Modelling The Transport Of Momentum And Oxygen In An Aerial-Disk Driven Bioreactor Used For Animal Tissue Or Cell Culture

K.Y.S. Liow[1], G.A. Thouas[2], B.T. Tan[1], M.C. Thompson[3] and K. Hourigan[2,3]

[1] School of Engineering, Monash University, Selangor, Malaysia
[2]Division of Biological Engineering, Engineering, Monash University, Melbourne, Australia
[3]Department of Mechanical and Aerospace Engineering, Monash University, Melbourne, Australia

***Abstract*— This study considers the momentum transport and oxygen transfer in a modified stirred tank bioreactor. The design is novel in the sense that the impeller is positioned above the culture medium (instead of being suspended inside it). This design has potential benefits of enhanced gas transfer, reduced possibility of contamination, and better access to the culture medium. Computational fluid dynamics modelling is used to simulate the gas and fluid flow in the bioreactor. A rotation rate of 60 to 240 rpm (corresponding to the laminar regime) was adopted. Results show that the flow in the medium is swirl-dominant with an induced secondary flow in the meridional plane consisting of a steady and robust recirculation bubble. As the Reynolds number is increased beyond ~427, we observe the formation of an additional smaller toroidal-bubble at the bottom wall. This bubble bears some resemblance to the vortex breakdown topology commonly found in confined swirling flows. In terms of the oxygen distribution, oxygen transfer from the gaseous phase into the culture medium is enhanced through forced diffusion taking place across the air-medium interface. For the Reynolds number range studied there is clear dominance of convection over diffusion in the transport of oxygen from the air-medium interface and throughout the culture medium.**

***Keywords*— bioreactor, computational fluid dynamics, oxygen concentration, tissue engineering**

I. Introduction

Research in the area of bioreactor process hardware technology in biomedical engineering continues to receive prominent attention ([1],[2]), particularly in relation to the industrial-scale production of mammalian cell lines. While the use of bioreactors has been fairly well established in certain industries (e.g., wastewater, food processing, etc), it remains important to consider the physiological requirements of mammalian cells toward the *in-vivo* condition. Two critical physiological parameters relevant to larger bioreactor volumes with high tissue densities, include oxygen transfer and mechanically-induced shear at the cell walls.

The adverse susceptibility of animal cells to high levels of shear has been well described [3] and significant efforts have also been made to improve the efficiency of oxygen diffusion [4]. While it is accepted that methods such as agitation or gas sparging can enhance oxygen diffusion and nutrient transfer, this often comes at the expense of increased levels of shear [5]. Therefore a well-correlated relationship between the mechanical stimulation of a bioreactor and the oxygenation requirements of particular cell type remains crucial in bioreactor optimization studies.

The objective of this study is to computationally model in detail the transport of momentum and oxygen taking place in a novel small-scale bioreactor in which the culture medium is agitated by an aerial impeller that is positioned directly above it (see Figure 1a). This study forms a part of ongoing investigations by biological engineers at Monash University, to investigate the feasibility and practical application of laminar stirred-tank bioreactors for cell and/or tissue culture. From a fluid dynamics perspective, characterization of the flow in a laminar bioreactor based on a lid-driven cylinder have been performed extensively, both experimentally and numerically ([6],[7],[8]). The design itself was originally based on explorations of the possibility of applying vortex breakdown theory to provide a controlled, low-shear and well-mixed environment within the bioreactor chamber.

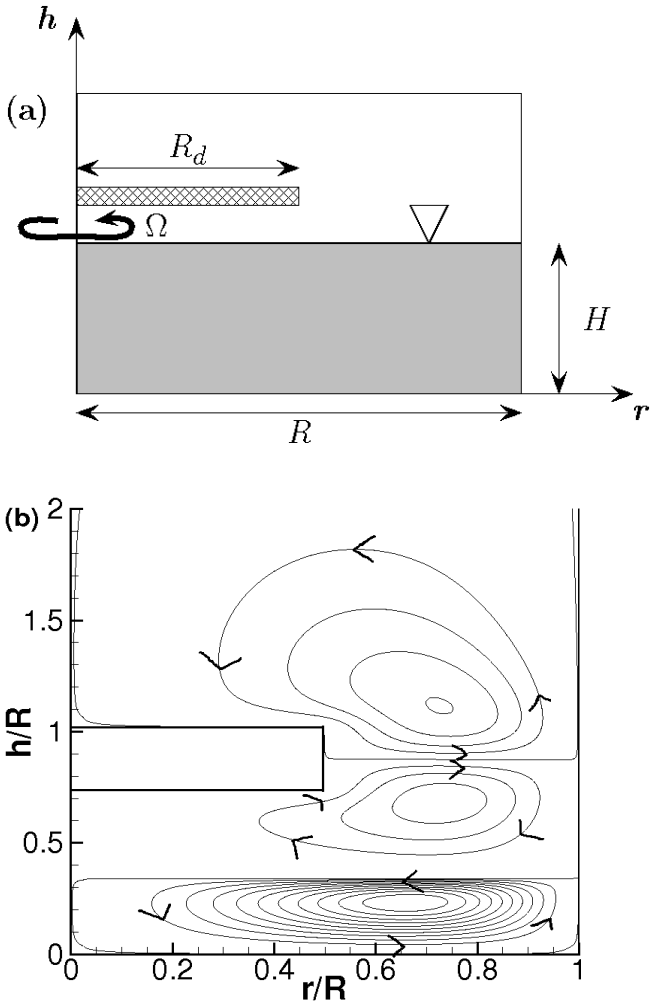

Figure 1: (a) Schematic of the aerial bioreactor in the meridional plane, (b) representative streamlines of the flow.

In terms of cell culture, two prototype configurations in which the laminar bioreactor concept have been successfully tested are 5~ml cylindrical vessels with a partially-submerged disk [9], as well as a non-submerged disk [10].

In this study, the latter configuration (see Figure 1) is considered in further detail. An instantaneous snapshot of the streamline pattern highlighting the induced secondary flow in both the gaseous and aqueous phases is shown in Figure 1b. Note that the results and discussion will be mainly confined to the aqueous phase, e.g., the culture medium in the remainder of the paper. Specifications of the rig and flow are shown in Table 1. The Reynolds number is based on the rotation speed of the impeller, radius of the impeller, and the physical properties of *air*.

II. Numerical methods

The commercial CFD software package, FLUENT, is used to simulate the flow and oxygen transport in the aerial bioreactor. The unsteady solver is used whereby the incompressible Navier Stokes equations (INSE) are temporally marched from initially zero flow to steady state. The axisymmetric form of the INSE are solved on the meridional plane (see Figure 1) with the assumption of axisymmetry. The volume-of-fluid (VOF) technique is used to model the air/culture-medium interface.

Table 1: Specifications and aspect ratio (AR) of the bioreactor

Parameter	Range
Cell consumption of oxygen	$1.0\text{-}1.2*10^{-17}$ mol O_2/cell.s
Cell density	$5*10^{10}$ cells/m^3
Cylinder radius, R	17.65 mm
Fluid height, H	6 mm
Fluid density, ρ	998 kg/m^3
Impeller radius, R_d	15.43 mm
Impeller thickness	5 mm
Impeller height (from base)	13 mm
Impeller speed	60-240 rpm
Bioreactor AR, H/R	0.339
Impeller AR, R_d/R	0.872
Reynolds number, Re	183-731

As expected for a CFD investigation, a series of meshes was tested to ensure the flow is well resolved. In terms of spatial resolution, the grid points were concentrated around the impeller and cylinder walls. While it is largely anticipated that the inter-phase boundary would remain flat (given that the Froude number is << 1), nevertheless particular attention was paid to resolving the interface boundary to prevent the development of non-physical oscillations. Further details on the numerical techniques used in this study can be found from [7] and [8].

III. Results and discussion

Figure 2 shows steady-state plots of the normalized stream-function in the meridional flow at three rotation speeds. As seen in Figure 1b, there is a clockwise induced recirculation region in the gaseous phase lying directly underneath the

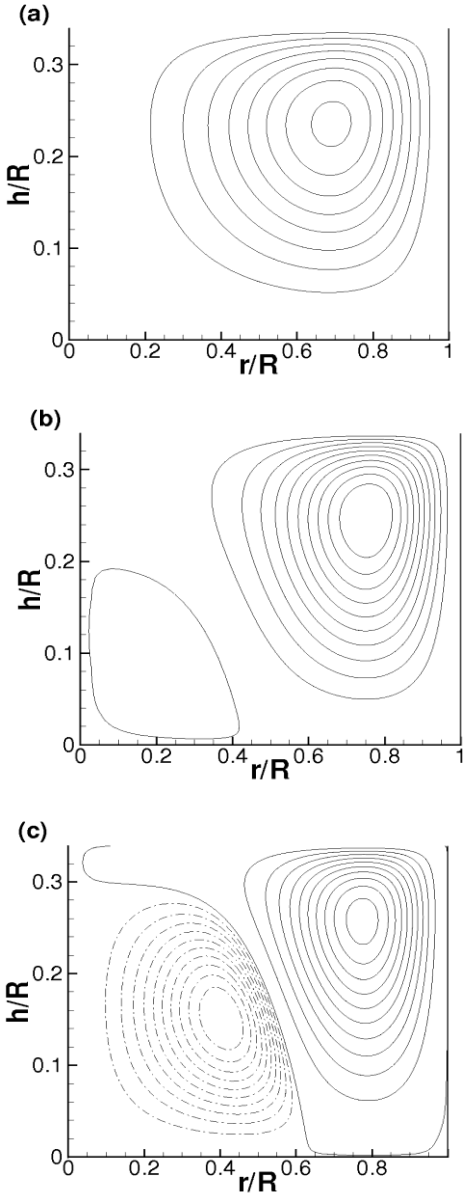

Figure 2: Normalised streamfunction for impeller rotation speed of (a) 60 rpm, (b) 180 rpm, and (c) 240 rpm. The solid lines represent the clockwise rotation with range of 0-1*10^{-5}, while the dashed-dot lines represent the counter-clockwise rotation with range of -8*10^{-5}-0. The intervals are equi-spaced.

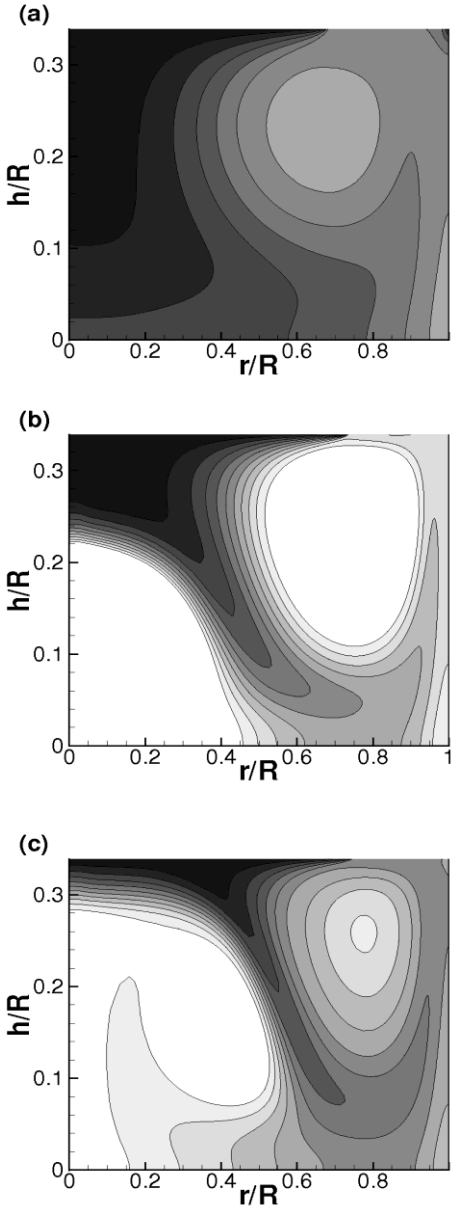

Figure 3: Normalised dissolved oxygen (DO) concentration in the medium phase for impeller rotation speed of (a) 60 rpm, (b) 180 rpm, and (c) 240 rpm. The range is the same for each plot: 0.993 < DO < 1.0.

impeller. The centrifuging effect of the air induces a counter-clockwise recirculation bubble in the culture medium. The concentration of the streamlines at the radial location of r/R ~ 0.7-0.8 along the interface boundary suggests significant transport of angular momentum across the air- medium interface.

As the rotation speed is increased, it is observed that an initially narrow and weaker bubble rotates in the opposite direction is formed, being located at the bottom wall. Similar to the situation found in confined swirling flows undergoing vortex breakdown [11], this bubble is located along the axis of rotation. Figure 2c shows that while the main recirculation region remains relatively steady from Re=180 to 240, a widening of the *narrow* bubble can be observed.

The number of streamlines inside the bubble indicates an increase in the circulation of the fluid. In addition, the stagnation point of the bubble also moves upwards towards the air-medium interface.

Figure 3 shows the steady-state normalized dissolved oxygen (DO) concentration in the medium phase at three rotation speeds. The dissolved oxygen (DO) clearly shows a strong association of oxygen transport with the dominant counter-clockwise recirculation pattern of the flow. Figure 3a shows that the presence of DO at the base (where cell colonies are expected to congregate in practice) is convection-dependent. In contrast, transport via diffusion is much weaker, judging by the relatively low concentration of DO at the centre of the main recirculation region. At the lowest speed of 60 rpm, it is observed that the oxygen concentration is concentrated mainly along the axis of rotation. Note that the diffusivity of oxygen is approximately 500 times lower than the kinematic viscosity of water. The induced flows within the fluid medium clearly indicate the flow is driven by convection, hence the transport of oxygen will clearly be dominated by advection by the flow for the chosen operating condition range.

As the rotation speed is further increased resulting in the appearance of the axial bubble, there are two distinct regions of low DO concentration, which correspond to the respective centres of the main recirculation zone and the axial bubble (refer to Figure 3b). The streamfunction plot shown in Figure 2b suggests that oxygen from the interface is mainly convected within the medium along a narrow path with negligible diffusion across the streamlines. In contrast, Figure 3c shows an overall improvement in the oxygen diffusion to the two recirculation zones as Re is increased. However, the oxygen concentration within the second bubble remains lower as it is relatively weaker in comparison to the main recirculation region.

IV. Conclusions

In this study, computational modelling of the flow and oxygen transport in a small-scale stirred tank bioreactor that is driven by an aerial disk has been performed. The results show that oxygen diffusion across the air/culture-medium interface is enhanced by advection of the oxygen rich interface fluid by the induced secondary flow in the culture medium. In addition, this study has provided some insight into the development of the vortex breakdown bubble (commonly found in confined swirling flows) and the resulting effect of the presence of the bubble to the overall oxygen transport within the bioreactor chamber.

References

1. Martin I, Wendt D and Heberer M (2004) The role of bioreactors in tissue engineering. Trends Biotechnol. 22(2):80-86.
2. Martin Y, and Vermetter P (2005) Bioreactor for tissue mass culture: Design, characterization and recent advances. Biomaterials 26:7481-7503.
3. Chisti Y (2001) Hydrodynamic damage to animal cells. Crit. Rev. Biotechnol. 21:67-110.
4. Volkmer E, Drosse I, Otto S et al. (2008) Hypoxia in static and dynamic 3D culture systems for tissue engineering of bone. Tissue Eng Part A 14(8):1331-1340.
5. Koynov A, Tryggvason G, Khinast JG et al. (2007) Characterization of the localized hydrodynamic shear forces and dissolved oxygen distribution in sparged bioreactors. Biotechnol Bioeng. 97(2):317-331.
6. Dusting J, Sheridan, J. and Hourigan, K. (2006) A fluid dynamics approach to bioreactor design for cell and tissue culture. Biotechnology and Bioengineering 94(6), DOI: 10.1002/bit.20960.
7. Liow KYS, Tan BT, Thouas GA et al. (2008) CFD Modelling of the steady state momentum and oxygen transport in a bioreactor that is driven by an aerial disk. Modern Physics Letters (B) `in press'.
8. Liow KYS, Tan BT, Thompson MC et al. (2007) In silico characterization of the flow inside a novel bioreactor for cell and tissue culture. 16th Australasian Fluid Mechanics Conference, Gold Coast, Australia, 2007.
9. Thouas GA, Sheridan J, and Hourigan K (2007) A bioreactor model for mouse tumour progression. J. Biomed Biotech, Article ID 32754.
10. Thouas GA, Thompson MC, Contreras K et al. (2008) Combined improvement of oxygen diffusion and high-density cancer formation in a modified mini-bioreactor. In Proceedings of the 30th Annual International IEEE Engineering in Medicine and Biology Society Conference, Vancouver, British Columbia, Canada, 2008, pp 3586--3589.
11. Lopez JM (1989) Axisymmetric vortex breakdown in an enclosed cylinder flow. Lecture notes in physics. Springer, Berlin.

Author: Keith Liow
Institute: Monash University (Sunway campus)
Street: Jalan Lagoon Selatan
City: Selangor
Country: Malaysia
Email: keith.liow@eng.monash.edu.my