Biochemical Engineering Journal 45 (2009) 41–47

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Efficient oxygen transfer by surface aeration in shaken cylindrical containers for mammalian cell cultivation at volumetric scales up to 1000 L

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ARTICLE INFO

Article history:
Received 5 September 2008
Received in revised form 30 January 2009
Accepted 5 February 2009

Keywords:
Bioreactor
Agitation
Animal cell culture
Oxygen transfer
Scale-up
Aeration

ABSTRACT

Cylindrical containers agitated by orbital shaking are being developed as simple and cost-effective bioreactor systems for the cultivation of mammalian cells. Here the oxygen transfer capacities of containers with nominal volumes from 50 mL to 2000 L were determined, and the operating parameters influencing oxygen transfer were investigated. In general, the shaking speed necessary for efficient oxygen transfer diminished as the container size increased. At shaking speeds suitable for the growth of shear-sensitive cells, k_La values between 10 and 30 h^{-1} were typically achieved in small-scale (<1 L nominal volume) containers at shaking speeds above 120 rpm. A k_La value of 8 h^{-1} was measured at 75 rpm in a 200-L container with a working volume that was 50% of the nominal volume. In a 2000-L container with a working volume of 1000 L, a moderate k_La of 3 h^{-1} was obtained with a shaking speed of only 47 rpm. The free-surface area in 50-mL and 30-L containers was determined by photographic image analysis and computational fluid dynamic (CFD) simulation, respectively. The results showed that the increase in k_La at higher shaking speeds was mainly due to an increased k_L value, highlighting the dominant effect of free-surface turbulence on gas transfer in orbitally shaken containers. The results demonstrated the feasibility of orbital shaking technology for the cultivation of mammalian cells at scales up to 1000 L.

1. Introduction

Due to their capacity for proper protein folding, assembly, and post-translational modification, cultivated mammalian cells have become the dominant host for the production of recombinant therapeutic proteins [1]. The large-scale (>10 L) cultivation of mammalian cells in suspension is usually performed in stirred-tank bioreactors, but their high investment and maintenance costs limit their availability to many potential users. In contrast, for small- (<1 L) and medium-scale (1–10 L) applications, the choice of cell culture containers is more diverse with the availability of multiwell plates, shake flasks, roller bottles, spinner flasks, and hollow fiber reactors [2]. In the past decade, disposable, single-use bioreactors, especially the WAVE bioreactor based on a novel mixing principle, have been widely accepted for many applications at scales up to several hundred liters [3]. These disposable bioreactors have proven to be more flexible and less expensive than the traditional stainless steel stirred-tank bioreactors. More recently, cultivations of mammalian cells in orbitally shaken cylindrical and square-shaped containers with working volumes typically in the range of 5 mL to 30 L have been reported [4–9]. Mammalian cell cultivation has also been successfully performed in 200- and 2000-L single-use plastic bags secured in cylindrical containers agitated by orbital shaking [10]. For successful scale-up and process control with these novel bioreactors knowledge of hydrodynamic phenomena along with quantitative information about the physical parameters influencing the cells are necessary in order to assure reproducibility.

One of the most critical parameters for judging the suitability of a reactor for the cultivation of suspension cells is the oxygen transfer rate (OTR) between the gas and liquid phases. The volumetric mass transfer coefficient (k_La expressed in h^{-1}) describes the nature of oxygen transfer within the reactor and serves as an important parameter for reactor design and scale-up [11]. Standard and baffled shake flasks (Erlenmeyer flasks) for bacterial cultures

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doi:10.1016/j.bej.2009.02.003

have k_La values of 10–100 h^{-1} at filling volumes corresponding to 10–20% of the nominal volume [12–14]. Compared to bacteria, cultivated animal cells have a relatively low demand for oxygen due to the lower cell densities achieved. The k_La values of sparged stirred-tank reactors for mammalian cell cultivation are typically 1–10 h^{-1} while spinner flasks have k_La values about 2 h^{-1} [15,16]. By comparison, WAVE bioreactors have k_La values less than 4 h^{-1} at working volumes below 100 L [17]. Unfortunately, there is little information on k_La values for orbitally shaken reactors for mammalian cell cultivation.

For orbitally shaken reactors, surface aeration is preferred to sparging. This choice highlights the crucial impact of the free liquid surface on oxygen transfer in these containers. Since suspension cell cultures are low viscosity water-like Newtonian fluids, a defined interfacial surface is expected under certain operating conditions. Although some reports implied a better oxygen transfer capacity for shaken square-shaped containers as compared to cylindrical ones [6,9], more regular and predictable flow patterns are generated in the latter at all volumetric scales. This property makes cylindrical containers the preferred choice for shaken cultures [4,9,10]. Basic engineering investigations on shaken cylindrical reactors have been performed on containers with working volumes less than 10 L [18–20], but a systematic investigation on gas–liquid mass transfer in this type of reactor has not been published. Since the conical wall of a standard shake flask has a completely different flow pattern compared to that developed in a shaken cylindrical container, the empirical or semi-empirical gas–liquid mass transfer correlations obtained for shaken flasks are not applicable to the other system [11,13,21,22].

In this report, the k_La values of shaken cylindrical containers from the mL scale to the 2000-L scale were determined. Operating parameters having an impact on oxygen transfer including shaking diameter, shaking speed, working volume, and container size were investigated. The results are expected to facilitate the establishment of operating conditions for orbitally shaken bioreactors and to improve their geometrical design to achieve optimal animal cell culture performance.

2. Materials and methods

2.1. Equipment

General geometric and material information about the cylindrical containers used in this study is provided in Table 1. For the largest volumetric scales, inflated custom-designed 200- and 2000-L cell culture bags (Platinum UltraPAK™, Lonza Verviers, Verviers, Belgium) were inserted into the respective containers and agitated on custom-built shakers (Adolf Kühner AG, Birsfelden, Switzerland) with shaking diameters (d) of 5 cm (200-L shaker) and 10 cm (2000-L shaker). The smaller containers were agitated on a Model ES-W shaker (Adolf Kuhner AG) with a shaking diameter of 5.0 cm or a KS 250 shaker (IKA Werke, Staufen, Germany) with a shaking diameter of 2.0 cm as indicated in the text.

2.2. k_La determination

Non-invasive dissolved oxygen (DO) measurements were executed using an OXY-4 oxygen meter (PreSens GmbH, Regensburg, Germany) with a PSt3 fiber-optic oxygen minisensor (Presens GmbH). The non-invasive minisensor patch had a thickness of 1 mm, a diameter of 4 mm, and a response time (t_{90}, the time for 90% of the change in signal to occur) of 10 s. The sensor was glued 1–5 cm from the bottom of the container and calibrated with water at a controlled temperature according to the manufacturer's instructions. The k_La was determined in water at room temperature by the dynamic gassing-out method [13,23]. Briefly, the air in the container was displaced by nitrogen to remove oxygen in the liquid phase, and the gas phase above the liquid was then replaced by air. The container was orbitally agitated and the DO was measured over time to determine the k_La according to the mass balance equation (1) in which C^* is the oxygen saturation concentration [mg L^{-1}] and C is the oxygen concentration at time t [h].

$$\mathrm{OTR} = \frac{dC}{dt} = k_La(C^* - C) \quad (1)$$

k_La was calculated from Eq. (2) after derivation from Eq. (1).

$$k_La = \frac{1}{(t2 - t1)} \ln \frac{(C^* - C1)}{(C^* - C2)} \quad (2)$$

All the k_La values reported here represented the average of at least two independent measurements. All containers were left uncovered so that gas transfer resistance from covers or closures did not need to be considered.

2.3. Computational fluid dynamics (CFDs) to simulate the free-surface

The two-phase free-surface flow developed inside orbitally shaken cylindrical containers was simulated by solving the Reynolds Averaged Navier–Stokes (RANS) equations using the FLUENT 6.3 software package (Fluent Inc., Lebanon, NH). The free-surface dynamics were tracked with a Volume of Fluid (VOF) model, the most commonly used method for the simulation of free-surface flows with complex interfaces. The liquid phase was assumed to have the physical properties of water. The structured grid was generated using Gambit™ software (Fluent Inc.). To obtain a high quality mesh, a block-structured topology was adopted. Several simulations with different grid discretizations and time-step resolutions were performed to define an acceptable compromise between accuracy and computational cost. The simulation duration was typically 60 s with a time step of 10^{-3} s. This grid had approximately 5×10^5 nodes and each simulation required around 8 d on 16 processors.

Table 1
Properties of shaken cylindrical containers used in this study.

Nominal volume (L)	Source	Material	Inner diameter (cm)	Vessel height to diameter ratio
0.05[a]	Sartorius AG	Polypropylene	2.7	3:1
0.5	Schott Glass	Glass	7.8	3:2
1	Schott Glass	Glass	9.2	3:2
5	In-house	Polymethyl methacrylate	17.2	3:2
30	In-house	Polymethyl methacrylate	28.7	3:2
200[b]	Plastomatic AG	Low density polyethylene	60	3:2
2000[b]	Plastomatic AG	Low density polyethylene	130	3:2

[a] The 50-mL tube (Satorius AG, Göttingen, Germany) has a conical bottom with an angle of 45°.
[b] Single-use polyethylene bioprocessing bags of 200- and 2000-L were inflated and fitted into plastic cylindrical containers with a conical bottom having an angle of 10°.