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Stress-sensitive nutrient consumption via steady and non-reversing dynamic shear in continuous-flow rotational bioreactors☆

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ABSTRACT

Stress-sensitive biological response is simulated in a modified parallel-disk viscometer that implements steady and unidirectional dynamic shear under physiological conditions. Anchorage-dependent mammalian cells adhere to a protein coating on the surface of the rotating plate, receiving nutrients and oxygen from an aqueous medium that flows radially and tangentially, accompanied by transverse diffusion in the z-direction toward the active surface. This process is modeled as radial convection and axial diffusion with angular symmetry in cylindrical coordinates. The reaction/diffusion boundary condition on the surface of the rotating plate includes position-dependent stress-sensitive nutrient consumption via the zr- and $z\Theta$-elements of the velocity gradient tensor at the cell/aqueous-medium interface. Linear transport laws in chemically reactive systems that obey Curie's theorem predict the existence of cross-phenomena between scalar reaction rates and the magnitude of the second-rank velocity gradient tensor, selecting only those elements of ∇v experienced by anchorage-dependent cells that are bound to protein-active sites. Stress sensitivity via the formalism of irreversible thermodynamics introduces a zeroth-order contribution to heterogeneous reaction rates that must be quenched when nutrients, oxygen, chemically anchored cells, or vacant active protein sites are not present on the surface of the rotating plate. Computer simulations of nutrient consumption profiles via simple nth-order kinetics (i.e., $n=1,2$) suggest that rotational bioreactor designs should consider stress-sensitivity when the shear-rate-based Damköhler number (i.e., ratio of the stress-dependent zeroth-order rate of nutrient consumption relative to the rate of nutrient diffusion toward active cells adhered to the rotating plate) is greater than ≈25% of the stress-free Damköhler number. Rotational bioreactor simulations are presented for simple 1st-order, simple 2nd-order, and complex stress-free kinetics, where the latter includes a 4th-order rate expression that considers adsorption/desorption equilibria via the Fowler–Guggenheim modification of the Langmuir isotherm for receptor-mediated cell–protein binding, accompanied by the formation of receptor complexes. Dimensionless parameters are identified to obtain equivalent stress-free nutrient consumption in the exit streams of 2-dimensional creeping-flow rotational bioreactors and 1-dimensional laminar-flow tubular bioreactors. Modulated rotation of the active plate at physiological frequencies mimics pulsatile cardiovascular flow and demonstrates that these rotational bioreactors must operate above the critical stress-sensitive Damköhler number, identified under steady shear conditions, before dynamic shear has a distinguishable effect on bioreactor performance.

1. Introduction

Rotational shear in conventional viscometers is useful to stimulate the proliferation of anchorage-dependent cells and identify the critical angular velocity that induces cell–protein detachment. Larger cell–protein binding energies cause detachment to occur at higher angular velocities of the rotating plate and at radial positions that are farther from the rotation axis. These characteristics of steady-shear rotational viscometers are incorporated into a unique two-dimensional creeping-flow bioreactor to quantify the effects of viscous shear on rates of chemical reaction in stress-sensitive systems, such as anchorage-dependent cells attached to a protein layer on the surface of the rotating plate. Continuous flow of nutrients radially outward from the rotation axis is employed to avoid problems associated with a discontinuous nutrient environment when batch systems are regenerated with fresh feed at regular intervals. Assistance from non-

☆ This manuscript recognizes the life of Prof. Naz Karim's sister, Rashida Muhiuddin, who dedicated her time on this planet to serve and represent millions of people in Muktagacha, Bangladesh. She died suddenly in May 2008. Furthermore, this manuscript commemorates the 100th anniversary of the birth of Alphonse Vincent Belfiore on May 8th, 1909.

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equilibrium thermodynamics provides a fundamental approach to describe stress-dependent nutrient consumption at the cell/aqueous-medium interface. Under isotropic conditions where the transport coefficients are scalars, flux-*i* is coupled to force-*j* if the tensorial ranks of flux-*i* and force-*j* are the same or if they differ by an even integer [1–3]. This classic theorem for flux-force relations is known as the Curie restriction in isotropic systems, proposed by P. Curie in 1903 [1]. As a consequence of Curie's theorem in N-component systems, via the transport-phenomena-based rate of entropy production per volume of fluid, there are N first-rank tensorial fluxes that are coupled to N first-rank tensorial forces via linear laws [3]. Soret diffusion and Dufour conduction represent examples of these couplings between vector fluxes and driving forces in heat and mass transfer [4,5]. Curie's theorem predicts that scalar rates of production of the mass of species *i* due to chemical reaction should be coupled to a scalar representation of the velocity gradient tensor, in addition to any convective enhancement resulting from a reduction in the mass transfer boundary layer thickness adjacent to the active surface. This formalism is employed to construct heterogeneous rates of nutrient consumption and mammalian cell proliferation that are stimulated by viscous shear [6]. There are practical examples where tensile stress stimulates the response of smooth muscle cells [7], compressive stress stimulates nutrient consumption by bone cells [8,9], and shear accelerates the proliferation of endothelial cells [6]. Hence, one constructs the *magnitude of the velocity gradient tensor*, not its *trace* or determinant, to quantify *shear*-stress-sensitive rates of nutrient consumption by selecting only those elements of ∇v that act across the surface of the rotating plate where anchorage-dependent cells bind to proteins. This methodology introduces a zeroth-order contribution to heterogeneous reaction rates that must be quenched via *Heaviside step functions* when nutrients, oxygen, chemically anchored cells, or vacant active protein sites are not present on the surface of the rotating plate. The primary motivation for this investigation includes (i) the use of conventional rotating-disk viscometers that exhibit position-dependent viscous shear at the active surface where anchorage-dependent cells consume nutrients, (ii) comparisons between steady shear in geometrically dissimilar tubular and rotational bioreactors, and (iii) implementation of dynamic shear in the rotating-disk geometry via angular velocity modulations at physiological frequencies to simulate pulsatile cardiovascular flow.

2. Stress-sensitive rotational bioreactor model

2.1. Schematic representation of the rotational bioreactor

The conditions in this creeping-flow parallel-disk configuration combine two classic problems from Newtonian fluid dynamics, as illustrated in Fig. 1. Solid-body rotation of the upper plate at $z=2B$ induces either steady (i.e., $A=0$) or dynamic (i.e., $A=\Omega$) tangential fluid motion. The introduction of fresh nutrient feed that enters the bioreactor through the center of the rotating shaft at $r=R_{\text{inlet}}$ is responsible for flow radially outward. Nutrient depletion in the exit stream at the outer edge of both plates (i.e., $r=R_{\text{Plate}}$) is predicted via integration of the microscopic nutrient mass density profile with respect to Θ (i.e., $0\le\Theta\le2\pi$) and z (i.e., $0\le z\le2B$).

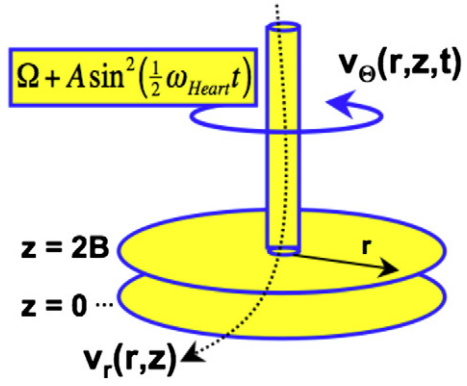

Fig. 1. Schematic illustration of the parallel-disk bioreactor with 2-dimensional creeping flow and non-reversing dynamic shear at the surface of the rotating plate, where cells are seeded at $z=2B$. Steady shear is obtained when the peak-to-peak amplitude A of angular velocity modulations vanishes. The lower plate at $z=0$ is stationary and inert.

2.2. Receptor-mediated cell–protein binding

Active poly(amino acid) sites are identified by favourable protein conformations within an aqueous layer on the surface of the rotating plate (i.e., $z=2B$) that expose functional groups which participate in interactions with cell receptors. Attachment of cell receptors to these protein sites is described by the Fowler–Guggenheim modification of the Sipps isotherm [10–12];

$$\Theta_{\text{Cell}} = 1-\Theta_{\text{Vacant}} = \frac{\left\{K_{\text{Cell}}(T)[\rho_{\text{Cell}}]_{z=2B}\exp(-\varphi\Theta_{\text{Cell}})\right\}^{1/\lambda}}{1+\left\{K_{\text{Cell}}(T)[\rho_{\text{Cell}}]_{z=2B}\exp(-\varphi\Theta_{\text{Cell}})\right\}^{1/\lambda}} \quad (1)$$

where Θ_{Cell} represents the fraction of active sites occupied by cells, Θ_{Vacant} is the vacant site fraction, ρ_{Cell} is the local cell surface density (i.e., mass of free and bound cells per unit area of protein-coated surface), and K_{Cell} is the temperature-dependent adsorption–desorption (i.e., association) equilibrium constant with dimensions of length squared per mass. The statistical thermodynamic derivation [11,12] of Eq. (1) accounts for interactions between adsorbed cells on adjacent active sites via the formation of receptor complexes. The cell–cell interaction energy Ξ is negative to simulate chemical bonding, and $\varphi=\Xi/(k_{\text{Boltzmann}}T)$. Cell–cell attraction and the formation of chemical bonds between receptors (i.e., $\Xi<0$, $\varphi<0$) increases Θ_{Cell} at the same cell mass density [13]. Hence, stronger chemical bonds between adjacent receptors, due to the formation of receptor complexes, and stronger cell–protein binding energies increase cell fractional surface coverage. One recovers the Sipps isotherm from Eq. (1) when the interaction energy between receptor complexes on adjacent active sites vanishes (i.e., $\varphi=0$). The Sipps exponent (i.e., $1/\lambda$) on cell mass density in Eq. (1) corresponds to the Hill coefficient. The Hill equation for protein–ligand binding [14], which is mathematically similar to the Sipps isotherm in heterogeneous catalysis, describes the equilibrium fraction of active protein sites occupied by ligands (i.e., cell receptors). One recovers the Langmuir isotherm when the Hill coefficient λ^{-1} is unity for non-cooperative binding. Hill coefficients greater than unity (i.e., $0<\lambda<1$) correspond to cooperative protein–cell binding, where protein conformational changes occur after the first cell receptor docks to permit subsequent docking with greater affinity.

2.3. Stress-free rate of nutrient consumption

Within reasonable physiological limits, a 4th-order *stress-free* heterogeneous reaction rate for nutrient consumption is expressed in terms of (i) nutrient and oxygen mass densities near the surface of the rotating plate at $z=2B$, (ii) surface coverage fraction of cells on active sites, Θ_{Cell}, which is related to cell mass density via Eq. (1), and (iii) vacant site fraction, Θ_{Vacant}, which is required for cells to consume nutrients aerobically and increase their mass density via chemisorption as a monolayer on the protein-active surface. Hence, the complex *stress-free* rate of nutrient consumption, with dimensions of nutrient mass per surface area per time, is;

$$-R_{A,\text{SurfaceRx}} = k_{\text{Surface}}\{\rho_{\text{Nutrient}}\}_{z=2B}\{\rho_{\text{Oxygen}}\}_{z=2B}\Theta_{\text{Cell}}\Theta_{\text{Vacant}} \quad (2)$$
$$\Theta_{\text{Cell}}+\Theta_{\text{Vacant}}=1$$