This item was submitted to [Loughborough's Research Repository](#) by the author.
Items in Figshare are protected by copyright, with all rights reserved, unless otherwise indicated.

Numerical simulation of coupled cell motion and nutrient transport in NASA's rotating bioreactor

PLEASE CITE THE PUBLISHED VERSION

<http://dx.doi.org/10.1016/j.cej.2014.08.077>

PUBLISHER

© Elsevier

VERSION

AM (Accepted Manuscript)

PUBLISHER STATEMENT

This work is made available according to the conditions of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence. Full details of this licence are available at:
<https://creativecommons.org/licenses/by-nc-nd/4.0/>

LICENCE

CC BY-NC-ND 4.0

REPOSITORY RECORD

Chao, Tzu-Chieh, and Diganta Bhusan Das. 2014. "Numerical Simulation of Coupled Cell Motion and Nutrient Transport in Nasa's Rotating Bioreactor". figshare. <https://hdl.handle.net/2134/15857>.

Numerical simulation of coupled cell motion and nutrient transport in NASA's rotating bioreactor

Tzu-Chieh Chao, Diganta B. Das*

Department of Chemical Engineering, Loughborough University, Loughborough LE11 3TU, Leicestershire

*Author to whom correspondence should be addressed (Email: D.B.Das@lboro.ac.uk; Tel.: 00441509222509; Fax: 00441509223923)

Abstract

Rotating bioreactor, such as the NASA bioreactor, which was designed by the National Aeronautics and Space Administration (NASA), USA, can be used to mimic micro-gravity conditions and simulate the effects of microgravity on cells growth. The cell growth in the bioreactor depends on the nutrient availability which in turn depends on the cell density and distribution within the bioreactor. In this work, we use a numerical model of suspended particle motion to simulate the cell motion and distribution in a specific variant of the NASA bioreactor, namely, the high aspect ratio vessel (HARV) bioreactor. The nutrient distribution in the bioreactor is simulated based on a convection-diffusion-reaction supplemented by laboratory experiments aimed at obtaining the required data. We present the modelling framework in this paper and discuss the most salient simulated results. For example, the simulation results show that the distributions of the cells in the bioreactor appear as concentric circles and that the cells density is higher in the middle of the HARV bioreactor. These cell distributions imply that they may accumulate in the middle of the bioreactor at sufficiently high cell density. The results also demonstrate that the concentration of nutrient is fairly uniform in the bioreactor but decreases slightly from the outer radius of the HARV bioreactor to the inner radius. This is possibly caused by the higher consumption of glucose due to the higher cell density in the middle of the radius. We expect that the modelling framework in this paper would help optimize the culture conditions for the cells in HARV bioreactors.

Keywords: Cell culture; mathematical models; diffusion; nutrient transfer; rotating bioreactor

Nomenclature

Symbol	Description
a	Particle diameter of cells (m)
C	Nutrient concentration (mol/m^3)
c_s	Mass fraction of solid cells particle (-)
D	Diffusion coefficient (m^2/s)
d	Cells density (mol/m^3)
f_h	Hindered setting function (-)
g	Gravitational constant (m/s^2)
$\vec{J}_s$	Particle flux (1/s)

$\vec{J}_{sc}$	Particle flux contributed to particle interaction (1/s)
$\vec{J}_{s\mu}$	Particle flux contributed to spatial variation (1/s)
k	Empirical coefficient based on experimental result (m ³ /mol)
k_c	Correlation coefficient of nutrient consumption (m ³ /mol)
k_e	Cell doubling time coefficient (1/s)
k_{sc}	Coefficient of particle flux contributed to particle interaction (-)
k_{μ}	Coefficient of particle flux contributed to spatial variation (-)
L	Thickness of HARV bioreactor (m)
P	Pressure of fluid flow (Pa)
r	Radial co-ordinate
r_c	Reaction rate of nutrient consumption (mol/m ³ s)
T	Time (s)
u	Mean fluid velocity (m/s)
$\vec{u}_s$	Velocity of solid particle (m/s)
$\vec{u}_{st}$	Setting velocity in stokes law (m/s)
$\vec{u}_{slip}$	Relative velocity between solid and liquid phases (m/s).
$\vec{u}$,	Velocity of fluid in rotating bioreactor (m/s)
$\vec{u}_r$	Velocity of bioreactor wall rotating (m/s)
V_p	Volume of solid particle (m ³)
X	Cartesian coordinate x direction
y	Cartesian coordinate y direction
μ	Average viscosity of particle and medium mixture
μ_f	Viscosity of pure liquid medium
μ_c	Coefficient of nutrient consumption (1/s)
P	Average density of particle and fluid mixture (kg/m ³)
ρ_f	Density of pure fluid (kg/m ³)
ρ_s	Density of solid particle (kg/m ³)
ρ_f°	Phase density of liquid medium (CCM) (kg/m ³)
ρ_s°	Phase density of solid particle (kg/m ³)
τ_w	Shear stress (kg/ms ²)
Ω	Rotational speed (rad/s)
Φ	Volume fraction of particle (-)
Φ_{max}	Maximum packing volume fraction (-)
Φ_s	Initial solid particle volume fraction (-)
$\dot{\gamma}$	Magnitude of shear tension rate (1/s)

28 Acronyms

Acronyms	Definition
CHO	Chinese hamster ovary cell
HARV	High aspect ratio vessel
NASA	National Aeronautics and Space Administration
RWPV	Rotating-wall perfused-vessel
RWV	Rotating-wall vessel
STLV	Slow turning lateral vessel

1. Introduction

The rotating wall vessel (RWV) bioreactor is the first generation bioreactor which was developed by the National Aeronautics and Space Administration (NASA), USA, to protect cultured cells from high shear forces generated during the launch and the landing of space shuttles. The RWV bioreactor is popularly known as the NASA bioreactor. The cell/tissue growth environment in this type of bioreactor shows special features such as partial microgravity and continuous cell suspension in a fluid which provide a number of desired conditions for three-dimensional development of artificial tissues, e.g., low shear-stress, high mass transfer rate and good fluid mixing. Due to these features, the NASA bioreactor is regularly used for studying different aspects of cell culture. Several variants of the RWV bioreactor have been reported [1–4], such as rotating-wall perfused vessel (RWPV) bioreactor, slow-turning lateral vessel (STLV) bioreactor and high aspect ratio vessel (HARV) bioreactor to complete a number of previous experiments under microgravity [1,5–9]. Among these variants, the HARV bioreactor shows a good potential for application for cell culture. This is due to the high ratio of radius-to-depth of the bioreactor and large area of gas (e.g., oxygen) permeable membrane, which allows the bioreactor to provide higher mass transfer and concentration of the gas in the cultured environment than the other variants. Although there have been some previous attempts to develop computational models to simulate the cell motion [4] or fluid and nutrients transport [10,11] in HARV bioreactor, most of these studies focus on simulating the effects of specific factors such as cells growth, fluid flow and single cell trajectory within the bioreactor, or different geometries of bioreactor [1–3,12]. Therefore, it seems that there is a lack of well-established modelling framework for simulating transient cell distribution within the bioreactor, particularly, for small cells, such as Chinese hamster ovarian (CHO) cells. Furthermore, there is a general lack of studies that deal with simulating the coupled behavior between the cell and nutrient distributions in these bioreactors. In addressing these issues, we report a mathematical framework in this paper, which focuses on determining the fluid flow and transient distribution of cells in the HARV bioreactor while growing cells under specified operating conditions (e.g., angular frequency). We define that the cells cultured in the HARV bioreactor (Figure 1) appear as suspended micron-particles (non-deformable) and apply a particle suspension model to simulate the cell movement during cell cultivation. Our results into the relationships between these parameters provide important understanding of the nutritional requirement in the HARV bioreactor, which, as stated above, is a specific variant of the NASA bioreactor.

2. Methodology

The cell growth and distribution inside the bioreactors in general and HARV bioreactor in specific depend on a number of inter-related processes such as the fluid dynamics, mixing, cell growth kinetics and convective and diffusive mass transport [1–6,13]. Our approaches to modelling these processes for the purpose of this paper are discussed briefly below.

2.1. Cell distribution

To simulate the cell distribution, we define that the cells are non-deformable spherical micro-particles suspended in a Newtonian fluid medium, namely, a cell culture medium (CCM). In other words, we

start by defining that there are two-distinct phases in the bioreactor, namely, the cells (solid phase) and CCM (fluid phase). The distribution of the cells in the suspension is defined to be influenced by the fluid velocity field and, conversely, the velocity field is affected by the cells' buoyant movement. The developed model then uses a continuum approach, which in turn is defined to rely on a constitutive equation [14] to simulate the cell and fluid movement in the HARV bioreactor.

Following on from this background, a two-dimensional model based on a two-phase suspension model [15] is applied where the fluid medium and cells are considered to be two different phases. For the purpose of the simulations, we define that the content in the bioreactor vessel is well mixed. It is also defined that the vessel is placed symmetrically about the centre line of the bioreactor where we neglect the fluid motion along the horizontal direction (z-axis) in order to simplify the simulations (Figure 1).

Given the above assumptions, the following momentum equation is used to describe the conservation of momentum of the liquid medium within the HARV [16,17]:

$$\rho \frac{\partial \vec{u}}{\partial t} + \rho(\vec{u} \cdot \nabla) \vec{u} = -\nabla p - \nabla \cdot [\rho c_s(1 - c_s) \overrightarrow{u_{slip}} \cdot \overrightarrow{u_{slip}}] + \nabla \cdot [\mu(\nabla \vec{u} + \nabla \vec{u}^T)] + \rho \vec{g} \quad (1)$$

In equation (1), the $\vec{g}$ term represents a body force acting on the cells where the force is written as a positive term. In the scalar components of the equation, the vertical component of the gravity vector is set as a negative term. On the other hand, the components of the gravity vector in the other directions are set to zero.

In equation (1), the mixture density (ρ) is expressed as:

$$\rho = (1 - \Phi_s) \rho_f + \Phi_s \rho_s \quad (2)$$

According to Subia et al. [13], treating the suspended micro-particles as a single continuum requires considering the variable viscosity with different particle concentrations. We adopt the same consideration in this paper and apply the following correlation (equation 3) which Krieger [16] and Leighton and Acrivos [17] proposed for the relative viscosity with variable cell volume fractions. In equation (3), μ represents the effective mixture viscosity ($N \cdot s / m^2$) according to a Krieger-type expression:

$$\mu = \mu_f \left(1 - \frac{\Phi_s}{\Phi_{\max}} \right)^{-2.5 \Phi_{\max}} \quad (3)$$

In this case, the value of $\Phi_{\max}$ depends on the uniformity of cell's shape (defined as spherical in this work) and size, the structure of the packed configuration. The range of maximum volume fraction can vary from 0.52 for simple cubic packing to 0.74 for face centred cubic packing. To simplify the model complexity, we define that the maximum volume fraction is 0.68 for the purpose of this work, which is

the high-shear limit value used by Krieger [16]. For experimental data collection, the $\Phi_{\max}$ parameter is considered as an adjustable variable.

The continuity equation for mass of the mixture is described by the following formula:

$$(\rho_s^\circ - \rho_f^\circ)[\nabla \cdot (\Phi_s(1 - c_s)\overrightarrow{u_{slip}})] + \rho_f^\circ(\nabla \cdot \vec{u}) = 0 \quad (4)$$

The transport equation for the solid-phase (cell) volume fraction in the bioreactor is given as:

$$\frac{\partial \Phi_s}{\partial t} + \nabla \cdot (\Phi_s \overrightarrow{u_s}) = 0 \quad (5)$$

Where the solid-phase (cells) velocity $\overrightarrow{u_s}$ is defined as $\overrightarrow{u_s} = \vec{u} + (1 - c_s)\overrightarrow{u_{slip}}$. Inserting $\overrightarrow{u_s}$ with equation (5), the equation can be rearranged as:

$$\frac{\partial \Phi_s}{\partial t} + \nabla \cdot (\Phi_s(\vec{u} + (1 - c_s)\overrightarrow{u_{slip}})) = 0 \quad (6)$$

Instead of using $\overrightarrow{u_{slip}}$, Rao et al. [15] have used the particle flux $\vec{J}_s$ in terms of particle movement in the continuity equation and the conservation equation. Using the same approach, we write the conservation equation for cells as follows:

$$\frac{\partial \Phi_s}{\partial t} + \nabla \cdot (\vec{u}\Phi) = -\frac{\nabla \cdot \vec{J}_s}{\rho_s^\circ} \quad (7)$$

Due to the density difference between the cells and the cell culture medium, an assumption is made which is that the cell and the fluid are incompressible by each phase. The flux term $\vec{J}_s$ can then be introduced into the continuity equation as follows:

$$\nabla \cdot \vec{u} = \frac{\rho_s^\circ - \rho_f^\circ}{\rho_s^\circ \rho_f^\circ} \nabla \cdot \vec{J}_s \quad (8)$$

Where, the relationship between $\overrightarrow{u_{slip}}$ and $\vec{J}_s$ is determined by combining them with the continuity equation for the solid particles' relative velocity. The relationship is as follows:

$$\overrightarrow{u_{slip}} = \frac{\vec{J}_s}{\Phi_s d(1 - c_s)} \quad (9)$$

Equation (7) is dissolved and equation (8) is inserted in place of the divergence of velocity. Hence, the conservation equation for the cell volume fraction can be rearranged as:

$$\frac{\partial \Phi}{\partial t} + \vec{u} \nabla \cdot (\Phi) = \frac{\rho_s^\circ - \rho_f^\circ}{\rho_s^\circ \rho_f^\circ} \nabla \cdot \vec{J}_s = \frac{\rho}{\rho_s^\circ \rho_f^\circ} \nabla \cdot \vec{J}_s \quad (10)$$

In a flow problem involving the cells, how the cell concentration varies with time must be known in order to evaluate the total stress. According to the studies by Subia et al [14], the evolution equation for particle volume fraction, shown as the above equation, can be derived by considering several mechanisms, which include Brownian motion, sedimentation, hydrodynamic particle interactions and gradients in suspension viscosity.

Based on the assumption that neglecting the effects of Brownian motion and sedimentation (e.g., see, Subia et al. [13]) the diffusive flux of the cells is written as follows:

$$\vec{J}_s = \vec{J}_{s\mu} + \vec{J}_{sc} \quad (11)$$

In consistent with Leighton and Acrivos [17] and Phillips *et al.* [18], we apply a scaling argument for the diffusive particle flux as follows:

$$\vec{J}_{sc} = -a^2 \Phi^2 k_{sc} \nabla(\dot{\gamma} \Phi) \quad (12)$$

And

$$\vec{J}_{s\mu} = -a^2 \Phi^2 k_{\mu} \nabla(\ln \mu) \quad (13)$$

These equations show how the particle flux is driven not only by the gradient of concentration but also by the gradient of shear stress.

Zhang and Acrivos [19] proposed that one should include a sedimentation term in equation (11) by using a hindered settling function to take into account the buoyancy effect on the sedimentation of the particles in the liquid. Following Rao et al. [14], we can get the governing equation of the flux of the cells by combining the above three equations (11-13) so as to incorporate the effect of hindered sedimentation in the model:

$$\frac{\vec{J}_s}{\rho_s} = -[\Phi D_{\Phi} \nabla(\dot{\gamma} \Phi) + \Phi^2 D_{\mu} \dot{\gamma} \nabla(\ln \mu)] + f_h \vec{u}_{st} \Phi \quad (14)$$

Where D_{Φ} and D_{μ} are the rearranged coefficients resulting from multiplying the squared particle diameter with K_c and K_{μ} . In consistent with Rao et al. [14], these empirical coefficients are determined as follows:

$$D_{\Phi} = 0.41 a^2 \quad (15)$$

$$D_{\mu} = 0.62 a^2 \quad (16)$$

$$\vec{u}_{st} = \frac{2a^2(\rho_s - \rho_f)}{9\mu} \vec{g} \quad (17)$$

Where $\vec{u}_{st}$ is the settling velocity that is assumed for a spherical particle surrounded by a pure fluid. $\vec{u}_{st}$ is actually the Stokes velocity vector which is derived from a force balance and is composed in

terms of the diameter of a single particle, the density difference between the fluid and the particle, the mixture viscosity and the gravity acceleration.

As shown in equation (14), we use a hindered settling function, f_h while incorporating the effect of sedimentation. In the work of Rao *et al.* [15], this hindered settling function was found to reduce the occurrence of physical shocks that would occur near the zone of maximum cell packing which in turn was found to give realistic-looking profiles for the maximum packing zone. In consistent with the previous studies, we apply the same form of hindered settling equation in this work:

$$f_h = \frac{\mu_f(1-\Phi_{\text{average}})}{\mu} \quad (18)$$

In equation (14), $\dot{\gamma}$, i.e., the magnitude of a shear-rate tensor, is directly related to the second invariant of the shear-rate tensor. The shear-rate tensor is given as:

$$\tilde{\gamma} = (\nabla \vec{u} + \nabla \vec{u}^T) \quad (19)$$

To determine the magnitude of shear-rate tensor $\dot{\gamma}$, we employ a generalized measure of shear rate $\dot{\gamma}$ by direct analogy with two-dimensional flows. The magnitude of the shear tensor $\dot{\gamma}$ is given as:

$$[\dot{\gamma} = \sqrt{\frac{1}{2}(\tilde{\gamma} \cdot \tilde{\gamma})} = \sqrt{\frac{1}{2}(4u_x^2 + 2(u_y + v_x)^2 + 4v_y^2)}] \quad (20)$$

By combining equations (14)-(18) and, (20), equation (10) can be rearranged as below:

$$\frac{\partial \Phi}{\partial t} + \vec{u} \nabla(\Phi) = \frac{\rho_f - \rho_s}{\rho_s \rho_f} \nabla \cdot \rho_s \left\{ [0.41a^2 \Phi \nabla(\dot{\gamma} \Phi) + 0.62a^2 \Phi^2 \dot{\gamma} \nabla(\ln \mu)] + \Phi \frac{2\mu_f a^2 (1-\Phi_{\text{average}})(\rho_s - \rho_f)}{9\mu^2} \vec{g} \right\} \quad (21)$$

We assume that the fluid flow is a Newtonian flow, the flow condition is laminar and the effective viscosity of the fluid flow is constant in the mixture medium. The boundary conditions of the horizontal surface introduce the shear stress τ_w , which is caused by the rotation of the mixture in the Newtonian fluid's velocity field, is given as follows:

$$\tau_w = \frac{4\mu_f(\overline{u_w} - \bar{u})}{l} \quad (22)$$

With the centre at point (0, 0), the fluid velocity $\vec{u_r}$ at any arbitrary point within the bioreactor is given as:

$$\vec{u_r} = \omega \cdot (y, -x) = \frac{2\pi \cdot \text{rpm}}{60} \cdot (y, -x) \quad (23)$$

To simplify the boundary conditions for the simulations, we define that there is no cell penetration and dispersion on the wall. Also, the fluid velocity $\vec{u}$ is equal to the rotation velocity $\vec{u}_r$ on the wall boundary. The suspension velocity satisfies the no-slip condition at all walls.

2.2. Nutrient transport

The growth of the cells in the bioreactor requires several different chemical components, such as oxygen, amino acids and glucose [20,21]. However, we only consider one solute, namely glucose, in this paper. In previous studies [22,23], the cell growth kinetics were found to be affected by the concentration of the nutrient. In consistent with these studies, the cell growth rate is determined by the distribution of cell density simulated by the above mathematical analysis and by the distribution of nutrient concentration. In this paper, the glucose is considered as an ideal solute that has no volume. It is also assumed that there is no additional glucose supplied or lost in the bioreactor throughout the cell growth process. The governing equation is given as a convection–diffusion equation:

$$\frac{dC}{dt} + u \cdot \nabla C = D_f \cdot \nabla^2 C + r_c \quad (24)$$

r_c , the nutrient consumption rate, is determined by the metabolism of the cells. In consistent with the experimental results of Altamirano *et al.* [24], the nutrient consumption rate of the cell is specified as a constant as follows:

$$r_c = -\mu_c \cdot d \quad (25)$$

μ_c , the nutrient consumption coefficient, is determined by experimental data in this work.

2.3. Growth kinetics

In this work, the exponential growth model is applied to simulate the CHO cells' growth kinetics. In view of the effects of the mass transport of nutrients on cell growth kinetics, the mechanism of cell growth is coupled with the governing equations for nutrient transport. However, to simplify the verification mechanism for the cell growth, we define that the metabolism rate of cells is constant and that there is a single nutrient: glucose. By making these assumptions and roughly comparing the hypotheses for growth kinetics to the experimental data collected by Altamirano *et al.* [24], the nutrient influent mechanism is considered as a first-order kinetic reaction. In accordance with the previous study on general cell growth kinetic model by Almquist *et al.* [25], we have modified the mass balance equation as a cell kinetic model and apply the first order nutrient consumption by the cell and an exponential cell growth into the general model. For the purpose of this paper, the following exponential growth kinetics is used to define transient cell growth:

$$\frac{\partial d}{\partial t} = k \cdot k_e \cdot C \cdot d_o \cdot e^{k_e t} = k_c \cdot C \cdot d_o \cdot e^{k_e t} \quad (26)$$

2.4. Finite element model (FEM) model development

In this work, the model of suspended cell movement and the medium's fluid dynamics are simulated in a two-dimensional model structure in commercially available modelling software COMSOL. Figure 2 shows a numerical mesh of the 2D geometry of the HARV bioreactor. The figure demonstrates the cross-section of the surface geometry of the HARV model, divided into a number of small divisions and elements over which the numerical solution scheme is implemented. The radius of the model may be different for different types of bioreactor. However, we use the experimental data from the cultivation experiment, where the diameter of the HARV bioreactor is applied as 0.1 m. The meshing parameters (Figure 2) are set in order to obtain an accurate measure of the concentration changes in the HARV bioreactor, where minimum and maximum values of 1.25×10^{-5} m and 0.0037 m are set. The mesh is generated using three-node triangular Lagrangian elements. This mesh is chosen due to the high gradient of concentration that occurs throughout the bioreactor and the precise simulations of local concentrations of cells and nutrients.

2.5. Data collection for numerical simulations

2.5.1 Material and method of cells growth experiment

This cell growth kinetics for the simulation is determined based on in-house experimental data. In order to calculate the doubling time coefficient, k_e , the in-house experiments were operated as follows. Chinese hamster ovarian (protein free) (CHO) cells were purchased from the Health Protection Agency (HPA), UK. The HARV bioreactor was placed in a humidified incubator under a 95% air: 5% CO_2 atmosphere and temperature of 37°C . The cells were seeded in a 55 ml capacity vessel at a cell density of 3×10^5 viable cells/ml at 37°C and the vessel was connected to the motor on the bioreactor. The rotational speed of the bioreactor was set to 7.5 rpm. The cells density was monitored at every 24 hours by counting cells via haemocytometer while the nutrient concentration was maintained as a constant by adding the nutrient medium every 24 hours.

2.5.2 Growth kinetics coefficient

Using the experimental data collected by Altamirano *et al.* [24], the cell growth rate and metabolism in their batch culture condition are applied to satisfy our assumption of exponential cell growth in the HARV reactor. The reason we have chosen to use the data from this paper is because it contains these data for the cells we have chosen for the purpose of this paper, namely the CHO cells. The nutrients may affect the growth kinetics of the CHO cells. However, in our simulations, we only consider one nutrient and its effects on cell growth in order to simplify the simulation process. To eliminate the effects of other nutrients on cell growth, the experimental data collected from 5mM glucose concentration solution is applied in our simulation. As the metabolites of CHO cells are considered to be constant with different concentrations of glucose, the metabolic rate per volume of CHO cells is given as per equation (24). The coefficient of the CHO cells' glucose metabolites is calculated as $k_c \approx 1.0 \times 10^{-13}$ mole/cell · hr, where k_c is derived from the experimental data from Altamirano *et al.* [24].

2.5.3 Doubling time coefficient

This cell growth kinetics for the simulation is determined based on in-house experimental data. The following steps were conducted to collect the data for the simulations. A HARV bioreactor was placed in a humidified incubator under a 95% air: 5% CO₂ atmosphere and at a temperature of 37°C. Cells were then seeded in a 55ml capacity vessel at a density of 3×10^5 viable cells/ml in the cell culture medium at 37°C. The vessel was connected to the motor on the bioreactor and a rotational speed of 7.5 rpm was selected. During the experiment, we assume that no aggregate is formed and there is no need to increase the rpm as cells are grown. We then identify the growth kinetics based on the assumption of exponential growth and the relationship between nutrient diffusion and cell growth. The data on the transient cell density from our experiments are shown in Figure 3.

Based on our assumption of exponential cell growth and the comparison of the experimental data from Altamirano *et al.* and our experiments, the growth kinetics of the cells in terms of cell density (d) is calculated using the modified exponential growth from equation (27). In our experiments, the rotation speed is set at a constant 7.5 rpm. The initial cell density d_0 is 3×10^5 viable cells/ml and the doubling time of the cells is 1.95 days. Based on the experimental data, the following coefficient is obtained: $k_e = 4.2 \times 10^{-6} (\text{s}^{-1})$.

In view of the initial conditions used (as described in the cell distribution section) for the cultivation experiment, the cell density is considered as well mixed and distributed throughout the whole domain. The medium parameters applied in the cell growth kinetics and metabolisms are based on the assumption of the glucose medium described in the cell distribution section. Hence, the initial concentration of glucose used is 5.5 mole/m^3 and is considered as having equal distribution throughout the whole domain.

3. Results and Discussions

3.1. Validation of numerical results

The validation of the numerical simulation is conducted by simulating and qualitatively comparing experiments of Pollack et al. [4]. For this purpose, the HARV parameters in Pollack et al.'s model are applied in the developed model where the vessel volume is approximately 55ml where the diameter of the disk is defined to be about 10 cm. This simulation is conducted to identify the cell movement in the early stage of cell culture and to compare the simulation results with previous experimental results [4]. In this modelling validation, the initial condition of the suspended cell is considered to be that they are micro-particles suspended in the upper region of the bioreactor. Subsequently, we observe if the developed model can produce the swirling motion of the cells observed in the bioreactor by Pollack et al. [4], as follows.

To compare the simulation results with the experimental results under consistent/similar conditions, the experimental parameters are made consistent with previous study [4], whereby the cell diameter is defined to be 1 mm, the density is 1200 kg/m^3 and fluid is distilled water with a density of 1000 kg/m^3 . The rotation speed is set at 10 rpm. It is assumed that the cells are well dispersed at the initial

condition, with a constant volume fraction of 0.5. The initial conditions for the cells' volume fraction distribution are shown in Figure 4 (a).

The results show that the cells are likely to move towards the wall, which happens when the central volume fraction slightly decreases and the volume fraction is higher on the left side. In the experiment by Pollack et al. [4], the results of the micro-carrier movement for higher-density particles show sedimentation of the cells near the wall. This is caused by the high centrifugal force caused by the high density difference between the particles and the fluid medium; thus, the particle distribution is more significant, as shown in Figure 4. The simulation results in Figure 4(b) show that for the cell with a density higher than that of the medium fluid, the cells density tend to be higher at the outer region of the radius of the bioreactor. The high volume fraction of the cells shown in the left section may cause high-frequency collisions between the cells and the vessel wall. These results are almost the same as the experimental results in the study of Pollack et al. [4]. For the cells with a lighter density than the medium fluid, the simulation results can be found in Figure 4(c). In this operation, the initial volume fraction of the cells is set as 0.5, responding to this change, the results represent a reverse condition compared to previous simulations. They show that the volume fraction in the inner region of the radius increased, whilst a high volume fraction appeared in the right vessel wall. These simulation results are also consistent with the experimental results in previous research.

It is not possible for the initial cell distribution to be well mixed due to the different densities of the cells and the medium fluid. For the cells with a lower density than the medium fluid, the cell volume fraction should be allocated in the top of the bioreactor, where all cells are floating in the medium fluid. The modelling results using this assumption as a new initial condition are shown in Figure 5. We find that the experimental results of Pollock et al [4] are almost the same as predicted in this paper. The lighter cells are first mixed in the outer radius of the bioreactor and then the cell density slowly increases in the inner part of the bioreactor. The simulated results shown in Figure 5 are similar to the previous experiment results [4], as shown in Figure 6. The early stage of cell movement is considered as a helix structure, and the lighter cells move towards the centre of the HARV bioreactor. The validation of the simulation result provides a better understanding of the cells movement in HARV and enhances the reliability of the performance of our modelling framework in the simulation of cells movement and distribution.

3.2. Cells Distribution and Motion

In the above simulation (based on Pollack et al.'s experiment [4]), the cell movement has a constant cell number and a constant initial condition for the suspended cells. However, in the real experiment in HARV, the volume fraction of the cells is relatively small at the beginning, and the cell density increases and decreases with time for the same population of cells. To incorporate this behaviour of the culturing conditions with growing cells, we apply in-house experimental data from culturing CHO cells in higher-speed rotation conditions. Due to the small cell size and stable growth kinetics, CHO cells were a perfect split-growth cell to choose as a culturing target. The cultivation conditions of the

experiment are introduced in the data collection section (section 2.5.2). Some typical cell distribution simulation results are shown in Figure 7. The right bars of the figures indicate the cell density.

In the simulations, the initial condition for our culturing experiment is not considered to be a cell suspension condition, which means the well-mixed CHO cells condition is introduced as the initial condition. Because of this, when we inject the cells and the medium into the HARV bioreactor, the cells and the fluid medium are well mixed. Also, the HARV bioreactor is placed horizontally until the rotating process begins. However, our experimental data does not completely fit with the requirements of this model simulation. Due to the lack of precise experimental data relating to the cell density, the viscosity and the parameters of the CHO cells and the culture medium ex-cell etc, CHO (Sigma-Aldrich, UK), we make the assumption that the culture medium is 5.5 mole/m³ glucose. The physical parameters of the CHO cells are taken from both experimental assumptions in our experiment and from some experimental data in Ye *et al.* [26] and Abdullah *et al.* [27], in order to determine the values of the model parameters used for cell growth kinetics. Hence, the relationship between cell density, d , and the cell volume fraction, Φ , could be defined as the following equation:

$$\Phi = \frac{1}{6} \pi d a^3 \quad (28)$$

The parameters of the model are shown in Table 1. The simulation results show that the distributions of cells appear as concentric circles and that the cells density is higher in the middle of the HARV bioreactor. These cell distribution results imply that the cells may accumulate in the middle of the HARV bioreactor at sufficiently high cell density. In a 24-hour cell distribution experiment, Pollack *et al.* [4] also found that the microcarriers aggregated in the centre of the HARV bioreactor. Comparing these results to those of the present experiment, the different distribution conditions are significant. One possible reason for the distribution difference is the different sizes of the cells. Because the effect of drag force is larger on smaller cells than on bigger cells, smaller cells are more affected by the diffusion and convection caused by the fluid velocity field. Another possible reason is the slight density difference between the fluid and the cell, which eliminates the effect of the buoyancy force on the cells.

A typical snap-shot of velocity distribution inside the bioreactor is shown in Figure 8. This result implies that, due to the small cell volume and parameters of the cells, the effects of the drag force on the suspended cells in the rotation velocity condition are negligible. Therefore, the rotation velocity distribution is not found to vary significantly as cells grow and it can be considered as a stable rotation condition throughout the cell growth process. The purpose of presented model of cells distribution and motion is to provide the evolution of cell distribution over time during the cell cultivation in HARV. On the basis of these simulation results, we could then determine nutrient transport behaviour and cells growth kinetics with the help of developed tool for simulating coupled cell growth and nutrient transport. Importantly, the simulator can be used to control the parameters of cells cultivation, such as, rotating speed, scaffold density etc., for designing experiment in the future.

3.3. Nutrient transport behaviour

As stated earlier, we only consider one specific nutrient used for cell growth and metabolism, namely, glucose, where the diffusion equation (24) is applied to simulate the glucose transport. The metabolite of CHO cells is r_c , which is taken as a constant rate consumption of individual cells (discussed in the cell cultivation section). The diffusion coefficient is defined to be isotropic in the HARV bioreactor for the purpose of this paper and is set as $5.4 \times 10^{-10} \text{ m}^2\text{s}^{-1}$ [26]. The nutrient transport boundary conditions for the above simulation are considered as non-flux and slip rotating wall. Also, the velocity field of the fluid medium u is simulated by the momentum equation for suspended cells (1) as shown in Figure 8. The simulation results for the glucose transport are derived from the aforementioned parameters, the aforementioned equations and the metabolites of CHO cells, which will be described in the next section on cell cultivation. Figure 9 shows the distributions of glucose from the first day to the fourth day. The right bar represents the concentration of glucose.

The distributions of glucose at each time point show similar results. The glucose concentration is fairly homogeneous for in the HARV bioreactor due to the minimal differences in the nutrient concentration distribution throughout the culturing rotation. However, the results (Figure 9) demonstrate that the concentration of glucose decreases from the outer radius of the HARV bioreactor to the inner radius, and the concentration distribution is especially significant in the middle section. This is possibly caused by the higher consumption rate of glucose, which contributes to the higher cell density distribution in the middle of the radius.

3.4. Numerically determined cell growth

In real cell cultures experiment, the cell growth kinetics is determined on the basis of many factors and the complex interplay of a number of other parameters such as nutrient concentration distribution. The cell density in a bioreactor may also be experimentally determined at different time. A similar exercise may be conducted using our simulation as well where the average cell density in the bioreactor can be determined numerically as shown in Figure 10. Comparing the simulation results and the results of the experiments done by Altamirano *et al.* [24], the findings show similar growth conditions relating to CHO cell density. The growth condition in the simulation results is almost equal to that in the experimental results shown in Figure 2, which shows that the doubling time from the cultivation experiment and the growth kinetics applied in the higher nutrient concentration condition resemble the real cell growth condition. Also, the rotating bioreactor simulation shows better growth rates compared to the batch reactor, which may be caused by the higher density of CHO cell in the HARV bioreactor than in the batch reactor. In their experiments, Altamirano *et al.* found that the CHO cells continued growing for 20–30 hrs after the nutrient had been completely consumed. On the other hand, the simulation results represent death/degeneration of the CHO cells during this time period. This may be due a lack of precise experiment results for HARV in our study. Nevertheless, the developed framework provides a possible way to simulate the culturing conditions of rotating bioreactors in lieu of experiments. In consideration of designing the bioreactor, our framework provides the capability to analyze different circumstances separately and control specific parameter for different designing purpose, such as, the geometry of bioreactor, the density of cells. Alternatively, the flexibility of the

developed model enables one to simulate the effects of various modelling parameters (such as growth kinetics) for a given circumstance.

4. Conclusion

The rotating bioreactor is a novel bioreactor used for tissue development and cell culturing. In previous research, the rotating bioreactor has been found to provide a well-mixed and three-dimensional cell culture environment with low shear stress and microgravity. The bioreactor also provides higher gas permeability and it has been shown in earlier work that this type of bioreactor has high potential for three-dimensional tissue developments. Hence, building a modelling framework to simulate the fluid dynamics, nutrient transport and cell growth kinetics in the bioreactor is important for further research and development of the bioreactor technology. In the developed mathematical model, we use a model of suspended particle movement to simulate the cell distribution in the HARV bioreactor. This is a specific variant of the NASA bioreactor. The nutrient transport behavior is simulated by coupling diffusion and convection equations. Combining those two features in the HARV bioreactor, we developed a model of cell growth kinetics for calculating the cell growth rate and the productivity of the cells. We have used in-house experimental data of transient cell density and doubling time for simulating the CHO cells' growth. We have then compared the simulated results with cell cultivation experiment from the literature where we have observed that the simulated results can produce swirling motion of the cells in the HARV bioreactor. By performing this qualitatively comparison, we could confirm the reliability of the developed model. The results of the cell distribution simulation imply that the distribution in the HARV bioreactor depends on the cell, such as the cell density and type of cells. The level of cell aggregation may increase with larger-sized cells and with higher density differences between the cells and the fluid medium. However, some parameters used in this simulation are defined by assumptions as they were not available at the time of simulation. To collect more precise simulation results, further studies are needed. Nevertheless, the aim of this paper, i.e., to develop a simulation tool for coupled cell motion and nutrient transport in HARV bioreactor have been achieved. The framework provides the capability to analyze different circumstances and control specific parameters for given designing purpose, such as, the geometry of bioreactor, the density of cells. Alternatively, the flexibility of our model enables one to simulate the effects of various modelling parameters (such as growth kinetics).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Begley, C. M.; Kleis, S. J. RWPV bioreactor mass transport: earth-based and in microgravity. *Biotechnology and bioengineering* **2002**, *80*, 465–76.
2. Begley, C. M.; Kleis, S. J. The fluid dynamic and shear environment in the NASA/JSC rotating-wall perfused-vessel bioreactor. *Biotechnology and bioengineering* **2000**, *70*, 32–40.

- 460 3. Rivera f Solano, cell K cells, model of the mass transpo
461 bioreactor operated in micro gravity. *Biotechnology and bioengineering* **2006**, 94, 495–504.
- 462 4. Pollack, S. R.; Meaney, D. F.; Levine, E. M.; Litt, M.; Johnston, E. D. Numerical model and experimental
463 validation of microcarrier motion in a rotating bioreactor. *Tissue engineering* **2000**, 6, 519–30.
- 464 5. Freed, L. E.; Vunjak-Novakovic, G. Microgravity tissue engineering. *In Vitro Cellular & Developmental*
465 *Biology - Animal* **1997**, 33, 381–5.
- 466 6. Nickerson, C. a; Ott, C. M.; Wilson, J. W.; Ramamurthy, R.; LeBlanc, C. L.; Höner zu Bentrup, K.;
467 Hammond, T.; Pierson, D. L. Low-shear modeled microgravity: a global environmental regulatory signal
468 affecting bacterial gene expression, physiology, and pathogenesis. *Journal of Microbiological Methods* **2003**,
469 54, 1–11.
- 470 7. Sytkowski, a J.; Davis, K. L. Erythroid cell growth and differentiation in vitro in the simulated microgravity
471 environment of the NASA rotating wall vessel bioreactor. *In vitro cellular & developmental biology. Animal*
472 **2001**, 37, 79–83.
- 473 8. Schwarz, R. P.; Thomas, J.; Wolf, D. A.; Texas, D. A. W. Cell culture for three-dimensional modeling in
474 rotating-wall vessels: An application of simulated microgravity. *Journal of tissue culture methods* **1992**, 14, 51–
475 57.
- 476 9. Ingram, M.; Techy, G. B.; Saroufeem, R.; Yazan, O.; Narayan, K. S.; Goodwin, T. J.; Spaulding, G. F. Three-
477 dimensional growth patterns of various human tumor cell lines in simulated microgravity of a NASA bioreactor.
478 *In vitro cellular developmental biology Animal* **1997**, 33, 459–466.
- 479 10. Cummings, L. J.; Waters, S. L. Tissue growth in a rotating bioreactor. Part II: fluid flow and nutrient
480 transport problems. *Mathematical medicine and biology : a journal of the IMA* **2007**, 24, 169–208.
- 481 11. Cummings, L. J.; Sawyer, N. B. E.; Morgan, S. P.; Rose, F. R. a J.; Waters, S. L. Tracking large solid
482 constructs suspended in a rotating bioreactor: A combined experimental and theoretical study. *Biotechnology*
483 *and bioengineering* **2009**, 104, 1224–34.
- 484 12. Lappa, M. Organic tissues in rotating bioreactors: fluid-mechanical aspects, dynamic growth models, and
485 morphological evolution. *Biotechnology and bioengineering* **2003**, 84, 518–32.
- 486 13. Nauha, E. K.; Alopaeus, V. Modeling method for combining fluid dynamics and algal growth in a bubble
487 column photobioreactor. *Chemical Engineering Journal* **2013**, 229, 559–568.
- 488 14. Subia, S. R.; Ingber, M. S.; Mondy, L. A.; Altobelli, S. A.; Graham, A. L. Modelling of concentrated
489 suspensions using a continuum constitutive equation. *Journal of Fluid Mechanics* **1998**, 373, 193–219.
- 490 15. Rao, R.; Mondy, L.; Sun, A.; Altobelli, S. A numerical and experimental study of batch sedimentation and
491 viscous resuspension. **2002**, 483, 465–483.
- 492 16. Krieger, I. M. Rheology of monodisperse lattices. *Advances in Colloid and Interface Science* **1972**, 3, 111–
493 36.
- 494 17. Leighton, D.; Acrivos, A. The shear-induced migration of particles in concentrated suspensions. *Journal of*
495 *Fluid Mechanics* **1987**, 181, 415–439.
- 496 18. Phillips, R. J.; Armstrong, R. C.; Brown, R. A.; Graham, A. L.; Abbott, J. R. A constitutive equation for
497 concentrated for shear-induced particle migration suspensions that accounts. *Development* **1992**, 4, 30–40.

- 498 19. Zhang, K.; Acrivos, A. Viscous resuspension in fully developed laminar pipe flows. *International Journal of*
499 *Multiphase Flow* **1994**, *20*, 579–591.
- 500 20. Schäpper, D.; Stocks, S. M.; Szita, N.; Lantz, A. E.; Gernaey, K. V. Development of a single-use
501 microbioreactor for cultivation of microorganisms. *Chemical Engineering Journal* **2010**, *160*, 891–898.
- 502 21. Krichnavaruk, S.; Loataweesup, W.; Powtongsook, S.; Pavasant, P. Optimal growth conditions and the
503 cultivation of *Chaetoceros calcitrans* in airlift photobioreactor. *Chemical Engineering Journal* **2005**, *105*, 91–98.
- 504 22. Waters, S. L.; Cummings, L. J.; Shakesheff, K. M.; Rose, F. R. a J. Tissue growth in a rotating bioreactor.
505 Part I: mechanical stability. *Mathematical medicine and biology : a journal of the IMA* **2006**, *23*, 311–37.
- 506 23. Lu, S.; Sun, X.; Zhang, Y. Insight into metabolism of CHO cells at low glucose concentration on the basis of
507 the determination of intracellular metabolites. *Process Biochemistry* **2005**, *40*, 1917–1921.
- 508 24. Altamirano, C.; Illanes, A.; Becerra, S.; Cairó, J. J.; Gódia, F. Analysis of metabolic behavior of CHO cells
509 in the presence of glucose plus galactose. In *2nd Mercosur Congress on Chemical Engineering*; 1997; pp. 1–8.
- 510 25. Almquist, J.; Cvijovic, M.; Hatzimanikatis, V.; Nielsen, J.; Jirstrand, M. Kinetic models in industrial
511 biotechnology - Improving cell factory performance. *Metabolic engineering* **2014**, *24*, 38–60.
- 512 26. Ye, H.; Das, D. B.; Triffitt, J. T.; Cui, Z. Modelling nutrient transport in hollow fibre membrane bioreactors
513 for growing three-dimensional bone tissue. *Journal of Membrane Science* **2006**, *272*, 169–178.
- 514 27. Abdullah, N. S.; Jones, D. R.; Das, D. B. Nutrient transport in bioreactors for bone tissue growth: why do
515 hollow fiber membrane bioreactors work? *Chemical Engineering Science* **2009**, *64*(1), 109-125.

517

Table 1. The parameters used for the simulation of cell distribution

Model parameter	Symbol	Value	Unit	Reference
Diffusivity of glucose	D_f	5.4×10^{-10}	$m^2 \cdot s^{-1}$	[26]
Concentration of glucose	C	5.5	$mole \cdot m^{-3}$	[26]
Density of fluid	ρ_f	1,050	$kg \cdot m^{-3}$	-
Density of CHO cells	ρ_s	1,000	$kg \cdot m^{-3}$	-
Average CHO cell diameter	a	5.0×10^{-6}	m	-
Viscosity of medium fluid	μ_f	0.5889	Pas	-
Rotation speed	-	7.5	rpm	-

518

519

Figure 1. A schematic diagram of the rotating bioreactor. This vessel rotates on its z-axis (shown in the diagram) [4].

Figure 2. Cross-section of the surface geometry of the HARV model. The diameter is 0.1m, and the model framework is built with a Cartesian coordinate system. The size of the mesh ranges from a minimum value of 1.25×10^{-5} m to a maximum value of 0.0037m.

Figure 3. In-house experimental data for CHO cell density as a function of time. The doubling time of CHO cells is 1.95 days, where the cell is culturing at a density of 3×10^5 viable cells/ml in at 37°C and a constant rotating speed at 7.5 rpm

Figure 4. (a) The initial conditions of the validation of the cell volume fraction distribution, according to previous experimental data [4], (b) The validation of denser particles' (1.200 g/cm³) volume fraction distribution after 100s clockwise rotation, (c) The particles' (0.800 g/cm³) volume fraction distribution after 100s rotation.

552

553

554

555

556

557

558

Figure 5. The cell volume fraction distribution (a) Time zero, when all particles are floating on the top of the bioreactor. (b), (c) and (d) bioreactor starts rotating; the cells are first mixed on the outer region of the radius and then the cell density slowly increases into the inner part of the bioreactor.

Figure 6. The microcarrier movement with the polymer microcarrier at a lighter density than the flow medium. The early stage of movement shows similar results to the simulated results in the suspended cell model [4]. This is for a vessel of 55ml volume which has a diameter of 0.1m.

565

566

567

568

569

570

Figure 7. The cell density distribution in the HARV bioreactor at (a) time =86,400s (1 day), (b) time =259,200s (3 days), (c) time = 432,000s (5 days), and (d) time = 604,800s (7 days).

571

572

573

574

575

Figure 8. A typical velocity field in the bioreactor throughout the whole cultivation; (a) shows the magnitude of the velocity field and (b) shows the velocity vectors.

583

584

585 Figure 9. The simulation results for the nutrient distribution of glucose for CHO cell
586 cultivation in the HARV bioreactor at (a) time=86,400s (1 day), (b) time=172,800s (2
587 days), (c) time=259,200s (3 days) and (d) time=345,600s (4 days)

588

Figure 10. Numerically determined average cell density as a function of time. These are averaged data based on the cell density at all numerical points in the bioreactors and therefore they are not compared directly to experimental measurements of the cell density as they are point data.