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A Study of the Coriolis Effect on the Fluid Flow Profile in a Centrifugal Bioreactor

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Abstract

Increasing demand for tissues, proteins, and antibodies derived from cell culture is necessitating the development and implementation of high cell density bioreactors. A system for studying high density culture is the centrifugal bioreactor (CCBR) which retains cells by increasing settling velocities through system rotation, thereby eliminating diffusional limitations associated with mechanical cell retention devices. This paper focuses on the fluid mechanics of the CCB system by considering Coriolis effects. Such considerations for centrifugal bioprocessing have heretofore been ignored; therefore a simpler analysis of an empty chamber will be performed. Comparisons are made between numerical simulations and bromophenol blue dye injection experiments. For the non-rotating bioreactor with an inlet velocity of 4.3 cm/s, both the numerical and experimental results show the formation of a teardrop shaped plume of dye following streamlines through the reactor. However, as the reactor is rotated the simulation predicts the development of vortices and a flow profile dominated by Coriolis forces resulting in the majority of flow up the leading wall of the reactor as dye initially enters the chamber, results confirmed by experimental observations. As the reactor continues to fill with dye, the simulation predicts dye movement up both walls while experimental observations show the reactor fills with dye from the exit to the inlet. Differences between the simulation and experimental observations can be explained by excessive diffusion required for simulation convergence, and a slight density difference between dyed and un-dyed solutions. Implications of the results on practical bioreactor use are also discussed.

Keywords

Coriolis effect; Centrifugal Bioreactor; High Density; Numerical Simulation

1. Introduction

The demand for recombinant proteins, specifically monoclonal antibodies (mAbs), has risen dramatically due to the increased requirement for both therapeutic and diagnostic applications (1–3). Traditional methods of mAb production are incapable of meeting production needs exceeding hundreds of kilograms per year. As such, two approaches to obtain higher mAb yields have been proposed: i) increasing cellular productivity, and ii) increasing volumetric productivity (3). The former involves modification of the growth environment, cellular genome, or other such approach; while increasing volumetric productivity can be achieved through increasing bioreactor cell density.

Traditional cultures of mammalian cells, such as batch and continuous stirred-tank reactors are limited to densities on the order of 10^6 cells/mL, and can reach as high as 10^7 cells/mL if

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cells are separated from spent medium and returned to the reactor (4–8). Further increases in cellular density necessitate cell immobilization to prevent wash-out as medium throughput must also be increased. Packed bed bioreactors (PBR) and hollow fiber bioreactors (HFB) both immobilize cells with the use of porous packing or membranes, increasing cellular densities to 10^8 cells/mL while maintaining cell viability (9–12). However, these reactors are commonly plagued by heterogeneous product formation and inactive portions of the biomass due to membrane fouling, mass transport limitations, and non-uniform gradients of inhibitors and nutrients (11, 13, 14).

Another type of bioreactor capable of maintaining cell densities above 10^8 cells/mL is the continuous centrifugal bioreactor (CCBR). The CCBR has been researched by Van Wie *et al.* (1991) and is similar to the Beckman Elutriation System and a centrifugal bioreactor patented by Kinetic Biosystems, Inc., with applications in remediation processes through immobilized bacterial culture (15–17). During operation the CCBR spins in the horizontal plane while medium is supplied to the rotating reactor at the point furthest from the center of rotation, as shown in Fig. 1, through flexible tubing and the use of an anti-twister mechanism (18). Limitations associated with other high density bioreactors are circumvented through the use of non-mechanical cell immobilization. Within the CCBR cells are retained by balancing centrifugal forces with opposing buoyancy and drag forces. Consequently, diffusional limitations are eliminated as medium must flow around each cell to result in a drag force opposite the centrifugal force, thus providing convective transport of nutrients and wastes to and from cells. This results in a uniform environment which can be controlled with dilution rate allowing maintenance of optimal growth conditions for each individual cell during culture.

Of particular interest in this paper is the unique fluid flow profile within the CCBR which results from the Coriolis effect on momentum transport due to reactor rotation. Rotating systems ranging from cooling passages in modern turbine blades to rotating wall bioreactors, rotating pipes and fluidized beds all have a unique fluid flow profile due to Coriolis forces (19–22). The Coriolis effect is the result of a fictitious force acting at right angles to the axis of rotation and the local velocity vector, the force is termed fictitious as it is a deflecting force which does no work on a material element (23). The change in motion attributed to the Coriolis force is opposite the direction of motion, thus a system rotating anticlockwise will produce a Coriolis force to the right in an attempt to restore a material element to its original position upon the projected path of a circle, completed with the same frequency as that of system rotation (23). This paper will compare experimental observations with numerical simulation results for a CCBR operating at conditions typical of those for cell culture to understand the contribution of Coriolis forces to the CCBR's fluid flow profile. The implications of this modeling effort in the CCBR without cells will be discussed in addition to the framework for future more complex studies including various cell types and population densities.

2. Theory

The phenomenon examined in this paper is the laminar flow profile in the plane normal to the axis of rotation in a conical shaped CCBR. Solid body rotation where motion is referenced to axes rotating along with the bulk fluid gives rise to Coriolis and centrifugal forces, both of which must be assumed to act on the fluid (23). The three dimensional Navier-Stokes equation for incompressible fluid flow in a system rotating about a vertical axis is shown in Eq. 1:

$$\frac{\partial u}{\partial t} + u \cdot \nabla u = -\frac{1}{\rho} \nabla P - 2\Omega \times u + \mu \nabla^2 u \quad (1)$$

where u is the velocity field vector, ρ is the density, Ω is the angular velocity of the system, μ is the viscosity of the fluid, and P is the modified pressure accounting for both gravitational and centrifugal forces, as shown in Eq. 2:

$$P \equiv p - \frac{1}{2} \rho \Omega^2 r^2 - \rho g z \quad (2)$$

where p is the background pressure field, r is the distance from the axis of rotation, and z is distance in the opposite direction to the earth's gravitational field (20, 23, 24). Of particular interest in the application of the CCBP is the Coriolis term, $-2\Omega \times u$, and how this acceleration is translated into a force which drives fluid flow within the CCBP during operation.

Coriolis forces result from a combination of fluid velocity and the angular velocity of the system, resulting in the object following a curved path as viewed from the rotating reference frame. This paper will examine a CCBP void of cells showing how Coriolis forces deflect the flow profile of a stationary system and results in a unique fluid flow profile that will need to be considered when working with high cell density reactor cultures.

3. Materials and Methods

3.1 Solution Preparation

Two liters of pH 7.0 buffer solution were made through combination of 1 L of 100 mM potassium dihydrogen phosphate, 582 mL of 100 mM sodium hydroxide, and 418 mL of deionized water (25). This solution was split in half and Bromophenyl Blue (BPB) was added to one of the solutions to a final concentration of 3×10^{-5} M providing a dark contrast solution with which the flow profile within the CCBP chamber could be visualized.

3.2 Visualization of Flow Profile

The equipment used for this research was a COBE® Spectra™ Apheresis System (Gambro BCT Inc., Lakewood, CO) manufactured for use as a blood separation device. The platelet elutriation chamber supplied as part of the Cell Purging tubing set (Gambro BCT Inc., Lakewood, CO) was replaced with a similar smooth-walled chamber cut out of a rectangular piece of acrylic. The acrylic block has two threaded bolt holes on either side of the chamber for attachment to the rotor. During operation fluid supply and removal is through flexible tubing and the use of an anti-twister mechanism previously described by Van Wie *et al.* (15, 18).

Prior to starting reactor rotation, one inlet line was filled with the BPB solution to the point where the two inlet lines converge just before entering the reactor, as shown in Figure 1, while the other inlet line and the entire volume of the reactor were filled with the colorless pH buffered solution. Reactor rotation and fluid flow were started and steady state was reached after about one minute, after which BPB solution was delivered to the reactor at a set inlet flow velocity. Flow profiles were visualized using a variable frequency strobe light and recorded using a Cannon Digital Rebel XT camera (Cannon U.S.A., Inc., Lake Success, NY).

3.3 Simulation Simplification and Setup

The 3D geometry needed for mathematical simulation was created in COMSOL 3.3 by drawing one half of the 2D projection in the x–y plane, Fig. 2 (inset). The 2D figure has corresponding radii and heights to that indicated in Fig. 2. This 2D drawing was then rotated 360 degrees about the reactor midline to create a 3D representation of the conical reactor in COMSOL.

The COMSOL physics used for modeling fluid flow was that of the Incompressible Navier-Stokes approach applied to 3D geometry. Within the subdomain settings of this fluid flow physics, the density and dynamic viscosity were set to that of water at 25 °C assuming these properties were not appreciably changed by the dilute salt solution. The Coriolis forces $F_x = -2\Omega\rho u_y$ and $F_y = 2\Omega\rho u_x$ were added into the sub-domain settings panel as volume forces resulting from the Navier-Stokes Equation for a rotating frame of reference, Eq. 1; density was added to F_x and F_y to coincide with the format of the conservation equation used by COMSOL. All boundary conditions were specified as indicated in Table 1. The inlet boundary condition varied between 0 – 16 cm/s and was specified based on inlet volumetric flow rates and cross-sectional areas used in CCBP practice. A pressure of zero was specified at the outlet boundary which specifies fluid outflow is determined by the influx of material and is particularly appropriate when the outflow may not be normal to the boundary. An example of a mesh configuration used in the Navier-Stokes simulation is shown in Fig. 2 and consists of 86,037 total nodes. The fluid flow simulation was solved for inlet velocities of 0–16 cm/s and RPM in the 0–1200 range. To represent experimental procedures, a transient convection and diffusion mass balance was added to the 3D geometry in COMSOL.

The convection and diffusion portion of the numerical model was coupled to the steady state Navier-Stokes solution and was used to represent the transient flow of dye at steady state through the reactor as was done in experimental procedures. The fluid flow and mass balance simulations were solved independently. First, the Navier-Stokes steady state solution was found, and the resultant fluid flow profile was then coupled to the convection diffusion module by equating the velocity variables of the two modules. The convection and diffusion mass balance was then solved, resulting in mass convection along steady state fluid flow streamlines. Finally, before solving the mass balance an isotropic diffusion coefficient of $5 \times 10^{-10} \text{ m}^2/\text{s}$ was specified for BPB as reported by Kakizak *et al.* (26). The boundary conditions for the convection and diffusion physics were defined as stated in Table 1. A constant concentration of BPB was specified at the inlet while a convective flux condition was assumed at the outlet.

The convective flux boundary condition assumes all mass passing through this boundary is due to convection, thereby eliminating transport due to diffusion. A Peclet number is determined by using an outlet velocity of 4.3 cm/s, an outlet diameter of 0.13 cm as the characteristic length, and the diffusion coefficient specified above. The Peclet number value thereby determined is 1×10^5 indicating that mass transport due to convection is much larger than that due to diffusion, which supports the use of the convective flux boundary condition at the outlet. This boundary condition is particularly useful when an outlet concentration is unknown.

As shown in Table 1 the mesh used in the simulation of the convection and diffusional mass balance consists of more nodes (431,402) than that used in the solution of the Navier-Stokes equation because there are fewer independent variables resulting in a less complex system of equations. The transient mass balance has an initial condition of zero BPB throughout the reactor and at time zero a constant concentration of BPB is allowed to enter the reactor and

travel along Navier-Stokes solution streamlines. This provided a numerical representation for comparison with experimental observations.

4. Results and Discussion

4.1 Fluid Flow Simulation

Streamlines depicting the steady-state solution of fluid flow through the CCBP at a constant inlet velocity of 4.3 cm/s and various rates of rotation are shown in Fig. 3 and 4. Streamlines depicting the numerical results of the fluid flow are viewed along the z-axis, the axis parallel to that of rotation; all streamlines throughout 3D geometry are visible although the perspective directly along the z-axis gives the appearance of only a single plane. Shown in Fig. 3 is the effect of Coriolis forces on flow through the reactor as rotor speed is increased. However, once a flow pattern dominated by Coriolis forces is established further increasing the RPM has little effect on the overall flow profile, as shown in Fig. 4.

The importance of Coriolis forces on a fluid flow profile within a system can be estimated through evaluation of the dimensionless Rossby number. The Rossby number is the ratio of the magnitudes of inertial forces to Coriolis forces and is defined as $U/L\Omega$; where U is a representative velocity, L is the unit length over which velocity varies, and Ω is the angular velocity (23). Coriolis forces are likely to have minimal effect when the Rossby number is much greater than unity, however Coriolis forces will contribute significantly to a system's flow pattern when the Rossby number is much less than one. Taking an inlet velocity of 4.3 cm/s and assuming uniform flow across the reactor cross-section a velocity of 9.4×10^{-3} cm/s will result at the widest point in the reactor which has a diameter of 2.73 cm. Using the diameter as the unit length scale the Rossby number will be 3×10^{-4} and 5×10^{-5} for 100 and 600 RPM, respectively; indicating Coriolis forces will have a significant role in determining the overall fluid flow profile in the CCBP.

As seen in the 0 RPM case, Fig. 3, the resulting profile shows laminar streamlines moving from the inlet to the outlet of the reactor as would be expected for fluid passing through an object with diverging walls. Streamlines show two distinct vortices just past the inlet, shown with arrows in Fig. 3 (0 RPM), which are actually part of a torus-shaped vortex ring around the central axis and the inlet stream. This vortex is the result of a laminar jet of fluid entering the reactor with a velocity of 4.3 cm/s which results in a Reynolds number of 54 within the inlet tube leading to the reactor. The vortex indicating the presence of the laminar jet is first observed at an inlet velocity of 3.8 cm/s in numerical simulations. Increasing the fluid velocity to 10 cm/s will result in a jet of fluid passing directly through the reactor and a vortex spanning its entire length, Reynolds numbers for each inlet velocity are 48 and 127, respectively.

Shown in the center pane in Fig. 3 is the steady state solution for 10 RPM. At this low RPM the effects of Coriolis forces on the flow profile can be visualized. Deflection of streamlines to the left can be attributed to Coriolis forces by confirming the direction of the force for clockwise rotation and fluid flow toward the axis of rotation, in which case the right-hand rule indicates fluid movement will be deflected to the left. At 10 RPM the streamlines appear to be deflected toward the left wall of the reactor as the walls diverge. At the widest point in the reactor the streamlines predict fluid movement from the left to the right before exiting the reactor. The Rossby number for an inlet velocity of 4.3 cm/s and 10 RPM is 3×10^{-3} when calculated as before. Observing the results at this low RPM indicate Coriolis forces are not yet dominating the flow pattern and inertial forces still contribute to the observed flow profile.

Increasing system rotation to 100 RPM streamlines indicate that immediately after entering the reactor fluid is forced to the left wall, as a result of the increased magnitude of the Coriolis force. Fluid movement in the region of the reactor with the largest cross-section moves in a continuous path from the left wall to the right wall of the reactor along a skewed “N” shaped path before flowing out of the reactor. Finally, although there is minimal flow directly above the inlet as Coriolis forces have caused the majority of flow along the left wall of the reactor, at both 10 and 100 RPM a slowly rotating vortex is predicted in this region as indicated with the arrow.

The development of vortices at the widest cross-section of the reactor becomes apparent when comparing the results of 100 RPM with the higher RPMs shown in Fig. 4. The flow profile remains largely unchanged as RPM increases from 100 to 300; fluid enters the reactor, flows up the leading wall, transverses the reactor to the trailing wall at the widest cross-section, before flowing out of the chamber. This overall flow profile remains largely unchanged as system RPM is increased even further, as shown in Fig. 4. The major change in the flow profile as RPM is increased is the vortices shown with arrows in Fig. 4 become progressively more well defined.

Initially, at low RPM the vortices are not clearly defined, but their development can be seen as RPM is increased from 100 to 600. As suggested by Batchelor (1967) because the Coriolis force is linear in velocity and tends to change the direction of all velocity components at the same rate; an element whose motion is dominated by the Coriolis force moves in a circular path where the projected circle is completed with a frequency of the same order as that of system rotation (23). Therefore, as the system rotation frequency is increased, material elements complete their circular path faster adding definition to the vortices.

A similar situation occurs when system rotation is held constant and inlet velocity is varied. Simulations show that as inlet flow velocity is varied from 1.1 cm/s to 16.4 cm/s, inlet tube Reynolds number varies between 14 and 220, and the resultant flow profile differs very little from that that simulated in Fig. 4 (600 RPM). In the previous discussion decreasing rotation rate from 600 to 10 showed a marked change in the overall flow profile with respect to RPM, and a similar effect would be expected for fluid velocity as both RPM and fluid velocity are linear with respect to Coriolis force magnitude. However, decreasing fluid velocity at a constant rotation rate enhances the importance of Coriolis forces due a reduction of the Rossby number. Therefore, to increase the importance of inertial forces compared to Coriolis forces the Rossby number must be increased. A Rossby number near unity would diminish the effect of Coriolis forces, however it would require an inlet velocity on the order of 10^5 cm/s, in turn resulting in a turbulent system and invalidate the results presented in this paper. Consequently a change in fluid velocity over the range of possible operating conditions is not expected to have an effect on the observed fluid flow pattern within the CCBR.

4.2 Simulation Convergence

Convergence of the numerical solution was verified in the following ways: According to a theorem by Baker (1983), the error in a finite element solution converges to zero, as the number of elements increases without limit (27). Therefore, a solution obtained using an infinite number of nodes corresponds to the real solution, which can be found by extrapolation from a set of solutions using a large, but finite number of elements. This extrapolation is carried out at a single point in the domain by plotting the velocity against the inverse of the number of elements in several simulations with increasing numbers of elements. Then the y-intercept of this plot is found as the reciprocal of the number of elements goes to zero and is assumed to be the solution for an infinite number of elements

corresponding to the real solution. The difference between the projected solution and a given simulation solution is then assumed to be a measure of error at that point, and the error of the entire solution can be estimated by summing the error over multiple points chosen throughout the domain.

The formula for the normalized error of a particular solution with respect to the projected solution is given in Eq. 3,

$$\varepsilon_s = \frac{\sum_{i=1}^I \sqrt{\Delta U_i \cdot \Delta U_i}}{\sum_{i=1}^I \sqrt{U_{i,P} \cdot U_{i,P}}} = \frac{\sum_{i=1}^I \sqrt{(u_{i,P} - u_{i,S})^2 + (v_{i,P} - v_{i,S})^2 + (w_{i,P} - w_{i,S})^2}}{\sum_{i=1}^I \sqrt{u_{i,P}^2 + v_{i,P}^2 + w_{i,P}^2}} \quad (3)$$

where U is the velocity vector and ΔU is the difference between the velocity vector at the projected solution P and a simulation solution S , u , v and w are the individual velocity components in the x , y and z direction respectively, i represents an individual point, and I is the total number of discrete points within the domain for which the velocity vector is evaluated. The coordinates of the discrete points are independent of the computational mesh.

The projected solution for this system was found at 2,347 individual points using a linear regression to find the y -intercept. The velocity profile for CCBP settings of 600 RPM and 4.3 cm/s was solved using a total of ten different meshes with the number of nodes ranging from 786 to 86,000; where the y -intercept was estimated using the 5 highest mesh densities. Using this method the percent error in the solution was reduced from 38% using 786 nodes to 2.9% when 86,000 nodes were used. Following the same procedure for the non-rotating case and an inlet velocity of 4.3 cm/s yielded a similar result as percent error was reduced from 23% at 786 nodes to 1.6% at 86,000 nodes. The error associated with 44,500 nodes was 3.9% and 1.6% in the rotating and non-rotating case, respectively, and it can be seen that increasing the number of nodes above 44,500 did not substantially alter the numerical solution.

4.3 Experimental Comparisons

Coupling a transient convection and diffusion mass balance to the steady state Navier-Stokes simulation allows direct comparison with the transient dye injection experimental results. This comparison was initially made for the case of the non-rotating system; numerical and experimental results are shown 15 seconds after initial dye injection in Fig. 5A and Fig. 5B, respectively. Numerical results depict concentrations of dye at the middle of the reactor, a single x - y plane at $z=0$ is shown. Concentrations are reflected as a grayscale gradient from white to black corresponding to low (0 mol/m^3) and high (0.03 mol/m^3) concentrations of BPB dye. The gradient spans -1×10^{-4} to 0.03 mol/m^3 in Fig. 5 which includes non-real negative concentrations and concentrations greater than that of the inlet that result from numerical noise at individual nodes within the global mesh as the simulation attempts to close the mass balance.

In the comparison at 0 RPM it can be seen that the numerical solution, Fig. 5(A), shows that as dye enters the reactor little dispersions just past the inlet is observed as the dye is entering as a fluid jet, as previously discussed for an inlet velocity of 4.3 cm/s. Both the numerical, Fig. 5(A) and experimental observations, Fig. 5(B), indicate a narrow stream of dye entering the reactor before the dye cloud expands radially through momentum transport as the walls of the reactor diverge. However, neither dye injection result shows the presence of the

vortex created by the fluid jet. Under current conditions visualization of this vortex would be difficult as the amount of dye entering the vortex would not provide sufficient contrast until it appeared as dispersion due to the nature of the transient dye injection experiment. At later time points (not shown) dye follows steady state streamlines, Fig. 3(A), expanding radially until reaching the widest cross-section of the reactor then converging on the reactor exit, an observation consistent between both the numerical and experimental results.

During typical hybridoma culture, or other cultures with similarly sized cells, the CCBP operating conditions are set at a rotor speed of 600 RPM and an inlet velocity of 4.3 cm/s. These conditions can provide a cell density near 1×10^8 cells/mL; which can be maintained for weeks with adequate nutrient supply (15). Increasing rotor RPM at constant inlet velocity leads to a new equilibrium between centrifugal force and drag force which increases the overall culture density. However, as previously discussed, the overall flow profile remains largely unchanged within the standard cell culture operating conditions of 300–1200 RPM and 4.3 – 16.4 cm/s. Therefore, a single operating condition of 600 RPM and an inlet velocity 4.3 cm/s will be used for comparison of experimental results with the numerical simulation as the system rotates.

Results at 600 RPM and an inlet fluid velocity of 4.3 cm/s for the numerical solution of the transient dye injection experiment are shown in Fig. 6. The simulated solution at 40 seconds shows dye enters the reactor and flows up the leading wall along streamlines depicted in Fig. 4 (600 RPM). At 70 seconds dye has reached the top of the reactor and follows streamlines around the vortex indicated with the upper arrow in Fig. 4 (600RPM), forcing dye back toward the inlet and the right wall of the reactor. Additionally at this time point dye is observed to the right of the reactor midline, just past the inlet, as diffusion has caused dye to enter streamlines moving toward the right wall of the reactor. At the final time point of 150 seconds, Fig. 6 (150s), the simulation indicates high concentrations of dye throughout the reactor save for the area containing the two vortices indicated with arrows in Fig. 4 (600 RPM). Additionally, dye that has reached the top of the reactor by 70 seconds is not directly entering streamlines exiting the reactor; instead a high concentration front has formed against streamlines moving along the right wall resulting in dye being pulled around the vortices shown in Fig. 4 (600 RPM).

Comparisons of numerical results with experimental observations exhibit similarities and differences. Experimental observations in Fig. 7 (40s) coincide with the simulation at 40 seconds, showing the inlet dye stream moving up the left wall of the reactor. Similarly at 70 seconds experimental results show dye continues to enter the reactor and flow up the left wall as a thin stream. However, rather than follow simulated streamlines around vortices as predicted in the numerical simulation, experimental results show dye completely filling the top portion of the reactor which continues until the entire reactor is dyed, as seen at 150 seconds, Fig. 7 (150s). At 150 seconds the majority of the reactor is colored indicating high concentrations of BPB throughout except for a small area along the right wall near the inlet. This small un-dyed area near the inlet does not persist and is just the last portion of the reactor to be dyed during experiments, making it unlikely dye is excluded from this region as the result of a vortex not seen in the simulation.

The most notable difference between the simulation and experimental observations is that the simulation predicts diffusion of dye away from the stream moving up the left wall of the reactor. In the simulation this diffusion results in dye moving up both walls, where as experimentally a sharp interface between the dyed and undyed fluid along the left wall is observed throughout the experiment. The sharp interface implies minimal diffusion during experimentation and that diffusion is over predicted in the simulation. As stated earlier the diffusion coefficient for BPB is small, 5×10^{-10} m²/s, however, COMSOL was unable to

find a solution without the use of artificial diffusion in the transient mass balance portion of the simulation. Isotropic artificial diffusion which increases diffusion near steep gradients, which are shown experimentally as the sharp dyed interfaces, was then used in the simulation making convergence possible.

COMSOL defines isotropic artificial diffusion, D_{art} , as

$$D_{art} = \delta_{ID} h |\beta| \quad (4)$$

where δ_{ID} is the isotropic diffusion tuning parameter, h is the local mesh size, and β is the magnitude of the convective velocity which can be found using the sum of the squares of the x , y , and z velocity components. The general transient mass balance on the species is then changed from its general form in Eq. 5 to include the artificial diffusion term as shown in Eq. 6

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = R - u \cdot \nabla c \quad (5)$$

$$\frac{\partial c}{\partial t} + \nabla \cdot (-(D + D_{art}) \nabla c) = R - u \cdot \nabla c \quad (6)$$

where D is the original diffusion within the system, c is concentration, R is a generation term, and u is the velocity vector. From Eq. 4 it can be seen that the amount of added artificial diffusion will not be globally uniform, but instead is locally dependant on the mesh size and convective velocity at a particular node in the system. As a result, COMSOL adds artificial diffusion only when needed to reach a solution. Using a tuning parameter of 0.1, the lowest value resulting in a simulation solution, the maximum artificial diffusion is on the order of $10^{-7} \text{ m}^2/\text{sec}$ which is needed primarily along the left wall of the reactor immediately past the inlet. Throughout the remainder of the reactor a minimum artificial diffusion of at least $10^{-8} \text{ m}^2/\text{sec}$ is needed. In either case the diffusion in the system is increased by multiple orders of magnitude over that of BPB alone. Thus increased diffusion due to artificial diffusion is likely leading to the presence of BPB along the right wall of the reactor which is not seen experimentally.

When comparing numerical and experimental results of dye movement through the reactor an important consideration is that simulation results show a single x - y plane at $z=0$ (middle of the reactor) and experimental observations are viewed along the z axis through the thickness of the reactor. Although only similar viewpoints can be shown results should be comparable according to the Taylor-Proudman theorem. For very small values of the Rossby number, the Taylor-Proudman theorem specifies that the derivatives of velocity in the axial direction become small, and thus particle velocity becomes 2D in the plane perpendicular to the axis of rotation (23, 28, 29). Therefore, all x - y planes independent of position along the z -axis should have the same concentration profile. Numerical results support the Taylor-Proudman theorem and upon viewing multiple planes along the z -axis the same concentration profile is observed, data not shown. Therefore, the full thickness view of the reactor in experimental results is likely not contributing to discrepancies with the numerical simulation results.

Discrepancies observed between the numerical solution and experimental observations are likely attributed to the following reasons. First, diffusion of dye within the mass balance simulation is several orders of magnitude larger than the actual diffusion of BPB due to artificial diffusion required for simulation convergence. Diffusion of dye across the reactor midline is seen in the simulation initially at 40 seconds and is far more apparent at later time points resulting dye flowing up the right wall of the reactor, whereas experimental observations show no diffusion of dye away from the inlet stream. It may be possible to eliminate artificial diffusion by increasing the number of mesh nodes within the 3D representation of the CCBR. However, this is not possible at the current time due to simulation complexity and current computer hardware limitations, as the number of nodes which can be solved in a particular simulation is limited by the amount of physical memory (16GB).

Second, it has been observed during experimentation that addition of BPB to the buffered salt solution reduced density when compared with the original solution. This was confirmed through visualization of dye moving through the reactor with the inlet and outlet rotated 90 degrees into the plane of Earth's gravity. If density remained constant with the addition of BPB the same results shown in Fig. 5 are expected. However, when the reactor is oriented with the inlet below the outlet, dye quickly rises as a thin stream; and if the orientation is reversed, inlet is above the outlet, dye fills the reactor as a single front across the entire cross section. These two observations lead to the conclusion that the solution containing BPB is less dense than the original buffer solution. The solution density difference may begin to explain why the reactor fills with dye from the exit to the inlet moving as a sharp front during experiments with system rotation as the less dense dyed solution is retained in the region with the lowest gravitational force. Despite these differences agreement between the numerical and experimental observations has been shown for the transient movement of dye through the reactor, particularly at initial time points before density and diffusion can affect results.

4.3 Implications in CCBR Bioprocessing

The importance of this work lies first in its implications for fluid mechanics of flow through rotating vessels. During operation with system rotation one might assume flow to be only slightly effected by system rotation. However, experimental observations indicate a radical change in the fluid flow profile over that observed without any rotation and this flow profile remains largely unchanged at all possible operation conditions for high cell density culture. With the use of simulation software such as the COMSOL package used in this work rapid assessment of a wide range of operating conditions is possible enhancing the understanding of system fluid dynamics and how the rotating and non-rotating systems differ.

This work also has CCBR bioprocessing implications for priming and flushing operations to change system concentrations of different nutrients or metabolites. The rate at which these operations can be accomplished is impacted by the amount of time it takes for the reactor to reach a steady state concentration coinciding with a new inlet concentration. One typically uses a residence time analysis to determine the required time for reactor concentrations to reach a new steady state. If we were to consider complete replacement of the CCBR contents in a plug flow system it would take one time constant or about 240 sec for the system to reach a new steady state at an inlet velocity of 4.3 cm/s. However, it is believed that a mixed flow is more likely as modeling and experimental results clearly indicate fluid enters the reactor and flows up the left wall, then cross the chamber before exiting which clearly is not plug flow behavior. Preliminary experiments to investigate mixing within the CCBR (data not shown) indicate a behavior similar to that of a CSTR where it will take three time constants to displace 95% of the reactor contents.

Second, this work has implications as to how the fluid flow profile impacts cell culture conditions and how the flow profile presented here will be affected by different cell culture parameters such as cell size and population density. For very small cells, such as bacteria, it is possible they will be entrained in fluid and either pass directly through the reactor along predicted streamlines or be trapped in vortices where cells are less likely to receive ample fresh medium needed for growth. Disrupting the CCBR flow profile that results because of the Coriolis effect with the use of a mesh or by other mechanical means may become necessary to retain small diameter cells for long term high density culture.

How larger mammalian cells are retained in the CCBR and their effect on the flow profile is of interest from a bioprocessing standpoint. Preliminary research has shown that a dense culture of mammalian cells is retained in the lower half of the reactor furthest from the center of rotation, while a fluid head space fills the remainder of the reactor. One of two hypotheses is likely to explain how high density cultures of cells are retained in the CCBR. First, if the fluid flow profile shown in Fig. 4 (600) does not change a circular flow pattern of cells may develop. Cells would be pulled up the left wall in the stream containing most of the fluid flow to the widest point where they begin to move across the reactor. As cells move toward the right wall the direction of the drag force is no longer opposite the centrifugal force and cells would begin to settle toward the inlet along the right wall of the reactor. After settling to near the inlet the cells would once again be pulled up the left wall creating a constant circulation of cells during culture. The second hypothesis would be that larger cells disrupt the simulated flow profile resulting in a more uniformly dispersed flow profile throughout the cell bed rather than the scenario where entrant fluid flows solely along the left wall. However, it is entirely possible that a more elaborate flow profile, similar to that shown in this manuscript, may be maintained in the fluid head space above the cells. Therefore, a future simulation study will be conducted in which cell sedimentation analysis for dense suspensions is added to the Coriolis force analysis presented here and compared with experimental cell cultures at varying operating conditions.

5. Concluding Remarks

The effect of Coriolis forces on the fluid flow profile within a rotating bioreactor has been studied through mathematical simulation and dye injection experiments. Numerical simulations at an inlet flow rate of 4.3 cm/s and various RPMs in a 12.5 mL CCBR chamber show the development of a fluid flow profile dominated by Coriolis forces as RPMs are increased from 0 to 100. Rotation speeds above 100 RPM result in a flow pattern largely unchanged with the exception of vortices becoming more defined. Additionally, it has been shown through a discussion of the Rossby number and numerical simulations that the inlet fluid velocity has little effect on the fluid flow profile at a particular RPM.

Coupling of the steady-state Navier-Stokes solution with a mass balance provides a method for direct comparison with transient dye injection experiments. Comparisons between the numerical and experimental results, particularly at early time points, show agreement in the how dye enters the reactor and flows up the leading wall due to Coriolis forces. Discrepancies arise at later time points when the dye has almost completely filled the reactor and can be attributed to experimental fluid density differences and excessive diffusion within the numerical simulation.

The diffusion of BPB is dominated by artificial diffusion in the simulation as it increases the diffusion coefficient of BPB by several orders of magnitude which is required for solution convergence. The use of artificial diffusion results in simulation prediction of dye movement up both walls of the reactor as dye diffuses into streamlines moving along the right wall; this is opposed to experimental observations where diffusion of dye from the inlet stream along

the left wall is never observed. Similarly, a slight difference in density between dyed and undyed solutions contributes to differences the final concentration profile of dye within the reactor. Experimentally, the reactor is filled with dye as a sharp front moves from the outlet to the inlet, likely due to a difference in solution density which is exaggerated in the presence of centrifugal acceleration; while the simulation predicts a low concentration of dye will be maintained within vortices over time.

In conclusion, these results show that the overall flow profile of an empty CCBR chamber is dominated by Coriolis forces over the range of operation typically used in high density culture. The mixing that occurs in the system has obvious implications for priming or flushing the reactor as the system behaves similar to a well stirred vessel requiring three time constants or more for adjustment of reactor nutrient concentrations during operation. Further investigation will be done on how the fluid flow profile described in this paper is affected by the presence of cells being cultured in the reactor. It is hypothesized that smaller microbial cultures will be affected more by Coriolis forces as they are carried along streamlines, possibly becoming trapped in vortices. For larger cells, it is expected that studies will reveal that larger sedimentation rates will serve to deflect streamlines affected by Coriolis forces leading to a more uniform flow around each cell resulting in a stable homogeneous fluidized bed of cells. In either case understanding how cells affect the surprising fluid flow pattern presented in this paper will provide valuable insight for predicting operating conditions capable of retaining a variety of different cells.

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Figure 1.

An illustration of the CCBR reactor and rotor (shaded region) showing the unit axis along with the direction of rotation. Inlet tubes feeding the reactor with BPB dyed solution (A) and colorless solution (B) join before the reactor inlet (C). Fluid flows through the reactor radially inward (D) resulting in Coriolis forces with direction to the left (E). The reactor outlet (F) as well as both inlet tubes are bundled and remain untangled during operation through the use of an anti-twister mechanism.

Figure 2.

A representation of the centrifugal bioreactor with the computational mesh used to find the Navier-Stokes numerical solution is shown. The total number of nodes and boundary conditions used in numerical simulations are found in Table 1. Radii for the identical inlet and outlet tubes and the widest part of the reactor are indicated, where all other radii vary linearly with height. Corresponding distances from the start of the inlet tube are also indicated. Rotating the 2D projection, inset, creates the 3D reactor for simulation in COMSOL.

Figure 3.

The steady state numerical solutions of the Navier-Stokes flow profile with constant inlet velocity of 4.3 cm/s and increasing system RPM is shown by streamlines depicting the development of a flow profile dominated by Coriolis forces. The size scale is indicated on the right of each figure in terms of meters from the start of the inlet tube of the reactor.

Figure 4.

Steady state numerical solutions of the Navier-Stokes flow profile indicating minimal alteration of the overall flow profile as RPM is increased above 100 RPM. The increasing definition and size of vortices results from Coriolis causing a material element to complete the projected circle with higher frequency at higher system RPM. Each solution is solved with a constant inlet velocity of 4.3 cm/s.

Figure 5.

A comparison of the numerical solution (A) and experimental results (B) as dye enters a non-rotating CCBR when viewed from a position parallel to the axis of rotation. Both the numerical and experimental results depict the same profile as dye follows streamlines through the reactor for a system without rotation.

Figure 6.

Numerical simulation of the convection and diffusion mass balance at various time points after dye is initially pumped into the reactor with an inlet velocity of 4.3 cm/s and a rotation rate of 600 RPM. The early time point shows dye following streamlines along the left side of the reactor, while later time points show significant diffusion of dye toward the reactor midline which is then pulled along streamlines moving up the right wall of the CCBR.

Figure 7.

Experimental results as dye is pumped with a constant inlet velocity of 4.3 cm/s into a reactor rotating at 600 RPM. As predicted by the simulation, dye initially moves up the left side of the reactor due to the Coriolis forces and then begins to fill from the top (exit) to the bottom (inlet) of the reactor.

Table 1

Boundary conditions and mesh Density for simulations COMSOL v3.3

Boundary Condition		# of Nodes
Navier Stokes		86,037
Inlet	y-Velocity 0 – 16 cm/s	
Outlet	Pressure 0 Pa	
All Other Boundaries	No Slip	
Convection & Diffusion		431,402
Inlet	Concentration 0.03 mol/m ³	
Outlet	Convective Flux ^a	
All Other Boundaries	Insulation/Symmetry	

^a Convective flux boundary conditions assumes $N_i \cdot n = C_i u \cdot n$