APPRAISAL OF FLUID FLOW IN A SHAKEN BIOREACTOR WITH CONICAL BOTTOM AT DIFFERENT OPERATING CONDITIONS

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Abstract. The flow dynamics in cylindrical shaken bioreactors of different conical bottom geometries is investigated by means of phase-resolved Particle Image Velocimetry. Amid the variety of shaken vessels used for bioprocess applications, a cylindrical bioreactor with a conical bottom geometry was selected to assess its potential application in three-dimensional cultures, and improve solid suspension in shaken systems. This work builds upon the study of Weheliye et al.(2013) for a flat bottom with the objective to evaluate the effects of conical shaped bottoms of different heights on the fluid flow under different operating conditions with water being the working fluid. The results provide evidence that the presence of the conical bottom affects the transition from laminar to turbulent flow documented by Weheliye et al. (2013). The conical bottom with the greatest height investigated extended significantly the range of speeds over which flow transition occurs, with high intensity vortical structures spanning over the entire height of the bioreactor at lower speeds than those reported for a flat bottom geometry. This combined with the observed higher levels of kinetic energy should provide more efficient mechanisms for solid suspension.

Keywords: Shaken bioreactor, conical bottom, mixing dynamics, PIV

1. INTRODUCTION

The pharmaceutical industry heavily relies on mammalian cells as host to manufacture biologics for the diagnosis and treatment of a variety of patient conditions, such as rheumatoid arthritis (Adalimumab), leukemia (Ofatumumab), colonrectal cancer (Panitumumab) (Walsh, 2014; Zhang, 2010). Process development studies are typically performed at small scale in orbitally shaken reactors (OSRs), either in microwell plate format or Erlenmeyer flasks, offering an effective solution to screen several conditions in parallel using small volumes. Once the optimal parameters are identified, the process is commonly scaled up for manufacturing in larger stirred tank bioreactors (STRs). The scale translation from OSRs to conventional stirred tank reactors currently represents a bottleneck at the process development stage due to the significant differences in geometry and mixing dynamics, and has generated interest in large scale OSRs for facilitating bioprocess development. Production scale OSRs are now on the market (see for example Kühner OrbShake) allowing users to select the same bioreactor geometry at multiple scales, thus facilitating scaling-up and simplifying regulatory approval.

The flow dynamics inside OSRs were first studied by Gardner and Tatterson (1992), where dye visualization techniques were employed to assess the variation of the homogenization time with increasing Reynolds number, *Re*. Büchs et al. (2001) were the first to report the 'out-of- phase' phenomenon present at certain conditions, when the liquid in the shaken flask does not move in synchronization with the shaker table. Recently, Weheliye et al. (2013), Ducci and Weheliye (2014) and Rodriguez et al. (2014; 2013) provided a thorough insight into the flow dynamics inside a cylindrical shaken bioreactor with a flat bottom. From their work phase-resolved velocity maps at low Froude number (*Fr*) showed the presence of two completely separated counter-rotating vortices when the free surface is at its maximum inclination on the vertical plane of measurement ($\varphi = 0^o$). As the shaking speed, *N,* was increased, the counter-rotating vortices, often denoted as toroidal vortex to characterize the three dimensionality of the flow, were observed to extend towards the bottom of the cylindrical bioreactor and increase in magnitude. When *N* was further increased, a mean flow transition was observed, with the flow being dominated by an axial vortex precessing around the axis of the bioreactor. It was also observed that, at conditions before the onset of the flow transition, the free surface could be approximated by an ellipse, whilst at greater *N,* after flow transition, the free surface was highly three dimensional. Weheliye et al. (2013) derived a scaling law, which allows to calculate the conditions at which flow transition occurs for different bioreactor sizes, orbital diameter, filling volume and fluid viscosity. The expansion of the toroidal vortical cell from the free surface to the bioreactor base with increasing shaker speed found by Ducci and Weheliye (2014) and Weheliye et al. (2013) provides an explanation for the results of Tissot et al. (2010) and Rodriguez et al. (2014; 2013), who reported the presence of faster mixing in regions close to the surface and a diffusion-dominated zone close to the cylinder bottom for low values of *N*. Rodriguez et al. (2013) investigated the effect of feeding location on mixing time, and reported slower mixing when feeding occured inside the toroidal vortex, whilst mixing time decreased when additions were performed elsewhere in the bioreactor.

The mentioned flow scaling law and fluid dynamics studies of shaken systems are limited to cylindrical and flask geometries, but several other designs have been proposed in the literature in an attempt to improve process mass transfer at different scales. For example, at small scale, a 6-petaled flower shape cross-section micro-titre plate geometry was found to be the most promising among all the proposed designs (Funke et al., 2009). At large scale, V_f=50-1500L Zhang et al (2008) proposed a novel bioreactor configuration, where helical tracks were positioned on the cylindrical walls resulting in 10-fold increases in mass transfer in comparison to unbaffled cylinders. Monteil et al. (2013) investigated a shaker tube with a bottom equipped with a downward cone, reporting that a higher shaking speed was required to obtain the same mixing times as a flat bottom cylinder having a similar diameter. The research of Tissot et al. (2010) and Rodriguez et al. (2014; 2013) highlighted the presence of poor mixing regions and low flow activity at the bottom of the vessel. This is a drawback for those applications based on microcarrier suspensions, which are essential for cultivation of cells not yet adapted to suspended cultures, such as legacy cell lines and stem cells. From this point of view the recent study of Pieralisi et al. (2015) has shown that microcarriers tend to accumulate at the centre of the bioreactor when a flat bottom geometry is considered.

Cells cultured in a bioreactor are subject to mechanical stresses due to the fluid dynamics developing within the system; however in the published literature no agreement has been reached on the qualitative and quantitative effects of flow shear stresses on mammalian cells and their metabolism. High shear forces at the air/liquid interphase in sparged bioreactors were reported to be detrimental to cell growth (Cherry and Hulle, 1992). Recent works by Nienow et al. (2013) proved that density and product titre of CHO cells were not affected by industrially relevant shear stress levels, both in laminar converging devices and turbulent bioreactors stirred by Rushton turbines. A low shear environment caused by the use of controlled agitation rates, on the other hand, has been reported to affect cell viability, growth and metabolism (Aloi and Cherry, 1996; Elias et al., 1995; Keane et al., 2003). The effect of sub-lytic shear stresses was evaluated for baby hamster kidney (BHK) cells in terms of cell morphology and viability (Ludwig et al., 1992) and critical shear stress levels of 0.8 to 1 Nm^{-2} were reported. In the prospect of stem cells grown in 3-dimensional microcarrier cultures, flow shear becomes crucial with respect to cell proliferation and lineage selection (Li et al., 2011). Hydromechanical damage on microcarrier-cultured cells arises from inter-particles collisions and from turbulent eddies whose characteristic dimension is similar to the microcarrier size (Cherry and Papoutsakis, 1988). Ismadi et al. (2014) investigated the effects of flow shear stresses on mouse induced pluripotent stem cells attached on microcarriers cultivated in spinner flasks and reported an agitation of 25 RPM and 7 days culture time as the optimal conditions. Nienow et al. (2014) developed a new method for the harvesting of human mesenchymal stem cell (hMSC) in a spinner flask. Their study indicates that intense agitation for a short period (7 mins) under the presence of a suitable enzyme can promote cell detachment without damaging the cells or affecting their attributes, with an overall harvesting efficiency above 95 %. Studies on the effect of shear stresses on mammalian and stem cell culture are generally limited to global considerations where cell population properties, such as product titre and cell viability, are related to global flow characteristics based on volume average velocity and shear stress. From this point of view a rigorous quantification of local shear stresses and their distribution in the bioreactor are crucial to better understand their effect on the process, and be able to design suitable operating conditions. This is especially important for novel technologies like stem cells and cells for therapy where shear sensitivity has not been fully investigated, and where the direction and magnitude of shear stresses have been found to have a major impact on cell expansion and differentiation into cell lineages (Sargent et al., 2010; Stolberg and McCloskey, 2009).

Studies on the flow in orbital shaken reactors have focused primarily on simple geometries, such as Erlenmeyer flasks or flat-bottomed cylinders. Efforts have been made to improve bioreactor performance through geometry modifications. Aeration, for example, was improved by the addition of baffles and/or modifications to the bioreactor wall and such changes have proven successful in increasing mass transfer rates at the liquid/air interface. Very few designs have been proposed aiming at improving solids suspension in OSRs. The critical agitation speed required for microcarrier suspension in Erlenmeyer flasks and cylindrical vessels was determined by Olmos et al. (2015). Pieralisi et al. (2015) measured just-suspended speed in cylindrical OSRs and found microcarriers settled at the centre of the vessel. In an attempt to address the aforementioned drawbacks of orbital shakers, different conical bottom geometries were investigated in this work to assess/compare their mean flow characteristics and potentials for solid suspension. A conical shaped bottom was employed in a cylindrical OSR in order to minimize the volume associated to poor mixing and flow recirculation (Rodriguez et al. (2013))..The shear rate levels were quantified for all bottom geometries considered as such levels are crucial to understand whether orbital shaken bioreactors can be successfully used for shear-sensitive cells, like stem cells and cells for therapy, either in suspension or adherent to microcarriers.

2. MATERIALS AND METHODS

The experimental apparatus comprised a glass cylinder with a transparent Perspex bottom characterized by an inner diameter, d_i, height, H, and wall thickness, t_c, of 100 mm, 250 mm and 5 mm, respectively, as shown in Figure 1. The cylinder was placed in a square acrylic trough to minimize refraction. A steel structure enclosed the trough and cylinder leaving the top of the cylinder open. The gap between the trough and glass cylinder was filled with water to minimise refraction at the cylinder curved wall. Three bottom geometries were investigated in this work: flat, conical A (cone height, h_{cone} = 5 mm; angle from the horizontal, α_{cone} = 6.3°) and conical B (cone height, h_{cone} = 15 mm; angle from the horizontal, α_{cone} = 18.4°). All the geometry characteristics are listed in Table 1. It should be noted that the conical bottoms used in this work were truncated cones, with a 10 mm upper face diameter. The bioreactor bottoms were manufactured on a lathe from a solid piece of Perspex and designed to be interchangeable on the cylindrical vessel. The manufacturing and polishing process allowed good optical access to the measurement area. Distilled water (ν = 10^{-6} m^2/s) was used as working fluid. For all the conditions investigated, a fixed fill volume, V_f = 393mL, was employed, corresponding to fluid heights at rest of h = 50 mm, 52 mm and 56 mm for the flat, conical A and conical B geometries, respectively. The bioreactor was secured to a benchtop L-SX shaker (Kühner AG Shaker) equipped with a Hall effect incremental encoder. The origin of the phase angular coordinate, φ, corresponded to the shaker being at the point furthest to the left along its clockwise circular orbit when seen from above. A magnet placed on the shaker drive triggered the Hall effect sensor at each revolution, when the vessel was at the phase angle $\varphi = 0$. The shaker rotational speed was varied in a range N = 80-130 RPM. The orbital diameter was d_o = 25 mm.

Bottom geometry	d_i, *[mm]*	h_{cone} *[mm]*	α_{cone} *[deg]*	d_o, *[mm]*	h, *[mm]*	N *[RPM]*
Flat	100	0	0	25	50	80-130
Conical A		5	6.3		52	
Conical B		15	18.4		56	

Table 1. Summary of bioreactor geometric parameters and operating conditions.

A two-dimensional Particle Image Velocimetry system (PIV) was employed to determine velocity characteristics and estimate the kinetic energy distribution and shear rates on a vertical plane of measurement passing through the axis of the cylindrical vessel. The PIV system comprised a green diode laser, a cylindrical lens, a mirror and an intensified camera, which were rigidly mounted on the tray of the shaker table. The laser had 532 nm wavelength and an output power of 300 mW. The intensifier in the camera amplified the photons entering the camera lens, allowing short exposure times (5 milliseconds) with the low power laser. The cylindrical lens created a 1 mm laser sheet. The mirror was placed below the bioreactor at a 45° angle so to reflect the laser sheet and illuminate the base along the vertical plane orthogonal to the camera. The timing box and magnetic encoder allowed to synchronize the image acquisition, and to obtain phase-resolved velocity data. Rhodamine-coated Polymethyl methacrylate spheres (50 μm) were used for the PIV measurements in conjunction with a 570 nm orange cut-off filter to minimize reflections of the laser.

Different sets of measurements were taken at angular positions $\varphi = 0^\circ$ and $\varphi = 270^\circ$, which are the most effective to characterize the flow. The same set up as that of Weheliye et al. (2013) was employed in this work so that the field of view corresponded to the r-z plane bisecting the cylinder along its longitudinal axis (see Figure 1). The data recorded with the intensified camera was processed using Dynamic Studio (Dantec Dynamics) with an adaptive correlation analysis of the full image for an initial interrogation window of 256×256 pixels and a final window of 32×32 pixels. The adaptive correlation method is a multi-scale correlation approach, where velocity vectors estimated from larger interrogation area are used as input to the next correlation step. In this way a preliminary velocity direction is estimated to compute the final vector fields corresponding to the final interrogation area. In the current analysis a 50% window overlap was used for a final resolution of 16×16 pixels, corresponding to an area of 1.3×1.3 mm^2. Five hundred image pairs were recorded for each experimental condition with a time delay of 10- 20 ms between the two images used to estimate the instantaneous vector field. Data processing was carried out using purposely written Matlab routines.

3. RESULTS AND DISCUSSION

The flow dynamics in a flat bottom cylindrical OSR have been described by Weheliye et al. (2013) and Rodriguez et al. (2013). This work builds upon those findings to investigate the effects of a conical bottom geometry on the flow in a cylindrical OSR. Section 3.1 investigates the impact of a conical bottom of two different heights on the development of the vortical structures described by Weheliye et al. (2013) and on the intensity of the space-averaged vorticity. The principal components of the strain rate tensor were estimated over the entire flow field for the flat and conical B bottom geometries and, together with the intensity and position of maximum strain rate, are reported in Section 3.2. Section 3.3 provides an account on how and to what extent conical bottom geometries affect the flow at a phase angle $\varphi = 270^{\circ}$, which is characterized by a strong radial flow responsible for moving the fluid between opposite sides of the bioreactor (Rodriguez et al., 2013). Section 3.4 shows how the conical bottom geometry affects the intensity of kinetic energy for increasing Froude number.

Different definitions of Froude number, based either on velocity or centrifugal acceleration, are available in the literature relevant to shaken systems. In this article the definition used by Buchs et al. (2001) and Ducci and Weheliye (2014), namely $Fr_{do} = 2\,\pi^2 N^2 d_o/g$, was adopted.

3.1 Effects of bottom design on flow dynamics and vorticity

Phase-resolved velocity fields and tangential vorticity contour maps were obtained for the three bottom geometries investigated. The velocity fields were measured at a phase angle $\varphi = 0^{\circ}$. The vertical plane of measurement bisected the cylindrical bioreactor into two halves. The phase-resolved velocity fields were calculated using Equation 1.

$$\langle \boldsymbol{u}(r,z,\varphi)\rangle = \frac{\sum \boldsymbol{u}(r,z,\varphi)}{N_{\varphi}}$$

Equation 1

Where $\boldsymbol{u}(r,z,\varphi)$ is the instantaneous velocity field on the plane of measurement (r,z) and $N\varphi$ is the number of velocity fields obtained for each angular position, φ; in this work 500 image pairs were used to calculate the phase average $\langle u\rangle$. The vorticity in the k^{th} direction, ω_k, defined in Equation 2, was calculated from phase-averaged velocity fields on a two-dimensional grid using a central differentiation scheme:

$$\omega_k = \left(\frac{\partial u_i}{\partial x_j} - \frac{\partial u_j}{\partial x_i}\right)$$

Equation 2

Where u_i and u_j indicate the velocity components in the i_{th} and j_{th} directions, respectively.

Velocity fields and vorticity contours are shown in Figures 2, 3 and 4 for flat, 5 mm and 15 mm conical bottom cylinders, respectively. In all cases the data shown were obtained at shaking speeds N= 85, 100, 110 and 115 RPM and Fr = 0.10, 0.14, 0.17, 0.18 (Figures a-d). All vorticity contour maps were scaled according to the colour bar included in Figures 2, 3 and 4 (d). Regions with red hues indicate a positive value of vorticity and counterclockwise rotation, whilst those in blue are associated to negative vorticity and clockwise rotation. Two counter-rotating vortical cells can be identified within the bulk of the fluid from all the vorticity contour maps considered. The cells represent a section of the toroidal vortex inclined below the free surface. In agreement with previous studies conducted using flat bottom bioreactors (Weheliye et al 2013), the size of the vortical cells increases and expands from the free surface towards the cylinder bottom as Fr is increased. Figure 2 (a) shows vortical cells present at a location close to the vessel walls and the free surface. As the shaking speed is increased the vortical cells increase in size and intensity, expanding toward the centre and bottom of the bioreactor (Figures 2a-d), reaching the bottom at shaking speeds $N > 110$ RPM and $Fr > 0.17$ (Figure 2d). The shaking speed at which flow transition occurs for the flat bottom bioreactor is in agreement with that indicated by Weheliye et al. (2013) for the geometrical configuration and operating conditions used in this study (h/d_i=0.5 and d_o/d_i= 0.25).

A similar vortical cell expansion was observed for the conical bottom bioreactors investigated, however some differences with the flat bottom geometry can be noted from Figures 3 and 4. The shaking speed at which the vortices extend to the bottom decreases as the inclination of the conical bottom is increased. It is clear from Figures 4 (a-d) that the vortices reach the base of the bioreactor at a shaking speed $N = 100$ RPM for the most inclined conical bottom studied in this work, while the same phenomenon occurs at $N = 110$ RPM and 115 RPM for the conical A and flat bottom designs, respectively. Despite the fact that the fully extended vortex configuration occurs at lower speeds when conical bottom geometries are used, Figures 4 (a-d) suggest that the vortical structures are still present at speeds higher than that associated to full vortex extension and the critical speed of the aforementioned mean flow transition is not affected by the bottom geometry. This could be due to the conical design influencing the shape of the vortical cells, pushing them closer to the vessel walls and away from each other reducing their interaction at the centre of the bioreactor.

Vorticity intensity increases as the shaking speed is increased for all bottom configurations investigated, as seen in Figures 2-4. The space-averaged non-dimensional vorticity, ω_θ*, is defined in Equation 3 and its variation with Fr is provided in Figure 5a. The spaced-average was carried out inside the area delimited by a non-dimensional vorticity threshold of $\omega_\theta > 0.1\ \pi N$, which is shown for reference in Figure 5b (conical A configuration, $N = 80$ RPM, $Fr = 0.09$).

$$\omega_\theta{}^* = \frac{\int_{A_{\omega=0.1\,\pi N\cdot}} |\omega_\theta| dA_\omega}{A_\omega}$$ **Equation 3**

As expected, the space-averaged non-dimensional vorticity, ω_θ*, increases with Fr for flat, conical A and B bottoms (Figure 5a). At shaking speeds corresponding to values of Fr below 0.12, similar values of ω_θ* were obtained for all geometries investigated. At these conditions the toroidal vortices are present only in proximity of the free surface and vessel walls, as shown in Figures 2a, 3a and 4a, respectively. The main flow does not extend to the bioreactor bottom hence no effect of the conical bottom was observed. However for $Fr > 0.12$ the rate of vorticity variation with Fr is more pronounced with increasing inclination of the conical bottom. Power curves fitted to the data points suggest a steeper increase of the non-dimensional space-averaged vorticity for the conical B geometry. This behavior is in agreement with the description of the phase-resolved flow provided above, and indicates that the vorticity of the two cells is affected by the bottom geometry only for speed sufficiently large to determine full expansion to the bioreactor base. The greater space-averaged vorticity would benefit solid suspension as the solid lifting force, $F_{Saffman} = \frac{\pi}{4} d_p^3 \frac{\rho_l}{2} C_s \left((u - v_p) \times \omega \right)$ (Derksen, 2003), is proportional to the slip velocity $(u - v_p)$ and flow vorticity (ω) with d_p and v_p being the solid diameter and velocity, ρ_l and u the viscosity and velocity of the liquid phase and C_s the lift coefficient.

3.2 Effects of bottom design on shear rates

The effect of shear on cells and product expression has been a topic of discussion since mammalian cells became one of the major expression host for the production of antibodies and other therapeutics, and an agreement has yet to be reached on whether fluid stresses have a significant impact (Cherry and Hulle, 1992; Keane et al., 2003; Ludwig et al., 1992; Nienow et al., 2013). It is clear, though, that a more rigorous quantification of fluid shear is needed in order, for example, to improve understanding of its effect on stem cells. Maximum shear and strain rate components have therefore been quantified in this work. The strain rate tensor, S_{ij}, for the 2D flow studied is defined in Equation 4:

$$S_{ij} = \begin{pmatrix} \frac{\partial u_i}{\partial x_i} & \frac{1}{2}\left(\frac{\partial u_i}{\partial x_i} + \frac{\partial u_j}{\partial x_j}\right) \\ \frac{1}{2}\left(\frac{\partial u_i}{\partial x_i} + \frac{\partial u_j}{\partial x_j}\right) & \frac{\partial u_j}{\partial x_j} \end{pmatrix} \qquad \textbf{Equation 4}$$

Where indices i and j indicate the radial and axial directions, respectively. The strain rate tensor is a measure of local rate of stretching, compression and shear. When a local reference system aligned with the principal axes of S_{ij} is considered, the highest rate of stretching and compression can be determined. These are found from the principal components (eigenvalues) S_1 and S_2 of the strain rate tensor in Equation 4. Positive values of S_1 and S_2 indicate stretching whilst negative values relate to compression (Bouremel et al., 2009; Davidson, 2004). In this work only two velocity components were measured, therefore the S_3 component of the strain rate tensor in the k direction orthogonal to the plane of measurement (i.e. azimuthal direction) was estimated using the continuity equation (Equation 5):

$$S_3 = -S_1 - S_2 \qquad \textbf{Equation 5}$$

The principal components of the strain rate tensor, S_1 and S_2, for the conical B geometry at Fr = 0.14 (N = 100 RPM) are shown in Figures 6 (a) and (b), respectively. It should be noted that the principal component S_1 is negative across the entire field of view, and therefore it provides an estimate of the compression rates experienced by the cells. The direction of compression is not given in Figure 6 (a), but it is locally aligned with the direction of the first eigenvector of the local strain rate tensor. From the contour plot the highest compression rate is found at the margins of the counter-rotating vortices. The normal stress S_2, which is always positive and is associated to local stretching rates, reaches its maximum in the lower section of the toroidal vortices, at $|r/d_i|$= 0.1-0.5 and z/d_i = 0.1-0.2, in proximity of the tips of the conical bottom upper surface.

The maximum shear rate, τ_{max}, experienced at a specific location, can be found from the principal components of the strain rate tensor according to Equation 6:

$$\tau_{max} = \frac{\left(S_i - S_j\right)_{max}}{2} \qquad \textbf{Equation 6}$$

Where indices i and j are selected based on the maximum difference between any two principal components S_1, S_2, S_3. The contour plots of the phase-resolved maximum shear rates on the vertical plane ($\varphi = 0^{\circ}$) at Fr = 0.14 (N = 100 RPM) for flat, conical A and B geometries are shown in Figures 7 (a-c), respectively. This Fr number corresponds to in-phase flow condition for all the geometries investigated (according to the definition given by Weheliye et al., 2013), but the toroidal vortices extend to the bottom of the vessel only for the conical B configuration (as determined from Figures 2b, 3b and 4b). Values as high as $\tau_{max} = 0.13\pi N$ can be observed in Figure 7 (a) at the periphery of the toroidal vortical cells (shown in Figure 2b). The maximum shear rate of Figure 7 (a), $\tau_{max} = 0.2\pi N$ occurs at $r/d_i = 0.5$ in correspondence to the cylinder axis. This was probably due to inaccuracies in the PIV measurements as tracer particle agglomerates were visually observed at such location. The lowest τ_{max} were found at the core of the cells ($|r/d_i| = 0.2$, $z/d_i = 0.2$ -0.3), where quasi-solid body rotation occurs. Similarly, the maximum shear rates for the conical A and B bottoms are characterized by low values at the core of the vortical cells and higher shear rates at the periphery (Figures 6b-c). The maximum intensity of the shear rate occurs at the interfaces between the vortices and the fluid above the upper conical surface. Whilst Figure 7 (a) shows two distinct low shear regions in correspondence to the vortical cell cores, a third region of low τ_{max} can be observed for conical geometries A and B, above the truncated cone tip. When observing the high shear regions rising at the margins of the counterclockwise and clockwise vortices, it appears that all of them are locally orthogonal to bioreactor base, i.e. vertical for the flat bottom (in Figure 7a) and outwards inclined in Figure 7 (b-c) for the conical bottom geometries. The maximum shear rate obtained in Figures 7 corresponds to dimensional shear stresses of 0.001 Nm^{-2}. As a reference stem cell proliferation is affected by shear stress greater than 0.1 Nm^{-2} (see Toh et al. 2011) while mammalian cells damage occurs at value higher than 0.15 Nm^{-2} (Elias et al., 1995), which are well above those reported in this work.

The variation of the average maximum shear stress, τ^*_{max}, with Fr is shown in Figure 8 for the three bottom configurations investigated. The asterisk denotes the space-average of the absolute value of τ_{max} over the vertical plane of measurement. Outlier values of τ^*_{max} were not included in the space-average as caused by erroneous velocity vectors close to the free surface. For all cases τ^*_{max} increases with Fr. Similarly to Figures 7 (a-c), the conical B geometry presents higher values of τ^*_{max} for most Fr, as the toroidal vortices are more compressed towards the sides of the vessel, resulting in a more intense deformation rate. The maximum shear stress, τ^*_{max}, increases up to $Fr = 0.17$, then a sharp decrease can be observed, after which τ^*_{max} increases again with Fr. This can be explained by the onset of the out-of-phase flow transition, where the tangential velocity component in the direction orthogonal to the plane of measurement is the most significant.

3.3 Effects of bottom design on local flow direction

A better understanding of the bioreactor flow can be gained from Figure 9 (a) where vertical profiles of the phase-averaged radial velocity component are plotted for a phase angle $\varphi = 270^\circ$. In this figure the vertical profiles associated to a flat and conical B bottom geometries are compared at two radial coordinates, $r/d_i = 0$ and 0.25, indicated in Figure 9 (c), for two shaking speeds, $N = 80$ and 100 RPM. In Figure 9 (a) the non-dimensional velocity is on the abscissa, with magnitude increasing from right to left, while the distance from the vessel bottom is plotted on the ordinate axis. As expected the intensity of the radial velocity increases when moving closer to the free surface for the profiles plotted. It is evident that the two profiles for the flat and conical B bottom differ at a shaking speed $N = 100$ RPM, because at this regime the flow has nearly developed for both geometries all the way to the bottom of the reactor, and it is therefore affected by the bottom geometry. This does not occur when the lowest speed is considered as for both configurations the vortical flow has developed over a small region below the free surface, and therefore the differences in bottom geometries are less relevant. It can be observed that the curves seem to be shifted vertically between flat and conical B bottoms, this being more apparent at the higher shaking speed considered. It should be noted that both sets of measurements were obtained for the same filling volume and therefore the maximum height of the conical bottom was higher than the flat one. This implies that points at the same height are further away from the free surface, which is the flow driving mechanism, for the conical bottom than for the flat one. The same data are presented in Figure

9 (b), where a normalized axial coordinate with origin on the free surface $(z\text{-}h)/d_i$ is considered. The new axial coordinate system shown in Figure 9 (c) is more effective at capturing the similarity between the flow of the two geometries. From Figure 9 (b) it is evident that according to this reference system the vertical profiles of the two configurations show an excellent match, particularly in proximity of the free surface where the local flow is less affected by the difference in bottom geometry.

The mean flow at a phase angle $\varphi = 270^\circ$ consists of fluid motion from one side to the other of the bioreactor. Figure 10 shows the velocity field for $Fr = 0.14$ ($N = 100$ RPM) for flat (white vector field) and conical B bottom geometries (black vector field). As can be seen in the inset of Figure 10, the velocity fields present differences in magnitude and direction between the two geometries. The contour describes the angle difference α_{angle} between the velocity fields of the flat bottom and conical B geometry, with a clockwise direction change in red and counterclockwise direction change in blue. The regions where the flow is most affected by the different bottom geometry are close to the conical bottom, with the 'upstream' side at the right of the cone experiencing a more significant change in velocity direction in comparison to the 'downstream' on the left. The condition $Fr = 0.14$ presents toroidal vortices expanded to the bioreactor bottom, as described in Figure 4c, hence a large region of the flow is affected by the conical B geometry. The velocity ratio U/U_c was estimated to further assess the effect of the bottom geometry on the velocity field, where U and U_c are the velocity magnitude for the flat and conical B bottom geometries, respectively. A relation was sought between the change in α_{angle} and the change in velocity magnitude, and the results of this analysis are shown in Figure 11 for $N = 100$ RPM ($Fr = 0.14$). The U/U_c data shown in Figure 11 are limited to those points where the $|\alpha_{angle}| > 10^\circ$. The distribution of the data points on the positive α_{angle} axis, upstream side, is characterized by a narrower spread of U/U_c in comparison to the data points associated to negative α_{angle}, downstream side (Figure 11). This implies that the upstream flow is mainly deflected, as indicated by the larger region with angle variation (cf. Figure 10), but its velocity magnitude is less affected (cf Figure 11). The opposite occurs downstream of the conical bottom where the region associated to a significant deflection is smaller, but its velocity distribution is broader and U/U_c is larger.

3.4 Effects of bottom design on energy content

Similarly to Rodriguez et al. (2013), in this work the velocity field is described using the Reynold's decomposition, $u(r, z, t) = \langle u(r, z, \varphi)\rangle + u_i'(r, z, t)$,t), with $\langle u(r, z, \varphi)\rangle$ being related to flow periodic variations and $u_i^{'}(r, z, t)$ taking into account random fluctuations. The spaced-averaged non-dimensional kinetic energy of the random velocity fluctuations, $k_{rz}'^{*}$, was evaluated for the bioreactors under investigation according to the 2D approximation of Equation 7:

$$k_{rz}'^{*} = \frac{1}{A}\int_A \frac{3}{4}\left(\langle u_r'^2\rangle + \langle u_z'^2\rangle\right)dA \qquad \textbf{Equation 7}$$

Where $\langle u_r'^2\rangle$ and $\langle u_z'^2\rangle$ are phase-resolved terms of the random velocity fluctuations, and A is the area of the measurement field. The variation of $k_{rz}'^{*}$ with Fr for all geometries under investigation is shown in Figure 12 at a phase angle $\varphi = 0^\circ$. Low values of kinetic energy $k_{rz}'^{*}$ were measured for $Fr = 0.9\text{-}0.14$ ($N = 80\text{-}100$ RPM), followed by a sharp increase peaking at $Fr = 0.17$. Above $Fr = 0.17$ $k_{rz}'^{*}$ drops to low values. This can be explained by considering that at $Fr = 0.17$ the flow transition to a precessional vortex with a vertical axis occurs (see Weheliye et al., 2013). Above the critical flow-transition speed the main velocity component is aligned with the tangential direction which cannot be directly estimated from the vertical plane measurements presented in this work. The results presented in Figure 12 confirm that flow transition occurs at the same Fr numbers for all geometries considered. It can also be noted that the measured values of k'^{*}_{rz} for all geometries were comparable up to Fr = 0.14, whilst for Fr >0.17 lower values of kinetic energy were found for the conical B geometry when compared to the flat bottom (approximately 35% smaller). This suggests that the phase-resolved flow at conditions close to N_{crit} presents more stable vortical structures, with fewer velocity fluctuations for the conical configuration.

4. CONCLUSIONS

In the present study the effect of the implementation of a conical bottom design in an orbital shaken bioreactor has been extensively discussed. Two truncated conical bottoms of h_{cone} = 5 mm and 15mm were used with an OSR and the resulting fluid dynamics investigated in detail and compared with those obtained for a flat bottom configuration. The impact of the bottom geometry on the general flow characteristics at a phase angle $\varphi = 0^{\circ}$ was investigated first. It was found that the flow in cylindrical bioreactors with conical bottoms is characterised by toroidal vortices expanding towards the bottom with increasing Fr, as is the case for a standard flat configuration, while for the cone with the highest inclination examined (conical B) the vortices reached the bottom at a lower Fr. Despite this difference the Froude number, Fr_{cr}, associated to the flow transition did not vary from the values reported for a flat bottom bioreactor. The space-averaged vorticity increased for all bottom geometries with Fr, however ω_θ^* increased at a higher rate in the conical B geometry when $Fr > 0.12$. This was explained by considering that for this configuration the interaction between the vortical cells and the bioreactor bottom starts to occur at lower speeds. The proposed designs were also evaluated in terms of shear rates. It was found that the most intense deformations occurred at the periphery of the toroidal vortices, with low shear rate values present at the centre of the vortical cells. For a given Fr, the vortical cells are closer to the vessel base with the conical bottom resulting in higher maximum shear rates, τ_{max}, when the conical B configuration was analysed. The use of the conical geometry resulted in an area of low shear rates at $r/d_i = 0.5$ as the inclined surfaces of the conical bottoms modified the shape of the vortical cells, resulting in the regions of largest shear deformation being orthogonal to the cone external walls. The space-averaged maximum shear rate variation indicated that the mean shear rate within the vessel was similar for all geometries at the same Fr, although slightly higher values were found for the highest cone. When the designs were evaluated at a different measurement angle, $\varphi = 270^{\circ}$, which is characterized by a strong radial flow from one side to the other one of the bioreactor, it was observed that the region with the greatest change in velocity direction were in proximity of the inclined surfaces of the conical bottoms. The flow 'upstream' of the conical bottom experiences larger deflection with similar velocity magnitude between flat and conical B geometries, whilst the region 'downstream' of the conical bottom resulted in a greater range of velocity ratios. Finally, the variation of the kinetic energy content of the random (i.e. cycle-to-cycle) velocity fluctuations shows that the energy content increases with Fr for all designs and the Fr associated with flow transition does not change.

In this work a rigorous methodology was established, that allows the fluid dynamics characterisation of alternative bioreactor designs. The use of a conical geometry at the bottom of a cylindrical OSR allows the achievement of similar flow dynamics conditions at a lower N in comparison to a flat bottom bioreactor. This has important implications for shear-sensitive cultures requiring solids suspension, as the new bioreactor design can be operated at low shaking speeds, resulting in low shear rates, without compromising the quality of the suspension. It is also noteworthy that the proposed designs could be beneficial for microcarriers suspension, as higher lifting forces due to increased vorticity levels should occur in conical bottom reactors, thus avoiding the accumulation of solids at the centre of the bioreactor bottom and probably requiring a lower just suspended speed in comparison to flat bottom configurations.

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Abbreviations		
2D	Two -Dimensional	
PIV	Particle Image Velocimetry	
OSR	Orbitally Shaken Reactor	
STR	Stirred Tank Reactor	
CHO	Chinese Hamster Ovary cells	
hMSC	Human mesenchymal stem cell	
Greek Symbols		**Units**
φ	Phase angle of the table	^{o}
α_{cone}	Angle of the conical bottom from the horizontal	^{o}
α_{angle}	Angle difference between velocity fields	^{o}
ω_θ	Vorticity in the tangential plane	s^{-1}
ω_θ^*	Space-averaged non-dimensional vorticity	s^{-1}
τ_{max}	Maximum shear rate	s^{-1}
τ_{max}^*	Space-averaged maximum shear rate	s^{-1}
Roman Symbols		**Units**
d_i	Inner diameter of the cylinder	m
d_o	Orbital diameter	m
g	Gravitational acceleration	$m\ s^{-2}$
h_{cone}	Height of the truncated conical bottom	m
h	Fluid height	m
Fr_{crit}	Critical Fr for flow transition	-
S_{ij}	Strain rate tensor	s^{-1}
S_n	Principal components of the strain rate tensor	s^{-1}
$k_{ij}'^*$	Space average of the kinetic energy due to the random fluctuations in the i_{th} and j_{th} direction	$m^2\ s^{-2}$
N	Shaker table rotational frequency	s^{-1}
N_{crit}	Critical N for flow transition	s^{-1}
t_c	Cylinder wall thickness	m
u_i	Velocity component in the i_{th} direction	$m\ s^{-1}$
u_i'	Random velocity fluctuation in the i_{th} direction	$m\ s^{-1}$
V_{fill}	Fill volume	L

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Figure 3. Phase-resolved non-dimensional velocity fields and contours of the tangential component of vorticity, ω_θ, for conical A bottom geometry at (a-d) *Fr* = 0.10, 0.14, 0.17, 0.18 (*N* = 85, 100, 110, 115 RPM).

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