MIXING AND FLUID DYNAMICS CHARACTERISTICS IN SINGLE-USE BIOREACTORS FOR IMPROVED DESIGN AND SCALABILITY

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The pharmaceutical industry is at the forefront of the production of antibodies using mammalian cell-based cultures, with single-use technologies gaining prominence in the manufacturing process. Since the development of the first rocking bag bioreactor in 1997, other novel designs have been developed such as orbitally shaken, two and three dimensionally rocked, pneumatically driven, in addition to inflated cylindrical stirred bags. These have the potential to address new applications like expansion of adult stem cells for allogeneic therapies approaches by providing sufficient mixing while controlling maximum shear stress levels. Rigorous fluid dynamics studies are needed to understand the flow behaviour at different operating conditions, be able to determine meaningful dimensionless characteristics and establish robust scaling laws. A combination of different advanced analytical techniques were used to determine mixing and velocity characteristics and the validity of the approach is demonstrated by three case studies looking at different bioreactor flows relevant to bioprocessing.

In this work, phase-resolved Particle Image Velocimetry (PIV) and high frequency visual fluid tracking were used to investigate the flow pattern and mixing characteristics of a geometrical mimic of a Sartorius 2L CultiBag at various rocking speeds and fill volumes. Fluid velocity and rate of dissipation of the turbulent kinetic energy were found to significantly vary with rocking speed. Under specific experimental conditions, wave formation was observed, which corresponded to high gas transfer rates from the headspace into the liquid phase. Higher rocking speeds caused the fluid to move proportionately out of phase with respect to the platform. Dimensional comparisons of fluid velocities with similar volume conventional bioreactors suggest that similar fluid dynamics characteristics can be achieved between rocked and stirred bioreactor configurations.

Large scale orbitally shaken bioreactors employ the agitation principle of shaken flasks and microwell plates, providing a single-use upstream process thus facilitating scaling-up and simplifying regulatory approval. In this case study the mixing and flow dynamics in a cylindrical orbitally shaken bioreactor with conical bottoms of different heights was evaluated. The rationale for a conical bottom is to ease the suspension of cells or microcarriers for adherent cells applications. This study builds upon previous works of the research group (Weheliye et al 2013, Rodriguez et al. 2013, Rodriguez et al. 2014, Ducci and Weheliye, 2014) for flat bottom reactors, where increases in Froude number were found to determine a mean flow transition and to increase the turbulence levels. PIV and Dual Indicator System for Mixing Time, DISMT, combined with advanced image processing were employed to assess the mixing performance in the bioreactor with a conical bottom and the findings offer a novel approach to design the next generation of products and improve scaling methodologies for cell therapy applications involving microcarriers' suspensions.

Thirdly, a thorough experimental study of the flow within the Millipore 3L CellReady stirred reactor was conducted combining PIV with a biological study into the impact of fluid dynamic characteristics on cell culture performance. PIV measurements conveyed a degree of fluid compartmentalisation resulting from the up-pumping impeller. Both impeller tip speed and fluid working volume had an impact upon the fluid velocities and spatial distribution of turbulence within the vessel. Cell cultures were conducted using the GS-CHO cell-line and a significant reduction in recombinant protein productivity was found at conditions corresponding to the highest Reynolds number tested in this work.