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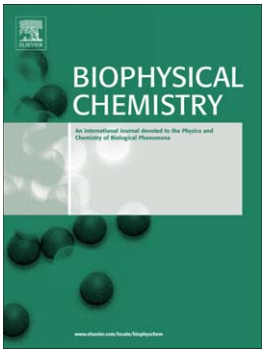

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Tubular Bioreactor Models that Include Onsager-Curie Scalar Cross-Phenomena to Describe Stress-Dependent Rates of Cell Proliferation

Laurence A. Belfiore & M. Nazmul Karim***

**Department of Chemical & Biological Engineering*
**Colorado State University, Fort Collins, Colorado USA 80523*
**presently at; Department of Materials Engineering & Industrial Technologie*
University of Trento, via Mesiano 77
38050 Trento, ITALY

***Department of Chemical Engineering*
Texas Tech University, Lubbock, Texas USA 79409

Abstract

The theory of heterogeneous catalysis in chemical reactors is employed to simulate laminar flow through tubes at large mass transfer Peclet numbers in which anchorage-dependent cells (i) adhere to a protein coating on the inner surface at $r = R_{wall}$, (ii) receive nutrients and oxygen from an aqueous medium via transverse diffusion toward the active wall, and (iii) proliferate in the presence of viscous shear at the cell/aqueous-medium interface. This process is modeled as convective diffusion in cylindrical coordinates with chemical reaction at the boundary, where chemical reaction describes the rate of nutrient consumption. The formalism of irreversible thermodynamics is employed to describe an unusual coupling between viscous shear, or velocity gradients at the cell/aqueous-medium interface, and rates of nutrient consumption. Linear transport laws in chemically reactive systems that obey Curie's theorem predict the existence of cross-phenomena between fluxes (i.e., scalar reaction rates) and driving forces (i.e., second-rank velocity gradient tensor) whose tensorial ranks differ by an even integer--in this case, two. This methodology for stress-dependent chemical reactions yields an additional zeroth-order contribution, via the magnitude of the velocity gradient tensor, to heterogeneous kinetic rate expressions because nutrient consumption and cell proliferation are stress-sensitive. Computer simulations of nutrient consumption suggest that bioreactor designs should consider stress-sensitive reactions when the shear-rate-based Damköhler number (i.e., defined for the first time in this study as the stress-dependent zeroth-order rate of nutrient consumption relative to the rate of nutrient diffusion toward active cells adhered to the tube wall) is greater than 10-20% of the stress-free Damköhler number. Models of bioreactor performance are presented for simple 1st-order, simple 2nd-order, and complex chemical kinetic rate expressions, where the latter considers adsorption/desorption equilibria via the Fowler-Guggenheim modification of the Langmuir isotherm for cell-protein docking on active sites, accompanied by cell-cell attraction. Stress sensitivity is magnified in physically realistic cell-based tubular bioreactors with complex stress-free kinetic rate expressions relative to simulations with simple first- and second-order kinetics.

Key words: Convective diffusion, bioreactor design, stress-dependent reactions, irreversible thermodynamics, Curie's theorem, scalar cross-phenomena.

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*Author to whom all correspondence should be addressed; belfiore@engr.colostate.edu

Introduction

The thermodynamics of irreversible processes provides a fundamental approach to describe effects of viscous shear on rates of chemical reactions in stress-sensitive biosystems, such as anchorage-dependent cells attached to a protein layer on the inner wall of a tube. 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, even though he may never have stated it or proved it [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 the velocity gradient tensor, but this coupling is typically discarded based on physical rather than mathematical arguments [2], even though experiments have not been designed to investigate this unusual coupling [1]. If Curie's theorem is interpreted rigorously, then it becomes possible to describe quantitatively how velocity gradients at the cell/aqueous-medium interface influence the scalar rate of nutrient consumption (via the magnitude of the velocity gradient tensor), because there is a connection between nutrient consumption and anchorage-dependent (i.e., endothelial) cell proliferation, where the latter is stimulated by viscous shear [6]. *This phenomenon is not simply described by larger nutrient flux toward the wall at higher shear rates via reduction in the mass transfer boundary layer thickness external to adsorbed cells on the tube wall,* because convective enhancement of molecular fluxes is not considered in Curie's theorem [3]. The Curie theorem also suggests that molecular momentum flux should be coupled to chemical potential differences, but this coupling is not necessarily useful, due to the heterogeneous nature of nutrient consumption and cell proliferation on protein-coated surfaces where cells are attached. There is no consumption of nutrients in the bulk aqueous solution. However, chemical potential differences between species at the cell/aqueous-medium interface could provide a contribution to viscous shear stress at the tube wall that might affect laminar flow velocity profiles, but this coupling has not been considered in this contribution. The next section outlines eleven assumptions of a biotubular reactor model for the consumption of nutrients by anchorage-dependent cells bound to protein-coated surfaces. Then, non-equilibrium thermodynamics is employed to describe rates of nutrient consumption in biological systems that are stimulated by viscous shear at the tube wall.

Theoretical Considerations

Assumptions. Continuous-flow heterogeneous tubular bioreactors with cylindrical cross-section are described within the framework of the following assumptions.

1) Fluid flow is steady, laminar, incompressible, and Newtonian. In some cases, entrance effects might have a significant influence on mass transfer and overall performance of individual channels. This problem is circumvented, and the steady flow assumption is realized in

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practice, by passing the nutrient mixture through a tube with no cells adhered to the wall, prior to entering the bioreactor.

2) The convective diffusion mass transfer equation is solved for pseudo-binary mixtures of nutrient A and product B.

3) The diffusional flux of nutrients toward the cell/aqueous-solution interface is governed by Fick's law with a concentration-independent binary molecular diffusion coefficient. Thermal (i.e., Soret), pressure, and forced diffusion are neglected relative to concentration diffusion.

4) Nutrient diffusion via Fick's law in the primary direction of flow (i.e., z-direction) is negligible compared to convective mass transfer. This assumption for *ideal* tubular reactors is justified at large mass transfer Peclet numbers. However, surface diffusion of cells [7] on protein-coated surfaces in the z-direction is influenced strongly by viscous shear.

5) Mass transfer, nutrient consumption, and cell proliferation occur with minimal temperature changes (i.e., all processes occur at 37^0C). Hence, physical properties of the aqueous mixture and the active surface, such as overall mass density, viscosity, ordinary molecular diffusion coefficients in the aqueous nutrient mixture and for cell mobility on protein-coated surfaces, kinetic rate constants for stress-free and stress-dependent rates of nutrient consumption, and the adsorption/desorption equilibrium constant for cell-protein binding do not vary throughout the reactor.

6) The rate of nutrient consumption and cell proliferation contains stress-free and stress-dependent contributions. The mathematical form of this rate law is consistent with the principles of irreversible thermodynamics, subject to physiological constraints.

7) Monolayer cell proliferation on *active sites* at the tube wall is described by the Sipps isotherm [8];

$$\Theta_{Cell} = \left\{K_{Cell}(T)\rho_{Cell}(r = R_{wall})\right\}^{1/\lambda}\Theta_{Vacant} = \frac{\left\{K_{Cell}(T)\rho_{Cell}(r = R_{wall})\right\}^{1/\lambda}}{1+\left\{K_{Cell}(T)\rho_{Cell}(r = R_{wall})\right\}^{1/\lambda}} \quad (1)$$

where Θ_{Cell} represents the fraction of these active sites occupied by cells, Θ_{Vacant} is the vacant site fraction, ρ_{Cell} is the local cell surface density (i.e., mass of 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. Active sites are identified by favourable protein conformations within the aqueous layer at the tube wall that promote cell-protein docking. The Sipps exponent on mass density in Equation (1) corresponds to the Hill coefficient. The Hill equation for protein-ligand binding [9], which is mathematically similar to the Sipps isotherm in heterogeneous catalysis, describes the equilibrium fraction of active protein sites occupied by ligands (i.e., cells), and it reduces to the Langmuir isotherm when the Hill coefficient λ^{-1} is unity for non-cooperative binding. Hill coefficients greater than unity correspond to cooperative protein-cell binding, where protein conformational changes occur after the first cell receptor docks to permit subsequent docking with greater affinity. Within reasonable physiological limits, the 4th-order *stress-free* heterogeneous reaction rate for nutrient consumption and cell proliferation depends linearly on

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(i) nutrient mass density at the wall, $\rho_{Nutrient}(r=R_{wall})$, with dimensions of nutrient mass per volume of the aqueous phase, (ii) oxygen mass density at the wall, $\rho_{Oxygen}(r=R_{wall})$, with dimensions of mass per volume of the aqueous phase, (iii) surface coverage fraction of cells on active sites, Θ_{Cell}, which is related to cell mass density via the Sipps isotherm, and (iv) 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 protein-coated surfaces. Hence, the complex *stress-free* reaction rate, with dimensions of nutrient mass consumed per surface area per time, is;

$$R_{SurfaceRx} = k_{Surface}\{\rho_{Nutrient}\}_{r=R_{wall}}\{\rho_{Oxygen}\}_{r=R_{wall}}\Theta_{Cell}\Theta_{Vacant}$$
$$\Theta_{Cell} + \Theta_{Vacant} = 1 \qquad (2)$$

The kinetic rate law is a mathematical expression that describes the rate of nutrient consumption at the cell/aqueous-solution interface. The analog in heterogeneous catalysis is a dual-site chemical-reaction rate-controlling mechanism in which nutrients and dissolved oxygen from the aqueous medium are consumed by protein-bound cells such that nutrients and oxygen do not occupy active protein sites [3].

8) The Sipps isotherm, or the Hill equation, that describes fractional surface coverage by cells on active protein sites via protein-ligand docking contains sufficient flexibility to include the effects of cell binding energy and protein conformation on the adsorption/desorption equilibrium constant (i.e., association constant). Larger exothermic binding energies (i.e., greater affinity) are consistent with an increase in the activation energy for desorption (i.e., same barrier energy but a deeper potential well in the adsorbed state), a decrease in the kinetic rate constant for desorption, an increase in the equilibrium constant for protein-ligand binding, and an increase in the equilibrium fraction of active protein sites that are occupied by cell receptors. Statistical thermodynamics is employed to describe the effect of protein conformation on the association equilibrium constant K_{Cell} in the Hill equation for protein-ligand interactions. Starting from the grand partition function Z for mixtures, a few important conformations (i.e., states) that proteins adopt (i.e., alpha-helix, beta-sheet, etc.) are simulated on a tetrahedral lattice using *trans, gauche+, and gauche-* projections for backbone bonds in an N-segment chain (i.e., $N \approx 7$). The energy of each single-chain conformation is approximated via pairwise intramolecular interactions between non-bonded segments that occupy adjacent lattice sites, because these nearest neighbor interactions on a lattice mimic van der Waals interactions in the continuum, whereas bonding interactions are quite different. Flexibility to exclude a group of unfavourable states is incorporated into Z when proteins do not adopt the appropriate conformation for efficient docking, and the connection between K_{Cell} and Z is generated via the *statement of chemical equilibrium* with assistance from statistical thermodynamics for species chemical potentials. This quantitative relation between K_{Cell} and Z reveals the following trend; the affinity between cells and proteins decreases when the protein conformation is not optimum. The association equilibrium constant K_{Cell} decreases and approach zero as the protein conformation becomes less favourable until "docking" (i.e., protein-ligand binding) is no longer feasible.

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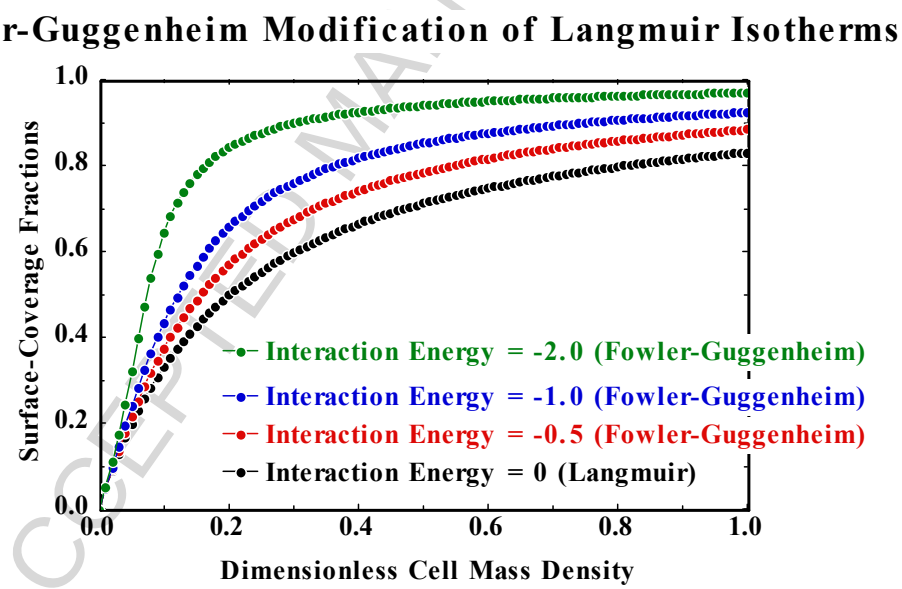
9) Interactions between adsorbed cells on adjacent active sites are described by the Fowler-Guggenheim modification [10] of the Sipps isotherm. If Ω represents the cell-cell interaction energy (i.e., $\Omega<0$ is attractive, $\Omega>0$ is repulsive), $\varphi = \Omega/(k_{Boltzmann}T)$, and $k_{Boltzkmann}$ is Boltzmann's constant, then the fraction of active protein sites occupied by interacting cells is given by;

$$\Theta_{Cell} = \frac{\left\{K_{Cell}(T)[\rho_{Cell}]_{r=R_{wall}} \exp(-\varphi\Theta_{Cell})\right\}^{1/\lambda}}{1+\left\{K_{Cell}(T)[\rho_{Cell}]_{r=R_{wall}} \exp(-\varphi\Theta_{Cell})\right\}^{1/\lambda}} \qquad (3)$$

Cell-cell attraction and the formation of chemical bonds between receptors (i.e., $\Omega<0$, $\varphi<0$) increases Θ_{Cell} at the same cell mass density [11]. Hence, stronger attraction between cells and stronger cell-protein binding energies increase cell fractional surface coverage, as illustrated in Figure#1.



Figure#1

Effect of attractive cell-cell interactions on fractional surface coverage by cells bound to active protein sites, according to the Fowler-Guggenheim modification of the Sipps isotherm when the Hill coefficient is unity for non-cooperative adsorption. The dimensionless cell-cell interaction (i.e., attractive) energy φ is provided in the legend. Non-interacting cells adsorbed on adjacent active sites are described by the Langmuir isotherm (i.e., lowermost curve for non-cooperative cell-protein binding, $\varphi=0$). Dimensionless cell mass density on the protein-coated surface at the tube wall is defined by Eq. (15), and the dimensionless adsorption/desorption (i.e., association) equilibrium constant is $\kappa = 0.5R_{wall}K_{Cell}\rho_{Nutrient,Bulk}(z=0) = 5$, as defined by Eq. (20).

10) Surface diffusion [7] is invoked to describe cell mobility within the aqueous protein layer that coats a biodegradable polymer at the tube wall, where cell receptor diffusivities range from 10^{-9}-10^{-10} cm^2/sec [22]. This phenomenon is required to develop relations between nutrient mass density at the cell/aqueous-solution interface and cell mass density on protein-coated surfaces. One invokes a balance between diffusion and reaction at the boundary (i.e., tube wall, stationary or rotating plate in rotational viscometers, etc.). Nutrients diffuse toward the reactive

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boundary and are consumed. At steady state, this nutrient balance is connected to the rate of cell proliferation, followed by surface diffusion of cells away from the site of nutrient consumption. If angular symmetry is reasonable, such that cell mass density in the aqueous protein coating at the tube wall is independent of the polar angle in cylindrical coordinates, then the desired relation between radial diffusion of nutrients within the mass transfer boundary layer and surface diffusion of adhered cells in the z-direction is given by;

$$-\frac{1}{\varepsilon_{Nutrient}} D_{Nutrient} \left\{ \frac{\partial \rho_{Nutrient}}{\partial r} \right\}_{r=R_{wall}} = \frac{2 D_{Cell}}{\varepsilon_{Cell} R_{wall}} \left\{ \frac{d\rho_{Cell}}{dz} \right\} \qquad (4)$$

Viscous shear stress τ_{rz} at the cell/aqueous-solution interface influences cell motility along the surface in the direction of bulk flow. In general, nutrients exhibit 3-dimensional diffusion and cells diffuse in two coordinate directions within the protein coating on the inner wall of the tube. The dimensionality of the diffusion process is embedded in the number of independent spatial variables required to construct the Laplacian of mass density in the convective diffusion equation [i.e., see Eqs. (11) & (12)], where all diffusivities have dimensions of length^2/time [21,22]. Reasonable approximations (i.e., Pe_{MT}>>1 and angular symmetry) suggest that nutrients diffuse primarily in the radial direction and viscous shear influences cell motility on the surface in the z-direction. Nutrient molecular flux on the left side of Equation (4) has dimensions of mass per area-time, because this surface-related process occurs everywhere within the aqueous medium and $\rho_{Nutrient}$ is a volumetric mass density. The appropriate use of cell surface density ρ_{Cell} in Fick's first law on the right side of Equation (4) requires an additional factor (i.e., the active-surface-area-to-volume ratio = $2/R_{wall}$) because surface diffusional mass flux that incorporates ρ_{Cell} in Fick's law yields dimensions of mass per *length*-time. Using 2nd-order-correct *backward* differences for both derivatives, except for $d\rho_{Cell}/dz$ at the reactor inlet, the previous relation provides an expression for ρ_{Cell} at the current axial position *z;*

$$\frac{2}{R_{wall}} \{3\rho_{Cell}(z) - 4\rho_{Cell}(z-\Delta z) + \rho_{Cell}(z-2\Delta z)\} =$$
$$\frac{\varepsilon_{Cell} D_{Nutrient} \Delta z}{\varepsilon_{Nutrient} D_{Cell} \Delta r} \{-3\rho_{Nutrient}(r=R_{wall},z) + 4\rho_{Nutrient}(r=R_{wall}-\Delta r,z) - \rho_{Nutrient}(r=R_{wall}-2\Delta r,z)\} \qquad (5)$$

where $\varepsilon_{Cell}/\varepsilon_{Nutrient}$ (i.e., ≈ 20-40%) represents the ratio of cell mass produced relative to nutrient mass depleted during nutrient consumption and cell proliferation, considering inefficiencies in nutrient consumption and use of a fraction of this energy generated by consumption to support cell motility in the protein-coated layer at the tube wall. If there are no nutrient gradients in aqueous media, then nutrient transport toward adhered cells will not occur, these cells would subsequently die (not immediately), and cell movement would cease. There might be a time lag between these processes, but there should be a connection between nutrient transport via diffusion toward the active surface and subsequent cell movement via surface diffusion. A recent development [12] mentions inconsistencies between surface diffusion of cells and the assumption of adsorption/desorption equilibrium for fractional coverage of active protein sites due to cell-protein docking, as described by the isotherm in *Assumption#9.* It might be possible to circumvent this potential inconsistency by replacing the total cell surface mass density ρ_{Cell} in the aqueous protein layer with the surface density of unbound cells (i.e.,

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$\rho_{Cell}\Theta_{Vacant}$) to quantify surface diffusion via Fick's law, because *free* cells have significantly more mobility than those that are chemisorbed. However, according to *Assumption#7,* anchorage-dependent cells only consume nutrients when they are docked on conformationally-favourable (i.e., active) protein sites, so reaction/diffusion stoichiometry at the tube wall rigorously applies to bound cells, not free cells.

11) There exists a relation between nutrient and oxygen diffusional mass fluxes toward cells adhered on active protein sites at the tube wall, according to the cascade of physiological reactions that occur. Typically, stoichiometric relations among reactants occur volumetrically throughout the system. However, the heterogeneous nature of this bioreactor design restricts the applicability of stoichiometry between nutrients and oxygen to the active wall at $r=R_{wall}$, where consumption occurs. The same type of stoichiometry applies rigorously to reactants and products in porous catalytic pellets, but the homogeneity assumption that neglects the internal structure of the catalyst allows one to apply stoichiometry volumetrically [3]. At the well-defined cell/aqueous-medium boundary (i.e., $r=R_{wall}$), the appropriate relation between diffusional mass fluxes, with dimensions of mass per area-time is;

$$\frac{1}{\varepsilon_{Nutrient}} D_{Nutrient} \left\{ \frac{\partial \rho_{Nutrient}}{\partial r} \right\}_{r=R_{wall}} = \frac{1}{\varepsilon_{Oxygen}} D_{Oxygen} \left\{ \frac{\partial \rho_{Oxygen}}{\partial r} \right\}_{r=R_{wall}} \qquad (6)$$

Since each side of Equation (6) depends on axial position *z*, integration from the reactor inlet at z=0 to any position downstream at the wall yields the following mass density of oxygen which is required for quantitative evaluation of the rate of nutrient consumption;

$$\rho_{Oxygen}(r=R_{wall},z) = \rho_{Oxygen,Inlet}(z=0) - \frac{\varepsilon_{Oxygen} D_{Nutrient}}{\varepsilon_{Nutrient} D_{Oxygen}} \left\{ \rho_{Nutrient,Inlet}(z=0) - \rho_{Nutrient}(r=R_{wall},z) \right\} \qquad (7)$$

Ordinary molecular diffusion coefficients for nutrients and oxygen in the aqueous medium scale inversely with the square-root of molecular weight [13], $\varepsilon_{Oxygen}/\varepsilon_{Nutrient}$ (i.e., ≈ 2-4%) represents the ratio of oxygen mass depleted relative to nutrient mass depleted during nutrient consumption, and within reasonable physiological limits, the inlet mass density of dissolved oxygen is approximately 5-7% of the inlet nutrient mass density.

Mathematically correct form of the coupling between scalar reaction rates and the velocity gradient tensor

It is necessary to address the correct quantitative construction of reaction rates, or rates of nutrient consumption and cell proliferation, affected by viscous shear stress at the cell/aqueous-medium interface. Mathematical inconsistencies must be avoided if scalars and second-rank tensors are coupled. Curie's theorem suggests that couplings exist between fluxes and forces whose tensorial ranks differ by 2, where these fluxes and forces are identified by terms in the transport-phenomena-based rate of entropy generation per unit volume of fluid [3]. Several possibilities exist to construct mathematically consistent rate expressions. The scalar reaction rate is coupled linearly to scalar chemical potentials, also known as the *affinity* [2] (i.e., diagonal contribution), and the velocity gradient tensor ∇v (i.e., off-diagonal contribution). If the corresponding coupling coefficients are

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scalars, as expected for isotropic systems, then one should employ a scalar *invariant* of the velocity gradient tensor because these invariants of ∇v are independent of the choice of the coordinate system used to express this tensor [13]. If ∇v is denoted by *g,* then 2 other tensors are defined by g^2 = g•g and g^3 = g•{ g•g} = g•g^2. Three independent scalar invariants of the velocity gradient tensor are constructed by taking the trace of g, g^2, and g^3, based on summing the diagonal elements of these second-rank tensors [13]. In terms of all 9 elements of the velocity gradient tensor $\{g_{ij}\}$, these three independent scalar invariants, as well as two others [13], are defined by Equation (8). Only the first of these five scalar invariants of the velocity gradient tensor is linear in elements of ∇v, but shear components are not considered in *Trace{g}* and ∇•**v** vanishes for incompressible flow. Hence, for stress-sensitive nutrient consumption in biological systems that exhibit incompressibility, it might be necessary to identify one of the other non-vanishing invariants in Equation (8) and take its square root or cube root to obtain a useful expression that is <u>linear</u> in g_{ij}. However, all of the other possible invariants of the velocity gradient tensor, which are non-zero for generalized incompressible flow, vanish for simple one-dimensional shear flow in which there is only one non-zero element of the velocity gradient tensor.

$$
\begin{gathered}
Trace\{g\} = \sum_i g_{ii} = \nabla \bullet v \\
Trace\{g \bullet g\} = Trace\{g^2\} = \sum_{i,j} g_{ij} g_{ji} \\
Trace\{g \bullet [g \bullet g]\} = Trace\{g^3\} = \sum_{i,j,k} g_{ij} g_{jk} g_{ki} \\
\frac{1}{2}\left[\left(Trace\{g\}\right)^2 - Trace\{g^2\}\right] \\
\frac{1}{6}\left[\left(Trace\{g\}\right)^3 - 3\left(Trace\{g\}\right)\left(Trace\{g^2\}\right) + 2Trace\{g^3\}\right] = \text{determinant of g}
\end{gathered}
\tag{8}
$$

Another possibility is to construct the *magnitude of the velocity gradient tensor,* defined by the square-root of the double dot product of the velocity gradient tensor with the transpose of the velocity gradient tensor [13];

$$
\textit{Magnitude of the velocity gradient tensor};\ \left|\nabla v\right| = \sqrt{\frac{1}{2}\{\nabla v\} : \{\nabla v\}^T} = \sqrt{\frac{1}{2}\sum_{i,j} g_{ij}^2} \tag{9}
$$

Reactive mixing in flame combustion was simulated using the square-root of the magnitude of the rate-of-strain tensor [14]. The factor of 0.5 under the square-root guarantees that the magnitude of a symmetric 2nd-rank tensor reduces to its only independent off-diagonal element when all other elements vanish. However, one must recognize that ∇v is not a symmetric 2nd-rank tensor. Fitts [1] suggested that the correct form of the linear *off-diagonal* coupling between scalar reaction rates and the velocity gradient tensor should contain a *double-dot product of a second-rank "rate-coefficient tensor" with the velocity gradient tensor.* In other words, if forces and fluxes have tensorial ranks that differ by an even integer *n*, then the coupling coefficient that relates these fluxes to their conjugate forces should be a tensor of rank *n* [1]. This approach requires several scalar rate coefficients, unless the shear flow is extremely simple with one non-zero element in ∇v, such that only one non-

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zero element is required in the 2nd-rank rate-coefficient tensor. For one-dimensional tube flow, the same functional form of the off-diagonal flux-force relation between reaction rates and viscous shear is obtained by employing (i) a scalar Onsager off-diagonal coupling coefficient γ and the magnitude of the velocity gradient tensor, or (ii) the double-dot product of a second-rank rate-coefficient tensor with the velocity gradient tensor. Only one off-diagonal coupling coefficient is required, due to the simplicity of the shear flow. It should be emphasized that when homogeneous chemical reactions occur *volumetrically* throughout the system, all nonzero scalar elements of $\nabla\mathbf{v}$ contribute to stress-sensitive consumption of reactants within a representative volume element via scalar cross-phenomena in the thermodynamics of irreversible processes, as described in the next section. However, cells bind to favourable protein conformations in the aqueous coating on the inner wall of a tube, and nutrient consumption is a *surface-related process*. Consequently, it is only necessary to consider those components of $\nabla\mathbf{v}$ that cells experience across the active surface at $r=R_{wall}$. If necessary, the *magnitude of the velocity gradient tensor* at the tube wall is modified to reflect this fundamental difference between the effects of stress on homogeneous volumetric reactions vs. heterogeneous surface reactions.

Linear law for stress-dependent rates of nutrient consumption

If one focuses on fluxes and driving forces that appear as products in the rate of entropy generation per unit volume, from the transport-phenomena-based equation of change for fluid entropy [3], then Curie's theorem suggests that the scalar flux $-r_A$ in binary mixtures, better known as the rate of consumption of nutrients with dimensions of nutrient mass per surface area per time, possibly due to several chemical reactions, should depend linearly on both scalar and 2nd-rank tensor driving forces. For pseudo-binary systems that are not far removed from *equilibrium,* the appropriate *linear law* that satisfies Curie's restriction is written in the following form;

$$-r_A = \sum_{j\ \mathrm{Rx's}} \left(-\upsilon_{\mathrm{Aj}}\right) R_{j,SurfaceRx} = \xi_{A1}\frac{1}{T}\varphi_A + \xi_{A2}\frac{1}{T}\sqrt{\frac{1}{2}\{\nabla v\}:\{\nabla v\}^T}$$

$$\varphi_A = \frac{\mu_A}{MW_A} - \frac{\mu_B}{MW_B}$$

$$\text{Onsager coupling coefficients; } \xi_{A1} \text{ and } \xi_{A2}$$

$$\xi_{A1} = \alpha T; \xi_{A2} = \gamma T$$

Generalized kinetic rate law for stress-enhanced cell proliferation on active surfaces (10)

$$R_{SurfaceRx} \underset{\upsilon_{\mathrm{Aj}}=-1}{\overset{\text{1 chemical reaction}}{\Rightarrow}} \alpha\left\{\frac{\mu_A}{MW_A} - \frac{\mu_B}{MW_B}\right\} + \gamma\sqrt{\frac{1}{2}\sum_{i,j} g_{ij}^2}$$

$$g_{ij} = \{\nabla v\}_{ij\text{-element in the } 3x3 \text{ velocity gradient matrix evaluated at } r=R_{wall}}$$

Linear approximations in the thermodynamics of irreversible processes that adequately describe transport phenomena under most experimental conditions are not very satisfactory for chemical

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reactions [2]. In general, large deviations from linear behaviour are observed for chemical reactions. Nonlinearity can be introduced in the reaction rate expression by focusing on the diagonal contribution due to *stress-free* chemical kinetics, not the contribution from viscous stress. Hence, the diagonal (i.e., scalar) contribution to the previous kinetic rate expression [i.e., Eq. (10)], based on differences between chemical potentials (i.e., *the affinity*), is modified by a complex stress-free nonlinear rate law that represents a subset of the Hougen-Watson models for heterogeneous catalysis [3] and biochemical reactions. The linear cross-term should be appropriate under low-shear conditions in the laminar or creeping flow regimes. Specific nutrient/cell combinations are characterized by the kinetic rate constant in the numerator of the stress-free rate law and the Onsager off-diagonal coupling coefficient γ. Adsorption of cells on specific substrates is described by the adsorption/desorption equilibrium constant that appears in (i) the stress-free rate law based on the Sipps or Langmuir isotherm for chemisorption and (ii) the stress-free Damköhler number defined below [see Eqs. (19) & (20)]. It should be emphasized that the linear laws of irreversible thermodynamics do not support the inclusion of shear-rate dependence in the stress-free kinetic rate constant (i.e., ξ_{A1} or α) for nutrient consumption in stress-sensitive systems. This is analogous to the fact that temperature-dependent diffusivities in Fick's first law (i.e., concentration diffusion) are not adequate to describe thermal diffusion, because an additional term that contains the gradient of temperature is required for coupled heat and mass transfer [4,5].

The Onsager off-diagonal coupling coefficient (ξ_{A2} or γ) that connects reaction rates to viscous stress must contain sufficient flexibility to distinguish between stress-enhanced and stress-hindered situations. This is accomplished by using an empirical relation between ξ_{A2} or γ and Poisson's ratio υ, which is consistent with the general trend that favours enhanced catalytic activity when the distance between individual metal atoms increases on "stretched" surfaces. For example, when NO adsorbs on ruthenium, dissociation occurs preferentially at edge dislocations [15,16]. Rates of dissociative adsorption can be several orders of magnitude higher in the vicinity of these defects. This effect is supported by scanning tunneling microscope images that reveal a higher concentration of chemisorbed nitrogen near defects on the ruthenium surface. The connection between Poisson's ratio and stress-enhanced vs. stress-hindered chemical reactions contains some elements of generality. Poisson's ratio is less than or equal to 0.5 for all materials, indicative of either volume increase or no volume change, respectively, in response to tensile stress. There are practical examples where tensile stress stimulates the growth of muscle cells [17], compressive stress stimulates the growth of bone cells [18,19], and shear accelerates the proliferation of endothelial cells [6]. These effects can be summarized by the change in system volume due to tension, compression, or shear, via the contribution from Poisson's ratio (i.e., $1\text{-}2\upsilon$), except when systems are truly incompressible such that $\upsilon = 1/2$.

The primary objective of this research contribution is to develop stress-dependent kinetic rate expressions for bioreactor design that are consistent with Curie's restriction in the thermodynamics of irreversible processes. Parametric studies based on computer simulations allow one to identify a critical shear-rate-based Damköhler number that is approximately (i) 10-20% of the stress-free Damköhler number for simple n^{th}-order kinetics (i.e., n=1,2) and (ii) 1% of the stress-free Damköhler number for complex cell-based kinetics. In addition to the form of the kinetic rate law that is coupled to the laminar flow Newtonian velocity gradient at the cell/aqueous-medium interface, correct predictions of nutrient consumption in tubular bioreactors with anchorage-dependent cells at the wall must consider Onsager-Curie scalar coupling between reaction rates and the magnitude of the velocity gradient tensor when the shear-rate-based Damköhler number exceeds its critical value.

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Physically realistic simulations can be compared with experimental data to extract true kinetic rate constants and off-diagonal coupling coefficients for nutrient consumption. This methodology is useful to design similar bioreactors with different geometries when diffusional limitations are not severe, such that nutrient consumption is governed primarily by kinetics instead of rates of diffusion toward the active sites.

Mass Transfer Equation

Bioreactor performance is established by calculating the mass density of nutrients from a steady state microscopic mass balance that accounts for axial convection and transverse diffusion. Chemical reaction occurs only at the interface between adhered cells and the nutrient solution. Hence, nutrient consumption appears in the boundary conditions, but not in the mass balance that applies volumetrically throughout the homogeneous flow channel [3]. The generalized steady state mass transfer equation for convective diffusion is written in vector form;

$$v \bullet \nabla \rho_{Nutrient} = D_{Nutrient} \nabla \bullet \nabla \rho_{Nutrient} \qquad (11)$$

where ***v*** is the mass-average velocity of the aqueous nutrient mixture, $\rho_{Nutrient}$ is the nutrient mass density, and $D_{Nutrient}$ is a concentration-independent molecular diffusion coefficient of nutrients in aqueous solution. In cylindrical coordinates, nutrient mass density in aqueous media obeys the following partial differential equation at large mass transfer Peclet numbers when axial diffusion is negligible compared to axial convection;

$$v_z(r) \frac{\partial \rho_{Nutrient}}{\partial z} = D_{Nutrient} \frac{1}{r} \frac{\partial}{\partial r} \left\{ r \frac{\partial \rho_{Nutrient}}{\partial r} \right\} \qquad (12)$$

Boundary conditions. It is mathematically feasible to account for nutrient consumption near the well-defined cell/aqueous-medium interface in straight channels with circular cross-section [3]. A qualitative description of the boundary conditions is based on a steady state mass balance over a differential surface element. Since convective transport vanishes and viscous shear achieves a maximum at the cell/aqueous-medium interface [13], the rate of nutrient transport toward the surface via molecular mass transfer is balanced by the rate of nutrient consumption that contains stress-free and stress-dependent contributions, stimulating anchorage-dependent cell proliferation. The mathematical description of this boundary condition is;

$$-D_{Nutrient} \left\{ \frac{\partial \rho_{Nutrient}}{\partial r} \right\}_{r=R_{wall}} = k_{cell,Surface} \{\rho_{Nutrient}\}_{r=R_{wall}} \{\rho_{Oxygen}\}_{r=R_{wall}} \Theta_{Cell} \Theta_{Vacant} + \gamma \sqrt{\frac{1}{2} \sum_{i,j} g_{ij}^2} \qquad (13)$$

Both processes have dimensions of nutrient mass per area per time, and the Onsager coefficient γ has dimensions of nutrient mass per area of active protein sites that exhibit the appropriate conformation for cell-protein binding. For one dimensional laminar flow through tubes, the only non-zero element of the velocity gradient tensor that cells experience at the tube wall is; $g_{rz} = \{\partial v_z/\partial r\}_{r=Rwall} = -4\langle v_z \rangle_{Average}/R_{wall}$. Symmetry, or zero-flux, is invoked along the centerline of the tube.

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Zeroth-order rates of chemical reaction and modified boundary conditions at the active surface. The stress-dependent contribution to the rate of nutrient consumption in the boundary condition at the tube wall can be classified as a zeroth-order reaction because it does not exhibit *explicit* dependence on the mass density of any species. However, this term must be neglected when nutrients are not available for immediate consumption by anchorage-dependent cells due to extreme diffusion-limited conditions in the aqueous medium. In other words, zeroth-order rate laws must vanish in the absence of reactants, even though the simple mathematical expression for the rate law reduces to the same temperature-dependent "constant" for any nutrient mass density [3]. This conceptual problem with the mathematical form of zeroth-order chemical reactions can be circumvented with assistance from *Dirac delta functions,* $\delta\{\rho_{Nutrient}(r=R_{wall})\}$, which assume a value of unity when nutrients vanish at the active sites, and zero otherwise. The modified version of the diffusion/reaction boundary condition at the tube wall [i.e., Eq. (14), below] "turns off" the stress-dependent contribution to nutrient consumption when reaction is sufficiently faster than radial diffusion at large Damköhler numbers and nutrients do not exist at the active surface;

$$-D_{Nutrient}\left\{\frac{\partial \rho_{Nutrient}}{\partial r}\right\}_{r=R_{wall}} = k_{cell,Surface}\{\rho_{Nutrient}\}_{r=R_{wall}}\{\rho_{Oxygen}\}_{r=R_{wall}}\Theta_{Cell}\Theta_{Vacant} + \gamma\left[1-\delta\{\rho_{Nutrient}(r=R_{wall})\}\right]\sqrt{\frac{1}{2}\sum_{i,j} g_{ij}^2}$$

Use of the Dirac delta function in the modified boundary condition essentially terminates nutrient consumption when nutrients are completely consumed and they cannot reach the active surface due to extreme diffusional resistance in the mass transfer boundary layer. Three additional factors that contain Dirac delta functions should be included in the zeroth-order stress-dependent term of Equation (14) to account for the absence of (i) cells and vacant sites on the active surface, and (ii) oxygen within the cells, after a reasonable time lag when cells can no longer proliferate anaerobically. These effects are included in the dimensionless form of the previous reaction/diffusion boundary condition for complex cell-based kinetics [see Eq. (19)]. Computations that exclude delta-functions in the stress-dependent contribution to nutrient consumption are vulnerable to the prediction of unrealistic negative species concentrations at the active boundary.

Dimensionless equations for viscous flow in tubes with stress-dependent rates of nutrient consumption at the wall

Dimensionless variables and parameters for n^{th}-order irreversible chemical kinetics; These design equations include convection, transverse diffusion, and simple n^{th}-order surface-based chemical reaction that depends on nutrient mass density at the tube wall. The problem description contains three important dimensionless parameters; (i) the mass transfer Peclet number, Pe_{MT} (i.e., rate of convective mass transfer divided by rate of molecular mass transfer), (ii) the ordinary Damköhler number, $\beta_{0,nth\text{-}order}$, for stress-free heterogeneous chemical reactions (i.e., stress-free reaction rate divided by rate of molecular mass transfer), and (iii) the Damköhler number β_{Stress} for stress-dependent heterogeneous chemical reactions at the cell/aqueous-medium boundary (i.e., stress-dependent rate of reaction divided by the rate of nutrient diffusion toward adhered cells). A factor of $R_{wall}/2$ in the dimensional scaling factor for cell mass density represents the volume-to-surface ratio of the tubular reactor. It is required because nutrient mass density is volumetric within the aqueous medium, whereas cell mass density is a surface-related concentration within the aqueous protein coating on the tube wall. Notice that the mass transfer Peclet number and the stress-dependent Damköhler number in Equation (15) are not completely independent, due to the fact

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that each one contains the average fluid velocity. Even though Pe_{MT} and β_{Stress} can be the focus of systematic parametric simulations, independently, the analogous experimental studies are extremely challenging because $\langle v_z \rangle_{Average}$ appears in both dimensionless numbers. If the tube radius R_{wall} is chosen to dimensionalize the radial *r* and axial *z* position variables, then;

$$\begin{aligned}
&\textit{Radial independent}\ \text{variable};\ r = \eta R_{wall} \\
&\textit{Axial independent}\ \text{variable};\ z = \zeta R_{wall} \\
&\textit{Nutrient mass density};\ \rho_{Nutrient} = \rho_{Nutrient,Bulk}(z=0)\Psi_{Nutrient} \\
&\textit{Oxygen mass density};\ \rho_{Oxygen} = \rho_{Nutrient,Bulk}(z=0)\Psi_{Oxygen} \\
&\textit{Cell mass density};\ \rho_{Cell} = \frac{1}{2}R_{wall}\rho_{Nutrient,Bulk}(z=0)\Psi_{Cell} \\
&\textit{Mass transfer Peclet number};\ Pe_{MT} = \frac{\langle v_z \rangle_{Average} R_{wall}}{D_{Nutrient}} \\
&\textit{Stress - free Damkohler number}\ (n^{th} - order);\ \beta_{0,n^{th}-order} = \frac{k_{n,Surface} R_{wall} \{\rho_{Nutrient,Bulk}(z=0)\}^{n-1}}{D_{Nutrient}} \\
&\textit{Stress - dependent Damkohler number};\ \beta_{Stress} = \frac{2\sqrt{2}\gamma \langle v_z \rangle_{Average}}{D_{Nutrient}\rho_{Nutrient,Bulk}(z=0)}
\end{aligned} \tag{15}$$

Dimensionless mass transfer equation. In the laminar flow regime at large mass transfer Peclet numbers, the partial differential equation with variable coefficients that must be solved for dimensionless nutrient mass density is;

$$2Pe_{MT}\{1-\eta^2\}\frac{\partial \Psi_{Nutrient}}{\partial \zeta} = \frac{1}{\eta}\frac{\partial}{\partial \eta}\left\{\eta \frac{\partial \Psi_{Nutrient}}{\partial \eta}\right\} = \frac{\partial^2 \Psi_{Nutrient}}{\partial \eta^2} + \frac{1}{\eta}\frac{\partial \Psi_{Nutrient}}{\partial \eta} \tag{16}$$

The axial derivative (i.e., with respect to z, or ζ) is written using first-order correct finite differences, whereas both radial derivatives on the right side of Equation (16) are implicit at the new axial position using second-order correct finite-difference analogs.

The "starting" profile via simplified analysis of the reaction/diffusion boundary condition at the tube wall. Numerical solution of the mass transfer equation for nutrient mass density begins at an extremely small nonzero value of $z = z_{start}$, not at the inlet where $\rho_{Nutrient}(r,z=0) = \rho_{Nutrient,Inlet}$ [i.e., $\Psi_{Nutrient}(\eta=1,\zeta=0) = 1$] for all radial positions in the circular flow cross-section. This is achieved by solving for dimensionless nutrient mass density at the wall, $\Psi_{Nutrient}(\eta=1,\zeta_{start})$, via finite difference analysis of the reaction/diffusion boundary condition when nutrient mass density at all other radial positions assumes its inlet value of unity. For simple n^{th}-order rates of stress-free nutrient consumption, one writes;

$$\left\{\frac{\partial \Psi_{Nutrient}}{\partial \eta}\right\}_{\eta=1,\zeta_{start}} \approx \frac{\Psi_{Nutrient}(\eta=1,\zeta_{start}) - \Psi_{Nutrient}(\eta=1-\Delta\eta,\zeta_{start})}{\Delta\eta} = -\beta_{0,n^{th}-order}\Psi^n_{Nutrient}(\eta=1,\zeta_{start}) - \beta_{Stress} \tag{17}$$

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The general philosophy behind this approach is that one should begin the numerical algorithm with assistance from an analytical solution that exhibits significant concentration gradients normal to the active wall [3], so that cells are fed by transverse diffusion.

Dimensionless boundary conditions. Both types of Damköhler numbers appear in the reaction/diffusion boundary condition at the tube wall where cells bind to active protein sites. For simple n^{th}-order stress-free kinetics that depend only on nutrient mass density at the cell/aqueous-medium interface, the appropriate boundary conditions are;

$$\Psi_{Nutrient} = 1 @ \zeta = \zeta_{start}, 0 \le \eta < 1$$
$$\left\{ \frac{\partial \Psi_{Nutrient}}{\partial \eta} \right\}_{\eta=0} = 0 \qquad (18)$$
$$-\left\{ \frac{\partial \Psi_{Nutrient}}{\partial \eta} \right\}_{\eta=1} = \beta_{0,n^{th}-order} \Psi^{n}_{Nutrient}(\eta=1,\zeta) + \left[1 - \delta\{\Psi_{Nutrient}(\eta=1,\zeta)\}\right]\beta_{Stress}$$

Modified equations for nutrient consumption and cell proliferation at the tube wall. It is necessary to modify the stress-free Damköhler number and the reaction/diffusion boundary condition at $r=R_{wall}$ when the physiological aspects of heterogeneous nutrient consumption and subsequent cell proliferation are included in this bioreactor model. All of the other equations remain unchanged, but a few additional parameters are required to characterize the inlet conditions at z=0, diffusivity ratios, association equilibrium constant for cell-protein interactions, and the cell-cell energy of attraction when receptors on adjacent protein sites form chemical bonds. As presented in *Assumption#7,* if the rate of aerobic nutrient consumption by cells adhered to active protein sites at the tube wall is;

$$R_{SurfaceRx} = k_{cell,Surface} \{\rho_{Nutrient}\}_{r=R_{wall}} \{\rho_{Oxygen}\}_{r=R_{wall}} \Theta_{Cell} \Theta_{Vacant} \qquad (2)$$

with dimensions of nutrient mass per area-time, then the dimensionless diffusion/reaction boundary condition at $r=R_{wall}$, dimensionless kinetic rate law $R^*_{Nutrient}$, stress-free Damköhler number $\beta_{0,Cells}$, and nutrient/cell diffusion coefficient ratio δ are given by Equation (19), below. Four Dirac delta functions exclude the stress-sensitive zeroth-order reaction rate from the boundary condition at the active wall when nutrients and oxygen do not exist within cells under extreme diffusion-limited conditions. These delta functions stipulate that cells and vacant cites must be present when aerobic proliferation is stimulated by shear stress.

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$$-\left\{\frac{\partial \Psi_{Nutrient}}{\partial \eta}\right\}_{\eta=1} = \beta_{0,Cells} R^*_{Nutrient}(\eta=1,\zeta)$$

$$+\left[1-\delta\{\Psi_{Nutrient}(\eta=1,\zeta)\}\right]\left[1-\delta\{\Psi_{Oxygen}(\eta=1,\zeta)\}\right]\left[1-\delta\{\Theta_{Cell}\}\right]\left[1-\delta\{\Theta_{Vacant}\}\right]\beta_{Stress}$$

$$R^*_{Nutrient}(\eta=1,\zeta) = \frac{\Psi_{Nutrient}(\eta=1,\zeta)\Psi_{Oxygen}(\eta=1,\zeta)\{\Psi_{Cell}(\eta=1,\zeta)\exp(-\varphi\Theta_{Cell})\}^{\frac{1}{\lambda}}}{\left[1+\{\kappa_{Association}(T)\Psi_{Cell}(\eta=1,\zeta)\exp(-\varphi\Theta_{Cell})\}^{\frac{1}{\lambda}}\right]^2} \quad (19)$$

$$\beta_{0,Cells} = \frac{k_{cell,Surface}(T)\{\kappa_{Association}(T)\}^{\frac{1}{\lambda}} R_{wall}\rho_{Nutrient,Bulk}(z=0)}{D_{Nutrient}}$$

$$\delta = \frac{D_{Nutrient}}{D_{Cell}}$$

The dimensionless temperature-dependent association equilibrium constant $\kappa_{Association}(T)$ that characterizes cell-protein interactions is;

$$\kappa_{Association}(T) = \frac{1}{2} R_{wall} K_{Cell}(T)\rho_{Nutrient,Bulk}(z=0) \quad (20)$$

The Criterion for Optimal Bioreactor Performance

The most effective bioreactor design depletes nutrients and proliferates cells most rapidly at a given axial position z. This comparison among nutrient mass density profiles must be performed at identical stress-free and stress-dependent Damköhler numbers, which measure the rate of nutrient consumption relative to the rate of nutrient diffusion toward the wall. The optimal conditions correspond to the smallest bulk nutrient mass density at position z, averaged over the entire cross-sectional area (i.e., $S_{Cross\text{-}Section}$) of the tubular reactor. The velocity-weighted bulk nutrient mass density is defined as follows;

$$\rho_{Nutrient,Bulk}(z) = \frac{\iint_{S_{Cross-Section}} v_z(r)\rho_{Nutrient}(r,z)dS}{\langle v_z \rangle_{Average} S_{Cross-Section}} = 4\int_{\eta=0}^{1} \rho_{Nutrient}(\eta,z)\{1-\eta^2\}\eta d\eta \quad (21)$$

Computer Simulations

Effect of the shear-rate-based Damköhler number on nutrient consumption for first-order and second-order irreversible heterogeneous surface reactions. Bulk nutrient mass density profiles as a function of tubular reactor length were generated at constant values of the mass transfer Peclet number (i.e, Pe_{MT} = 50) and stress-free Damköhler number (i.e., $\beta_{0,nth\text{-}order}$ = 1). For

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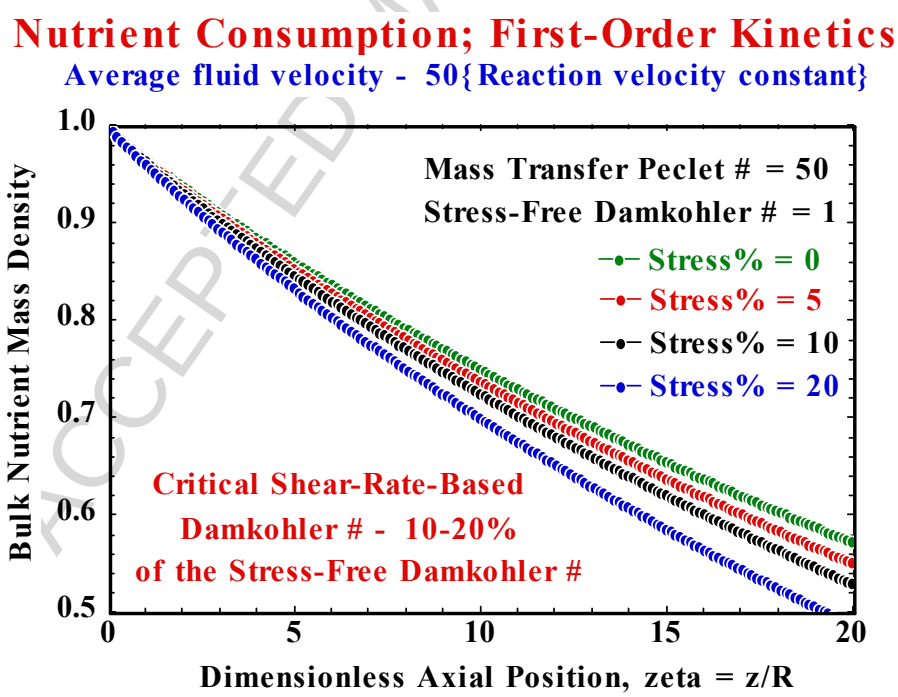
stress-free 1st-order irreversible chemical kinetics, this combination of Pe_{MT} and $\beta_{0,1st\text{-}order}$ corresponds to a mass-average fluid velocity that is 50-fold larger than the stress-free kinetic rate constant (i.e., reaction-velocity constant) for heterogeneous nutrient consumption. When the length-to-diameter ratio of the tubular configuration is $L_{PFR}/\{2R_{wall}\}$ = 10 on the right side of the graphs below, this corresponds to a residence time $\tau_{Residence}$ that is 80% of the characteristic time constant ω for chemical reaction, where $\tau_{Residence}$ and ω (i.e., for simple n^{th}-order nutrient consumption) are defined as;

$$\tau_{\text{Residence}} = \frac{L_{PFR}}{\langle v_z \rangle_{Average}}; \quad \omega = \frac{R_{wall}}{2k_{n,Surface}\left\{\rho_{Nutrient,Bulk}(z=0)\right\}^{n-1}} \qquad (22)$$

Hence, reasonable nutrient consumption is predicted in Figure#2 when L_{PFR}/R_{wall} = 20 and $\tau_{Residence}$ = 0.80ω, but less nutrient consumption is predicted for stress-free 2nd-order irreversible kinetics in Figure#3 relative to stress-free 1st-order kinetics in Figure#2. This occurs because only event must occur, based on the presence of nutrients at the tube wall, for stress-free consumption via 1st-order kinetics, whereas two events must occur simultaneously for stress-free consumption via 2nd-order kinetics.



Figure#2

Dimensionless bulk nutrient mass density profiles in tubular bioreactors at various shear-rate-based Damköhler numbers for 1st-order irreversible nutrient consumption. The *stress%* legend should be interpreted as the ratio of the shear-rate-based Damköhler number to the stress-free Damköhler number. Parameters: dimensionless step size in the radial direction, $\Delta\eta$ = 0.010; dimensionless step size in the axial direction, $\Delta\zeta$ = 0.128.

Even though the critical Damköhler number is reported rather subjectively in Figure#2, bulk nutrient mass density profiles seem to be outside of the range of experimental uncertainty when the shear-rate-based Damköhler number is approximately 20% of the stress-free Damköhler number. A similar

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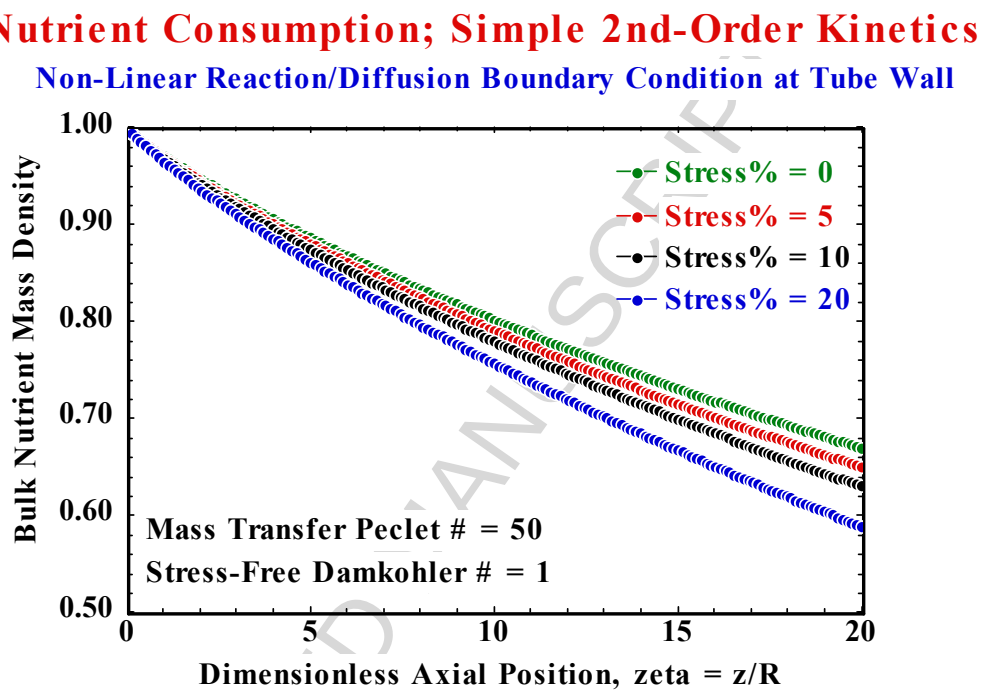
conclusion is obtained for simple 2nd-order kinetics in Figure#3 when the balance between radial diffusion and chemical reaction at the tube wall yields a nonlinear algebraic relation that must be solved together with the linear equations that are generated by finite-difference analysis of the convective diffusion mass transfer equation. Except for reaction order *n*, all other dimensionless parametric values are identical in Figures 2 and 3.



Figure#3

Dimensionless bulk nutrient mass density profiles in tubular bioreactors at various shear-rate-based Damköhler numbers for simple 2nd-order irreversible nutrient consumption that depends only on nutrient concentration at the active tube wall. The *stress%* legend should be interpreted as the ratio of the shear-rate-based Damköhler number to the stress-free Damköhler number. Parameters: dimensionless step size in the radial direction, $\Delta\eta$ = 0.010; dimensionless step size in the axial direction, $\Delta\zeta$ = 0.128.

Parametric sensitivity for complex cell-based rates of nutrient consumption. These simulations require the declaration of several dimensionless parameters, due to the complexity of the kinetic rate law that describes nutrient consumption. It was necessary to increase the ratio of the stress-free Damköhler number relative to the mass transfer Peclet number from 2% in Figures 2 and 3 to 50% in Figure#4, to achieve reasonable conversion of nutrients when the length-to-diameter ratio of the tubular configuration is $L_{PFR}/\{2R_{wall}\}$ = 10 on the right side of the graph, below. Bulk nutrient mass density profiles in Figure#4 suggest that the system is much more sensitive to viscous shear at the cell/aqueous-medium interface when nutrient consumption is modelled as a cascade of four sequential events, as described above in *Assumption#7*. Recent studies of fluid flow in and around 3-dimensional scaffolds, with average velocities between 10^{-3} cm/s and 10^{-2} cm/s that correspond to maximum wall shear stresses on the order of 3 *x* 10^{-2} Pa, describe extensive proliferation of osteoblast-like cells [20].

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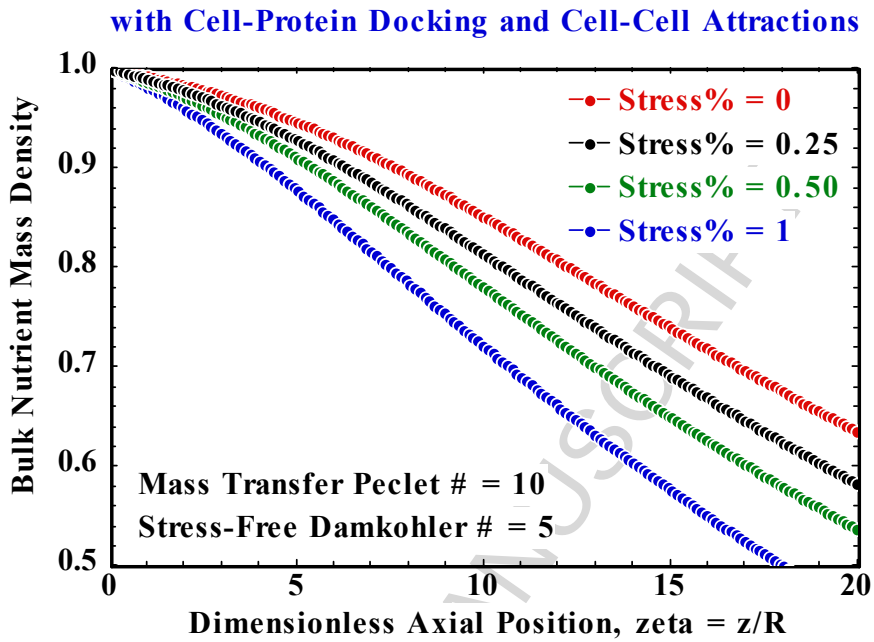

Figure#4

Dimensionless bulk nutrient mass density profiles in tubular bioreactors at various shear-rate-based Damköhler numbers for complex rates of nutrient consumption. The *stress%* legend should be interpreted as the ratio of the shear-rate-based Damköhler number to the stress-free Damköhler number. The critical shear-rate-based Damköhler number in these simulations is at least one order of magnitude smaller, relative to those for simple n^{th}-order kinetics (i.e., n = 1,2). Dimensionless parameters: step size in the radial direction, $\Delta\eta$ = 0.010; step size in the axial direction, $\Delta\zeta$ = 0.128; Hill coefficient, $1/\gamma$ = 1; association equilibrium constant for cell-protein docking, κ = 0.50; cell-cell attractive interaction energy, φ = -0.50; nutrient molecular weight = 300 daltons; nutrient-cell diffusivity ratio, $\delta = D_{Nutrient}/D_{Cell}$ = 3;
inlet mass ratio of oxygen to nutrients, $\rho_{Oxygen}(z=0)/\rho_{Nutrient}(z=0)$ = 0.07 (i.e., 7%);
mass ratio of cells seeded on the tube wall at z=0 to nutrients in the inlet stream,
$2\rho_{Cell}(z=0)/\{R_{wall}\rho_{Nutrient}(z=0)\}$ = 0.10 (i.e., 10%);
ratio of mass of oxygen consumed to mass of nutrients consumed by cells adhered on protein-coated surface,
$\varepsilon_{Oxygen}/\varepsilon_{Nutrient}$ = 0.04 (i.e., 4%);
ratio of mass of cells produced to mass of nutrients consumed by cells adhered on protein-coated surface,
$\varepsilon_{Cell}/\varepsilon_{Nutrient}$ = 0.25 (i.e., 25%);

Self-consistent check of the numerical solutions via the "quasi-macroscopic" mass balance. It is not unreasonable to suspect that truncation errors in the numerical approximation of 1st- and 2nd-derivatives might accumulate in the computational scheme used to integrate the convective diffusion mass transfer equation with diffusion and reaction at the boundary. In the absence of exact analytical solutions, particularly for *complex cell-based kinetics*, the following internal self-consistent approach was employed to verify accuracy of the numerical results. The steady state microscopic mass transfer equation for heterogeneous tubular bioreactors is integrated over a differential control volume (i.e., $dV=2\pi rdrdz$, $0\le r\le R_{wall}$) that is expanded to include the entire cross-sectional area for flow. Volume integrals are converted to surface integrals via Gauss' law, where the appropriate differential surface elements are (i) $2\pi rdr$ (i.e., $0\le r\le R_{wall}$) for convective transport of nutrients in the z-direction, and (ii) $2\pi rdz$ for radial diffusion toward the tube wall. Diffusional flux of nutrients at the tube wall is replaced by the consumption rate $R_{SurfaceRx}$ at $r=R_{wall}$ via the diffusion/reaction boundary condition to obtain the following quasi-macroscopic nutrient balance;

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$$\left\langle v_z \right\rangle_{Average} \pi R_{wall}^2 \left\{ \rho_{Nutrient,Bulk}(z+dz) - \rho_{Nutrient,Bulk}(z) \right\} = -R_{SurfaceRx}(r = R_{wall}, z) 2\pi R_{wall} dz \quad (23)$$

Since the control volume dV and the corresponding surface elements that surround dV remain differentially thick in the z-direction, one obtains an ordinary differential equation that describes how bulk nutrient mass density, defined by Equation (21), changes with axial coordinate *z*, or dimensionless axial coordinate ζ. The analogous equations, presented below in dimensional and dimensionless form, are applicable for incompressible fluids with constant physical properties at high mass transfer Peclet numbers, such that axial diffusion is negligible relative to convective transport of nutrients in the primary flow direction [3];

$$\left\langle v_z \right\rangle_{Average} \pi R_{wall}^2 \left\{ -\frac{d\rho_{Nutrient,Bulk}}{dz} \right\} = 2\pi R_{wall} R_{SurfaceRx}(r = R_{wall}, z)$$
$$-\frac{d\Psi_{Nutrient,Bulk}}{d\zeta} = \frac{2}{Pe_{MT}} \left\{ \beta_{0,Cells} R^*_{Nutrient}(\eta = 1, \zeta) + \beta_{Stress} \right\} \quad (24)$$

Finite-difference solutions of the convective diffusion mass transfer equation with diffusion and reaction at the boundary yield a discrete local nutrient mass density profile, $\rho_{Nutrient}(r,z)$, that is (i) evaluated at $r=R_{wall}$ (i.e., $\eta=1$) to predict rates of nutrient consumption by anchorage-dependent cells attached to a protein-coated layer at the tube wall, and (ii) integrated over the cross-section of the flow channel to obtain the velocity-weighted bulk nutrient mass density, $\rho_{Nutrient,Bulk}(z)$. Then, $\rho_{Nutrient,Bulk}$ is differentiated numerically with respect to axial coordinate z to obtain the quantity on the left side of Equation (24). All finite-difference solutions presented in this investigation exhibit internal self-consistency and satisfy the quasi-macroscopic mass balance to within 0.05%, via the ratio of the left-side to the right-side of Equation (24).

Unusual characteristics of this bioreactor analysis. There are several aspects of this investigation that deviate significantly from the traditional design of ordinary chemical reactors. (1) Kinetic rate expressions that describe nutrient consumption are modified for stress-sensitive reactions, based on the formalism of non-equilibrium thermodynamics. (2) Stress-sensitive zeroth-order contributions to the rate of nutrient consumption are quenched via Dirac delta functions when cells are starved of either nutrients or oxygen due to extreme diffusion-controlled conditions within the mass transfer boundary layer adjacent to the aqueous protein coating on the tube wall. (3) The adsorption isotherm for monolayer cell binding to protein-coated surfaces, based on cell mass density in the vicinity of the active surface rather than in the mass transfer boundary layer or in the bulk aqueous medium, is modified to account for attraction between cells on adjacent protein sites and the formation of chemical bonds between receptors. (4) Surface diffusion of cells within the aqueous protein coating on the tube wall, strongly influenced by viscous shear in the primary flow direction, is invoked to relate cell mass density on the surface to nutrient mass density in the mass-transfer boundary layer adjacent to the tube wall. (5) The complete problem description is developed in terms of mass density, not molar density, because cell physiology and the cascade of events that describe nutrient consumption are treated rather simplistically.

Conclusions

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Theoretical analysis and computer simulations for heterogeneous bioreactors are described from the viewpoint of nutrient consumption in laminar tube flow. Simple 1st- and 2nd-order stress-free kinetics require either one or two events to occur simultaneously, based on the presence of nutrients, for consumption to spawn proliferation. Under these conditions, stress-sensitive ratios of consumption rate to diffusion rate should be on the order of 10-20% of the analogous stress-free ratio before bioreactor designs must consider mechano-sensitive zeroth-order terms in the overall rate of nutrient consumption. Complex cell-based rates of nutrient consumption require a cascade of events to occur. The simplistic model described in this study includes at least 4 events in the stress-free consumption rate, where each event reduces the magnitude of the rate expression. Consequently, stress-sensitive ratios of consumption rate to diffusion rate on the order of 1% of the stress-free ratio have a significant effect on nutrient consumption in more realistic tubular bioreactor simulations. The critical shear-rate-based Damköhler number for zeroth-order stress-sensitive reactions is an order of magnitude smaller in bioreactors with complex cell-based rates of nutrient consumption relative to bioreactors with simple 1st- or 2nd-order stress-free kinetics.

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Nomenclature

D_{Cell}	surface diffusion coefficient for cells on protein-coated surfaces; length^2/time
$D_{Nutrient}$	ordinary molecular diffusion coefficient of nutrients in aqueous mixtures; length^2 per time
D_{Oxygen}	ordinary molecular diffusion coefficient of dissolved oxygen in aqueous mixtures; length^2 per time
g	velocity gradient tensor (i.e., $\nabla\mathbf{v}$)
g_{ij}	ij-element of the velocity gradient tensor, evaluated at $r=R_{wall}$, which is not symmetric
$k_{n,Surface}$	kinetic rate constant for stress-free heterogeneous rate of nutrient consumption via simple n^{th}-order reaction that depends only on nutrient mass density at the wall; {volume/mass}$^{n-1}$•length/time
$k_{cell,Surface}$	kinetic rate constant for stress-free heterogeneous rate of nutrient consumption based on complex cell-based kinetics; {volume/mass}•length/time
K_{cell}	adsorption/desorption (i.e., association) equilibrium constant; cm^2/gram

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MW_i	molecular weight of species i
Pe_{MT}	mass transfer Peclet number
r	independent radial variable, measured transverse to the flow direction
Δr	step size in the radial direction, between grid points
$R_{SurfaceRx}$	stress-free heterogeneous rate of nutrient consumption; mass per area-time
R_{wall}	radius of the biotubular reactor
T	absolute temperature
$\mathbf{v}$	velocity vector
$\nabla\mathbf{v}$	velocity gradient tensor
$\{\nabla\mathbf{v}\}^T$	transpose of the velocity gradient tensor
v_z	z-component of the local fluid velocity vector
$\langle v_z \rangle_{Average}$	cross-section averaged z-component of the velocity vector
z	independent spatial variable measured in the primary flow (i.e., axial) direction
Δz	step size in the axial direction, between grid points
z_{start}	value of z where the numerical simulations begin
Z	grand partition function for protein conformations in mixtures

Greek symbols

∇	gradient operator
$\beta_{0,nth\text{-}Order}$	stress-free Damköhler number for nutrient consumption by n^{th}-order kinetics
$\beta_{0,Cells}$	stress-free Damköhler number for nutrients comption by complex cell-based kinetics
β_{Stress}	stress-dependent Damköhler number for nutrient consumption by zeroth-order kinetics
δ	nutrient/cell diffusivity ratio, with a factor of $2/R_{wall}$ for dimensional consisency
$\varepsilon_{Cell}/\varepsilon_{Nutrient}$	ratio of cell mass produced relative to nutrient mass depleted during nutrient consumption and cell proliferation
$\varepsilon_{Oxygen}/\varepsilon_{Nutrient}$	ratio of oxygen mass depleted relative to nutrient mass depleted during nutrient consumption
φ	dimensionless cell-cell interaction energy in the Fowler-Guggenheim modification of the Sipps isotherm
φ_A	affinity of species A in binary mixtures; chemical potential difference
κ	dimensionless association equilibrium constant for cell-protein docking
λ	exponent in the Sipps isotherm; inverse of the Hill coefficient; λ=1 for non-cooperative cell-protein binding; $0<\lambda<1$ for positive cooperativity; $\lambda>1$ for negative cooperativity.
μ_i	chemical potential of species *i*
η	dimensionless spatial coordinate in the radial direction, r/R_{wall}
υ	Poisson's ratio which describes the extent of lateral contraction upon extension
Ψ_{Cell}	dimensionless mass density of cells on protein-coated surfaces
$\Psi_{Nutrient}$	dimensionless mass density of nutrients in aqueous solution
$\Psi_{Nutrient,Bulk}$	dimensionless bulk mass density of nutrients in aqueous solution
Ψ_{Oxygen}	dimensionless mass density of dissolved oxygen in aqueous solution
ρ_{Cell}	mass density of cells on protein-coated surfaces; grams/cm^2
$\rho_{Nutrient}$	mass density of nutrients in aqueous solution; grams/cm^3
$\rho_{Nutrient,Bulk}(z=0)$	bulk velocity-weighted area-averaged inlet mass density of nutrients
ρ_{Oxygen}	mass density of dissolved oxygen in aqueous solution; grams/cm^3
$\tau_{Residence}$	average residence time, $z/\langle v_z \rangle_{Average}$
Θ_{Cell}	fractional coverage by anchorage-dependent cells on protein-coated surfaces

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Θ_{Vacant}	fraction of sites on protein-coated surfaces that are not occupied by cells
ω	characteristic time constant for n^{th}-order irreversible chemical reaction
Ω	cell-cell interaction energy in the Fowler-Guggenheim modification of the Sipps isotherm
ξ_{A1}	Onsager diagonal coefficient (i.e., αT) that couples the affinity (i.e., φ_A) to the rate of nutrient consumption
ξ_{A2}	Onsager off-diagonal coefficient (i.e., γT) that couples the magnitude of the velocity gradient tensor to the rate of nutrient consumption
ζ	dimensionless independent spatial variable measured in the primary flow direction, z/R_{wall}

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