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Flow Modelling in Medium-Scale Stirred-Tank Bioreactors featuring Large Free Surface Deformations

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Abstract

The effect of impeller speed on the flow in an Eppendorf New Brunswick Scientific (NBS) 5 L stirred-tank reactor (STR) filled with 2.2 L of water is studied in this paper. The Computational Fluid Dynamics (CFD) model constructed for this study features a free surface model within a Large Eddy Simulation (LES), and is proven by comparison with Particle Image Velocimetry (PIV) to generate high-fidelity solutions to the free surface flow. Operation beyond 60 rpm is characterized by a sizeable central free surface depression that increases in size with increasing impeller speed. The CFD model has enabled 60 rpm and below to be identified as impeller speeds for which the free surface is essentially flat hence enabling it to be simplified to a rigid wall for efficient single-phase LES.

Introduction

Stirred-tank reactors (STRs) have a long history of successful use as bioreactors for fermentation of mammalian cell lines and other products, and are now being considered for human stem cell applications. Human stem cells are known to be sensitive to their chemomechanical environment, making the excellent mixing and chemical composition control that can be achieved in STRs particularly appealing for stem cell bioprocessing [1,2]. Mixing in STRs intense enough to also achieve suspension of microcarriers for rapid and scalable stem cell culture is also accompanied by shear stress imposition onto the cells, which can lead to cell damage and reduced viability [1]. Evidence from other bioreactor types furthermore shows that stresses at lower levels can result in unintended changes in cell function such as precursors to cell differentiation [3]. For reliable rapid undifferentiated stem cell expansion, stresses in STRs must be able to be realistically characterized, to ensure that mixing and stem cell suspension do not come at the expense of stress-induced reductions in stem cell viability and changes in stem cell behaviour.

Turbulent mixing prevails in most STRs, such that idealizations of the flow with simplified mathematical models are basically inadequate for quantifying stresses in them. As a result, the complexity associated with the Computational Fluid Dynamics (CFD) required to investigate stresses in STRs is substantial. Recent studies [4-6] have successfully used Large Eddy Simulation (LES) to resolve the key transient vortical flow structures, to better capture the spatiotemporal variability of the fluid microenvironment in which stem cells function in STRs. The validated LES models of the STR flows in those studies featured a single fluid phase for the liquid culture and a rigid free surface. In this manner, the computation essentially becomes one of single-phase CFD, enabling more efficient and reliable LES to simulate flow durations long enough for good turbulence statistics to be generated with commercial CFD software. [It is noted that Lagrangian particle tracking to model microcarriers and stem cells in suspension were also included in [4-5].] The rigid free surface assumption can only be valid if the free surface in an operating STR does not significantly deform away from horizontal. Even then, free surface flows have intricate mean flows, transients and turbulence asymptotics associated with the approach to the free surface from the liquid side as well as from the gas side.

As STR systems are upscaled, the ability of process control to achieve fine-scale real-time control over flow structure to ensure a flat free surface diminishes. As such, CFD modelling of STR flows would ideally progress to enable the interface kinematics and its interactions with STR bath dynamics to be captured as a natural part of the solution process. A first free surface LES model of the flow in a medium-scale STR used for stem cell culture was briefly presented in [6]. The current study presents the free surface LES model in more detail, and uses the model to study the effect of impeller speed on the free surface and the liquid bath flow. This study uses a 5 L Eppendorf New Brunswick Scientific (NBS) STR that contains 2.2 L of liquid, and is agitated by a three-blade 100 mm diameter pitched-blade impeller operating in upward-pumping mode – the same configuration as in [6]. That predecessor study only considered impeller operation at 60 rpm, which gave early confidence that the rigid free surface assumption was valid. Until now, the characteristics of the flow near the free surface have remained unknown, along with the sensitivity of the assumption's validity to small changes in STR configuration and operation (whether it be impeller speed, size or submersion, among other parameters). The current paper addresses those questions by considering impeller speeds up to 150 rpm. This is an operating speed that is well above normal levels for stem cell bioprocessing, but may be increasingly relevant into the future as it is used in a similar half-filled 5 L NBS STR systems by Rafiq et al [7] as part of a detachment protocol based on trypsinization and agitation.

Experimental Model

The NBS 5 L STR considered in the current work features a vessel that is cylindrical but with a rounded bottom edge, of 155 mm internal diameter. The 45° pitched-blade impeller is 100 mm in diameter and 73.5 mm in maximum vertical extent, with the three blades mounted onto a 20 mm radius shaft at impeller level, sweeping a horizontal arc of 95° and positioned with their bottom-most tips 44.5 mm above the bottom. Above impeller level, the impeller shaft is 9.5 mm in diameter. The only other internal geometry feature in the STR is a thin gas sparger ring that descends down near the STR wall but then makes a right angle and forms a ring underneath the impeller; the ring is closer to the bottom than the impeller blades.

In the experiment, the NBS 5 L STR is partially filled with 2.2 L of distilled water, to yield a quiescent free surface height of 132.5 mm above the bottom. The bath was then seeded with Solohill polystyrene microparticles to a concentration of 0.0007 mg/mL. Motion in the STR was driven by a New Brunswick Eppendorf direct drive agitation motor. Two different impeller speeds were experimentally investigated for the current study – 150 rpm, but also 60 rpm which was related to the scenario investigated in [6].

Particle Image Velocimetry (PIV) was the primary fluid flow diagnostic technique used in the experimental study; details of the laser system are provided in [6]. For the velocity vector maps, the analysis window size was 64×64 pixels, with 75 percent consecutive window overlap, and 64 instantaneous velocity fields were generated, sufficient for an ensemble-averaged velocity field representative of the fully developed flow in the STR. The instantaneous images also captured the free surface position, with subsequent image processing yielding free surface levels in the fully developed flow.

Mathematical Model

The ANSYS-CFX 14.5 package [8] is used in the work to simulate the flow within the STR. In stem cell bioprocessing, it is usual to prefer hypoxic condtions in culture as compared to normoxic conditions. The early bioprocess design associated with the related project work is relying on mass transfer at the free surface to maintain dissolved oxygen levels in the liquid bath. As a result, gas sparging is neglected in the current work. While the flow also features microcarrier particles as inclusions, they are sufficiently dilute when suspended, meaning Eulerian-Eulerian multiphase flow modelling in CFX does not require the use of the inhomogeneous model to represent interdispersed phases. The focus of the current work is on ensuring the flow model can accurately capture liquid bath stresses in this STR configuration that is upscaled relative to the spinner-flask studies of [4-5]. For this purpose, the Lagrangian particle tracking used to model microcarrier stem cells motions in [4-5] is neglected. The multiphase flow problem in the STR for the most part becomes a free surface flow problem, enabling the homogeneous free surface model in CFX to be used to track the free surface. In CFX, the homogeneous free surface model is a CFX version of a Volume-of-Fluid (VOF) method that fits within the algebraic VOF paradigm involving compressive differencing (as compared to geometric VOF).

For this highly turbulent flow, Reynolds-Averaged Navier-Stokes (RANS) approaches to turbulence modelling are generally unacceptable for generating predictions to stress exposures experienced by microcarriers, because of unacceptable smearing out of spatiotemporal fluctuations. Our work in generating simulation-based models of STR flows therefore is based on Large Eddy Simulation, which we have seen in previous single-phase CFD studies does a far better job of capturing transient vortical structures in the flow [4-6], and also enables turbulent dispersion to be captured without additional RANS-based models just for the dispersion. Such assertions of course only apply when good SGS modelling approaches are used within the LES. This study uses the LES-WALE SGS model [9], which was used with success previously in single-phase LES.

Results

Experimental results at 150 rpm

The use of an 150 rpm impeller speed results in the formation of a sizeable free surface depression around the impeller shaft. Figure 1 indeed shows the depression to descend in depth to below the upper impeller tip level. The depression overall appears symmetric from the camera angle used in this study, although lop-sidedness and occasional extremes in downward penetration can occur. These deviations from symmetry can be attributed to interactions of water-sided flow structures with free surface deformations associated with waves and sloshing.

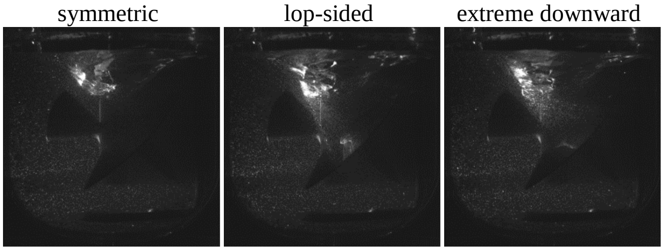


Figure 1. Free surface depressions caused by 150 rpm impeller speed through a vertical slice in the NBS 5 L STR.

Instantaneous snapshots of the velocity vector maps generated by the PIV setup show the flow to be highly turbulent. A relatively large vortex (roughly the impeller radius in size) is a consistent recurrence in the PIV images, but other than that the flow structure is highly variable and rarely consistent between frames. [Such fluctuations in flow structure between time-frames are smeared out by the time-averaging associated with RANS turbulence modelling, making these results an early justification for consideration of time-resolving LES from the outset.]

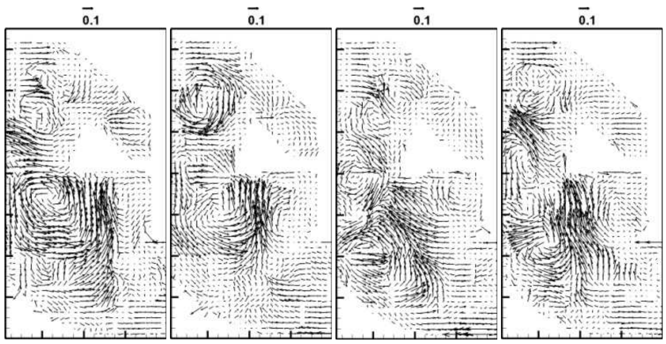


Figure 2. Sample instantaneous velocity vector maps in the vertical laser light sheet, as measured experimentally by PIV.

Free surface flow LES at 150 rpm

For 150 rpm operation, using the CFX combination of centered differencing for momentum advection, LES-WALE for SGS turbulence capturing and the homogeneous model for free surface capturing was unable to preserve stability. As such, the current study proceeded with the use of a high-resolution scheme for momentum advection instead. A grid dependence study of this model configuration was performed using multiple meshes – a coarse mesh based on a body mesh cell spacing in the liquid bath of 6 mm and featuring of 266,613 modes, a medium mesh based on 4 mm body mesh spacing and featuring 871,456 nodes, and a fine mesh based on a 2 mm body spacing and featuring 1,600,281 nodes. Simulations on all meshes were started from a previous common solution associated with steady-state STR operation. Figure 3 shows the grid dependence of the velocity vector maps at the same instant after restart. The medium and fine mesh solutions capture vortical flow structure detail that is qualitatively realistic relative to Figure 2 (based on the count, size and circulation directions of the larger vortices). In contrast, the coarse mesh solution spuriously smears out the detail into one very large vortex. Figure 4 shows that higher resolution is able to sustain the capturing of the vortical flow structure detail.

With regards to CFD model numerics, it is noted that the velocity magnitudes in the instantaneous fine-mesh CFD frame in Figure 3 are generally larger than those in the PIV frames. While the exact cause of this difference has not been pinpointed as yet, Figure 4 does show that velocities at different instants can be smaller when more vortices prevail in the flow. The velocity vectors maps in Figure 3 and 4 are currently best used for qualitative comparison of flow structure. A single-phase adaptation of the current 150 rpm model is being pursued, which will enable LES-WALE to be used with centered differencing; this combination was seen at 60 rpm in [6] to result in better capturing of smaller-scale turbulent flow structure, hence also better quantitative comparisons between CFD and PIV.

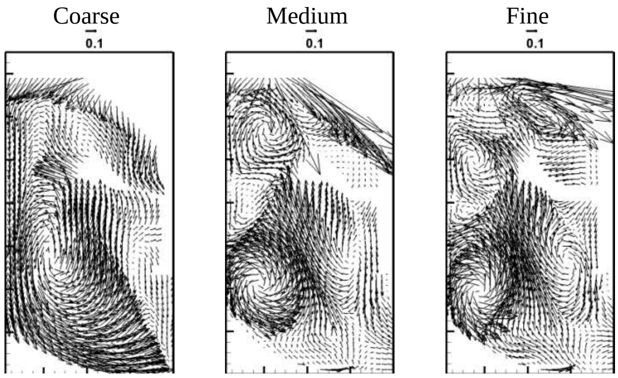


Figure 3. Comparison of free surface flow LES CFD frames at t = 0.89 s of steady-state operation, for different mesh resolutions. [The vectors near the free surface associated with the air-sided flow have not been filtered out.]

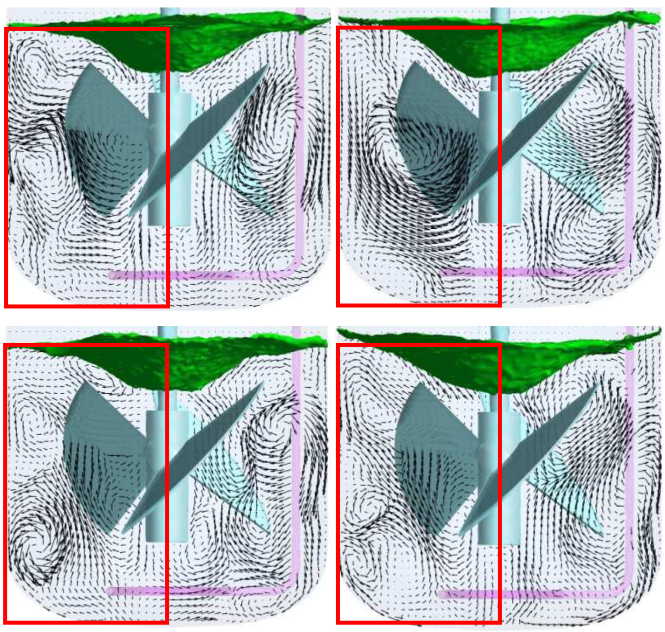


Figure 4. Sample instantaneous free surface profiles and velocity vector maps in the laser light sheet, as simulated by the CFD model. The red box represents the area of the flow overlapped by the PIV window.

Figure 4 also shows the corresponding free surface solution as represented by the $C = 0.5$ for the fractional-volume color function. The capturing of the free surface depression is realistic, and the quantitative correspondence shown in Table 1 that shows the depression depth to be predicted to within experimental uncertainty is a useful validation result. Exploring the free surface capturing by the LES model in more detail, Figure 5 at the same time instant shows the lower reaches of the free surface depression to be slightly more parabolic in shape in the fine mesh result (as compared to those for the medium and coarse meshes). This indicates that mesh resolution is important in resolving the near-free-surface turbulent flow energetics and free surface jump conditions, and contributes to the free surface representation in the fine-mesh solution having better correspondence with the profiles in Figure 1. This also makes the free surface position more sensitive to local flow conditions, thus explaining the larger standard deviation associated with the mean free surface depression level in Table 1.

Coarse mesh CFD	**Medium mesh CFD**	**Fine mesh CFD**	**Experiment**
20.71 ± 0.82	20.6 ±1.13	20.44 ±1.56	19.40 ±1.46

Table 1. Predictions of maximum free surface depression depth from the free surface flow LES CFD model of NBS 5 L STR, and comparison with experiment. The levels in the table are reported relative to the quiescent free surface level, and are in mm.

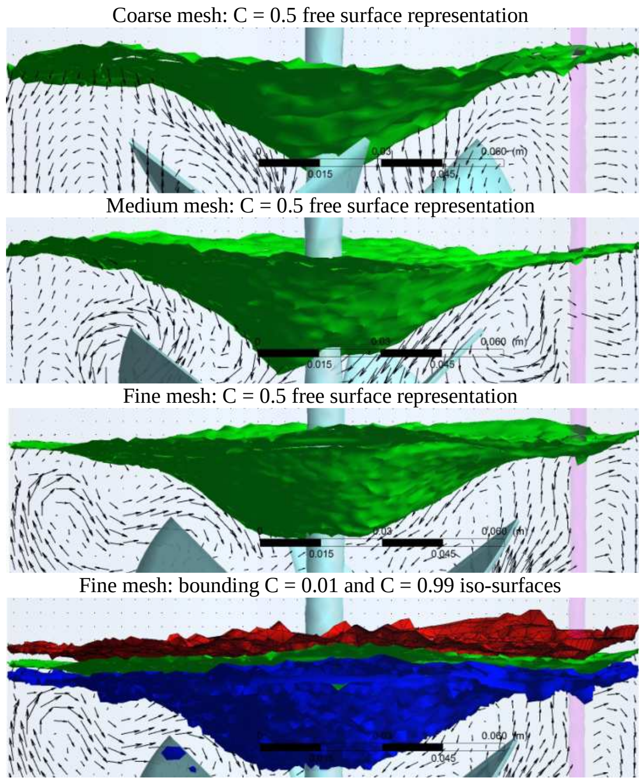
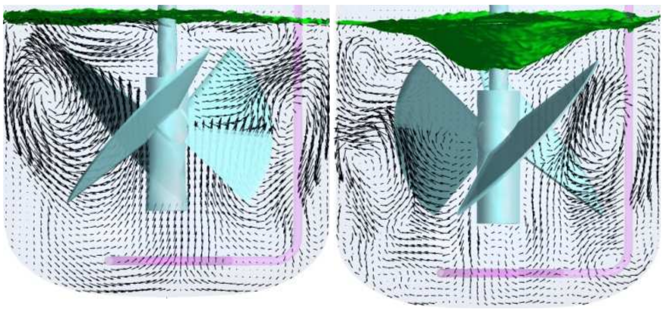


Figure 5. Grid dependence of sample instantaneous free surface profiles and velocity vector maps in the laser light sheet, as simulated by the CFD model: close-ups at t = 0.62 s. Bottom frame shows the smeared interface resulting from the use of algebraic VOF for interface tracking by the software.

The CFD uncertainties in the free surface depression levels in Table 1 are standard deviations over screen grabs during steady-state operation. The bottom panel of Figure 5 shows the interface smearing associated with the algebraic VOF treatment for the fine-mesh solution. This is somewhat equivalent to a measurement uncertainty associated with physical experiments, but is generally smaller than the standard deviation if the interface stays compact. Averaging of the free surface level was only done in the 0.5 s $\leq$ t $\leq$ 1.0 s interval because instability issues arose in the fine-mesh simulation at some stage after t = 1.0 s, with one of the early symptoms of the instability being interface smearing well beyond that shown in the bottom panel of Figure 5. In such a case, the measurement uncertainty is far larger than the uncertainty in the free surface location as computed by the standard deviation in the C = 0.5 isosurface (where C is the fractional volume indicator for the phase representation). A repeat simulation using a smaller time-step is planned in future work, but also noting the effect of smaller time-step on the feasibility of free surface flow LES for ergodic turbulent flow statistics. Computations for such statistics require long-time simulations on meshes that resolve the vortical structure of the flow.

Effect of impeller speed

The need for good mesh resolution and small timesteps motivates simplification of the STR liquid bath flow problem to single-phase using a rigid wall to model the free surface. Figure 6 compares the simulated free surface shapes at 60 and 150 rpm. The 60 rpm experiments associated with [6] confirm that there is no notable free surface depression in this case, and confirms the observation that the flat rigid free surface assumption is reasonable at 60 rpm but not reasonable at 150 rpm operation.



60 rpm 150 rpm

Figure 6. Sample instantaneous free surface shapes at 60 and 150 rpm.

To determine the range of validity for the flat rigid free surface simplification of the STR bath flow problem to single-phase LES, simulations were run using the medium mesh at impeller speeds of 60, 90, 120 and 150 rpm. Table 2 shows the free surface LES model to predict the abrupt appearance of the depression at an impeller speed somewhere between 60 rpm and 90 rpm, with the depth of the depression then tapering off as impeller speed rises above 90 rpm.

60 rpm	90 rpm	120 rpm	150 rpm
0*	10.50	12.67	20.6

Table 2. Predictions of maximum free surface depression depth from the free surface flow LES CFD model of NBS 5 L STR, as a function of impeller speed. [* on 60 rpm case denotes a lack of free surface depression.]

The main consequences of the Table 2 results are most relevant to the computational investigation procedure, in demonstrating the utility of the free surface LES model for due diligence in CFD, before using highly-resolved single-phase LES in earnest to generate turbulence statistics and predict stress exposure of stem cells in culture.

Conclusions

This paper shows that a free surface LES model based on available higher-end ANSYS-CFX options can quantitatively predict free surface shape and realistically predict flow structure in a highly agitated STR quite well. The model is predictive enough to enable the validity of the flat rigid free surface simplification to be assessed. Further work will consider the manner in which the free surface LES model results database can be interrogated to enable a rigid-wall model of the free surface depression to be imposed, for single-phase LES of high-rpm STR operation.

Acknowledgments

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