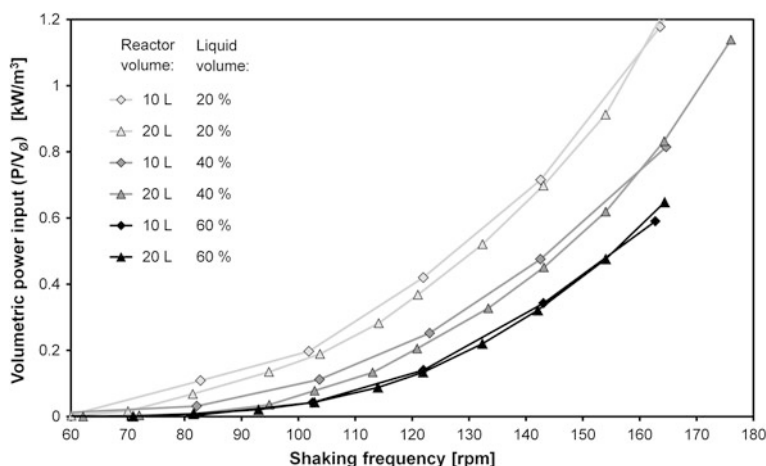


Fig. 5 Volumetric power input (P/V_ϕ) in cylindrical orbitally shaken reactors (Nalgene Clearboy); measured with a torque sensor using water at 25 °C; diameter of the 10 L reactor = 25 cm; diameter of the 20 L reactor = 28.6 cm; shaking diameter = 5 cm

cells. The first quantitative characterization of the oxygen transfer in orbitally shaken single-use reactors was reported in 2008 [15]. Comparatively low values for the oxygen transfer coefficient (k_{La}) of between 1 and 30 1/h were reported for conventional cylindrical vessels. A three- to five-fold increase in the oxygen transfer was achieved with a helical track attached to the inner wall of the cylindrical reactor [15]. However, there is a trade-off between the benefit of a higher oxygen transfer rate and the increased production costs for reactors with integrated helical track. Increased values for k_{La} were also reported for square and baffled reactor systems compared to non-baffled cylindrical reactors, but the improved mass transfer characteristics were accompanied by an inhomogeneous and undefined liquid flow that might hamper scale-up [15]. The influence of different shaking frequencies and filling volumes on k_{La} values in cylindrical orbitally shaken reactors was investigated in scales from 0.05 to 1,000 L. Sufficient oxygen transfer for cultivating mammalian cells was realized in culture volumes of up to 1,000 L [16]. The influence of different filling volumes, shaking frequencies and liquid properties on k_{La} values in cylindrical orbitally shaken reactors was recently investigated in scales from 50 mL to 200 L (manuscript in preparation).

Values for k_{La} of between 7 and 10 1/h are regarded as necessary in order that a sufficient amount of oxygen be delivered for cultivating mammalian cells [17, 18]. The dissolved oxygen tension (DOT) in the liquid phase changes during a normal batch-cultivation with constant aeration and agitation according to the cell density of the culture. A changing DOT has no influence on aerobic cell growth as long as oxygen is available in the liquid phase at a sufficient level and diffusion between liquid phase and cell wall is not hampered (e.g. due to cell aggregation, biopolymer production or filamentous growth). Consequently, the k_{La} value has no influence on cell growth as long as oxygen is available in the liquid phase at a nonlimiting level. However, a constant k_{La} value has been recently reported as an adequate means to keep the pH level constant during scale-up [18, 19]. The described effect is most likely caused by similar levels of dissolved carbon dioxide (CO_2) and not related to oxygen transfer. CO_2 transfer between the gas and liquid phase is much faster than oxygen transfer due to the higher solubility of CO_2 in aqueous solutions. In contrast to oxygen transfer, equilibrium conditions between gas and liquid phases usually prevail for CO_2 . Thus, the dissolved CO_2 concentration is mainly affected by the ventilation rate and not by the k_{La} value. This was recently shown in large-scale reactors for CHO cell cultivation where three- to four-fold increased CO_2 removal rates were achieved at a constant k_{La} value only by increasing the ventilation rate [20]. Hence, it is advisable to use a constant volumetric ventilation rate and not a constant k_{La} value to avoid pH shifts during scale-up of mammalian cell cultivations. Nevertheless, it is important to ensure that the k_{La} value is high enough during scale-up to prevent oxygen limitations, but solely a constant k_{La} is not a sufficient scale-up criterion.

In small-scale cultivations, k_{La} values for the 50 mL TubeSpin[®] system were reported in a range from 2 to 40 1/h depending on the filling volume and shaking frequency [21]. Figure 6 shows the pattern of the oxygen transfer rate (OTR) during a cultivation of *Nicotiana tabacum* BY-2 cells in 50 mL TubeSpin[®]

reactors using RAMOS [22, 23]. In the graph, the OTR in a conventional 250 mL shake flask was added as reference cultivation. Different filling volumes (V_L) were used in the TubeSpin[®] system to investigate different levels for the maximum oxygen transfer capacity (OTR_{max}). All cultures reached an oxygen limitation with the applied shaking frequency of 180 rpm as indicated with the dotted lines in Fig. 6. OTR_{max} levels are in the expected range according to reported k_La values for TubeSpin[®] reactor system [21]. Even if the oxygen supply at 180 rpm is not high enough to cultivate plant cells at the adjusted filling volumes without oxygen limitation, the OTR_{max} is still sufficient for cultivating mammalian cells. The results show that a detailed characterization of small-scale single-use systems is necessary to prevent unsuitable cultivation conditions during screening.

The oxygen transfer characteristics of conventional Erlenmeyer flasks were characterized in detail in different studies [24–26]. The rotating bulk liquid generates a thin liquid film on the hydrophilic glass wall that strongly contributes to the total oxygen transfer capacity [25]. This effect is reduced in single-use flasks made of polypropylene or polycarbonate due to the hydrophobic surface properties of the materials. Figure 7 shows a comparison between k_La values measured in flasks made of polycarbonate and those measured in conventional glass flasks. Values were determined by employing the Respiration Activity Monitoring System (RAMOS) using a sulfite oxidation reaction in the liquid phase to reduce the dissolved oxygen concentration [27]. Measured k_La values at 37 °C with the sulfite oxidation reaction are higher than values measured with medium for insect cell cultivation at 27 °C [28]. This was expected, as the diffusion coefficient for oxygen and thereby the k_La increases with increasing cultivation temperature. Oxygen supply in single-use flasks was about 30 % lower compared to oxygen transfer in conventional glass flasks (see Fig. 7). These significantly reduced k_La values in

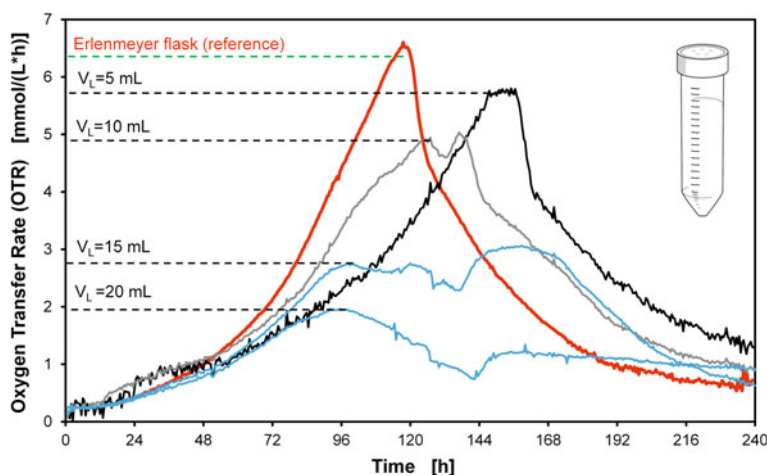


Fig. 6 Cultivation of *Nicotiana tabacum* BY-2 cells in Murashige and Skoog medium in 50 mL TubeSpin[®] reactors at a shaking frequency of 180 rpm with a shaking diameter of 5 cm

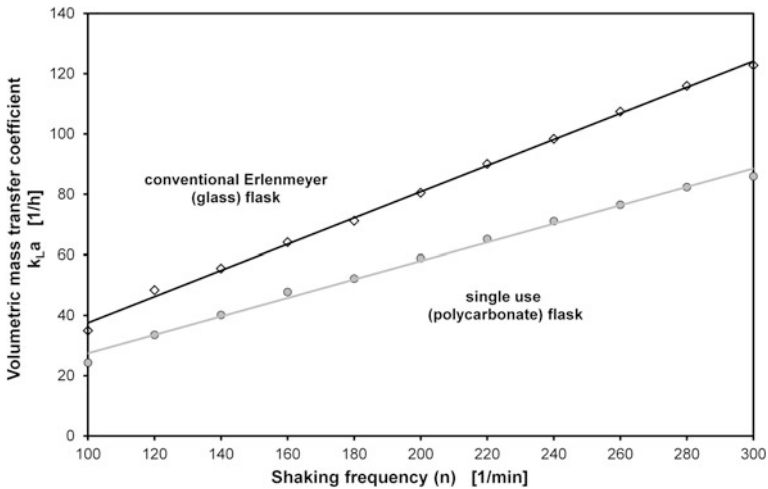


Fig. 7 Comparison of the volumetric mass transfer coefficient ($k_{L,a}$) in conventional glass flasks and single-use polycarbonate flasks; flask volume = 250 mL; filling volume = 50 mL; temperature = 37 °C; shaking diameter = 5 cm; sulfite solution with 0.5 M Na_2SO_3 ; 10^{-7} M CoSO_4 ; 0.1 M phosphate buffer; initial pH = 8; oxygen solubility $\text{Lo}_2 = 0.65 \text{ mmol}/(\text{L} \cdot \text{bar})$

single-use flasks make them unsuitable for cultivating aerobic yeast or bacteria cells with a high oxygen demand. However, $k_{L,a}$ values in single-use flasks are still high enough for cultivating mammalian or insect cells even at moderate shaking frequencies.

3.3 Mixing Performance and Out-of-Phase Operation

The first detailed characterization of the mixing performance in cylindrical orbitally shaken bioreactors with volumes ranging from 2 to 1,500 L was reported by Tissot et al. [29]. A calorimetric method was used to determine mixing times for different shaking frequencies and filling volumes. The best mixing efficiency was generated close to the reactor wall. Longer mixing times were reported in the center of the bulk liquid [29]. These findings concur with the fact that power transfer in shaken bioreactors occurs between the liquid bulk and the reactor wall. Consequently, local power input and mixing properties are at a maximum close to the wall and decrease with increasing distance from the wall. A comparison of mixing times in a cylindrical 30 L reactor using shaking diameters of 2.5 and 5 cm showed significantly higher mixing times with the smaller shaking diameter of 2.5 cm below frequencies of 115 rpm. Similar mixing times were detected for both shaking diameters for frequencies over 115 rpm [29]. This observation can be attributed to the critical shaking frequency that has to be exceeded to induce a circulating liquid flow in the reactor. The critical frequency describes the minimal

required shaking frequency to overcome inertial forces and to provoke liquid motion in the reactor [9]. An increase in the shaking diameter leads to a decrease in the critical frequency. This phenomenon is not comparable with the occurrence of out-of-phase operation conditions. In contrast to the critical frequency, the out-of-phase phenomenon occurs during the shaking process (also at high shaking frequencies) and leads to a breakdown of liquid motion. This phenomenon has been extensively characterized for shake flask bioreactors [30, 31]. Out-of-phase operation in orbitally shaken reactors is associated with a strong decrease in mixing performance, oxygen transfer, and power input. A comparison between the liquid distribution during in-phase and out-of-phase operation in cylindrical bioreactors is shown in Fig. 8.

As shown in Fig. 8, different shaking diameters were used with otherwise equal conditions. A metal ball rotating in a glass flask on the right side of the shaker thereby indicates the direction of the centrifugal force. With a shaking diameter of 2.5 cm, the liquid is oriented in the direction of the centrifugal force, thereby indicating in-phase operation (Fig. 8a). Here the liquid is evenly distributed, providing a large mass transfer area between gas and liquid phase. By contrast, with a shaking diameter of 1.25 cm and otherwise equal operating conditions, out-of-phase operation was observed (Fig. 8b). In this case, the liquid is no longer oriented in the direction of the centrifugal force, as indicated by the black rotating ball on the shaker. The strong reduction in the mass transfer and power transfer area triggers significantly lower mixing, power input, and oxygen transfer properties.

Out-of-phase operation occurs when frictional forces exceed centrifugal forces during shaking [30]. The most effective way to prevent out-of-phase operation is to

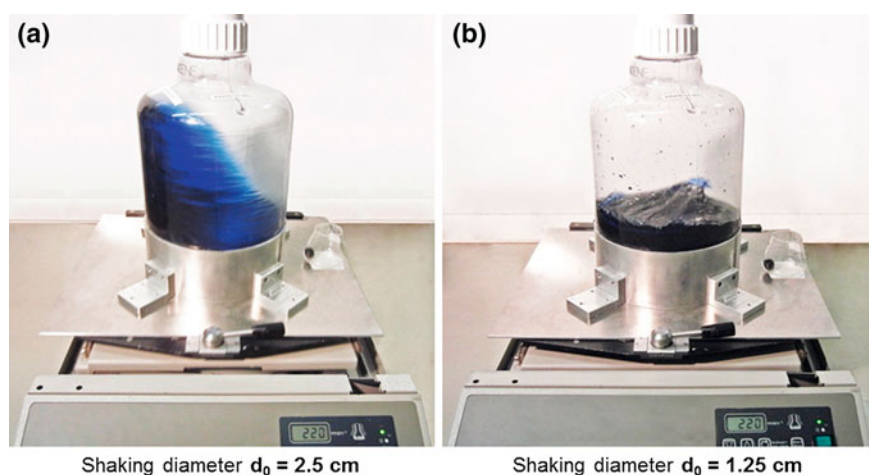


Fig. 8 Comparison between “in-phase” and “out-of-phase” operation in shaken cylindrical single-use reactors; reactor volume = 10 L; reactor diameter = 25 cm; filling volume = 2.5 L; shaking frequency = 220 rpm; dynamic viscosity = 0.984 mPas

increase the ratio between shaking and reactor diameter as demonstrated in Fig. 8. The influence of out-of-phase operation on volumetric power input (P/V_ϕ) and mass transfer ($k_L a$) is presented in Fig. 9. Please notice that a filling volume of 5 L was used for the measurements in Fig. 9 instead of the 2.5 L that were used in Fig. 8. Values for $k_L a$ were determined with a RAMOS for cylindrical reactors using a sulfite oxidation reaction in the liquid phase to reduce the dissolved oxygen concentration [27]. Furthermore, power input was measured with a torque sensor integrated in the shaker drive [9]. An abrupt decrease in the P/V_ϕ and $k_L a$ values was detected when the reactor with a shaking diameter of 1.25 cm reached out-of-phase conditions at 220 rpm. As previously described for shake flasks, a higher filling volume leads to a later occurrence of out-of-phase conditions [30]. Thus, at a shaking diameter of 1.25 cm and a shaking frequency of 220 rpm the reactor system with 5-L filling volume is still in-phase (Fig. 9), whereas the bioreactor with a 2.5 L filling volume is already out-of-phase (Fig. 8b). Reduced power input, mixing performance, and oxygen transfer combined with chaotic and non-reproducible cultivation conditions are all well-known characteristics of out-of-phase operation conditions in shake flasks [32]. Therefore, an adequate dimensioning of the shaking diameter according to the reactor scale is essential to prevent out-of-phase operation in orbitally shaken bioreactors.

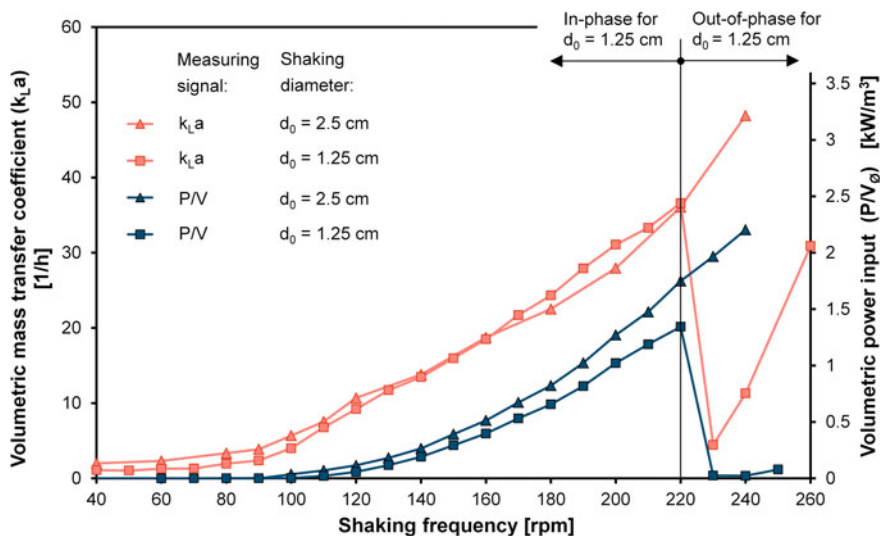


Fig. 9 Values for P/V_ϕ and $k_L a$ during “in-phase” and “out-of-phase” operation in a shaken single-use reactor; reactor volume = 10 L; reactor diameter = 25 cm; filling volume = 5 L; temperature = 25 °C; dynamic viscosity = 1.554 mPas; sulfite solution with 1 M Na_2SO_3 ; 10^{-7} M CoSO_4 ; 0.012 M phosphate buffer; initial pH = 8; oxygen solubility $\text{Lo}_2 = 0.56$ mmol/(L · bar)

4 Applications of Orbitally Shaken Single-Use Reactors

The first application of orbitally shaken single-use reactors was reported by Liu and Hong [33] for cultivating insect and animal cells. They monitored the number of viable cells during cultivation using orbitally shaken vessels in different scales and compared the results with values from a stirred tank reactor. For the first time, the scale-up from shake flasks to cylindrical shaken single-use bioreactors with culture volumes of up to 36 L had been successfully proven in this work.

The general suitability of orbitally shaken bioreactors for cultivating *Nicotiana tabacum* BY-2 cells growing in suspension was proven in cylindrical reactors with volumes of 20 and 50 L [34]. The successful utilization of square bottles for cultivating mammalian cells was first described by Muller et al. [35]. Comparable yields were reported between cultivations in square bottles of different size and cultivations in spinner flasks [35]. The first application and validation of the 50 mL TubeSpin® system for cultivating animal cells was reported by Jesus et al. [6]. Experiments were conducted with sealed and open ventilation membranes to investigate the influence of different ventilation rates on evaporation, pH and dissolved oxygen concentration. A sufficient oxygen supply and CO₂ removal rate was reported even for tubes that were entirely closed during a cultivation time of 4 days [6].

The successful application of the TubeSpin® system for the cultivation of mammalian cells was proven in several studies [36, 37]. Characteristics of the reactor system such as the cost-efficient design and easy handling make them suitable for a large number of parallel screening experiments. Consequently, the influence of 29 different cultivation media and 20 protein hydrolysates on growth and productivity of a CHO cell culture was investigated with the TubeSpin® system [37]. The effective application of the system for transient gene expression with CHO cells was also recently proven. Similar protein yields in the TubeSpin® system compared to standard stirred tank reactors were reported [36].

5 Conclusion and Outlook

Within the past 10 years, orbitally shaken single-use reactors have developed from the first proof of concept to established systems for upstream processing. Today, reactors are available in volumes ranging from 15 mL to 200 L, and the basic working principle has been substantiated up to reactor volumes of 2,000 L. Fundamental engineering parameters such as oxygen transfer, power input, mixing performance and hydromechanical stress have been investigated in several research studies. In addition, the applicability of orbitally shaken single-use reactors for cultivating animal, insect and plant suspension cells has been demonstrated on different scales. A major advantage of shaken single-use reactors compared to systems with a wave, rocking or stirred agitation is the very well-

defined liquid movement in the reactor and the fact that orbitally shaken bioreactors are commonly applied for screening and media optimization in small-scale systems. Transferring culture conditions from shake flasks or microtiter plates to orbitally shaken single-use reactors is greatly simplified due to similar characteristics with respect to hydromechanical stress, mixing and oxygen supply. The commonly accepted advantages of shaken bioreactors for small-scale systems such as simple and cost-efficient reactor design, easy handling and low hydromechanical stress are also essential requirements of single-use reactors. Despite the effort that has already been expended on characterizing shaken single-use reactors, further investigations are needed to exploit their full potential. In particular, a more detailed description of the fluid flow properties during shaking would be advantageous to allow a precise characterization of hydromechanical stress and out-of-phase operation. Nevertheless, orbitally shaken single-use reactors are already today a serious option.

References

1. Brod H, Vester A, Kauling J (2012) Opportunities and limitations of disposable technologies in biopharmaceutical processes. *Chem Ing Tech* 84(5):633–645
2. Reyes C, Pena C, Galindo E (2003) Reproducing shake flasks performance in stirred fermentors: production of alginates by *Azotobacter vinelandii*. *J Biotechnol* 105(1–2): 189–198
3. Seletzky JM, Noak U, Fricke J et al (2007) Scale-up from shake flasks to fermenters in batch and continuous mode with *Corynebacterium glutamicum* in lactic acid based on oxygen transfer and pH. *Biotechnol Bioeng* 98(4):800–811
4. Pena C, Millan M, Galindo E (2008) Production of alginate by *Azotobacter vinelandii* in a stirred fermentor simulating the evolution of power input observed in shake flasks. *Process Biochem* 43(7):775–778
5. Mehmood N, Olmos E, Marchal P et al (2010) Relation between pristinemycin production by *Streptomyces pristinaespiralis*, power dissipation and volumetric gas-liquid mass transfer coefficient, $k(L)a$. *Process Biochem* 45(11):1779–1786
6. De Jesus MJ, Girard P, Bourgeois M et al (2004) TubeSpin satellites: a fast track approach for process development with animal cells using shaking technology. *Biochem Eng J* 17(3):217–223
7. Büchs J, Maier U, Lotter S et al (2007) Calculating liquid distribution in shake flasks on rotary shakers at waterlike viscosities. *Biochem Eng J* 34(3):200–208
8. Büchs J, Maier U, Milbradt C et al (2000a) Power consumption in shaking flasks on rotary shaking machines: I. Power consumption measurement in unbaffled flasks at low liquid viscosity. *Biotechnol Bioeng* 68(6):589–593
9. Klöckner W, Tissot S, Wurm F et al (2012) Power input correlation to characterize the hydrodynamics of cylindrical orbitally shaken bioreactors. *Biochem Eng J* 65:63–69
10. Kato Y, Peter CP, Akgün A et al (2004) Power consumption and heat transfer resistance in large rotary shaking vessels. *Biochem Eng J* 21(1):83–91
11. Raval K, Kato Y, Büchs J (2007) Comparison of torque method and temperature method for determination of power consumption in disposable shaken bioreactors. *Biochem Eng J* 34(3):224–227
12. Raval K, Büchs J (2008) Extended method to evaluate power consumption in large disposable shaking bioreactors. *J Chem Eng Jpn* 41(11):1075–1082

13. Büchs J, Zoels B (2001) Evaluation of maximum to specific power consumption ratio in shaking bioreactors. *J Chem Eng Jpn* 34(5):647–653
14. Peter CP, Suzuki Y, Büchs J (2006) Hydromechanical stress in shake flasks: correlation for the maximum local energy dissipation rate. *Biotechnol Bioeng* 93(6):1164–1176
15. Zhang X, Stettler M, Reif O et al (2008) Shaken helical track bioreactors: providing oxygen to high-density cultures of mammalian cells at volumes up to 1000 l by surface aeration with air. *New Biotechnol* 25(1):68–75
16. Zhang X, Bürki C-A, Stettler M et al (2009) Efficient oxygen transfer by surface aeration in shaken cylindrical containers for mammalian cell cultivation at volumetric scales up to 1000 l. *Biochem Eng J* 45(1):41–47
17. Stettler M, Zhang X, Hacker DL et al (2007) Novel orbital shake bioreactors for transient production of CHO derived IgGs. *Biotechnol Prog* 23(6):1340–1346
18. Tissot S, Michel PO, Hacker DL et al (2012) k(L)a as a predictor for successful probe-independent mammalian cell bioprocesses in orbitally shaken bioreactors. *New Biotechnol* 29(3):387–394
19. Tissot S, Oberbek A, Reclari M et al (2011) Efficient and reproducible mammalian cell bioprocesses without probes and controllers? *New Biotechnol* 28(4):382–390
20. Mostafa SS, Gu XJ (2003) Strategies for improved dCO₂ removal in large-scale fed-batch cultures. *Biotechnol Prog* 19(1):45–51
21. Zhang X, Stettler M, De Sanctis D et al (2010) Use of orbital shaken disposable bioreactors for mammalian cell cultures from the milliliter-scale to the 1,000-liter scale. *Adv Biochem Eng/Biotechnol* 115:33–53
22. Anderlei T, Büchs J (2001) Device for sterile online measurement of the oxygen transfer rate in shaking flasks. *Biochem Eng J* 7(2):157–162
23. Anderlei T, Zang W, Papaspyrou M et al (2004) Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. *Biochem Eng J* 17(3):187–194
24. Henzler HJ, Schedel M (1991) Suitability of the shaking flask for oxygen supply to microbiological cultures. *Bioprocess Eng* 7(3):123–131
25. Maier U, Büchs J (2001) Characterisation of the gas-liquid mass transfer in shaking bioreactors. *Biochem Eng J* 7(2):99–106
26. Maier U, Losen M, Büchs J (2004) Advances in understanding and modeling the gas-liquid mass transfer in shake flasks. *Biochem Eng J* 17(3):155–167
27. Maier B, Dietrich C, Büchs J (2001) Correct application of the sulphite oxidation methodology of measuring the volumetric mass transfer coefficient k(L)a under non-pressurized and pressurized conditions. *Food Bioprod Process* 79(C2):107–113
28. Ries C, John G, John C et al (2010) A shaken disposable bioreactor system for controlled insect cell cultivations at milliliter-scale. *Eng Life Sci* 10(1):75–79
29. Tissot S, Farhat M, Hacker DL et al (2010) Determination of a scale-up factor from mixing time studies in orbitally shaken bioreactors. *Biochem Eng J* 52(2–3):181–186
30. Büchs J, Maier U, Milbradt C et al (2000b) Power consumption in shaking flasks on rotary shaking machines: II. Nondimensional description of specific power consumption and flow regimes in unbaffled flasks at elevated liquid viscosity. *Biotechnol Bioeng* 68(6):594–601
31. Büchs J, Lotter S, Milbradt C (2001b) Out-of-phase operating conditions, a hitherto unknown phenomenon in shaking bioreactors. *Biochem Eng J* 7(2):135–141
32. Büchs J (2001a) Introduction to advantages and problems of shaken cultures. *Biochem Eng J* 7(2):91–98
33. Liu CM, Hong LN (2001) Development of a shaking bioreactor system for animal cell cultures. *Biochem Eng J* 7(2):121–125
34. Raval KN, Liu C, Büchs J (2006) Large-scale disposable shaking bioreactors: a promising choice. *Bioprocess Int* 4(1):46–50
35. Muller N, Girard P, Hacker DL et al (2005) Orbital shaker technology for the cultivation of mammalian cells in suspension. *Biotechnol Bioeng* 89(4):400–406

36. Muller N, Derouazi M, Van Tilborgh F et al (2007) Scalable transient gene expression in Chinese hamster ovary cells in instrumented and non-instrumented cultivation systems. *Biotechnol Lett* 29(5):703–711
37. Stettler M, De Jesus M, Ouertatani-Sakouhi H et al (2007) 1000 non-instrumented bioreactors in a week. In: Smith R (ed) *Cell technology for cell products*. Springer, New York, pp 489–495

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