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Reactors and Reactions

5.1

Magnetic Resonance Microscopy of Biofilm and Bioreactor Transport

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5.1.1

Introduction

Bioreactors are important in both industrial and natural settings. Industrial applications in the biotechnological production of chemicals and materials for uses in pharmaceuticals and foods utilize both eukaryotic cells in animals, plants, fungi and algae and prokaryotic cells in bacteria. Bioreactor conditions relating to cell transport and activity are found in many natural and medical systems. Medical implants such as catheters serve as capillary bioreactors. In the Earth's subsurface microbial bacteria play a large role in geological and chemical processes in both fractured and porous media. Cellular organisms are present in bioreactors in two broadly defined "states", as free floating cells (planktonic) and as surface attached microcolonies (biofilms) [1]. Transport phenomena in bioreactors are typically dependent on advection dominated mixing which determines the flux of mass through the reactor. Mass nutrient transport from the bulk fluid to the biofilm or free floating cells, and byproduct transport from the biofilm or free cells are controlled by the mixing within the bioreactor. Two classical methods for quantification of transport in systems with convection are the mass transfer coefficient [2] and the Residence Time Distribution (RTD) [3]. Magnetic Resonance Microscopy (MRM) can generate maps of the spatial velocity distribution combined with non-spatially resolved propagator data to provide information to determine the efficacy of the mass transfer coefficient approach and to quantify the RTD.

The characterization of mixing in reactors has a long history in chemical engineering and a classic paper on the topic is that of Danckwerts, in which the concept of a residence time distribution was developed [4, 5]. This statistical characterization of the mean times spent within a reactor of length L , allows a quantification of the transport in complex reactor flows. A significant strength of the RTD approach is the ability to diagnose effects in packed beds, such as channeling [3]. In partitioned pipe mixers [6] and capillary based microfluidic

reactors [7, 8] chaotic advection [9] is responsible for mixing, and RTDs provide data on the complex mixing dynamics. MRM measurements of the bulk fluid flow in biofilm impacted capillary bioreactors show helical secondary flows qualitatively similar to those generated in curved microfluidic reactors [10]. MRM also provides for direct calculation of a modified scale dependent RTD, which is not identical to the RTD measured in the classical fashion but provides analogous information. The propagator contains all the information on the system dynamics and directly quantifies mixing. Calculating the RTDs from the propagators presents the data in a form familiar to the larger chemical engineering community and demonstrates the information that can be provided from MRM measured propagators [11, 12].

This chapter presents MRM data for capillary, packed bed and Couette Vortex Flow Reactor (VFR) types. A brief overview of the basic elements of the MRM techniques applied to study reactor flows in this chapter is given for completeness, but is in no way comprehensive. The RTD calculation from the propagator is discussed first, using the classic non-ideal capillary reactor undergoing Poiseuille flow with Taylor dispersion [13]. MRM velocity maps of secondary flows [14, 15] in a *Staphylococcus epidermidis* biofilm impacted capillary reactor are shown to demonstrate the limitations of mass transfer coefficients. The impact of the biofilm on the transport is quantified by the calculation of RTDs [15]. MRM characterization of the impact of bioactivity on porous media or packed bed reactors for the growth of *Pseudomonas aeruginosa* (FRD1 containing the plasmid pAB1) [16] is demonstrated using velocity maps and propagators [17] and the RTDs are calculated. Finally, the impact on the velocity field of a suspended *Fusobacterium nucleatum*, a rod like bacterium, in a Couette VFR is discussed.

5.1.2

Theory

5.1.2.1 Magnetic Resonance Microscopy (MRM)

MRM [18] involves the use of strong magnetic field gradients to investigate flow, diffusion and magnetic relaxation in matter on scales of the order of less than 100 μm spatial resolution. Information obtained from molecular motions over the entire sample or within the spatial resolution provides data on spatial scales down to 10 nm over timescales ranging from 10 μs to 1 s. Much of the potential of MRM lies not in its high resolution spin-density maps, but rather in the non-invasive, non-destructive spatially resolved flexibilities utilizing a range of physical and chemical molecular scale contrast mechanisms [18]. The most obvious contrast mechanism is spin density, or molecular density, to which chemical shift techniques can be applied to excite certain molecular species selectively, leading to the spatial mapping of a specific molecule. Alternatively, three-dimensional maps may be obtained where the pixel signal intensity is directly proportional to nuclear spin relaxation times (T_1 , T_2 or $T_{1\rho}$). Relaxation times are related to the rotational and translational freedom of the molecule and are most often used to distinguish more solid-like materials from more liquid materials, or to provide soft matter contrast when spin density is similar. The magnetic resonance (MR) signal may also be

encoded for translational molecular motion and quantitative spatial maps of self-diffusion and velocity fields can be obtained. A technique for combined velocity and T_2 relaxation maps has been introduced and rapid simultaneous transport and structure measurements will have significant application to bioreactors [19].

5.1.2.1.1 Transverse Magnetic Relaxation Maps

The MRM pulse sequence used to “image” the biofilm is an adaptation of a basic spin-warp slice selection sequence where the spins in the slice of interest ($z = -s/2$ to $s/2$) are excited at an initial time with a 90° radiofrequency (rf) pulse, Figure 5.1.1(a) [18]. Gradients in the x and y directions are stepped through such that for each gradient value the spins acquire a magnetization phase shift dependent on their location within that gradient. The signal is dephased and refocused at effective echo times of $1t_e, 2t_e, 3t_e \dots 8t_e$ [20]. The MR signal, $S(k_x, k_y, t_e)$ obtained at each echo time is the Fourier inversion of the spin density, $\rho(x, y, z)$ weighted by the average relaxation time parameter $T_2(x, y, z)$ at each image voxel.

$$S(k_x, k_y, t_e) = \int_{-s/2}^{s/2} \left[\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} [\exp\{-t_e/T_2(x, y, z)\} \rho(x, y, z)] \exp\{i2\pi(k_x x + k_y y)\} dx dy \right] dz \quad (5.1.1)$$

where $k_i = (2\pi)^{-1} \gamma G_i \tau_i$, and τ_i is the duration of the gradient in the i -direction, G_i . Fourier transformation of the MR signal provides the relaxation weighted spin density $\rho(x, y)$ spatially averaged over the slice thickness in z and the gradients can be applied along any coordinate axis.

5.1.2.1.2 Velocity Maps

Velocity maps are obtained using another adaptation of the basic spin-warp slice selection sequence, with a pair of gradient pulses added to encode for molecular motion [18]. The pulses are located either side of the refocusing 180° pulse, Figure 5.1.1(b). The first gradient pulse encodes the spins with a phase in magnetization dependent on their location at that point in time, and the second gradient pulse reverses this phase encoding. If a spin moves in the time period Δ , the spin retains a residual phase shift directly proportional to the displacement that has occurred in the time interval Δ . The MR spin echo signal normalized to eliminate relaxation effects, $E(k_x, k_y, q) = S(k_x, k_y, q)/S(k_x, k_y, q=0)$, is the Fourier inversion of the density of the spins weighted by the coherent motion

$$E(k_x, k_y, q) = \int_{-s/2}^{s/2} \left[\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} [\exp\{-i2\pi\Delta q_i v_i(x, y, z)\} \rho(x, y, z)] \exp\{i2\pi(k_x x + k_y y)\} dx dy \right] dz \quad (5.1.2)$$

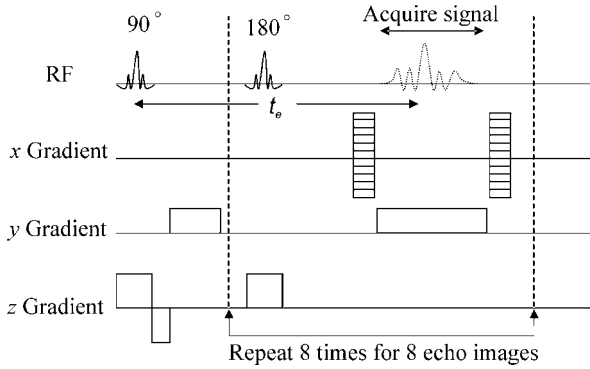


Fig. 5.1.1a

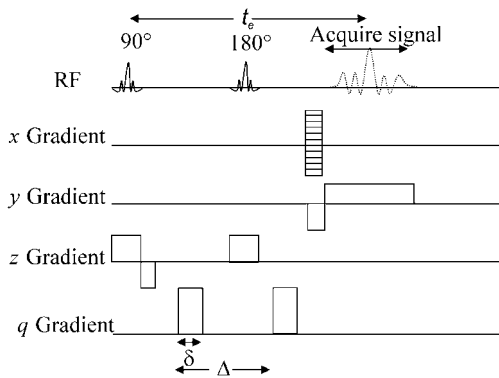


Fig. 5.1.1b

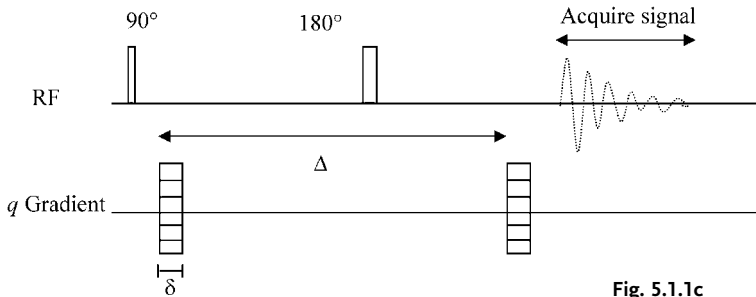


Fig. 5.1.1c

Fig. 5.1.1 (a) Pulse sequence or timing diagram indicating the application of radiofrequency excitation pulses and magnetic field gradients to generate a series of T_2 relaxation weighted images. To make a T_2 map the echo image is refocused eight times at different values of t_e . (b) Velocity map pulse sequence. To make a velocity map the echo image is repeated with two different values of the q gradient pulse. A velocity map is obtained by determining the phase shift at each image

voxel due to the motion sensitizing bipolar q -gradient and hence calculating the average displacement that occurred for those spins over the encoding time Δ . (c) Pulsed Gradient Spin Echo (PGSE) pulse sequence for measurement of the propagator, or displacement probability $\bar{P}_s(Z, \Delta)$. The above sequence samples all of q -space with positive and negative q -values in order to obtain all the data points needed to construct artifact free propagators.

where $q_i = (2\pi)^{-1}\gamma g_i \delta$, and δ is the duration of the velocity encoding gradient applied in the i -direction, g_i . A map of the residual phase shift at each spatial location can then be interpreted as an image of the average velocity over time Δ in each image pixel. Additional time averaging occurs as the velocity maps take approximately 10 min to acquire, so flow changes on a time scale shorter than this are averaged for each pixel providing the stationary velocity [21].

5.1.2.1.3 Propagator [Probability Density of Displacements $P(Z, \Delta)$]

In the pulsed gradient spin-echo sequence, Figure 5.1.1(c), only a pair of pulsed magnetic field gradients are applied so as to encode in the phase of the magnetization the location of all MR active spins at an initial time and then unwind that phase at a set time Δ later, generating phase shifts dependent on molecular dynamics over time Δ , from the entire sample. No spatially localized image data are obtained. The measured echo signal, $E(q, \Delta)$ is the Fourier inversion of the propagator averaged over the initial spin density $\rho(z)$,

$$E(q) = \int_{-\infty}^{\infty} P(Z, \Delta) \exp\{i2\pi q Z\} dZ \quad (5.1.3)$$

where $q = (2\pi)^{-1}\gamma g \delta$ is the Fourier reciprocal wavelength to displacement $Z = z' - z$ and $\bar{P}(Z, \Delta)$ the averaged propagator [18]. This allows a statistical measurement of the details of the dynamics over the entire sample to be obtained. The function can also be discussed in terms of the probability distribution of velocities for times Δ that are short relative to the time scale over which variations in velocity occur $\bar{P}(Z, \Delta) \equiv \bar{P}(v_z) = \bar{P}(Z/\Delta)$ [22, 23], an interpretation which provides the direct connection to the RTD.

5.1.2.2 Transport in Capillary and Vortex Flow Bioreactors

5.1.2.2.1 Mass Transfer Coefficients

Biofilms adhere to surfaces, hence in nearly all systems of interest, whether a medical device or geological media, transport of mass from bulk fluid to the biofilm–fluid interface is impacted by the velocity field [24, 25]. Coupling of the velocity field to mass transport is a fundamental aspect of mass conservation [2]. The concentration of a species, $c(r, t)$, satisfies the advection diffusion equation

$$\underbrace{\frac{\partial c}{\partial t}}_{\text{rate of change of mass}} + \underbrace{\nabla \cdot (vc)}_{\text{flux by advection}} = \underbrace{\nabla \cdot D \cdot \nabla c}_{\text{flux by diffusion}} + \underbrace{R}_{\text{rate of mass consumption or production}} \quad (5.1.4)$$

The spatial and temporal evolution of the concentration field is dependent on the velocity field vector $v(r, t)$, the diffusion tensor $D(r, t)$ and any reactions occurring in the system $R(r, t)$. Non-dimensionalization of Eqn. (5.1.4) generates the Peclet number, $Pe = v_{z, \max} l / D$, which represents the ratio of advective to diffusive

mass transport [2]. The velocity field is determined by the conservation of momentum, which for an incompressible Newtonian fluid is the Navier–Stokes equation

$$\left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \nabla \mathbf{v} \right) = -\frac{1}{\rho} \nabla p + \nu \nabla^2 \mathbf{v} + \mathbf{g} \quad (5.1.5)$$

For axial capillary flow in the z direction the Reynolds number, $Re = v_{z,max}l/\nu$ = “inertial force/viscous force”, characterizes the flow in terms of the kinematic viscosity, ν , the average axial velocity, $v_{z,max}$, and capillary cross sectional length scale, l , by indicating the magnitude of the inertial terms on the left-hand side of Eq. (5.1.5). In capillary systems for $Re < 2000$, flow is laminar, only the axial component of the velocity vector is present and the velocity is rectilinear, *i.e.*, depends only on the cross sectional coordinates not the axial position, $\mathbf{v} = [0, 0, v_z(x, y)]$. In turbulent flow with $Re > 2000$ or flows which exhibit hydrodynamic instabilities, the non-linear inertial term generates complexity in the flow such that in a steady state $\mathbf{v} = [v_x(x, y, z), v_y(x, y, z), v_z(x, y, z)]$.

The modeling of mass transport from the bulk fluid to the interface in capillary flow typically applies an empirical mass transfer coefficient approach. The mass transfer coefficient is defined in terms of the flux and driving force $J = k_c(c_{bulk} - c)$. For non-reactive steady state laminar flow in a square conduit with constant molecular diffusion D , the mass balance in the fluid takes the form

$$0 = -v_z(x, y) \frac{\partial c(x, y, z)}{\partial x^2} + D \left(\frac{\partial^2 c(x, y, z)}{\partial x^2} + \frac{\partial^2 c(x, y, z)}{\partial y^2} \right) \quad (5.1.6)$$

indicating that the mechanism for transport to the capillary surface is molecular diffusion not advection [26]. Non-dimensionalization with axial length, L , and non-axial length, l , leads to, $Pe_L l^2/L^2$, multiplying the advective term with the diffusive term of order 1. This leads to the natural introduction of the axial mass transfer Peclet number, $Pe_L = v_{z,max}L/D$. The axial flow impacts the diffusive transport from the bulk fluid to the surface in two ways; through the diffusive sampling of the streamlines (Taylor dispersion) and the inflow of fluid at bulk concentration. The mass transfer coefficient can be derived for rectilinear capillary flow and the dimensionless mass transfer coefficient, the Sherwood number (Sh), scales linearly with the Reynolds number and the Schmidt number (Sc), $Sc = \nu/D$, $Sh = k_{cL}/D \sim Re Sc = Pe_L$. Analytical solutions for boundary layer flows generate correlations of the form $Sh \sim Re^n Sc^m$, with $n = m = 1/2$ for fluid–fluid interfaces with no interfacial velocity gradient and $n = m = 1/3$ for fluid–solid interfaces with a velocity gradient at the interface [2]. Following the analytical results, empirical values of $n = 7/8$ and $m = 1/3$ are determined for turbulent flows and for capillary flow with $Re > 2000$ indicating a stronger dependence of the mass transfer coefficient on Re due to turbulent eddy mixing. Mass transfer coefficient scaling relationships for biofilm systems have been developed from both experiment [25, 27] and simulation [24, 28, 29]. Correlations of the form $Sh \sim Re^{1/2} Sc^{1/2}$ [28] and $Sh \sim Re^{1/3} Sc^{1/3}$ [24] have been fit to simulation data.

The classical mass transfer coefficient approach fails to incorporate the finer details of the advection field into the correlation in a quantitative fashion. Of significant issue is the fact that in many low Re systems, secondary flows with coherent structure, e.g., vortices, occur due to boundary induced inertial effects and the mass transport is altered by the presence of bulk advection in the x and y directions [29], an issue studied extensively in the analysis of fluid mixing [6]. The concepts of dynamical systems theory have been applied to characterize complex advection in mixing devices [6, 30]. Qualitatively two mass transport effects can occur. The secondary flows can increase homogenization, so the concentration field is uniform and all points in the bulk fluid are near to the average bulk concentration, leading to enhanced mass transport to the biofilm interface relative to a rectilinear flow. Regions of diffusion limited transport and depleted concentration, i.e., hydrodynamic and concentration boundary layers, at the biofilm–fluid interface are decreased. Alternatively, secondary flows can generate a heterogeneous concentration distribution due to fluid regions where regular motions inhibit exchange with the bulk fluid. Mass transport is then dependent on the interaction between the biofilm structure and the structure of the advection field as to whether regions of high or low concentration are in contact with the interface. Lattice–Boltzmann simulations have clearly elucidated these variations in local Sherwood numbers in heterogeneous biofilms [29].

In non-linear mixing flows, e.g., a partitioned pipe mixer, dynamical systems simulations lead to a picture of the flow given by the Poincare section, the time repetition surface of fluid particle trajectories. The advection field exhibits regions of low mixing and high flow regularity surrounded by stronger mixing regions of less ordered flow, referred to as islands of regularity within a chaotic sea [31]. A mixing strength parameter based on the ratio of secondary to axial advection rate is used to characterize the flow, $\beta \sim (v_{x,max}L)/(v_{z,max}l)$ and the amount of chaotic motion increases with β [6]. It is convenient to introduce a non-axial Peclet number, $Pe_l = v_x(\gamma),maxl/D$, and an effective mixing strength can be expressed in terms of a ratio of mass transfer Peclet numbers as $\beta \sim (Pe_l/Pe_L)(L/l)^2$.

5.1.2.3 TRANSPORT in Packed Bed Bioreactors

The modeling of mass transport in packed bed reactors applies the theory of dispersion [32]. The conservation of mass for the average concentration, $\langle c \rangle$ is derived

$$\underbrace{\frac{\partial \langle c \rangle}{\partial t}}_{\text{rate of change of average mass}} + \underbrace{\langle v \rangle \cdot \nabla \langle c \rangle}_{\text{flux by average advection}} = \underbrace{\nabla \cdot D^* \cdot \nabla \langle c \rangle}_{\text{flux by dispersion}} + \underbrace{R}_{\text{rate of average mass consumption or production}} \quad (5.1.7)$$

in terms of the average velocity $\langle v \rangle$ and the dispersion coefficient $D^*(r,t)$. The form of the dispersion coefficient arises from the averaging process and is related to the fluctuations in velocity about the mean. Several mechanisms of dispersion, mechanical, hold-up and Taylor dispersion, may be identified and scaling with Pe

developed [32]. RTDs are often used to characterize macroscale behavior of packed beds [3].

5.1.2.4 Residence Time Distribution (RTD)

The RTD quantifies the number of fluid particles which spend different durations in a reactor and is dependent upon the distribution of axial velocities and the reactor length [3]. The impact of advection field structures such as vortices on the molecular transit time in a reactor are manifest in the RTD [6, 33]. MRM measurement of the propagator of the motion provides the velocity probability distribution over the experimental observation time, Δ . The residence time is a primary means of characterizing the mixing in reactor flow systems and is provided directly by the propagator if the velocity distribution is invariant with respect to the observation time. In this case an exact relationship between the propagator and the RTD, $N(t)$, exists

$$N(L, t)dt = \bar{P}(v_z, \Delta)dv_z \quad (5.1.8)$$

where the reactor length, L , is considered to be the mean distance traveled by the spins over the observation time, i.e., $L = v_{z,av}\Delta$, and the residence time, t , of the spin is related to the velocity of the spin, v_z , by $t = L/v_z$ or $t = v_{z,av}\Delta/v_z$.

In most real systems the propagator transitions from the instantaneous velocity distribution, measured using short observation times, to an invariant form, measured for times greater than the asymptotic time. The asymptotic time is typically that at which all spins have experienced all velocity streamlines via mixing and dispersion mechanisms. A calculation of the RTD from a propagator measured in the pre-asymptotic region is most accurate at times around the residence time of the spins traveling at the mean velocity. If the RTD is displayed on a normalized time axis, t/Δ , then spins residing in the reactor for a time $t/\Delta = 1$ are traveling at the mean velocity, and spins residing for half or double this time are accurately represented by the RTD calculated from this propagator only if the velocity distribution does not change significantly over this observation time window. As demonstrated below, scale dependent RTDs calculated from propagators of velocity distributions that vary over Δ provide details of the mixing, even though they are not exactly the RTD of classical theory.

5.1.3

Reactors

5.1.3.1 Pure Fluid in a Non-ideal Circular Capillary Reactor

Two template examples based on a capillary geometry are the plug flow ideal reactor and the non-ideal Poiseuille flow reactor [3]. Because in the plug flow reactor there is a single velocity, v_0 , with a velocity probability distribution $P(v) = v_0^{-1}\delta(v - v_0)$ the residence time distribution for capillary of length L is the normalized delta function RTD(t) = $\tau^{-1}\delta(t - \tau)$, where $\tau = L/v_0$. The non-ideal reactor with the

parabolic spatial velocity distribution has a velocity probability distribution which is a hat function and the corresponding RTD indicates the large amount of fluid eluted rapidly by the maximum velocities. In the measurement of RTDs for a specified length of the Poiseuille flow capillary reactor, the radius, the average velocity and molecular diffusivity of the reactor fluid, determine the impact of Taylor dispersion as a mixing mechanism. The propagator data for Taylor dispersion of octane flowing in a 150 μm circular capillary [13] analyzed as a velocity probability distribution are shown in Figure 5.1.2(a). The data can be converted into the scale dependent RTDs shown in Figure 5.1.2(b). The natural scaling for the

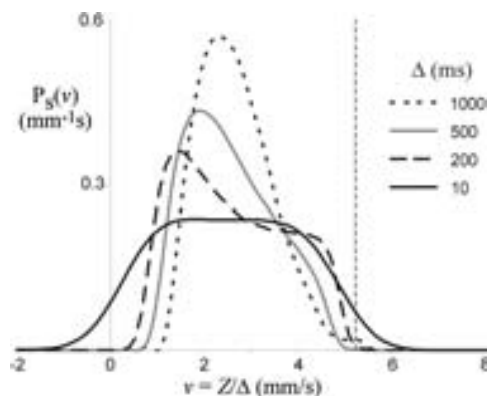


Fig. 5.1.2a

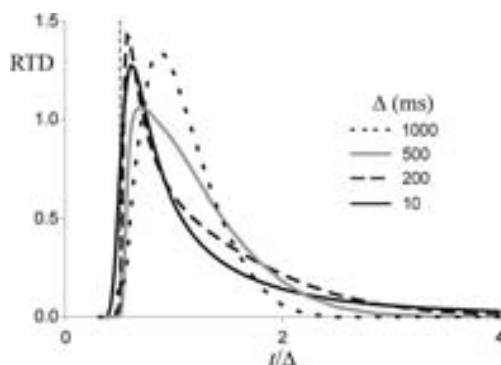


Fig. 5.1.2b

Fig. 5.1.2 Non-ideal capillary flow reactor (a) propagators [13] and (b) corresponding RTDs calculated from the propagator data. (a) The propagators indicate the distribution of average velocities over each observation time (Δ) ranging from 50 ms to 1 s. As the observation time increases the spins exhibit a narrowing distribution of average velocities due to the motional narrowing effect of molecular diffusion across the streamlines. The dashed vertical line represents the maximum velocity that would be present in the absence of molecular

diffusion. (b) The RTDs correspond to varying conduit lengths, L , related to the observation time for the propagator and the mean velocity of the spins, i.e., $L = v_z a \nu \Delta$ is the mean distance traveled by the spins in the observation time. The time axis of the RTD is normalized to the observation time so that a time $t/\Delta = 1$ indicates when spins traveling at the mean velocity will exit the conduit. The dashed vertical line represents the time at which the first spins would exit in the absence of molecular

RTD is the observation time Δ , so as to provide direct comparison between the RTDs calculated from propagators measured at varying displacement times. The impact of Taylor dispersion mixing on the propagator is manifested as a transition from the uniform velocity distribution represented by a hat function at an MR displacement observation time of 10 ms to the Gaussian distribution at 1000 ms [Figure 5.1.2(a)] [13]. Correspondingly the RTD is seen to transition from the 10 ms measurement, where a large amount of fluid is eluted at short times and a significant tail is present at long times due to the slow velocities near the capillary wall, to an RTD where the majority of material is eluted at an intermediate time with no significant tail at long times due to the mixing by Taylor dispersion. The data in Figure 5.1.2 clearly demonstrate that the ability to measure propagators by MR fully characterizes reactor performance in terms of the RTD.

It is important to note that an RTD depends on the length of the conduit analyzed, whereas the measured propagator depends on the observation time Δ of spin displacement evolution. The MRM measured RTDs are normalized about the average distance of conduit, L , sampled by the spins in time Δ , and scale dependent analysis of the variation of the RTD with L is possible. The RTD generated from propagators measured over longer observation times form the best representations of the true RTDs for longer conduit lengths. Of course the spin displacement is limited to the length of the rf coil, L_{rf} , so the MR displacement observation time is limited by the maximum velocity, $\Delta = L_{rf}/v_{z,max}$. Figure 5.1.3 provides a schematic diagram indicating the relationship between the high velocity tails of the MR measured propagator, displayed as a probability distribution of velocity, and the short time region of the RTD. Analogously the low velocity short displacement region of the propagator corresponds to the long time region of the RTD. The RTD can be used to characterize or troubleshoot reactor performance and can be calculated non-invasively from the MR measured propagator if the most relevant observation time is chosen relative to the conduit length of interest.

5.1.3.2 Biofilm Impacted Square Capillary Reactor

S. epidermidis is implicated in many medical implant infections. Mechanical heart valves, shunts, catheters and orthopedic devices are examples of implanted devices which are commonly infected by *S. epidermidis* biofilms [34]. These biofilms are

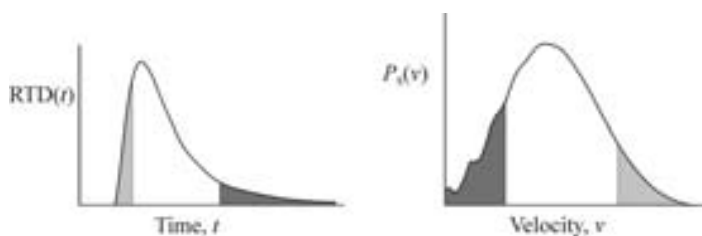


Fig. 5.1.3 Schematic of the relationship between the propagator and the RTD. The light gray shaded area indicates the 25% of spins traveling with the slowest velocities and the dark gray shaded area indicates the 25% of spins traveling at the fastest velocities.

often continuously subjected to high flow rates which cause the biofilm structure to develop high tensile strength [35]. Quantifying how biofilms interact with bulk fluid flow, or advection, is critical to modeling mass transport in biofilm systems [27], shear stress influences on the structure of biofilms [36] and detachment rates [29].

Measuring spatial distributions of velocity within bioreactors is difficult in part due to biofilm opacity. Particle tracking, where microscopic fluorescent spheres are tracked with confocal laser microscopy, is time and labor intensive and measures a tracer response to the flow [37, 38]. In systems where the velocity is of higher spatial dimension and/or time dependent, streamlines everywhere tangent to the Eulerian velocity, and streaklines followed by particles can diverge and a tracer measurement provides particle size dependent Lagrangian tracking, as opposed to the spatial distribution of the molecules composing the bulk flow field [39]. MRM accurately and non-invasively images spatial distributions of velocity fields in biofilm systems [40]. Recently, images of fluid flowing through a circular biofilm fouled capillary demonstrated the ability of MRM to image the axial flow and calculate shear force on the biofilm surface [41]. MRM measurements of 3D velocity components in a square glass capillary fouled with biofilm indicate significant non-axial flows are generated by the biofilm, and correlation between T_2 magnetic relaxation maps displaying biofilm structure with the corresponding velocity patterns provide data on the impact of biofilm growth on advective mass transport [14, 15]. These issues are of significant importance in biosensor and bioseparation applications based on microfluidics or “lab on a chip” technology [42].

5.1.3.2.1 Biofilm Capillary Reactor Materials

The bacterial species *S. epidermidis* strain 35984 is studied in these experiments, and is chosen for its medical importance and the fact that it forms thick opaque biofilms, hundreds of microns thick, in a relatively short amount of time. The biofilms cultured for these experiments were grown in 1 mm square glass capillaries with gravity driven nutrient flow [15]. The nutrient feed is 1/10th strength tryptic soy broth (DIFCO, Beckton Dickinson and Company) at a volumetric flow rate of 0.028 mL s⁻¹, corresponding to an average velocity in a clean capillary of 28 mm s⁻¹ with $Re = 90$. Note Re is calculated with $l = 1$ mm, as for a 1 mm square duct the hydraulic radius is 1 mm. After a no-flow inoculation period of 4 h to allow the suspended bacteria to settle and attach to the glass, the biofilm is allowed to grow for 48 h under these flow conditions. The biofilm sample is incubated at 37 °C during all stages of the growth process to simulate human body temperature. After the 48-h growth period, the biofilm sample was then loaded into the magnet for study. The time dependent growth data shown in Figure 5.1.4 are obtained using the same temperature and flow rate but with the bioreactor in the magnet 4 h after inoculation so that growth could be monitored [15].



Fig. 5.1.4 Time lapse T_2 maps of biofilm structures as a function of growth. The T_2 maps are collected at different stages in the growth of a biofilm at the time shown after initial inoculation. Light colors indicate water restricted within a biofilm and black is the bulk unrestricted water. Images are acquired in the absence of flow, however the flow direction for growth periods between images is left to right [15].

5.1.3.2.2 Biofilm Capillary Reactor MRM Experiment Details

A Bruker Avance DRX spectrometer networked to a 250-MHz superconducting magnet was used to conduct the MRM experiments. Microimaging was accomplished using a Bruker Micro5 probe and gradient amplifiers to produce magnetic field gradients up to 2 T m^{-1} . An rf coil with an internal diameter of 5 mm was used for all the MRM experiments. The medical contrast agent, Magnevist® (Berlex Laboratories) was added to the feed at a concentration of 0.6 mL per liter of nutrient feed in order to decrease the experiment times. Low concentrations of Magnevist have been shown to have no effect on the growth of the biofilms [43]. The MRM measurements are of the ^1H protons on the H_2O molecules in the bulk fluid and within the biomass. T_2 maps are generated using a slice-selection 2D multi-spin echo imaging sequence [Figure 5.1.1(a)] with the following parameter values: $T_R = 500 \text{ ms}$, $t_e = 10 \text{ ms}$, 8 echo images. Total acquisition time for each map was 5 min during which time the flow is stopped to enhance image resolution and avoid in-flow-out-flow artifacts. Velocity data are acquired for flow at $Re = 32$, using a spin-echo sequence with bipolar velocity sensitizing gradients [Figure 5.1.1(b)] with parameters of: $T_R = 2000 \text{ ms}$, $t_e = 20 \text{ ms}$, $g = 0$ and 100 mT m^{-1} , $\delta = 1 \text{ ms}$, $\Delta = 7 \text{ ms}$. Total acquisition time for the velocity maps is 17 min. The T_2 and velocity maps are averaged over a slice of thickness of 0.3 mm with a field of view (FOV) of $2.5 \text{ mm} \times 20 \text{ mm}$ with 64×128 pixels, for a spatial resolution of $39 \times 156 \mu\text{m}$ per pixel.

5.1.3.2.3 Biofilm Capillary Reactor Structure Evolution

MRM non-invasive monitoring of biofilms during growth provides the potential for extended studies of nutrient and biocide effects on biofilm structure and transport [15]. Figure 5.1.4 demonstrates the monitoring of biofilm structure in a capillary bioreactor over a 30-h period. The restricted water molecules within the biofilm are displayed in the light grey to white color range associated with low T_2 values, and

the free water is displayed as black [14, 15, 41, 44]. Note in the first image at 24 h the biofilm forms an approximately 100- μm thick layer of fairly uniform height. The biofilm contains hollowed regions near the capillary wall several of which are clearly visible slightly left of center at the bottom of the image. At 32 h after inoculation the biofilm thickness increases in a heterogeneous fashion with bumps of greater thickness. By 36 h after inoculation the biofilm exhibits significant tower like structures and heterogeneity. Calculation of roughness and surface enlargement from such images [41] can be made to quantify variation over time.

■AQ1■Regions of high molecular mobility, dark and low molecular mobility, bright, are heterogeneously dispersed throughout the biofilm for times of up to 55 h. The data clearly indicate the structural heterogeneity and further study is ongoing to determine if regions of low mobility due to cell clusters can be differentiated from dense EPS regions. An interesting point from the perspective of transport within the biofilm is the fact that what is directly measured is molecular mobility, from which biomass presence is inferred. It may be possible to analyze the data from a perspective such as volume averaging and attempt to correlate average material transport properties [45] to the measured spatial distribution of molecular mobility.

5.1.3.2.4 Biofilm Capillary Reactor Structure and Advective Transport

The cross sectional depth profile of a biofilm can be seen in the T_2 map for a slice perpendicular to the flow axis, Figure 5.1.5, showing the heterogeneity of the biofilm structure [15]. Quantitative velocity data, $v_i(z)$, are plotted as a function of the axial position for two positions in the x direction. The lines A and B drawn through the images indicate the x position of the data presented in the graphs to the right, and are chosen in the bulk fluid and at the biofilm–fluid interface. To construct a biofilm indicator function the T_2 data are masked, setting values associated with free water equal to zero and normalized by the maximum T_2 value. This provides a biofilm signal to be displayed as a value above zero where the biomass is present, with 1 the maximum within the image at the region of lowest T_2 , and zero in areas with no biomass [15]. All three components of velocity are normalized by the maximum z direction velocity so that the x and y components are displayed as a percentage of the axial velocity and are indicative of the mixing intensity [6].

Data for the bulk fluid, line A, indicate that v_z varies as a function of z but maintains a value near 0.75 of maximum velocity. The periodicity of v_x and v_y is clearly evident in the graph of line A and a 180° out of phase coupling of the components is seen with one positive when the other is negative. This indicates a preferred orientation to the plane of the oscillatory flow and this feature was seen in all the biofilms grown throughout this study. The secondary flow components are 0.1–0.2 of the maximum axial velocity and are spatially oscillatory. The significant non-axial velocities indicate non-axial mass transport has gone from diffusion dominated, $Pe_l = 0$, in the clean capillary, to advection dominated, $Pe_l \sim 2 \times 10^3$, due to the impact of the biofilm. For comparison, the axial Peclet number is

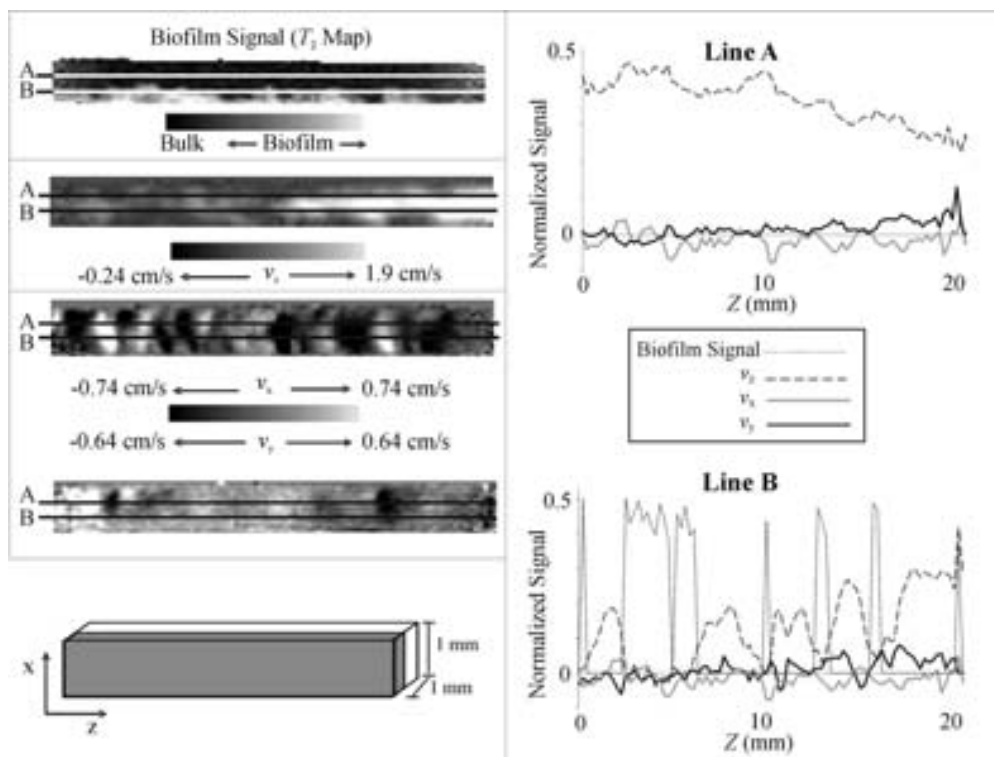


Fig. 5.1.5 Quantitative data on the correlation of biofilm and velocity for a slice perpendicular to the flow axis. The images on the left are from top to bottom: T_2 map, z velocity component, x velocity component and y velocity component. One dimensional profiles through lines A, in bulk fluid, and B, intersecting biofilm fluid interface, are shown on the right. The biofilm signal indicator, dotted grey line, has been normalized so that zero corresponds to no biomass and 1 corresponds to the highest

density of biomass. The velocity components are normalized by the maximum axial (z) velocity. Non-axial velocity components are significant at both heights A and B with maximum non-axial velocity $\approx 20\%$ of the axial maximum. In line A the 180° out of phase coupling of v_x , black line, and v_y , gray line can be clearly seen. Line B indicates the strong correlation between the biomass and axial (z) velocity near the interface [15].

$Pe_L \sim 2 \times 10^5$. Line B intersects areas covered by biomass and areas of only bulk fluid as indicated in the graph of the biofilm signal. Comparing the T_2 map and the graph, line B is seen to intersect a large biofilm clump from an axial position of 3–7 mm at a depth approximately $120 \mu\text{m}$ below the biofilm–fluid interface and is near the biofilm surface along the entire observed length [15]. ■AQ2■ This graph demonstrates that in areas where there is biomass, indicated by a value above zero on the thick black line, v_z , indicated by the dotted line, drops down to values less than 0.1 of the maximum. The axial velocity is negative at sufficient depth within the biomass, as in the region from 3 to 7 mm, as is evidenced by the axial velocity image with regions of small negative velocity within the biofilm. Negative velocities have been demonstrated in simulations [29] and intimated in lower resolution MRI

experiments [46]. The secondary velocity components v_x ,—solid black line and v_y —dashed line, are small within the biofilm and at the interface, and while not as regular as in line A, exhibit an out of phase spatial oscillation and provide amplitudes of 0.1 of maximum axial velocity. The spatial variations in the velocity field are significant over mm scales along the biofilm.

A perturbed helical flow structure of the advection field is indicated by the data. This is evidenced by velocity maps measured for consecutive slices perpendicular to the flow, Figure 5.1.6 [15]. Of greatest interest here are the secondary flow components. Note the onset of a vortex from slices 1 and 2 to a fully developed clockwise vortex flow in slice 3. In slices 4 and 5 the coherent vortex structure is rotated and decaying with axial position. It is important to remember that the velocity measured is for a displacement time Δ of 4 ms but time averaging over the image acquisition time of 10 min occurs due to encoding of information in each acquired signal, so that the spatial distribution velocity components measured are stationary [21]. This is clear visualization of the complex spatial distribution of flow structures which generates a corresponding variation in mass transport [29]. Recent simulations of the structure of secondary flows, Dean vortices, in curved micro-channels suggest that “switching” between flow patterns with varying numbers of vortices during axial transit generates a chaotic flow and has an impact on mixing [10]. Figure 5.1.6 indicates a “switching” type of event with strong negative v_x , and positive v_y , components in slices 1 and 2, transitioning to the coherent vortex in slice 3 and an inverted configuration in slices 4 and 5 relative to the flow upstream of slice 3, with positive v_x , and negative v_y [15]. The complexity of the advection field demonstrated indicates the limitation of the empirical mass transfer coefficient approach to mass transport in its inability to incorporate details of the secondary flows into models.

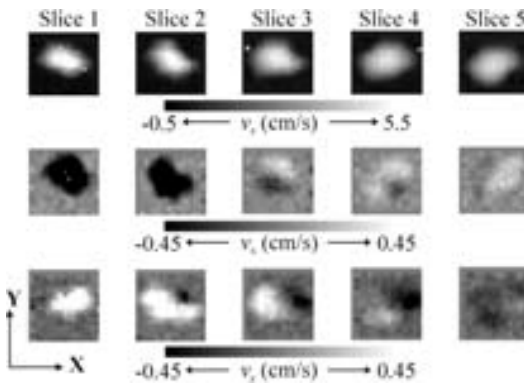


Fig. 5.1.6 These images display the three components of velocity in five consecutive slices through the transverse cross section of the capillary. The direction of bulk flow is from slice 1 to 5 with each slice 300- μm thick and contiguous. Positive axial velocity is out of the page. A negative x component represents flow

to the left. A negative y component represents flow down the page. The helical nature of the flow is clear with non-axial flow changing directions, through the sequence of slices and going through a coherent clockwise vortex flow in slice 3 [15].

5.1.3.2.5 Biofilm Capillary Reactor Propagators and Residence Time Distributions

Figure 5.1.7 shows the propagator of the motion measured for a clean and a biofilm impacted capillary [14, 15] and the residence time distributions calculated for each from these velocity distributions. The clean capillary gives an experimental propagator equal to the theoretical velocity distribution convolved with a Gaussian diffusion curve [14], as shown in Figure 5.1.2. For the flow around the biofilm structure note the appearance of a high velocity tail indicating higher probability of large displacements relative to the clean capillary. The slow flow peak near zero displacement results from the protons trapped within the EPS gel matrix where the primary transport mechanism is diffusive. The residence time distribution integrated over time $\int_0^{t'} N(L, t) dt$ gives the fraction of fluid that has been in a reactor of length L for less than time t' . In Figure 5.1.7 L is the average length traveled by the fluid in the time $\Delta = 15$ ms, i.e., $L = v_z \Delta$. The long tail in the biofilm propagator

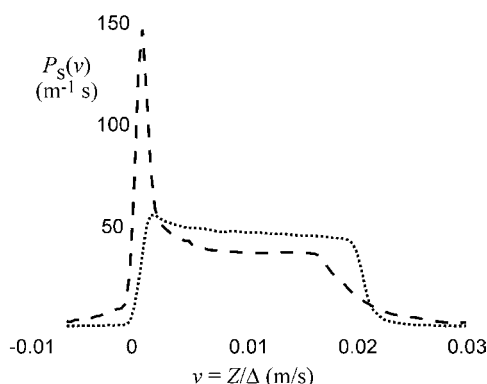


Fig. 5.1.7a

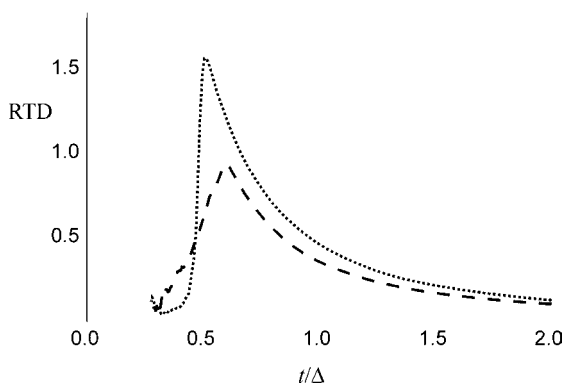


Fig. 5.1.7b

Fig. 5.1.7 (a) Propagators in both a clean square capillary (dotted) and for flow around a biofilm structure (dashed) for an observation time, $\Delta = 15$ ms. (b) Residence time distribution functions calculated from the propagator data shown in (a). The induction of a high

velocity tail due to biofilm fouling is indicated by an increase of spins exiting the conduit ahead of the mean velocity. The trapping of spins in the EPS matrix induces an increase in the zero velocity peak and is indicated by an increase in the tail of the RTD.

manifests itself as a feature at short residence times, whereas the large zero displacement peak in the biofilm propagator manifests itself as a long tail on the RTD [15].

5.1.3.3 Packed Bed Reactors

Packed bed bioreactors occur in a multitude of industrial and natural settings [40]. Industrial applications range from trickle bed reactors used in water filtration systems to hydrogel based enzymatic reactors for production of monoclonal antibodies from mammalian cells. The recovery of oil from or the remediation of contaminants within porous earth formations are strongly impacted by bioactivity and are in essence large packed bed bioreactors. In medical applications membrane and mechanical filtration devices experience biofouling and are variations of packed bed bioreactors. MR methods have found significant application in the study of transport in model and natural porous media due in large part to the ability to measure molecular dynamics in opaque systems [11, 40]. The characterization of porous media structure by molecular diffusion [47] and bulk flow [48, 49] are well established as are the ability to study the displacement and time dependence of the hydrodynamic dispersion [48, 50–53]. Recently the impact of bioactivity on scale dependent dispersion has been quantified and the theory of Continuous Time Random Walks (CTRWs) applied to describe the transition in dynamics from normal to anomalous fluid transport [17].

5.1.3.3.1 Packed Bed Reactor Materials

A 5-mm id porous media column packed bed reactor was constructed using 241- μm diameter monodisperse polystyrene beads (Duke Scientific 4324A). Experiments utilized a dual syringe pump (Pharmacia P-500), which allows controlled volumetric flow rates. The column, tubing and pump were sterilized by repeated treatments with an ethanol solution (70%-wt). For bacterial experiments, the column was then inoculated with a strain of *P. aeruginosa* (FRD1 containing the plasmid PAB1) [16] and allowed to sit for approximately 10 h under very low flow (10 mL h^{-1}) of a 1/10 $\times$ dilution of tryptic soy broth (TSB) media (Becton Dickinson) to allow initial bacterial attachment to the beads. The column was then aseptically transferred to the MR system. Either deionized water for the non-bacterial experiments or 1/10 $\times$ TSB media under constant stirring to ensure oxygenation was pumped into the column from bottom to top with the effluent collected in a waste carboy. Magnevist[®] (Berlex Laboratories) was added to the deionized water and 1/10 $\times$ TSB media at 0.05% v/v. The temperature of the column was held constant at 20 °C for all data collection. The temperature was increased daily for several hours in the bacterial experiments to 37 °C to encourage biofilm formation and growth.

5.1.3.3.2 Packed Bed Reactor MRM Experiment Details

The sample was placed in an 8-mm gradient coil in the Bruker Avance DRX250 spectrometer described previously. A sequence of MR pulse sequences were repeated over a 7–10 day period to monitor the effects of biofilm growth on velocity distributions, T_2 relaxation and averaged propagators [17, 54]. The MRM measurements are of the ^1H protons on the H_2O molecules in the bulk fluid and within the biomass. T_2 maps are generated using a slice-selection 2D multi-spin echo imaging sequence [Figure 5.1.1(a)] with the following parameter values: $T_R = 500$ ms, $t_e = 10$ ms, 8 echo images. Total acquisition time for each map was 40 min during which time the flow is stopped to enhance image resolution and avoid in-flow out-flow artifacts. Velocity data are acquired for flow at $Re = 0.9$, using a spin echo sequence with bipolar velocity sensitizing gradients [Figure 5.1.1(b)] with parameters of: $T_R = 2000$ ms, $t_e = 18$ ms, $g = 0$ and 80 mT m^{-1} , $\delta = 1$ ms, $\Delta = 6$ ms. Total acquisition time for the velocity maps is 70 min. The T_2 maps are averaged over a slice of thickness of 0.2 mm while velocity maps are averaged over a slice of thickness of 1.0 mm. Both T_2 and velocity maps were taken with a field of view (FOV) of $7.0 \text{ mm} \times 7.0 \text{ mm}$ with 128×128 pixels, for a spatial resolution of $55 \times 55 \mu\text{m}$ per pixel. The PGSE pulse sequence [Figure 5.1.1(c)] was used to measure the echo signal, $E(q, \Delta)$ and to compute the averaged propagators $P(Z/\Delta)$. The experimental parameters used for these experiments were $T_R = 2$ s and $\delta = 1$ ms. 128 q sampling points were used within the range of gradient values of $-g_{\text{max}}$ to $+g_{\text{max}}$ in order to detect both positive and negative displacements. The maximum gradient value, g_{max} , was chosen in order to prevent data wrap and varied depending on the value of Δ ranging from 250 mT m^{-1} for $\Delta = 300$ ms to 500 mT m^{-1} for $\Delta = 20$ ms. The total acquisition time for 128 point sampling was approximately 35 min.

5.1.3.3.3 Packed Bed Reactor Structure and Advective Transport

MRM images of the impact of biofilm growth on the pore scale velocity and magnetic relaxation time in the packed bed reactor for fixed volumetric flow rate are shown in Figure 5.1.8. The pore scale velocity images indicate greater spatial variability of the velocity due to biofilm growth 3 and 6 days after inoculation with *P. aeruginosa*. Increased regions of no flow due to biomass blocked pores generate high velocity in unclogged pores to conserve mass for the fixed flow rate. The transverse magnetic relaxation time images indicate the spatial distribution of the biomass growth by measuring enhanced relaxation due to the restricted motion of water molecules within the biomass [44]. On day 3 the impact on pore scale velocity is more significant than on the magnetic relaxation, demonstrating the sensitivity of transport processes to small amounts of biofilm growth.

5.1.3.3.4 Packed Bed Reactor Propagators and Residence Time Distributions

The propagators for both the clean reactor and a biofilm impacted reactor were measured at the same fixed volumetric flow rates of 1.67 mL min^{-1} corresponding to $Pe \sim 200$ based on the bead diameter. Figure 5.1.9 shows the propagator and

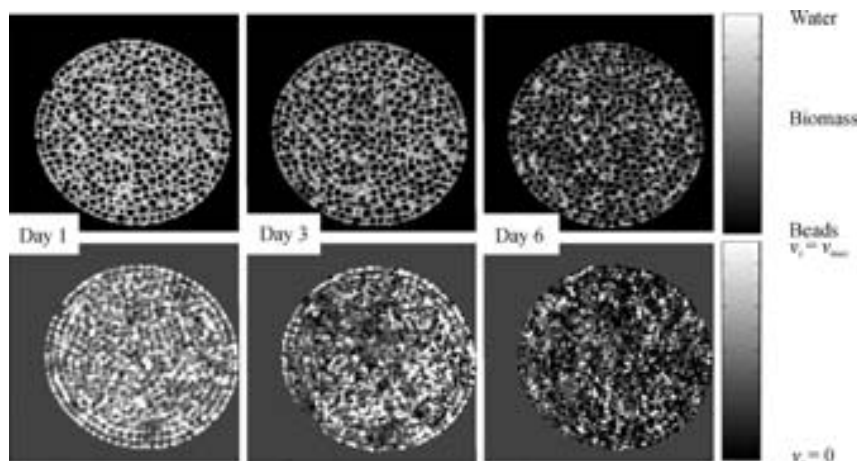


Fig. 5.1.8 MRM maps of velocity (bottom row) and T_2 magnetic relaxation (top row) as a function of biofilm growth time (left to right). Day 1 shows the clean porous media. Spatial resolution is $54.7 \mu\text{m}$ per pixel (128×128 pixels) in plane, so the data reflect pore scale spatial distributions of velocity over a $1000\text{-}\mu\text{m}$ slice and biomass over a $200\text{-}\mu\text{m}$ slice.

RTD calculated from these data for the clean packed bed reactor. The propagators in Figure 5.1.9(a) show the temporal transition in dynamics. A Cauchy [55] like distribution is seen at $\Delta = 20$ ms, indicative of the velocity distribution within single pore spaces, as during the observation time spins are primarily localized within single pores [17]. The dynamics transition to Gaussian at $\Delta = 300$ ms, due to the effect of spins sampling multiple pore spaces, indicating the impact of dispersion mechanisms on the dynamics [17]. The corresponding RTDs in Figure 5.1.9(b) show the transition from early to later primary efflux times and a decrease in the long time elution tails due to mixing as the observation time increases.

Figure 5.1.10 shows the impact of biofilm growth on the packed bed dynamics. The propagators, Figure 5.1.10(a), for a displacement observation time of $\Delta = 300$ ms for the clean bead pack and biofilm growth on days 3 and 6 show a strong transition from normal transport with Gaussian statistics to anomalous transport with non-Gaussian statistics [17]. The growth of the biofilm, day 3 and day 6, alters the dynamics with increased probability of small displacements, due to fluid that is entrained in the biomass and trapped in clogged dead end pores, and long tails in the distribution, due to the higher probability of large displacements in high permeability unclogged channels [17]. These features are also evident in the RTDs, Figure 5.1.10(b), with the trapped fluid in the biomass and dead end pores generating long time tails and the rapid flow on the backbone channels causing early time elution relative to the clean bead pack. This clearly demonstrates the classic channeling effect, for which RTDs have long been used to diagnose in packed bed reactors [3].

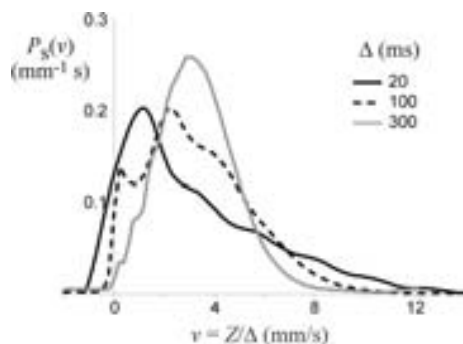


Fig. 5.1.9a

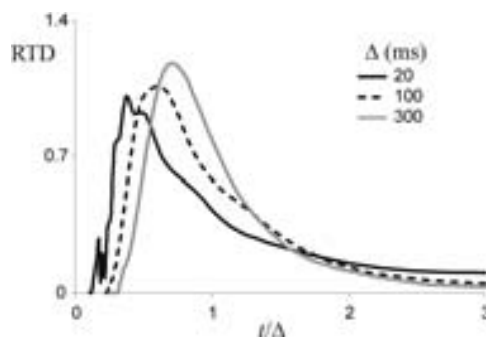


Fig. 5.1.9b

Fig. 5.1.9 (a) MR measured propagators and (b) corresponding calculated RTDs for flow in a model packed bed reactor composed of 241- μm monodisperse beads in a 5-mm id circular column for observation times, Δ , ranging from 20 to 300 ms. As the observation time increases,

the velocity distribution narrows due to the dispersion mechanisms of the porous media; this effect is observed in the RTDs as a narrowing of the time window during which spins will reside relative to the mean residence time as the conduit length is increased.

5.1.3.4 Vortex Flow Reactors (VFR)

Reactors which generate vortex flows (VFs) are common in both planktonic cellular and biofilm reactor applications due to the mixing provided by the VF. The generation of Taylor vortices in Couette cells has been studied by MRM to characterize the dynamics of hydrodynamic instabilities [56]. The presence of the coherent flow structures renders the mass transfer coefficient approaches of limited utility, as in the biofilm capillary reactor, due to the inability to incorporate microscale details of the advection field into the mass transfer coefficient model.

5.1.3.4.1 Vortex Flow Reactor Materials

The vortex flow reactor was a glass Couette cell driven by a Bruker RheoNMR system. The cell consisted of a stationary outer glass tube with an id of 9 mm and a rotating inner glass tube with an od of 5 mm, giving a gap of 2 mm. The Couette was filled with cylindrical bacterial cells, *F. nucleatum* ($\approx 2 \times 20 \mu\text{m}$), suspended in water at a concentration of $\approx 10^{11}$ cells mL^{-1} .

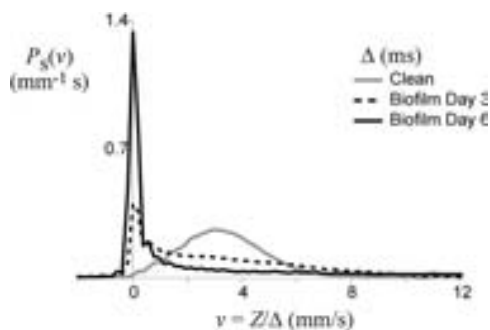


Fig. 5.1.10a

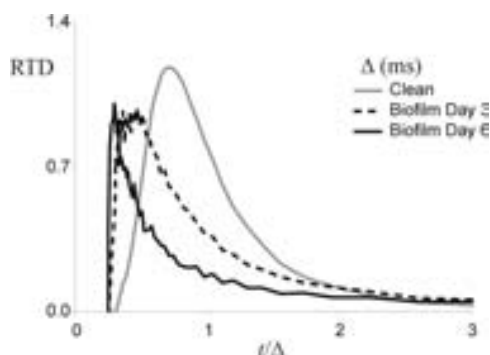


Fig. 5.1.10b

Fig. 5.1.10 (a) MR measured propagators and (b) corresponding calculated RTDs for flow in a model packed bed reactor composed of 241- μm monodisperse beads in a 5-mm id circular column for a fixed observation time of 300 ms and as a function of biofilm fouling. As the porous media becomes biofouled, a high velocity tail appears due to the formation of strong backbone flow paths, and a zero velocity peak forms due to entrapment of spins in the EPS gel matrix and dead-end pores. The RTD exhibits related features as the distribution of residence times increases with biofilm growth.

5.1.3.4.2 Vortex Flow Reactor MRM Experiment Details

The sample was placed in the Couette designed to fit in the 10 mm gradient coil of the Bruker Avance DRX250 spectrometer described previously. Velocity data are acquired for a rotation rate of 6 rps corresponding to a shear rate of 47 s^{-1} , using a spin echo sequence with bipolar velocity sensitizing gradients [Figure 5.1.1(b)] with parameters of: $T_R = 1000 \text{ ms}$, $t_e = 20 \text{ ms}$, $g = 0$ and 25 mT m^{-1} , $\delta = 0.5 \text{ ms}$, $\Delta = 10 \text{ ms}$. Total acquisition time for the velocity maps is 17 min. The velocity maps are averaged over a slice of thickness of 1 mm with a field of view (FOV) of $15 \text{ mm} \times 20 \text{ mm}$ with 64×128 pixels, for a spatial resolution of $23 \times 156 \mu\text{m}$ per pixel.

5.1.3.4.3 Vortex Flow Reactor Advective Transport

Detailed studies of VFRs under varying flow throughput conditions are ongoing and here we present preliminary data on the impact of a cell suspension on the

advection field under no flow through conditions. Figure 5.1.11 shows MRM velocity maps of pure water and cylindrical bacterial cells, *F. nucleatum*, ($\approx 2 \times 20 \mu\text{m}$), suspended in water at a concentration of $\approx 10^{11}$ cells mL^{-1} .

Quantitative velocity data for a fixed radius are shown in Figure 5.1.12 for each component of velocity. Several features are evident, in particular a shift in the z location of the maxima of all three components of velocity for the cell suspension toward the center of the Couette, relative to the pure fluid. A significant impact on mass transport is indicated by the strong increase in the maxima of the axial,

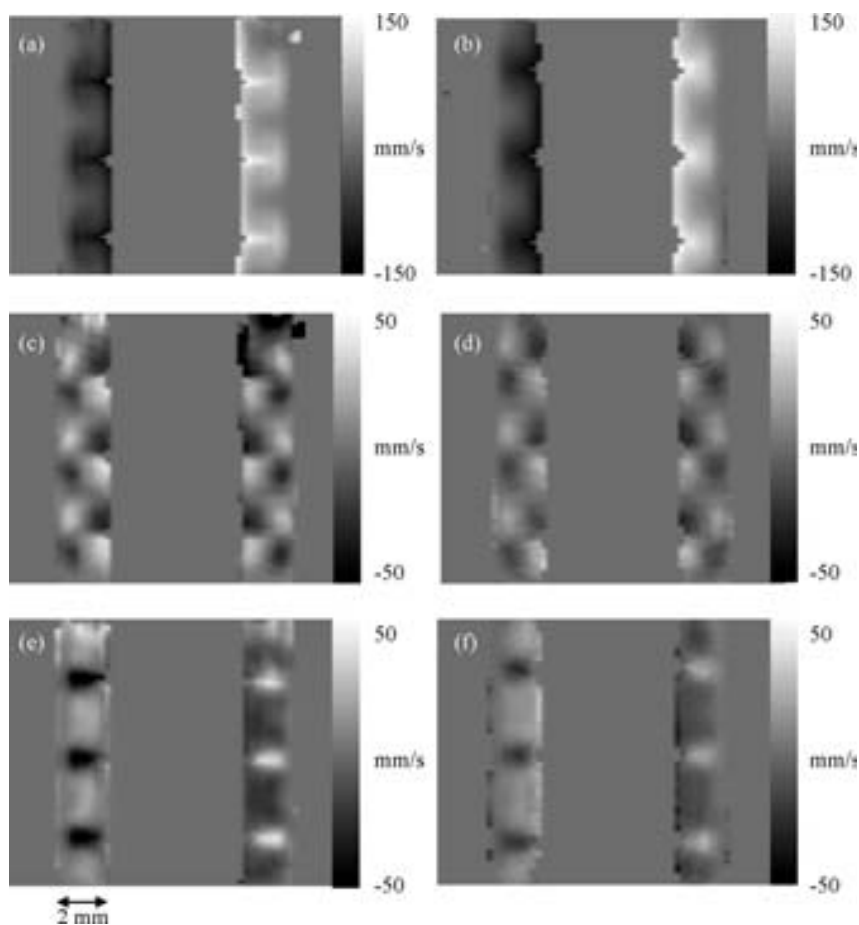


Fig. 5.1.11 MR velocity maps of a cross section through the center of a Couette cell with a 2 mm gap. The inner cylinder is rotating. (a) y component of velocity, v_y , corresponding to the tangential direction with flow out of the plane (black) and into the plane (white). (c) z component of velocity, v_z , corresponding to the axial direction with flow up (black) and down

(white). (e) x component of velocity, v_x , corresponding to the radial direction with flow left (black) and right (white). (b), (d) and (f) are the x , y and z components of the velocity, respectively, for cylindrical bacterial cells, *F. nucleatum* ($\approx 2 \times 20 \mu\text{m}$), suspended in water at a concentration of $\approx 10^{11}$ cells mL^{-1} .

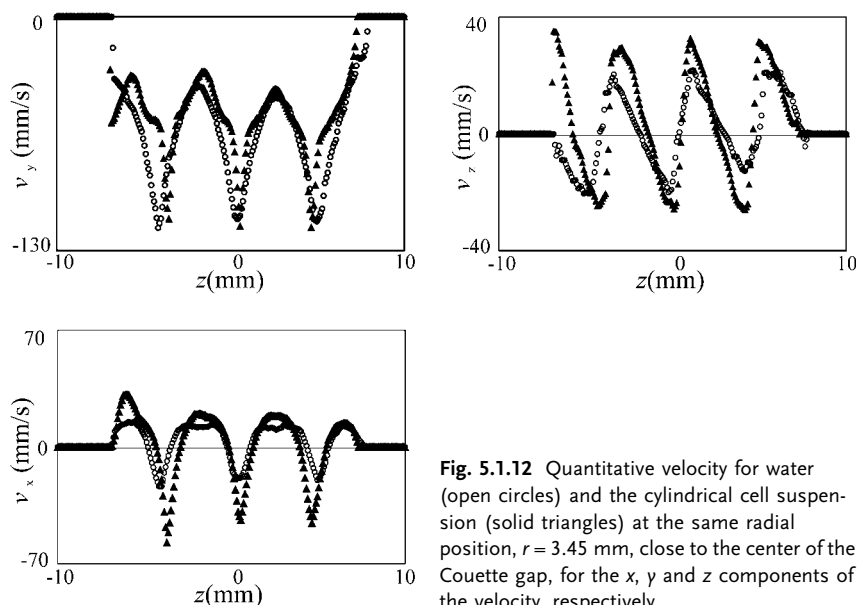


Fig. 5.1.12 Quantitative velocity for water (open circles) and the cylindrical cell suspension (solid triangles) at the same radial position, $r = 3.45$ mm, close to the center of the Couette gap, for the x , y and z components of the velocity, respectively.

Figure 5.1.12(b), and radial, Figure 5.1.12(c), components of velocity. This variation demonstrates the impact secondary flows can have on mass transport, which is not captured by a mass transfer coefficient approach dependent on the tangential or axial velocity based Re . The presence of the microbial cells generates stronger secondary flows, altering the mass transport. The development of modeling approaches which capture such effects is an ongoing area of research.

5.1.5

Conclusions

MRM methods have been demonstrated to provide data on the advective transport in capillary, packed bed and VF bioreactors. The correspondence between the MR measured propagators and RTDs has been demonstrated. While the exact correspondence holds only in the case of invariant velocity distributions, scale dependent RTDs can be calculated from time dependent propagators. This provides a clear connection between MR propagators and the classic RTDs used broadly in chemical engineering to design and troubleshoot reactors, indicating the strong potential for non-invasive MR based reactor process control devices. Biofilm growth is shown to generate secondary flows in capillary reactors of a magnitude up to 20% of the axial velocity on low Re flows. This calls into question the ability of mass transfer coefficients based on scaling with axial velocity Re to capture the important features of the mass transport within the reactor. In VF of a microbial cell suspension a strengthening of the secondary flows due to the suspended cells is demonstrated. The data indicate the importance of incorporating microscale details of the advection field into mass transfer models. A particular application of the

ability to incorporate microscale advection data into macroscale mass transfer models is the potential to impact the design of microfluidic [42] based bioreactor systems where the spatial variability of the flow field controls bioactivity [57].

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