

TRANSPORT OF DISSOLVED AND PARTICULATE MATERIAL IN BIOFILM-LINED
TUBES AND CHANNELS

by

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DEDICATION

Dedicated to my father,
Dr. Robert S. Jackson,
1952–2013.

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ABSTRACT

This dissertation develops two models for biofilm-lined channels. The first model seeks to address the rate at which cells move in or out of the flow in a natural hot spring drainage channel. This is done by building a one- and then two-dimensional partial differential equation model of the stream. The model is parameterized using data gathered at Mushroom Spring in Yellowstone National Park in 2011 and 2012. Using this data, we predict erosion and adhesion rates at steady state in upper and lower regions of the stream. The second model describes the utilization of urea by biofilms in an artificial tube flow reactor. The goal of this model is to determine kinetic parameters for ureolytic biofilms. The model is created by deriving two coupled steady state ordinary differential equations, which are parametrized using experimental data. Once the model is fully described, an inverse problem is formulated and solved using a Markov Chain Monte Carlo method. From this model we obtain first order kinetic parameters for a particular strain of *E. coli*, and discuss results for Michaelis-Menten kinetics. These two model systems are linked by a set of intersecting elements. First, both models concern biofilm-lined channels. Second, in each model these biofilms are found in a streamflow system in which some component transfers from the flow to the biofilm or vice-versa. Third, both systems are represented by low dimensional mathematical models which seek to summarize complex physical behaviors using broad, summarizing parameters. Fourth, in both scenarios the parameters of interest are estimated by combining experimental measurements and mathematical modeling. Finally, error plays an important role in model efficacy. The effects of error are implicit in the first model, but explicitly analyzed in the second.

CHAPTER ONE

INTRODUCTION

When we humans look at life in the world around us we tend to focus on macroorganisms such as trees, grass, dogs, and whales. We tend not to think much about single-celled organisms. This, however, is a distinct oversight. The Prokaryote kingdom, which includes the domains Bacteria and Archaea, comprises an amount of biomass at least equal to that of plants and possibly much more, totaling perhaps half the biomass on the planet [1, 2]. Contrary to the popular view of free-floating (planktonic) cells, much of this unicellular biomass is attached to surfaces in structured, aggregate communities held together by a extracellular matrix of carbohydrates which is itself produced by the cells [3, 4]. Such communities are called “biofilms” or “microbial mats.” The ubiquity of biofilms in natural, industrial, and medical contexts make them an important area of study [3, 5, 6].

Mathematical modeling plays an important role in describing and understanding biofilm systems. The complexity of biofilm morphology and the diversity of biofilm friendly environments means that models can in general be quite complex. The focus in this dissertation, however, is on “simple” biofilm models which use one or two spatial dimensions. The reasons for this are straightforward. First, a 1-dimensional (1D) model is generally much cheaper computationally than a complex model, and may yield “good enough” results in a larger context. As biofilms are recognized as important components of many systems, modeling biofilm growth or activity can become just one component in a much larger model system. The ability to compute this component cheaply but sufficiently well could be an important part of solving the overall problem. Second, there may be model systems in which a 1D model is directly relevant. For instance, flow through a thin

pore or channel may be well approximated by a 1D model if the axial dimension is much smaller than the longitudinal dimension.

A focus on 1D biofilm modeling in many ways represents a return to early biofilm models [7–10]. Later models demonstrate increased interest in understanding the mechanisms underlying the complex structures observed in biofilms [11–13]. However, by not attempting to provide detail on the spatial structures of biofilms or the surrounding hydrodynamics, we can focus simpler models on obtaining information on other processes and conditions which may be of interest. This might include investigating the detachment or attachment rate of cells, or studying kinetic parameters which give us information about biofilm substrate utilization.

This dissertation is comprised of two separate biofilm related projects which are linked by a common set of intersecting interests. First, both projects concern biofilm-lined channels. Second, in each project these biofilms are found in a streamflow system in which some components transfers from the flow to the biofilm or vice-versa. Third, both systems are represented by low (1–2) dimensional mathematical models which seek to summarize complex physical behaviors using broad, summarizing parameters. Fourth, in both scenarios the parameters of interest are estimated by making use of experimental measurements in conjunction with the mathematical model. Finally, uncertainty or error plays a role in model efficacy. This is implicit in the results of the first model scenario, but explicitly taken into account in the second by means of a formal inverse problem specification. The first three of these points are illustrated by the following general overview of a particular biofilm model structure.

Flow in a biofilm-lined channel can be modeled as two coupled systems. Consider a long, thin channel stretching in some axial direction which we will denote the x -axis. This channel contains a concentration c of some substrate and a concentration u of planktonic cells. These two components could be modeled (in 1D) using diffusion-advection-reaction

equations as

$$\frac{\partial c}{\partial t} = d \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x} + r, \quad (1.1)$$

$$\frac{\partial u}{\partial t} = d_1 \frac{\partial^2 u}{\partial x^2} - v \frac{\partial u}{\partial x} + g, \quad (1.2)$$

where v represents the stream velocity, r a reaction rate, g a growth rate, and d and d_1 diffusivity of substrate and cells, respectively [14]. Now, consider the biofilm lining the channel. The biofilm is consuming substrate and growing in a dimension orthogonal to the axial stream direction. Call this dimension z . Then, inside the biofilm the change in substrate concentration, $\hat{c}(z)$, and biomass concentration, $\hat{u}(z)$, could be written as

$$\frac{\partial \hat{c}}{\partial t} = d_2 \frac{\partial^2 \hat{c}}{\partial z^2} + r_1, \quad (1.3)$$

$$\frac{\partial \hat{u}}{\partial t} = -\hat{v} \frac{\partial \hat{u}}{\partial z} + g_1, \quad (1.4)$$

where d_2 is the diffusivity of the substrate in the biofilm, $\hat{v}$ is the velocity of the particular components of the biofilm moving perpendicular to the sub-stratum, and g_1 is a growth rate function [10]. The key to creating a coupled system is to describe “interface conditions” that connect what is happening in the x -dimension to what is happening in the z -dimension. That is, we must describe how

$$u \leftrightarrow \hat{u},$$

$$c \leftrightarrow \hat{c},$$

where we use the double-headed arrow to indicate the transfer of cells or substrate from one model to the other.

In the two projects described below we look at two linked systems which use different mechanisms as interface conditions. In the first project we explore a system in which u and $\hat{u}$ are linked by lower boundary conditions for (1.2). In this hot spring system, the interface condition is provided by a net “erosion” or “entrainment” term, E , which we use to broadly characterize the transfer of cells between the flow and the mat. In the second project, we solve a steady state version of (1.3) in order to compute the flux of substrate into the the biofilm, which in turn provides an interface condition for (1.1).

The coupling of flow and biofilm systems such as those shown in equations (1.1) and (1.2) is an idea exemplified by a class of “Freter” models which originate in Rolf Freter’s 1983 model for *E. coli* populations in mouse intestines [15–18]. In this model, Freter and his colleges treated the mouse’s large intestine as a continuous stirred-tank reactor (CSTR), comprised of a free-floating, planktonic culture of bacterial cells as well as an attached wall-population. The model is comprised of a system of ordinary differential equations for substrate concentration, the unattached bacteria population, and the attached bacteria population. These three systems are linked by the idea that cells which multiply in the flow can only adhere to the wall if there is a free site available for attachment. Similarly, daughter cells of the wall-attached biofilm must either have an attachment site or move into the flow. That is, the availability of an attachment site provides the interface condition. Substrate, of course, is consumed by both bacterial populations.

The original Freter model has been extended in various ways since the early 1980’s. Ballyk and Smith in 1999 took the model from its original setting in a CSTR and adapted it to the plug flow reactor (PFR or “tube flow”) model of Kung and Baltziz [19, 20]. Ballyk and Smith proved that their model had two steady-states, namely, the complete washout of bacteria and the successful colonization of both wall and fluid by bacteria. Jones et al. in 2003 showed that these steady states existed in the CSTR setting as well [21]. In 2011

Masic and Eberl returned to Freter's CSTR setting, but replaced the "free site" requirement for wall attachment with a flux term derived from Wanner and Gujer's 1D biofilm model [10,22].

Mushroom Spring: A Streamflow Model:

The first model system we develop describes the physical dispersion of cells found in the water of an effluent channel of Mushroom Spring, an alkaline hot spring in the Lower Geyser Basin of Yellowstone National Park. This is a well studied system, and for more than 50 years a large amount of research has been done at Mushroom Spring investigating the microbial communities which live in this extreme environment. Over the years, much attention has been paid to characteristics of the biofilm mat lining the hot spring and its effluent channel, including studies of the ecological, metabolomic, and genetic composition of the mat (cf. [23–25]). In contrast, much less attention has been paid to the cells in water overflowing these communities, though in 1968 Brock and Brock did look at the effects that shading the effluent channel had on the number of cells observed in the flow [26].

Unlike the majority of this previous work, our model focuses on the transport of cells to and from the water of the channel to the bacterial mat lining the bottom. To the best of our knowledge, this is the first attempt to quantify the erosion and deposition of cells in a hot spring streamflow environment. This model takes its basic inspiration from oceanographic sedimentation models by Lavelle et al. in the 1980s and early 1990s [27,28]. However, some calculated parameter values are informed by stream models from the 1970s by Fischer [29] and Parker [30]. However, the hot spring channel we wish to study in Yellowstone National Park is much, much smaller than the ocean currents of interest to Lavelle, or the rivers of interest to Fischer. Thus, while these models share

some similarities to our hot spring model, our model is derived from a consideration of mass balance and is different in both form and use from these previous models.

Our hot spring model does not seek to model activity in the biofilm directly, but rather summarize the contribution of cells to the stream from the biofilm (or vice-versa) by a general entrainment or erosion parameter, E , which interacts with the stream through the lower boundary condition. Mass transport is achieved by turbulent and advective transport as well as settling and entrainment. In Chapter 2, a steady state 1D version of this model is derived and used to test several possible lower boundary conditions. In addition, this chapter introduces the basic mechanism for particle transport. For each boundary scenario, information about the implications for erosion and adhesion (represented by parameter E) is extracted from the model solution. In Chapter 3, a two dimensional (2D) streamflow model is derived and the existence of a steady state solution is shown. No longer analytically tractable, we describe a numerical scheme and apply it to compute E using parameters estimated from field data. A detailed description of the acquisition of this data through field experiments and the subsequent calculation of model parameters is also found in this chapter.

The Tube Reactor Model

In the second half of this dissertation we derive and use a tube reactor model, which has its roots in the class of Freter models described above. In this work we use the tube flow setting of Ballyk and Smith [19], but describe the wall population using a 1D biofilm model like Masic and Eberl [22]. Unlike either of these earlier models, however, we focus solely on the concentration of substrate by the wall population. We do not model the cells in the flow or the wall-attached cells at all. Rather, we assume these populations are at a steady-state and focus on the consumption of substrate. This is similar to the 1988 treatment of the breakdown of biodegradable mass in a biofilm lined stream by Gantzer

et al [14]. In Gantzer’s model the mass balance for a 1D steady state stream quality model is written

$$D \frac{\partial^2 S}{\partial x^2} - v \frac{\partial S}{\partial z} - r_b - r_f = 0,$$

for substrate S , diffusion D , velocity v , and rate of bulk and rate of biofilm degradation constants r_b and r_f . Gantzer writes $r_b = K_2 X_b S$ where K_2 is the “mixed second-order rate coefficient” following Baughman et al’s 1980 description [31], and X_b is the concentration of biomass in the water column. The r_f term is more germane to our model, as we do not track the planktonic population. In his model, Gantzer relates r_f to the flux of substrate into a biofilm by $r_f = Q_0 a$, where Q_0 is the representative flux of contaminate into the biofilm and a the biofilm-covered surface per total liquid volume. While Gantzer does not compute this flux himself, the references he cites does so by applying a first order kinetic model to a 1D biofilm model and computing the first derivative at the bulk biofilm interface.

In some ways, our unified tube reactor model takes an approach similar to Gantzer’s model. While we omit the r_b term, we do unify the tube and biofilm models using a flux term like Gantzer. However, we allow the kinetics in the linked biofilm problem to be completely general, though in application we focus on Michaelis-Menten kinetics. Also, while Gantzer goes on to substitute complicated mass transfer equations into his model and then tries to fit their coefficients using cobble- and pebble-lined flume experiments, we focus on estimating the Michaelis-Menten parameters for our microorganism by combining experimental data and make use of a formal inverse problem formulation to quantify error.

The derivation of our unified tube reactor model from a mass balance is the subject of Chapter 4. This takes place in two pieces. First, we derive the 1D wall-mounted biofilm model and discuss the solution of this model using various kinetic models for reactions

in the biofilm. The discussion of kinetics focuses on zero-, first-, and Michaelis-Menten kinetic models. Next, the tube reactor model is derived. After a discussion of boundary conditions and nondimensionalization, the two models are linked into a “unified” model by relating the tube-wise reaction term with flux at the bulk-biofilm interface we will use to parameterize the unified model. Following a discussion of the numerical implementation of the model, we conclude the chapter with two naive attempts at parameter estimation which motivate the more sophisticated methods outlined in Chapter 5.

In Chapter 5 we formulate an inverse problem aimed at using experimental data to find estimates for biofilm ureolytic kinetic parameters. We do this first by formulating and minimizing a nonlinear least squares (NLS) problem. Through a set of synthetic data examples, we show that this method is limited in utility as it makes a number of limiting assumptions about the data. After discussing various characteristics of the resulting cost space, we describe a fully Bayesian approach which requires the specification of likelihood and prior probability distributions. We then make use of Markov Chain Monte Carlo to sample from the resulting posterior distribution and estimate kinetic parameter values. After developing this method using synthetic data sets, we apply the method to a real data set. These results are briefly compared to those found in 2015 by Connolly et al. [32] which the same data but a completely different methodology.

It is worth mentioning at this juncture that as far as we can tell, inverse methods have only infrequently been applied to biofilm related problems and only recently at that. In fact, we are aware of only a handful of previous attempts to combine inverse methods and biofilm models. In 2010 Ma et al. [33] developed a model to describe the movement of macromolecules by diffusion in oral biofilms. In this work the authors fit curves to measurements of fluorescent intensity to estimate diffusion rates. Also in 2010, Rao et al. created a wastewater purification model for a biofilm reactor in which they formulated a NLS cost function which they minimized using an “ant colony optimization” (ACO) algo-

rithm [34]. In 2014 Chen-Charpentier and Stanescu used polynomial chaos methods to solve a medical biofilm model [35]. This allowed them to maximize a resulting likelihood function to estimate parameter values. Finally, in 2014 Kumar and Venkateswarlu use “Tabu Search,” a “metaheuristic problem-solving approach” to search for solutions to a biofilm sludge reactor using an initial solution [36]. Notably, none of these authors use MCMC or a method based on Bayesian inference as we do.

CHAPTER TWO

A ONE-DIMENSIONAL STREAMFLOW MODEL

In this chapter we introduce the first of two models constructed to describe the physical dispersion of cells found in water of an effluent channel of Mushroom Spring, an alkaline hot spring in the Lower Geyser Basin of Yellowstone National Park which is shown in Figure 2.1. In particular, we are interested in modeling the transport of cells to and from the bacterial mat lining the bottom of the channel. The model in this chapter is 1-dimensional, and we focus on the effect that various lower boundary conditions have on the specification of a net erosion rate. The physical transport of cells in the model is determined by the the processes of advection, turbulent transport, and settling, which are illustrated in Figure 2.2.



Figure 2.1: A photograph of Mushroom Spring, taken June 15, 2012, which shows the source pool as well as the biofilm lined effluent channel which is being modeled here.

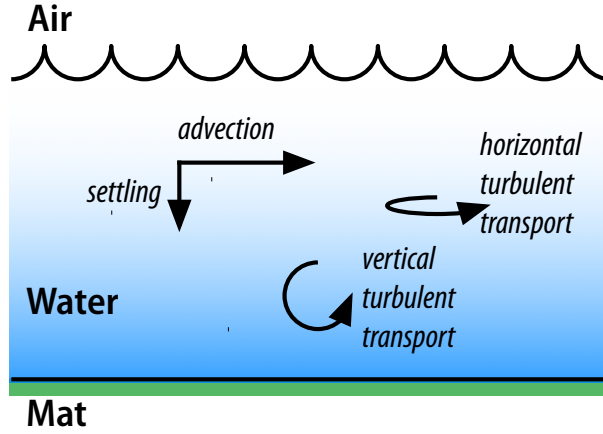


Figure 2.2: The transport of cells is determined by the the processes of advection, turbulent transport, and settling. Note that advection and turbulent transport are decomposed vertical and horizontal components. In the 1D model we will only consider the vertical modes of transport.

A One-Dimensional Streamflow Model

A Model for Turbulent Transport

Before beginning the 1D streamflow model derivation, we pause to discuss a common model for turbulent transport, namely, turbulent diffusion. In this model, turbulent transport is treated analogously to Fick's law of molecular diffusion or Fourier's law of heat conduction. Focusing on molecular diffusion, Fick's law results from the idea that transport due to molecular diffusion is the result of the random walk of molecules [29, p. 34]. Omitting the details, this means that the mass flux of molecules can be written (in one dimension) as

$$Q = -D_m \frac{\partial C}{\partial x}, \quad (2.1)$$

for flux Q and concentration C . The constant of proportionality D_m is called the molecular diffusivity. Modeling turbulent transport as turbulent diffusion treats turbulent flow as a random walk process on a much larger scale. This means that on a sufficiently large time

scale the mass flux of the average concentration $\bar{C}$ is commonly written (again in 1D) as

$$Q = -D_t \frac{\partial \bar{C}}{\partial x}, \quad (2.2)$$

for some turbulent diffusivity constant D_t [29, p. 65-71], [37, pp 92-95].

Whether or not (2.2) is a good model for turbulent transport relies on two assumptions. First, the system under consideration must exhibit turbulence which is statistically the same at every point and which does not vary over time. This is called stationary homogeneous turbulence. The second assumption is that the time scale of interest be longer than the time scale at which turbulent velocity appears correlated. While at short time scales turbulent velocities appear correlated, at longer time scales they appear uncorrelated and random. The scale of interest must be longer than this “integral” or “Lagrangian” time scale [29, p. 69], [38, p. 47]. When both these assumptions are met, the 1D advection diffusion equation for turbulent flow may be written as

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial x} = \frac{\partial}{\partial x} \left(D_t \frac{\partial C}{\partial x} \right) + \frac{\partial}{\partial x} \left(D_m \frac{\partial C}{\partial x} \right), \quad (2.3)$$

where C is the time-averaged concentration over some period longer than the integral time scale and u the fluid velocity. In general, $D_t \gg D_m$, so the final term is often neglected when modeling turbulent flow.

Model Mass Balance:

A one-dimensional streamflow model may be derived from a few assumptions and a careful mass balance, or may be viewed as a simplification of the 2D model, which will be introduced in a later chapter, where the rate of change of cell concentration in the x (downstream) direction is held constant. Proceeding directly with the mass balance, assume we have a channel lined with a steady state biofilm over which the fluid flows at

constant velocity v . Further, assume the stream is “infinitely long” in the sense it has been flowing far enough for any point downstream to be much the same as another. At some fixed but arbitrary location downstream the height of the water above the bacterial mat is a constant H . The concentration of cells in the water column may vary from height $z = 0$ to $z = H$, as forces of turbulent mixing and settling due to gravity move cells vertically in the column. Cells may adhere to the mat below, or be scoured off into the stream.

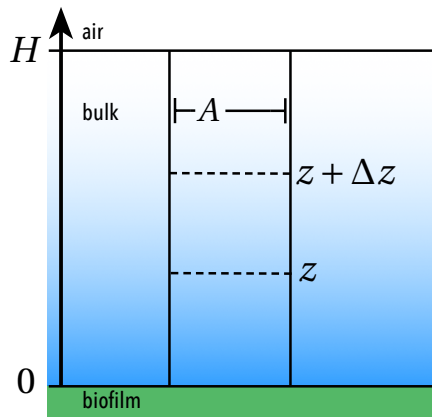


Figure 2.3: A 1D differential stream element.

To construct the mass balance, consider a short differential element from depth z to $z + \Delta z$ with constant cross sectional area A as illustrated in Figure 2.3. The steady state assumption gives the net accumulation of cells over time to be zero, but the constant gradient of cells in the horizontal direction acts as a source or sink depending on the sign. These cells are quantified by νc_x , which has units of flux and is applied over the volume of the element. Here c_x is constant, and the notation is chosen to suggest a concentration gradient in the x (stream) direction. This flux of cells from the streamflow is balanced by movement of cells within the element comprised of a flux Q of cells into the element at z , a flux of cells out of the element at $z + \Delta z$, and gravitational settling of cells at z and $z + \Delta z$,

which are proportional to settling velocity w . That is,

$$\nu c_x A \Delta z = Q(z)A - Q(z + \Delta z)A + wc(z)A - wc(z + \Delta z)A. \quad (2.4)$$

Dividing through by $A\Delta z$ and taking the limit as the element height goes to zero gives

$$\nu c_x = -\frac{dQ}{dz} - w\frac{dc}{dz}. \quad (2.5)$$

Here we model turbulent transport as turbulent diffusion, which means that $Q = -K_z \frac{dc}{dz}$ for turbulent diffusivity constant K_z . Making this substitution into the above equation yields

$$K_z c'' - wc' = \nu c_x, \quad (2.6)$$

where the prime indicates differentiation with respect to z .

Boundary conditions are also derived from flux balance. At the air-liquid interface, $z = H$, cells settle due to gravity or change position due to turbulence. Because no cells escape into the air above the stream and no cells enter the stream from the air, these forces must balance. These assumptions yield a mixed boundary condition given by

$$-K_z c'(H) + wc(H) = 0. \quad (2.7)$$

The lower boundary condition is more difficult to establish because different reasonable assumptions can be made which yield various conditions. We will explore three possible scenarios below.

The Non Dimensional Model. Before exploring the lower boundary condition we introduce the non dimensional model. Scale concentration c by c^0 , the source pool cell concentration, and spatial variable z by H , the height of the stream at the point

in question. Then $u = \frac{c}{c^0}$, and $Z = \frac{z}{H}$ for non dimensional variables u and Z . Then, using a prime to indicate differentiation with respect to Z , the ODE and upper boundary condition may be written as

$$\frac{1}{\text{Pe}} u'' - u' = \alpha, \quad (2.8a)$$

$$\frac{1}{\text{Pe}} u'(1) - u(1) = 0, \quad (2.8b)$$

where $\text{Pe} = \frac{wH}{K_z}$ is a Péclet number, and α is a non dimensional number given by $\alpha = \frac{vc_x H}{wc^0}$. Note that when settling dominates Pe is large, and when turbulent diffusion dominates it is small. In a similar way, the non homogeneous component α is a ratio comparing the source (or sink) flux with the settling flux due to gravity (given by wc^0), and is large when the source is large compared to the settling velocity and small when the opposite is true.

Case 1:

Returning to the lower boundary condition, a natural first approach is to mimic the flux balance at the top of the column. That is, to prescribe that turbulent flux and settling flux be balanced by a net “entrainment” or “erosion” term, E , which is positive when cells are eroding and negative when cells are adhering to the mat. Symbolically,

$$-Kc'(0) + wc(0) = E. \quad (2.9)$$

Non dimensionalizing using the same variable transformation as in (2.8) this boundary may be written as

$$\frac{1}{\text{Pe}} u'(0) - u(0) = -\beta, \quad (2.10)$$

where $\beta = \frac{E}{c^0 w}$ is the ratio between net entrainment and settling of the source concentration.

A Fredholm Alternative. Bringing together the ODE and upper boundary given by (2.8) and the lower boundary condition shown above, a 1D streamflow model is given by

$$\frac{1}{\text{Pe}} u'' - u' = \alpha, \quad (2.11a)$$

$$\frac{1}{\text{Pe}} u'(1) - u(1) = 0, \quad (2.11b)$$

$$\frac{1}{\text{Pe}} u'(0) - u(0) = -\beta. \quad (2.11c)$$

This non homogeneous boundary value problem can be written as

$$Lu = f, \quad (2.12a)$$

$$B_1 u = g_1, \quad B_2 u = g_2 \quad (2.12b)$$

for linear operators

$$L = D^2 - \text{Pe} D,$$

$$B_1 = B_2 = D - \text{Pe},$$

where D is the differential operator $\frac{d}{dz}$. Such a problem only has a unique solution if the corresponding completely homogeneous problem has only the trivial solution [39, pp. 196]. In this case, however, $Lu = 0$ has characteristic polynomial $r^2 - \text{Pe} r = 0$, and the

completely homogeneous problem has a nontrivial solution given by

$$u_h(Z) = \gamma_h e^{\text{Pe} Z}, \quad (2.13)$$

for an arbitrary constant γ_h . It follows then by the Fredholm Alternative Theorem that (2.12) has a solution if and only if the solvability condition

$$\int_0^1 f v dZ - J(u, v) = 0 \quad (2.14)$$

is met for all v satisfying the related homogeneous adjoint problem given by $L^* v = 0$, $B_1^* v = 0$, $B_2^* v = 0$ where $J(u, v)$ represents the boundary contributions.

In general, finding the adjoint operators L^* , B_1^* , and B_2^* can be quite tedious. In this case, however, the adjoint problem is made simple as results for general, second order operators are readily computed. An operator with the general form

$$L = a_2(x) D^2 + a_1(x) D + a_0(x)$$

has adjoint

$$L^* = a_2 D^2 + (2a_2' - a_1) D + (a_2'' - a_1' + a_0)$$

along with boundary operations given by

$$J(u, v) = a_2(vu' - uv') + (a_1 - a_2')uv.$$

Note that in these formulas a prime refers to differentiation with respect to the independent variable. Applying these formulas to the problem given in (2.11) yields adjoint

operators given by

$$L^* = D^2 - \text{Pe } D,$$

$$B_1^* = B_2^* = D.$$

It follows that the homogeneous adjoint problem $L^* v = 0$, $B_1^* v = 0$, $B_2^* v = 0$ is

$$K_z v'' + w v' = 0, \tag{2.15a}$$

$$v'(H) = v'(0) = 0, \tag{2.15b}$$

which is solved by $v = \gamma_0$ for arbitrary constant γ_0 .

A solution to the adjoint problem in hand, we can now invoke the Fredholm Alternative theorem. As stated above, (2.11) has a solution if and only if the solvability condition given by (2.14) holds. Substituting in the adjoint solution and the non homogeneity $f = \text{Pe } \alpha$, this solvability condition becomes

$$\int_0^1 \gamma_0 \alpha dZ = J(u, \gamma_2) \Big|_0^1, \tag{2.16}$$

where u satisfies (2.11). The left hand side of this equation is given by

$$\int_0^1 \alpha \gamma_0 dZ = \alpha \gamma_0, \tag{2.17}$$

as the argument is constant with respect to Z . The right hand side of (2.16) is given by

$$J(u, \gamma_0) \Big|_0^1 = \gamma_0 u' - \gamma_0 \text{Pe } u \Big|_0^1 = -\gamma_0 \beta. \tag{2.18}$$

Combining these results, the solvability condition for (2.11) is $\alpha = \beta$. Replacing these non dimensional constants with their definitions, this requirement reduces to

$$E = \nu c_x H. \quad (2.19)$$

Thus, a steady-state solution to our model does not exist with these boundary conditions unless the net difference between the concentration of cells in the column due to the source term and the erosion term is zero. While this is important as a solvability condition, this result also speaks to our goal of studying the effect of the lower boundary on the erosion rate. Namely, for this formulation of the model the erosion rate is completely specified by the height of the channel, H , and the source or sink term νc_x . Writing this another way, this says that $E = \int_0^1 \nu c_x dZ$ is the sum of the source/sink over the water column height. This specification is independent of the actual solution to the model and the resulting concentration profile. Before moving on, note that this solvability condition may also be found by integrating (2.11a) from $Z = 0$ to $Z = 1$ and applying the model boundary conditions from (2.11b) and (2.11c).

Solving Case 1. To solve (2.11a) we break the problem into a homogeneous and non homogeneous problem. The solution to the completely homogeneous problem was solved above and is given by

$$u_h(Z) = \gamma_h e^{\text{Pe} Z} \quad (2.20)$$

as shown in Equation 2.13. The particular solution may be found using the Method of Undetermined Coefficients. The ODE shown in the left hand side of (2.11a) is equal to a constant, and a constant satisfies the homogeneous ODE. The particular solution thus has the form

$$u_p(Z) = \gamma_1 Z + \gamma_0. \quad (2.21)$$

Substituting this result into the ODE gives $\gamma_1 = -\alpha$. Applying the upper boundary condition we find that

$$\gamma_0 = \alpha \left(1 - \frac{1}{\text{Pe}} \right). \quad (2.22)$$

Again updating the particular solution we have

$$u_p(Z) = -\alpha Z + \alpha \left(1 - \frac{1}{\text{Pe}} \right). \quad (2.23)$$

Applying the lower boundary condition to (2.23) does not provide any new information, but returns the solvability condition $\alpha = \beta$. Then, by superposition the solution to the model shown in (2.11) is

$$u(Z) = \gamma_h e^{\text{Pe}Z} + \alpha \left(Z - 1 + \frac{1}{\text{Pe}} \right). \quad (2.24)$$

This solution is composed of an exponential term with a coefficient that must be determined from initial data, and a linear term which is significant when α is large and not when α is small. The significance of the linear term follows logically from the fact that this term comes from the particular solution. The non homogeneous term α is large when νc_x is relatively large compared to $c^0 w$, that is, when the source is significant.

Case 2:

Other lower boundary conditions may be imagined, and in fact such may be preferred. One possible modification of the previous lower boundary condition is to separate the flux at the lower boundary into an upward erosive flux and a downward settling flux. Let w_d represent the depositional velocity [27, 40] and E the upward erosive flux. Then turbulent diffusion and settling in the water column must match erosion and depositional

flux at the lower boundary, that is

$$-Kc'(0) + wc(0) = E + w_d c(0). \quad (2.25)$$

If $-w_d < w < 0$ then the lower boundary is “attractive,” and if $w < w_d < 0$ then the boundary is “repulsive”. If $w_d = w$ the the boundary is neutral. We will most often make use of this last case. Nondimensionalizing as before using $u = \frac{c}{c^0}$ and $Z = \frac{z}{H}$, this boundary condition may be written as

$$u'(0) - (\text{Pe}_1 - \text{Pe}_2)u(0) = -\text{Pe}_1\beta, \quad (2.26)$$

where $\beta = \frac{E}{wc^0}$ as before. Here $\text{Pe}_1 = \frac{wH}{K_z}$ and $\text{Pe}_2 = \frac{w_d H}{K_z}$ are both Peclet numbers, one which compares settling velocity w to turbulence and the other which compares depositional velocity w_d to turbulence. Summarizing the system for convenience, Case 2 is described by

$$\frac{1}{\text{Pe}_1} u'' - u' = \alpha, \quad (2.27a)$$

$$\frac{1}{\text{Pe}_1} u'(1) - u(1) = 0, \quad (2.27b)$$

$$u'(0) - (\text{Pe}_1 - \text{Pe}_2)u(0) = -\text{Pe}_1\beta. \quad (2.27c)$$

Similar to case 1, the homogeneous solution to the ODE is given by

$$u_h(Z) = \gamma_h e^{\text{Pe}_1 Z} + \gamma_0. \quad (2.28)$$

However, in this case the completely homogeneous problem has only the trivial solution as the application of the upper boundary condition results in $\gamma_0 = 0$ and application of the lower boundary condition $\gamma_h = 0$. This means that Case 2 will yield a unique solution, unlike Case 1.

This unique solution may be found by once again using the Method of Undetermined Coefficients. As before, examination of the right hand side of (2.27a) shows a constant and thus we may suppose the particular solution is itself a constant. However, a constant also appears in the homogeneous solution so a factor of Z must be added. Thus, the solution to (2.27) is this given by

$$u_p(Z) = \gamma_h e^{Pe_1 Z} + \gamma_1 Z + \gamma_0 \quad (2.29)$$

for unknown coefficients γ_h , γ_1 , and γ_0 . Substituting this equation into the ODE yields

$$-\gamma_1 Pe_1 = Pe_1 \alpha \Rightarrow \gamma_1 = -\alpha.$$

Updating $u_p(Z)$ and substituting the result into the upper boundary condition gives

$$-\gamma_0 Pe_1 + \alpha Pe_1 - \alpha = 0 \Rightarrow \gamma_0 = \alpha \left(1 - \frac{1}{Pe_1}\right).$$

Again updating the particular solution with this result and substituting, this time into the lower boundary condition, yields

$$\gamma_h = \frac{\alpha}{Pe_1} - \alpha + \frac{Pe_1 \alpha}{Pe_2} - \frac{Pe_1 \beta}{Pe_2}.$$

Assembling these values, the solution to the model given in (2.27) is

$$u(Z) = \left(\frac{\alpha}{Pe_1} - \alpha + \frac{Pe_1 \alpha}{Pe_2} - \frac{Pe_1 \beta}{Pe_2} \right) e^{Pe_1 Z} - \alpha \left(Z - 1 + \frac{1}{Pe_1} \right). \quad (2.30)$$

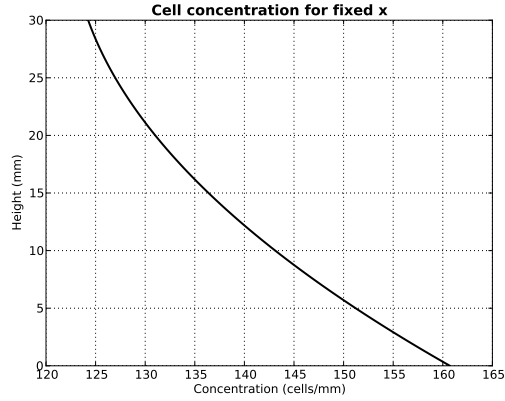


Figure 2.4: A plot of the Case 2 solution given by (2.30) for best-estimate model parameters. No estimates were available for erosion rate E or w_d so it was assumed that $E = -1.5\nu c_x H$ cells/s, and $w - w_d$ was taken to be -0.004 .

A Brief Comparison:

Recall that Case 1 required a solvability condition given by $\alpha - \beta = 0$, which in dimensional constants became $E - \nu c_x H = 0$, a requirement that the net vertical transport equal the horizontal transport at steady-state. This condition could be found by analyzing the adjoint problem or by integrating the ODE subject to the boundary conditions. While Case 2 has a unique solution and thus does not have a solvability requirement, an analogous statement can be derived by integrating the governing ODE (Equation 2.27a) subject to its boundary conditions (Equations 2.27b and 2.27c). Doing so gives

$$\frac{\text{Pe}_1}{\text{Pe}_2}(\alpha - \beta) = u(0). \quad (2.31)$$

Returning this equation to dimensional units, this is

$$(E + c(0)w_d) - \nu c_x H = 0. \quad (2.32)$$

This still says that at the boundary the net vertical flux must balance with the source/sink νc_x , but in this second case the vertical flux is decomposed into an erosive element and a depositional element. Focusing on the implications for E , in this case E does not depend only on the channel height H , but also on the concentration at the bottom of the channel and the depositional velocity. An increase in the depositional velocity or the concentration of cells at the bottom of the channel has the effect of increasing E . This makes physical sense given the steady-state assumption: if the concentration is not changing then additional deposition must be balanced by increased erosion.

Another comparison between Case 1 and Case 2 is possible when the source term νc_x vanishes. In this event, α goes to zero and the Case 2 solution reduces to

$$u(Z) = -\frac{\text{Pe}_1 \beta}{\text{Pe}_2} e^{\text{Pe}_1 Z} \Rightarrow c(z) = -\frac{E}{w_d} e^{\frac{wH}{K_z} \frac{z}{H}}. \quad (2.33)$$

Note that this will give positive cell concentrations in the case that $w_d < 0$, which is our usual assumption. Going further, this no-source solution is similar to the result when the same assumptions were made in the first model, which lead to a solution of $u(Z) = \gamma_h e^{\text{Pe} Z}$. That is, both cases yield an exponential concentration profile under zero source assumptions. In Case 1, the exponential coefficient was unspecified by the boundaries, but in Case 2 the coefficient is the ratio of erosive to depositional fluxes.

Case 2b (a common sub-case). We commonly assume that the depositional velocity and the settling velocity are the same, that is, that $w = w_d$. In the problem above, this assumption means that $\text{Pe}_1 = \text{Pe}_2$. Calling this value simply Pe , the solution shown in (2.30) simplifies to

$$u(Z) = \left(\frac{\alpha}{\text{Pe}} - \beta \right) e^{\text{Pe} Z} - \alpha \left(Z - 1 + \frac{1}{\text{Pe}} \right). \quad (2.34)$$

This same solution results from solving (2.27) with a simplified lower boundary as is readily shown. If $w = w_d$, then the settling and depositional fluxes at $z = 0$ cancel and the flux due to erosion and flux due to turbulence must balance, resulting in a lower boundary condition of

$$-Kc'(0) = E \Rightarrow u'(0) = -\text{Pe}\beta. \quad (2.35)$$

Replacing the lower boundary condition in (2.27) with this simplified lower boundary results in the solution shown in (2.34). Equating entrainment with mixing due to turbulence is sometimes a direct assumption used in oceanographic and atmospheric models [41]. In situations where the mechanism for bed deposition is settling velocity and the stream-bed neither attracts nor repels letting $w = w_d$ is an appropriate simplification [30]. In this scenario (2.32) becomes

$$E = \nu c_x H - c(0)w, \quad (2.36)$$

and erosion is again the balance of the source/sink and settling particles.

Case 3:

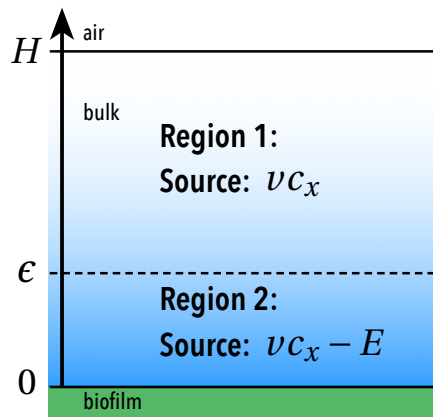


Figure 2.5: In Case 3 the domain is divided into two regions. Region 1 is has the same source and upper boundary conditions as in Cases 1 and 2. Region two, however, has an additional source term lacking in the previous cases and a no-flux lower boundary.

A final case is presented here which is somewhat different in spirit as compared with the first two. In this case, rather than specify the mechanics of how cells enter or leave the water column, we divide the domain into two regions, the lower of which has an additional source term and the top which does not. This is done by specifying a source term which is active only in the vicinity of the channel bottom. That is, we specify a no diffusion condition at $z = 0$ but introduce a source term which activates in a thin band of width ϵ at the bottom of the channel. If the upper boundary condition is the same flux balance as has been used in the previous two cases, such a model may be written as

$$K_z c'' - w c' = \nu c_x - \delta_\epsilon(z) E \quad (2.37a)$$

$$-K_z c'(H) + w c(H) = 0, \quad (2.37b)$$

$$c'(0) = 0, \quad (2.37c)$$

where

$$\delta_\epsilon(z) = \begin{cases} 0 & \epsilon \leq z \leq H, \\ \frac{1}{\epsilon} & 0 \leq z < \epsilon, \end{cases} \quad (2.38)$$

is a “tower” function of width ϵ and height $1/\epsilon$. Note that here ϵ has units of 1/length. This two-region domain is illustrated in Figure 2.5.

What happens to the solution of this system as the width of the delta-band goes to zero (that is, as ϵ vanishes)? The limit of this problem is analogous to having an impulse function providing cells at the lower boundary. To answer this question, we proceed by nondimensionalizing in the same manner as before. Set $u = \frac{c}{c^0}$ and $Z = \frac{z}{H}$. Then, let

$\text{Pe} = \frac{wH}{K_z}$, $\alpha = \frac{vc_x H}{wc^0}$, $\beta = \frac{E}{wc^0}$, and define

$$\delta_{\epsilon/H}(z) = \begin{cases} 0 & \frac{\epsilon}{H} \leq Z \leq 1, \\ \frac{H}{\epsilon} & 0 \leq Z < \frac{\epsilon}{H}. \end{cases} \quad (2.39)$$

Then the system can be written dimensionlessly as

$$u'' - \text{Pe } u' = \text{Pe } \alpha - \text{Pe } \delta_{\epsilon/H}(z) \beta \quad (2.40a)$$

$$u'(1) - \text{Pe } u(1) = 0, \quad (2.40b)$$

$$u'(0) = 0. \quad (2.40c)$$

We seek a solution to this problem which is as smooth as possible. In this case, we have a second order ODE and each region specifies one boundary condition. Thus, we expect one degree of freedom from each region in our piecewise solution. This is sufficient to specify a $C^1(0,1)$ solution. A solution in hand, we will be able to examine what happens as ϵ goes to zero.

Solving Case 3. The above system is solved in two pieces, one on each piece of the domain. For convenience name the upper region ($\frac{\epsilon}{H} \leq Z \leq 1$) “Region 1,” and the lower ($0 \leq Z < \frac{\epsilon}{H}$) “Region 2.” Then, beginning with the Region 1, the solution must satisfy

$$u'' - \text{Pe } u' = \text{Pe } \alpha, \quad (2.41a)$$

$$u'(1) - \text{Pe } u(1) = 0. \quad (2.41b)$$

This system was solved in case 1 above (cf. Equation 2.24). Recapitulating that solution for convenience, $u(Z)$ in Region 1 is given by

$$u(Z) = \gamma_h e^{\text{Pe } Z} + \alpha \left(Z - 1 + \frac{1}{\text{Pe}} \right). \quad (2.42)$$

In region 2 the solution must satisfy

$$u'' - \text{Pe } u' = \text{Pe } \alpha - \frac{\text{Pe } \beta H}{\epsilon}, \quad (2.43a)$$

$$u'(1) = 0. \quad (2.43b)$$

This problem has a homogeneous solution given by

$$u(Z) = \eta_0 + \eta_h e^{\text{Pe } Z}. \quad (2.44)$$

Proceeding once again by the Method of Undetermined Coefficients, we observe that the right hand side of (2.43a) is a constant but that a constant appears in the homogeneous solution. Thus, the particular solution must have the form $u_p(Z) = \eta_1 Z$. Substituting this into the governing ODE allows us to solve for η_1 as

$$\eta_1 = \frac{\beta H}{\epsilon} - \alpha. \quad (2.45)$$

Updating, by superposition the solution in Region 2 is

$$u(Z) = \eta_h e^{PeZ} + \left(\frac{\beta H}{\epsilon} - \alpha \right) Z + \eta_0. \quad (2.46)$$

Taking this result and applying the lower boundary condition, we find that η_h is given by

$$\eta_h = \frac{\alpha\epsilon - \beta H}{\epsilon Pe} \quad (2.47)$$

while η_0 remains unspecified.

Combining the results from Regions 1 and 2, the solution to (2.40) is given up to this point by

$$u(z) = \begin{cases} \gamma_h e^{PeZ} + \alpha \left(Z - 1 + \frac{1}{Pe} \right) & \frac{\epsilon}{H} \leq Z \leq 1, \\ \left(\frac{\alpha\epsilon - \beta H}{\epsilon Pe} \right) e^{PeZ} + \left(\frac{\beta H}{\epsilon} - \alpha \right) Z + \eta_0 & 0 \leq Z < \frac{\epsilon}{H}, \end{cases} \quad (2.48)$$

To solve for the two remaining constants, γ_h and η_0 , we enforce continuity of u and Ku' at $Z = \frac{H}{\epsilon}$. Denoting the solution in Region 1 as u_1 and the solution in Region 2 by u_2 , this means that we require

$$\lim_{Z \rightarrow \frac{H}{\epsilon}^+} u_1(Z) = \lim_{Z \rightarrow \frac{H}{\epsilon}^-} u_2(Z), \quad (2.49)$$

and

$$\lim_{Z \rightarrow \frac{H}{\epsilon}^+} u_1'(Z) = \lim_{Z \rightarrow \frac{H}{\epsilon}^-} u_2'(Z). \quad (2.50)$$

Computing these limits yields

$$\gamma_h = \frac{\alpha}{Pe} - \frac{\beta H}{Pe\epsilon} + \frac{\beta H}{Pe\epsilon} e^{-\frac{Pe\epsilon}{H}}, \quad \text{and} \quad \eta_0 = \alpha - \beta + \frac{\beta H}{Pe\epsilon} - \frac{\alpha}{Pe}. \quad (2.51)$$

With these, we can now assemble the complete solution in Case 3. This solution is

$$u(z) = \begin{cases} \left(\frac{\alpha}{\text{Pe}} - \frac{\beta H}{\text{Pe}\epsilon} + \frac{\beta H}{\text{Pe}\epsilon} e^{-\frac{\text{Pe}\epsilon}{H}} \right) e^{\text{Pe}Z} + \alpha \left(Z - 1 + \frac{1}{\text{Pe}} \right) & \frac{\epsilon}{H} \leq Z \leq 1, \\ \left(\frac{\alpha\epsilon - \beta H}{\epsilon\text{Pe}} \right) e^{\text{Pe}Z} + \left(\frac{\beta H}{\epsilon} - \alpha \right) Z + \alpha - \beta + \frac{\beta H}{\text{Pe}\epsilon} - \frac{\alpha}{\text{Pe}} & 0 \leq Z < \frac{\epsilon}{H}. \end{cases} \quad (2.52)$$

As stated above, our interest in this solution is in what happens as ϵ vanishes. A useful limit in this investigation is

$$\lim_{x \rightarrow 0} \frac{e^{ax} - 1}{x} = a. \quad (2.53)$$

Now, note that applying this limit results in

$$\lim_{\epsilon/H \rightarrow 0} \gamma_h = \frac{\alpha}{\text{Pe}} + \frac{\beta}{\text{Pe}} \lim_{\epsilon/H \rightarrow 0} \left(\frac{e^{-\text{Pe}\frac{\epsilon}{H}} - 1}{\frac{\epsilon}{H}} \right) = \frac{\alpha}{\text{Pe}} - \beta. \quad (2.54)$$

Thus, in the limit the Region 1 branch of the solution becomes

$$u(Z) = \left(\frac{\alpha}{\text{Pe}} - \beta \right) e^{\text{Pe}Z} - \alpha \left(Z - 1 + \frac{1}{\text{Pe}} \right). \quad (2.55)$$

The limit of the lower (Region 2) branch of the solution is more complicated and requires one fact in addition to the limit given in (2.53). This is the realization that as $\epsilon \rightarrow 0$, $z \rightarrow 0$. Equivalently, this means that as $\epsilon \rightarrow 0$, $z \rightarrow \epsilon$. This follows because in Region 2, $0 \leq z < \frac{\epsilon}{H}$. Then, again using $u_2(Z)$ to represent the the Region 2 portion of (2.52),

$$\lim_{\epsilon/H \rightarrow 0} u_2(Z) = \lim_{\epsilon/H \rightarrow 0} \frac{\beta}{\text{Pe}} \left(\frac{1 - e^{\text{Pe}\epsilon}}{\frac{\epsilon}{H}} \right) + \beta H + \alpha - \beta = \alpha - \beta. \quad (2.56)$$

Note that this limit is just Equation 2.55 evaluated at $z = 0$. That is, in the limit as $\epsilon \rightarrow 0$, the solution to Case 3 is Equation 2.55, which is in turn the solution to Case 2b. In other

words, a very thin source layer at the bottom of the channel yields an equivalent solution to the case where the settling and depositional fluxes cancel and entrainment is due to turbulent mixing. This means that in the limit, Case 3 gives the same result as Case 2b for E , namely, that E depends on the concentration of cells at the stream-bottom and the settling velocity w as shown in (2.36).

Conclusion:

A 1D model which requires an infinitely long stream and a constant change in horizontal cell concentration is obviously limited in utility. However, this discussion has allowed us to introduced the basic mechanisms of particle transport in a stream-flow situation, as well as take a first look at the implications several lower boundary conditions have for erosion rates. Based on this exploration, in the next chapter we expand this model to two dimensions and use boundary conditions similar to those in Case 2 and 2b, which were also the limiting boundaries in Case 3. Once we have developed the 2D model we will be able to consider real data obtained at Mushroom Spring in Yellowstone National Park.

CHAPTER THREE

A TWO-DIMENSIONAL STREAMFLOW MODEL

In this chapter we extend the work done in the 1D model chapter by expanding the streamflow model into two dimensions. After we derive a 2D model from a consideration of mass balance, we show the existence of a steady-state solution using separation of variables and a Rayleigh quotient estimation of the eigenvalues. Next, we look at a numerical implementation for the steady-state model. That done, we test the model output using artificial parameter values and describe a method by which we can solve for the erosion/adhesion rate. After this, we discuss the parameterization of the model using a combination of field data and calculation and, after summarizing the relevant parameters, apply the model to data from Mushroom Spring and estimate stream erosion and adhesion rates.

The 2D Streamflow Model:

This model, like the 1D model described previously, is based on conservation of mass. In addition to conservation, we make the additional important assumption that the channel is two dimensional. We will track the movement of cells in the downstream direction, x , and in the vertical direction, z , but ignore the possibility of cell movement perpendicular to the banks of the channel in the transverse direction (which we would label the y direction were it included). This may be conceptualized by assuming that the fluid flow and cell concentrations are identical in slices perpendicular to the banks.

A Mass Balance:

To compute the mass balance, consider a rectangular differential element in the flow ranging from x to $x + \Delta x$ in the horizontal direction and z to $z + \Delta z$ in the vertical

direction (cf. Figure 3.1). Coordinates are oriented so that location $x = 0$ represents the beginning of the stream at the source pool, while $x = L$ is the furthest downstream location of interest along the stream. In the other dimension, $z = 0$ is located at the bottom of the stream on the surface of the bacterial mat, and $z = H(x)$ is the height of the stream. Total accumulation rate of cells in this region is given by $\Delta x \Delta z \frac{\partial c}{\partial t}$, where $c(x, z, t)$ represents the average concentration of cells per unit area in the region.

Cells move in and out of the region by advection and turbulent transport in both the vertical and horizontal directions. Given a constant horizontal velocity of v , cells advect into the region on the left with rate $vc(x, z, t)\Delta z$, and advect out of the region at the right with rate $vc(x + \Delta x, z, t)\Delta z$. In the same way, w represents the gravitational settling velocity of cells in the flow, oriented so that when w is positive cells migrate upward, and when w is negative they settle downward. Net advection in the vertical dimension is given by the difference between $wc(x, z + \Delta z, t)\Delta x$ and $wc(x, z, t)\Delta x$. Turbulent transport, here modeled as turbulent diffusion, also induces a flux that moves cells in and out of the differential element. Let Q represent this flux at a given point. Then turbulent transport induces a flux across the left boundary of $Q(x, z, t)\Delta z$ and across the right a

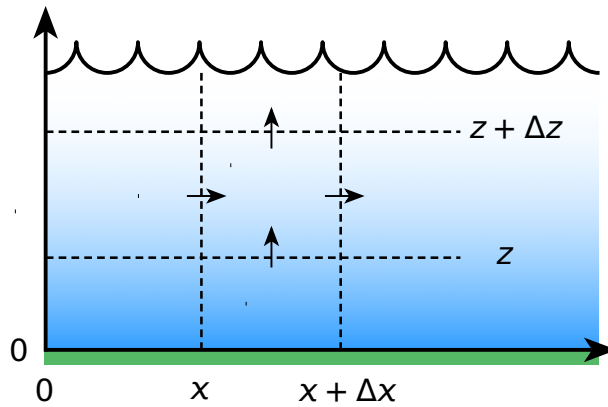


Figure 3.1: A differential stream element.

flux of $Q(x + \Delta x, z, t)\Delta z$. Thus, the flux of cells transported across the top boundary of the region is $Q(x, z + \Delta z, t)\Delta x$, and the flux across the bottom boundary is $Q(x, z, t)\Delta x$.

Combining these components into a mass balance requires the accumulation of cells to equal the net change in mass in the region. That is,

$$\begin{aligned} \Delta x \Delta z \frac{\partial c}{\partial t} = & -Q(x + \Delta x, z, t)\Delta z + Q(x, z, t)\Delta z \\ & - Q(x, z + \Delta z, t)\Delta x + Q(x, z, t)\Delta x - \nu c(x + \Delta x, z, t)\Delta z \\ & + \nu c(x, z, t)\Delta z - w c(x, z + \Delta z, t)\Delta x + w c(x, z, t)\Delta x. \end{aligned} \quad (3.1)$$

Dividing both sides by $\Delta x \Delta z$ and taking the limit as both Δx and Δz go to zero, this becomes

$$\frac{\partial c}{\partial t} = -\frac{\partial Q_1}{\partial x} - \frac{\partial Q_2}{\partial z} - \nu \frac{\partial c}{\partial x} - w \frac{\partial c}{\partial z}, \quad (3.2)$$

where Q_1 represents flux in the x -direction and Q_2 flux in the z -direction. Modeling turbulent transport as turbulent diffusion, let K_x be the diffusivity in the x direction, and K_z the vertical diffusivity in the z direction. Then $Q_1 = -K_x \frac{\partial c}{\partial x}$ and $Q_2 = -K_z \frac{\partial c}{\partial z}$. Substituting this result into (3.2) yields the model's governing partial differential equation,

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left(K_x \frac{\partial c}{\partial x} \right) + \frac{\partial}{\partial z} \left(K_z \frac{\partial c}{\partial z} \right) - \frac{\partial}{\partial x} (\nu c) - \frac{\partial}{\partial z} (w c). \quad (3.3)$$

Boundary Conditions:

Boundary conditions for the influent and effluent ends of the channel are chosen to be as simple as possible while also being natural for data collection, which will be described in detail in a later chapter. These considerations lead us to use Dirichlet conditions at each end of the stream where cell concentration is specified. Such boundary

conditions could be written as

$$c(0, z, t) = A(z, t), \text{ and } c(L, z, t) = B(z, t), \quad (3.4)$$

where $x = 0$ is the upstream source of the stream and $x = L$ indicates the end of the region of interest at the downstream end of the stream. Our expectation is that such boundary conditions will change very slowly, if at all, on the time-scale of the transportive mechanics of the system. Thus, we generally drop the time dependence in the notation. In the above specification $A(z)$ is a concentration profile at the source, and $B(z)$ the same at the downstream end of the domain. These functions are generally taken to be not only time-independent, but constants with respect to z as well. The values for these constants will be determined experimentally.

At times we will use a second, alternate downstream boundary condition given by

$$\frac{\partial c(L, z, t)}{\partial x} = 0. \quad (3.5)$$

In this case, we assume that the concentration at $x = L$ is some fixed constant given z , the value of which may be unknown. This formulation will be used in a numeric scheme to solve for cell erosion and adhesion rates.

Upper and lower boundary conditions are derived by a consideration of flux balance rather than comparison to experiments. At the air-bulk fluid interface cells can settle by gravity or be drawn down by turbulent transport. However, no cells can enter the region from the air above the stream, and no cells can escape into the air. Due to these considerations, an appropriate upper boundary condition would be a flux balance where

flux due to settling and flux due to turbulent transport sum to zero. That is,

$$-K_z \frac{\partial c}{\partial z}(x, H, t) + wc(x, H, t) = 0, \quad (3.6)$$

where H is the height of the water column at the point in question. Note that this is the 2D version of the same upper boundary conditions as was used in the 1D problem shown in equation (2.7).

Boundary conditions at the bottom, bulk fluid-biofilm boundary are derived similarly. Flux to the water column at the bottom of the stream is described as the sum of contributions from depositional and erosional forces. Let w_d represent the rate of deposition, or “depositional velocity” [27, 40]. We will often assume that w_d equals w but this need not be the case. At the bottom of the water column ($z = 0$) cells settle due to gravity at velocity w and may be transported by turbulence. These forces are balanced by E , a sediment erosion or entrainment rate [27, 28] and the depositional flux. Symbolically, this is

$$-K_z \frac{\partial c}{\partial z}(x, 0, t) + wc(x, 0, t) = E + w_d c(x, 0, t). \quad (3.7)$$

Note that a positive value of E adds mass to the water column (erosion) while a negative value represents an adhesional flux. In addition, note that this is again the 2D analog of the lower boundary condition used in Case 2 of the 1D model (cf. equation (2.25)).

Summary of the General 2D Model:

The model described above is here summarized for convenience. The general 2D model, which will be used as a basis for subsequent simplifications and assumptions, is given by

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left(K_x \frac{\partial c}{\partial x} \right) - \frac{\partial}{\partial x} (vc) + \frac{\partial}{\partial z} \left(K_z \frac{\partial c}{\partial z} \right) - \frac{\partial}{\partial z} (wc), \quad (3.8a)$$

$$-K_z \frac{\partial c}{\partial z}(x, H, t) + wc(x, H, t) = 0, \quad (3.8b)$$

$$-K_z \frac{\partial c}{\partial z}(x, 0, t) + (w - w_d)c(x, 0, t) = E, \quad (3.8c)$$

$$c(0, z, t) = A(z, t), \quad (3.8d)$$

$$c(L, z, t) = B(z, t), \quad \left(\text{or } \frac{\partial c}{\partial x}(L, z, t) = 0 \right), \quad (3.8e)$$

$$c(x, z, 0) = c_0(x, z). \quad (3.8f)$$

Assumptions. In the work that follows we will commonly make a number of simplifying assumptions about the model. To start, we will generally assume that K_x , K_z , v , and w are all constants with respect to both time and space. This is done in the interest of simplifying the model and simplifying parameter estimation. We will also commonly assume that the system is at a mathematical steady state, though only after showing the existence of such a solution in the next section. In a natural hot spring environment growth of the microbial mat could balance decay, or the mat could be expanding or shrinking. In addition to these possible changes, phenomena such as weather, the intrusion of wildlife, and shifts in geothermal activity all impact the site. However, our focus in this model is on particle transport, which has a timescale on the order of the velocity of the water flowing in the drainage channel. Other site changes, such as those listed above, operate on a much longer time scale and so consideration of these is omitted in favor of a steady-state assumption.

Separation of Variables:

In this section we propose a separable solution to (3.8) of the form

$$c(x, z, t) = \tilde{c}(x, z) + X(x)Z(z)T(t), \quad (3.9)$$

and derive the resulting four systems of equations which must be used to find that solution. Our motivation in seeking such a solution is to show that the time dependent terms decay over time, meaning that the steady state problem has a solution given by $\tilde{c}(x, z)$. In writing (3.9) we assume the solution is composed of a time-invariant component, $\tilde{c}(x, z)$, as well as a time-dependent component which separates into a product of functions, each of which depends on a single variable.

Substituting the separated solution (3.9) into the general model's governing ODE (3.8a) and multiplying through by $1/(XZT)$ yields

$$\begin{aligned} \frac{1}{T} \frac{dT}{dt} = \frac{K_x}{X} \frac{d^2 X}{dx^2} - \frac{\nu}{X} \frac{dX}{dx} + \\ \frac{K_z}{Z} \frac{d^2 Z}{dz^2} - \frac{w}{Z} \frac{dZ}{dz} + \left(\frac{K_x}{XZT} \frac{\partial^2 \tilde{c}}{\partial x^2} - \frac{\nu}{XZT} \frac{\partial \tilde{c}}{\partial x} \right. \\ \left. + \frac{K_z}{XZT} \frac{\partial^2 \tilde{c}}{\partial z^2} - \frac{w}{XZT} \frac{\partial \tilde{c}}{\partial z} \right). \end{aligned} \quad (3.10)$$

Note that the left-hand side of this equation depends on a single variable, t , while the right-hand side depends on three. This is possible only if each side equals a mutual constant. Call that constant α_1 . Then (3.10) becomes

$$\frac{1}{T} \frac{dT}{dt} = \alpha_1, \quad (3.11)$$

and

$$\begin{aligned} \alpha_1 - \left(\frac{K_x}{XZT} \frac{\partial^2 \tilde{c}}{\partial x^2} - \frac{\nu}{XZT} \frac{\partial \tilde{c}}{\partial x} \right. \\ \left. + \frac{K_z}{XZT} \frac{\partial^2 \tilde{c}}{\partial z^2} - \frac{w}{XZT} \frac{\partial \tilde{c}}{\partial z} \right) = \frac{K_x}{X} \frac{d^2 X}{dx^2} - \frac{\nu}{X} \frac{dX}{dx} + \\ \frac{K_z}{Z} \frac{d^2 Z}{dz^2} - \frac{w}{Z} \frac{dZ}{dz}. \end{aligned} \quad (3.12)$$

Again, looking at the left- and right-hand sides of (3.12), we note that the left-hand side depends on three variables, but the right-hand side only two. Thus, this is again only possible if both sides are equal to a mutual constant. Call this constant α_2 . Starting with the left-hand side of (3.12), we must have

$$\left(K_x \frac{\partial^2 \tilde{c}}{\partial x^2} - \nu \frac{\partial \tilde{c}}{\partial x} + K_z \frac{\partial^2 \tilde{c}}{\partial z^2} - w \frac{\partial \tilde{c}}{\partial z} \right) = (\alpha_1 - \alpha_2) XZT. \quad (3.13)$$

Now, $\tilde{c}$ was defined to be time-independent and a function of x and z only. Thus, to avoid contradiction we must have $\alpha_1 = \alpha_2$, or a constant $T(t)$. The equivalence of α_1 and α_2 means that

$$0 = K_x \frac{\partial^2 \tilde{c}}{\partial x^2} - \nu \frac{\partial \tilde{c}}{\partial x} + K_z \frac{\partial^2 \tilde{c}}{\partial z^2} - w \frac{\partial \tilde{c}}{\partial z}. \quad (3.14)$$

If $T(t)$ is constant then the model is already at steady state. Assuming there is a time dependence and that T is not constant, then from (3.14) we conclude that $\tilde{c}(x, z)$ satisfies the steady-state version of the model's governing ODE.

Returning to the the right-hand side of (3.12) with $\alpha_1 = \alpha_2$, we have

$$K_x \frac{1}{X} \frac{d^2 X}{dx^2} - \nu \frac{1}{X} \frac{dX}{dx} + K_z \frac{1}{Z} \frac{d^2 Z}{dz^2} - w \frac{1}{Z} \frac{dZ}{dz} = \alpha_1. \quad (3.15)$$

Rewriting this equation as

$$K_x \frac{1}{X} \frac{d^2 X}{dx^2} - \nu \frac{1}{X} \frac{dX}{dx} = \alpha_1 - K_z \frac{1}{Z} \frac{d^2 Z}{dz^2} + w \frac{1}{Z} \frac{dZ}{dz}, \quad (3.16)$$

we again argue that the only way a function of z can equal a function of x is if each are equal to another mutual constant. Call this value α_3 , and define $\alpha_1 - \alpha_3 = \alpha_4$. Then (3.16) can be rewritten as

$$K_x \frac{1}{X} \frac{d^2 X}{dx^2} - \nu \frac{1}{X} \frac{dX}{dx} = \alpha_3, \quad (3.17)$$

and

$$K_z \frac{1}{Z} \frac{d^2 Z}{dz^2} - w \frac{1}{Z} \frac{dZ}{dz} = \alpha_4. \quad (3.18)$$

To summarize the above variable separations clearly, we use a prime to indicate differentiation with respect to each equation's sole independent variable and rename the separation variable. Let $\alpha_1 = -\lambda$, $\alpha_3 = -\alpha$, and $\alpha_4 = -\beta$. Then, substituting the separated solution (3.9) into the model PDE results in three ODEs and one PDE given by

$$K_x \frac{X''}{X} - \nu \frac{X'}{X} = -\alpha, \quad (3.19)$$

$$K_z \frac{Z''}{Z} - w \frac{Z'}{Z} = -\beta, \quad (3.20)$$

$$\frac{T'}{T} = -\lambda, \quad (3.21)$$

and

$$0 = K_x \frac{\partial^2 \tilde{c}}{\partial x^2} - \nu \frac{\partial \tilde{c}}{\partial x} + K_z \frac{\partial^2 \tilde{c}}{\partial z^2} - w \frac{\partial \tilde{c}}{\partial z} \quad (3.22)$$

where constants of separation are α , β , and λ , for $\alpha + \beta = \lambda$. Note that these constants each have units of 1/time.

Boundary Conditions:

Boundary conditions for these three ODEs are found by substituting the separated solution (3.9) into the boundary condition of the general model and simplifying as much as possible.

The Upper Boundary. Plugging (3.9) into upper boundary condition (3.8b)

$$-K_z \frac{\partial c}{\partial z}(x, H, t) + wc(x, H, t) = 0$$

and dividing through by XT yields

$$\left(\frac{-K_z}{XT} \frac{\partial \tilde{c}}{\partial z}(x, H) + \frac{w}{XT} \tilde{c}(x, H) \right) + \left(-K_z \frac{dZ}{dz}(H) + wZ(H) \right) = 0. \quad (3.23)$$

From this we can derive boundary conditions for $Z(z)$ and $\tilde{c}(x, z)$ at $z = H$. First, note that the second grouping of (3.23) could equal a constant, say C_1 , as it contains only functions of z evaluated at H . However, if this were the case, then

$$\frac{-K_z}{XT} \frac{\partial \tilde{c}}{\partial z}(x, H) + \frac{w}{XT} \tilde{c}(x, H) = -C_1, \quad (3.24)$$

which implies that

$$-K_z \frac{\partial \tilde{c}}{\partial z}(x, H) + w\tilde{c}(x, H) = -C_1 XT. \quad (3.25)$$

Since $\tilde{c}$ is defined to be time independent, this equation requires that T is constant or that $C_1 = 0$. If T is constant then the steady-state exists trivially. Since we assume there is a

time dependence, it must be that $C_1 = 0$. Thus, we must have

$$-K_z \frac{dZ}{dz}(H) + wZ(H) = 0, \quad (3.26)$$

and

$$-K_z \frac{\partial \tilde{c}(x, H)}{\partial z}(H) + w\tilde{c}(x, H) = 0. \quad (3.27)$$

Here both Z and $\tilde{c}(x, z)$ satisfy the same upper boundary as the general model.

The Lower Boundary. Substituting (3.9) into lower boundary condition (3.8c)

$$-K_z \frac{\partial c}{\partial z}(x, 0, t) + (w - w_d)c(x, 0, t) = E,$$

and again dividing through by XT yields

$$\left(\frac{-K_z}{XT} \frac{\partial \tilde{c}}{\partial z}(x, 0) + \frac{w - w_d}{XT} \tilde{c}(x, 0) \right) + \left(-K_z \frac{dZ}{dz}(0) + (w - w_d)Z(0) \right) = \frac{E}{XT}. \quad (3.28)$$

In the same way as for the upper boundary condition, the second group of terms cannot equal a constant because the first group is time independent, but must be identically zero. Then, separating this equation following the same argument about time-independence as in the upper boundary condition, we get a boundary condition for $Z(z)$ and $\hat{c}(z)$ at $z = 0$ given by

$$-K_z \frac{dZ}{dz}(0) + (w - w_d)Z(0) = 0, \quad (3.29)$$

and

$$-K_z \frac{\partial \tilde{c}}{\partial z}(x, 0) + (w - w_d)\tilde{c}(x, 0) = E. \quad (3.30)$$

The Upstream Boundary. So far we have been able to extract boundary conditions for $\tilde{c}(x, z)$ and $Z(z)$ by simply evaluating the separated solution (3.9) in each boundary

condition and separating terms appropriately. The same process results in the separation of the upstream downstream boundary conditions as will be shown below. Substituting (3.9) into upstream boundary

$$c(0, z, t) = A(z)$$

results in

$$\tilde{c}(0, z) + X(0)Z(z)T(t) = A(z). \quad (3.31)$$

Again, we must have $X(0) = 0$. The argument is the same as given in (3.25): if $X(0) = C_1$ is constant then

$$\tilde{c}(0, z) = A - C_1 Z T, \quad (3.32)$$

which violates the time independence of $\tilde{c}$ unless $T = 0$ for all t . Thus, (3.31) separates into

$$X(0) = 0, \quad (3.33)$$

and

$$\tilde{c}(0, z) = A. \quad (3.34)$$

The Downstream Boundary. In looking for downstream boundary conditions we solve two cases: the case where we have a Dirichlet downstream boundary and the case where the downstream boundary is Neumann. The Dirichlet case follows the same method as the upstream boundary condition shown above, and results in

$$X(L) = 0, \quad (3.35)$$

and

$$\tilde{c}(L, z) = B. \quad (3.36)$$

In the Neumann case, substituting (3.9) into

$$\frac{\partial c}{\partial x}(L, z, t) = 0$$

gives

$$\frac{d\tilde{c}(L, z)}{dx} + ZT \frac{dX(L)}{dx} = 0, \quad (3.37)$$

which, given the time independence of $\tilde{c}$, must separate into

$$\frac{dX(L)}{\partial x} = 0 \quad (3.38)$$

and

$$\frac{\partial \tilde{c}(L, z)}{\partial x} = 0. \quad (3.39)$$

Three Eigenvalue Problems:

Equations 3.19 – 3.21 with their associated boundary conditions form three eigenvalue problems. The existence of a steady state solution depends on the values of those eigenvalues. In this section we will argue that the steady state solution does exist, and in fact is given by

$$c(x, z) = \tilde{c}(x, z). \quad (3.40)$$

To do this, we must argue that the additional term in (3.9) given by $X(x)Z(z)T(t)$ decays to zero as time increases.

We proceed by first solving for $T(t)$. Combining (3.21) with the initial condition from (3.8f) gives us the system to solve for $T(t)$. Denoting differentiation with respect to t with a prime for convenience, this system is given by

$$T' + \lambda T = 0, \quad (3.41a)$$

$$T(0) = c_0(x, z). \quad (3.41b)$$

The solution is readily seen to be

$$T(t) = c_0(x, z)e^{-\lambda t}. \quad (3.42)$$

It follows from this result that $X(x)Z(z)T(t)$ will only vanish if λ is strictly positive. Recall from the original separation of variables that $\gamma = \alpha + \beta$. Thus, to determine if λ is positive we must analyze the sum $\alpha + \beta$.

Two Sturm-Liouville Problems

To examine α and β we look at the eigenvalue problems for $X(x)$ and $Z(z)$. Bringing ODE (3.19) together with boundary conditions (3.33) and (3.38) and denoting differentiation with respect to x with a prime, the first eigenvalue problem is given by

$$K_x X'' - \nu X' + \alpha X = 0, \quad (3.43a)$$

$$X(0) = 0, \quad (3.43b)$$

$$X(L) = 0, \text{ or } X'(L) = 0. \quad (3.43c)$$

The second eigenvalue problem is very similar. Combining (3.20) with the boundary conditions from (3.26) and (3.29) gives us the system to solve for $Z(z)$. Denoting differentiation with respect to z with a prime for brevity, this is

$$K_z Z'' - w Z' + \beta Z = 0, \quad (3.44a)$$

$$-K_z Z'(H) + w Z(H) = 0, \quad (3.44b)$$

$$-K_z Z'(0) + (w - w_d) Z(0) = 0. \quad (3.44c)$$

Besides being eigenvalue problems, the ODEs (3.43a) and (3.44a) are Sturm-Liouville problems which may be put in “regular form”

$$\frac{d}{dx} \left[p(x) \frac{d\phi}{dx} \right] + q(x)\phi + \mu\sigma(x)\phi = 0, \quad (3.45)$$

where $p(x)$ and weight function $\sigma(x)$ are both greater than zero [42, p. 162]). This is done by multiplying through by an integrating factor. For example, in the case of (3.43a) multiplying through by $p(x) = \exp\left(-\frac{\nu x}{K_x}\right)$ allows us to write (3.43a) as

$$\frac{d}{dx} \left[e^{-\frac{\nu x}{K_x}} \frac{dX}{dx} \right] + \beta \left(\frac{1}{K_x} e^{-\frac{\nu x}{K_x}} \right) X = 0.$$

This is regular Sturm-Liouville form with $p(x) = \exp\left(-\frac{\nu x}{K_x}\right)$ and weight function $\sigma(x) = \frac{1}{K_x} \exp\left(-\frac{\nu x}{K_x}\right)$, both of which are strictly positive, and $q(x) = 0$. Equation 3.44a may be similarly transformed using an integrating factor of $p(z) = \exp\left(-\frac{wz}{K_z}\right)$. This is useful because Sturm-Liouville problems have many well understood properties which we will appeal to in order to find the sign of μ (i.e. α and β). These include properties such as the completeness and orthogonality of eigenfunctions and the ability to order the real, infinite set of eigenvalues. Thus, any piecewise smooth function can be represented by a generalized Fourier series composed of the eigenfunctions corresponding to eigenvalues $\mu_1 < \mu_2 < \dots < \mu_n < \dots$, where μ_n goes to infinity as n increases.

In addition to these properties, for a regular Sturm-Liouville problem any eigenvalue can be related to its eigenfunction using the Rayleigh quotient. The Rayleigh quotient is found by multiplying (3.45) through by ϕ and integrating. Since $\int_a^b \phi^2 \sigma dx > 0$ the result can be solved for μ . After applying integration by parts, this is

$$\mu = \frac{-p\phi \frac{d\phi}{dx} \Big|_a^b + \int_a^b \left[p \left(\frac{d\phi}{dx} \right)^2 - q\phi^2 \right] dx}{\int_a^b \phi^2 \sigma dx} \quad (3.46)$$

Now, this is not generally useful for finding the eigenvalue μ directly, since the eigenfunctions are also unknown, but can certainly be used for estimates. For instance, the minimum value of the Rayleigh quotient for all continuous functions satisfying the boundary conditions is the smallest eigenvalue [42, p. 191]. In our case, if the Rayleigh quotient is always positive then we may conclude that the eigenvalues are always positive. If eigenvalues α_n and β_n are positive, then λ_n is positive as well and $X(x)Z(z)T(t)$ will vanish at steady state.

It follows directly from (3.46) that $\mu \geq 0$ if

1. $-p\phi \frac{d\phi}{dx} \Big|_a^b \geq 0$, and
2. $q \leq 0$.

For both the X and the Z problem, given in (3.43) and (3.44), $q = 0$ so the second condition is met. It remains to check the boundary constraint in the first condition.

The X Problem. Computing the boundary constraint is simple for the X problem: since $X(0) = 0$ and $X(L) = 0$ in the Dirichlet case, and $X(0) = 0$ and $X'(L) = 0$ in the Neumann case,

$$-pXX' \Big|_0^L = -p(L)X(L)X'(L) + p(0)X(0)X'(0) = 0, \quad (3.47)$$

where $P(0) = 1$. Thus, the Rayleigh quotient tells us that $\alpha \geq 0$ and our increasing sequence of eigenvalues is nonnegative for (3.43). Going a step further, $\int_a^b p \left(\frac{d\phi}{dx} \right)^2 dx = 0$ only when $p = 0$ or $\frac{dX}{dx} = 0$. The first possibility is ruled out as p is an exponential function, and the second because if X is constant then $X = 0$ by the boundary conditions and we have only the trivial solution. Thus, $\alpha_n > 0$ for $n = 1, 2, \dots$ is a positive sequence of eigenvalues corresponding to the X problem.

The Z Problem. The boundary constraint shown above is more complicated in the Z case, given that the boundary conditions are more complex. The lower boundary condition, shown in (3.44c), can be written as

$$Z'(0) = \frac{w - w_d}{K_z} Z(0). \quad (3.48)$$

Similarly, the upper boundary condition shown in (3.44b) can be reorganized as

$$Z'(H) = \frac{w}{K_z} Z(0). \quad (3.49)$$

Substituting these equations into the boundary condition

$$-pZZ'|_0^H = -p(H)Z(H)Z'(H) + p(0)Z(0)Z'(0) \quad (3.50)$$

yields

$$-p(H) \left(\frac{w}{K_z} \right) (Z(H))^2 + p(0) \left(\frac{w - w_d}{K_z} \right) (Z(0))^2. \quad (3.51)$$

The first term is positive if $w < 0$ because $p(z) > 0$. That is, we need cells to settle ($w < 0$) to guarantee the positivity of this term. In the second term, however, the sign depends on

the sign of the quantity $w - w_d$. If $w - w_d \geq 0$ then the entire term is positive and (3.51) is positive. This means that in the case that $w_d \leq w < 0$, that $0 < \beta_1 < \beta_2 < \dots < \beta_n < \dots$ for all n . Since $w = w_d$ and $w < 0$ are typical assumptions (as discussed in the last chapter), we conclude that in typical situations all eigenvalues for the Z problem are positive.

A Steady State Solution. It follows then that if $w_d \leq w < 0$, that $0 < \alpha_n + \beta_m = \lambda_{n,m}$ for all n and m . By the completeness of the eigenfunctions we may write

$$c(x, z, t) = \tilde{c}(x, z) + \sum_{n=0}^{\infty} \sum_{m=0}^{\infty} C_{n,m} e^{-\lambda_{n,m} t} X_m(x) Z_n(z), \quad (3.52)$$

where $\lambda_{n,m} = \alpha_n + \beta_m$. Here X_n and Z_n represent the eigenfunctions corresponding to α_n and β_n , respective. Utilizing the weighted orthogonality of X_m and Z_n ,

$$C_{n,m} = \int_0^L \int_0^H (c_0(x, z) - \tilde{c}(x, z)) X_m(x) Z_n(z) \frac{e^{-\left(\frac{vx}{K_x} + \frac{wz}{K_z}\right)}}{K_x K_z} dz dx. \quad (3.53)$$

Since $\lambda_{n,m} > 0$, $T(t)$ decays exponentially and the steady state solution is given by

$$c(x, z) = \tilde{c}(x, z).$$

In the case that $w_d > w$ or $w \geq 0$ such a steady state solution may or may not exist. It could be that though the second term in (3.51) is negative that the entire sum shown in the same equation is still positive. Or, it could be that the positive minimum X eigenvalue α_1 is large enough that $\beta_1 + \alpha_1 = \gamma_1 > 0$ despite β_1 being negative. In either of these two cases $\lambda_n > 0$ for all n and a steady state would still exist. However, it is possible that $\left(\frac{w-w_d}{K_z}\right)(Z(0))^2$ be small enough that neither the addition of extra terms nor the addition of α_1 is sufficient to make γ_1 positive. In these cases, the time problem could have a non positive eigenvalue and there would be no steady state solution.

One physical meaning of the possible lack of a steady state solution is apparent from looking at the necessary condition $w_d \leq w < 0$. This condition could fail in two ways. First, it could be that $w_d > w$. This would correspond to a “repelling” boundary where particles settle in the water column but are not removed from the stream. If material continues to accumulate in the lower regions of the stream but horizontal advection and diffusion are not enough to transport the material out of the region, then the total mass in the stream would grow indefinitely and not approach a steady state. Second, if $w \geq 0$ we are not guaranteed a steady state solution. In this scenario, cells are neutral or buoyant and do not settle out of the water column at all. Again, this could lead to the continual accumulation of cells in the stream and preclude a steady state solution. Note that the boundaries do not play a role in determining the existence of a steady state solution because their action has been transferred entirely to $\tilde{c}(x, z)$, leaving only homogeneous boundaries on $X(x)$ and $Z(z)$. This follows from our assumption that the boundaries are constant in time.

In summary of the results of this section we give the time-independent system solved by $\tilde{c}(x, z)$. This is

$$0 = K_x \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x} + K_z \frac{\partial^2 c}{\partial z^2} - w \frac{\partial c}{\partial z}, \quad (3.54a)$$

$$-K_z \frac{\partial c}{\partial z}(x, H) + wc(x, H) = 0, \quad (3.54b)$$

$$-K_z \frac{\partial c}{\partial z}(x, 0) + (w - w_d)c(x, 0) = E, \quad (3.54c)$$

$$c(0, z) = A, \quad (3.54d)$$

$$c(L, z) = B, \left(\text{or } \frac{\partial c}{\partial x}(L, z) = 0 \right). \quad (3.54e)$$

Here we drop the tilde for convenience, and assume that K_x , K_z , ν , w , A , and B are constants. These are the assumptions and this is the system we will use for the remainder of this chapter.

A Numerical Scheme

The PDE described in (3.54) must be solved numerically. This section exhibits a numeric scheme to solve the the steady-state version of the general model shown in (3.8). In this scheme, second derivatives are approximated by central differences. First derivatives are approximated by simple first order upwind formulations. An upwind formulation was chosen because the advection terms are one sided. That is, the horizontal advection term is always positive, and the vertical advection due to gravitational settling is always negative. We proceed by computing the finite difference coefficients for the governing PDE followed by those for each boundary.

The rectangular domain comprised by $0 \leq x \leq L$ and $0 \leq z \leq H$ is discretized into $m + 2$ nodes in the x -dimension (horizontally) and $n + 2$ nodes in the z -dimension (vertically). For convenience we will denote boundaries as the “North”, “South”, “East”, and “West” boundaries, where North corresponds to $z = H$, South to $z = 0$, East to $x = L$, and West to $x = 0$. Thus, $c_{i,1}$ for $i = 1, 2, \dots, m + 1$ corresponds to the southern boundary of the domain. The northern boundary corresponds to nodes $c_{i,n+2}$, again for $i = 1, 2, \dots, m + 1$. The western boundary is indicated by $c_{1,j}$ and the eastern boundary by $c_{m+2,j}$ for $j = 2, 3, \dots, n + 1$. This discretization and boundary convention is illustrated in Figure 3.2.

The Interior:

The interior mesh points ($i = 2, 3, \dots, m + 1$ and $j = 2, 3, \dots, n + 1$) are treated by substituting the central difference and upwind approximations into the governing ODE,

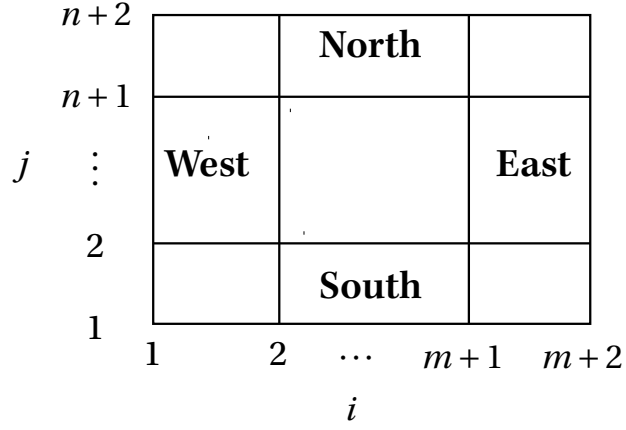


Figure 3.2: Finite difference grid schematic.

which yields

$$\begin{aligned}
 0 = K_z \left[\frac{c_{i,j+1} - 2c_{i,j} + c_{i,j-1}}{\Delta z^2} \right] + \\
 K_x \left[\frac{c_{i+1,j} - 2c_{i,j} + c_{i-1,j}}{\Delta x^2} \right] - v \left[\frac{c_{i,j} - c_{i-1,j}}{\Delta x} \right] \\
 - w \left[\frac{c_{i,j} - c_{i,j-1}}{\Delta z} \right]. \quad (3.55)
 \end{aligned}$$

Collecting like-indexed nodes, this may be written as

$$0 = \alpha_1 c_{i,j+1} + \alpha_2 c_{i,j} + \alpha_3 c_{i,j-1} + \alpha_4 c_{i+1,j} + \alpha_5 c_{i-1,j}, \quad (3.56)$$

where the coefficients are given by

$$\begin{aligned}
 \alpha_1 &= \frac{K_z}{\Delta z^2}, \\
 \alpha_2 &= -\frac{2K_z}{\Delta z^2} - \frac{2K_x}{\Delta x^2} - \frac{v}{\Delta x} - \frac{w}{\Delta z},
 \end{aligned}$$

$$\alpha_3 = \frac{K_z}{\Delta z^2} + \frac{w}{\Delta z},$$

$$\alpha_4 = \frac{K_x}{\Delta x^2},$$

and

$$\alpha_5 = \frac{K_x}{\Delta x^2} + \frac{v}{\Delta x}.$$

This treatment of the interior is completely standard. Only in the boundary conditions do we encounter terms that are unique to the problem at hand.

North and South Boundaries:

The discretization of the northern boundary is found by substituting the finite difference scheme into the steady state of the boundary condition given in (3.8b). Doing so results in the equation

$$-K_z \left[\frac{c_{i,n+2} - c_{i,n+1}}{\Delta z} \right] + w c_{i,n+2} = 0. \quad (3.58)$$

Multiplying this out and collecting like indexed nodes, this can be written as

$$\beta_1 c_{i,n+2} + \beta_2 c_{i,n+1} = 0, \quad (3.59)$$

where

$$\beta_1 = w - \frac{K_z}{\Delta z},$$

and

$$\beta_2 = \frac{K_z}{\Delta z}.$$

The southern boundary is very similar. Substituting the scheme into (3.8c) yields

$$-K_z \left[\frac{c_{i,2} - c_{i,1}}{\Delta z} \right] + (w - w_d)c_{i,1} = E. \quad (3.61)$$

Collecting like terms, this may be written as

$$\beta_3 c_{i,2} + \beta_4 c_{i,1} = E, \quad (3.62)$$

where

$$\beta_3 = -\frac{K_z}{\Delta z} = -\beta_2,$$

and

$$\beta_4 = \frac{K_z}{\Delta z} + (w - w_d).$$

In both of these cases i ranges from 1 to $m + 2$.

East and West Boundaries:

The east and west boundaries are quite simple. The west boundary is Dirichlet, so the finite difference scheme is just

$$c_{1,j} = A_j \quad (3.64)$$

for $j = 2, 3, \dots, n + 1$. Recall that we have two possible eastern boundary conditions. The Dirichlet case is nearly the same as the west boundary and is given by

$$c_{m+2,j} = B_j. \quad (3.65)$$

The Neumann case gives a slightly different east boundary, namely

$$c_{m+2,j} - c_{m+1,j} = 0. \quad (3.66)$$

Both of these representations hold for $j = 2, 3, \dots, n + 1$.

Numerically Preserved Quantities

This section contains a check of the above finite difference scheme. This check will verify that the chosen scheme matches the analytic presentation of the problem (at least to the order of the approximation). It will also provide a method for verifying that mass is conserved along the boundaries of the domain. This process will use two telescoping sums. The first sum corresponds to the upwind approximation. That is

$$\sum_{j=2}^{m+1} c_j - c_{j-1} = c_{m+1} - c_1. \quad (3.67)$$

The second sum comes from the second order central difference approximation of the the second derivate, and also telescopes as

$$\sum_{j=2}^{m+1} c_{j+1} - 2c_j + c_{j-1} = c_1 - c_2 - c_{m+1} + c_{m+2}. \quad (3.68)$$

These sums in hand, we begin by examining the governing PDE. Assuming a steady state and that the velocities and turbulent diffusivities are constant, the PDE is

$$0 = K_z \frac{\partial^2 c}{\partial z^2} - w \frac{\partial c}{\partial z} + K_x \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x}. \quad (3.69)$$

Integrating with respect over z and x this is

$$\int_0^L \int_0^H K_z \frac{\partial^2 c}{\partial z^2} - w \frac{\partial c}{\partial z} dz dx + \int_0^H \int_0^L K_x \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x} dx dz = 0. \quad (3.70)$$

Computing the inner most integrals on each term, this reduces to

$$\begin{aligned} \int_0^L \left(K_z \frac{\partial c}{\partial z}(x, H) - wc(x, H) \right) - \left(K_z \frac{\partial c}{\partial z}(x, 0) - wc(x, 0) \right) dx \\ + \int_0^H \left(K_x \frac{\partial c}{\partial x}(L, z) - vc(L, z) \right) - \left(K_x \frac{\partial c}{\partial x}(0, z) - vc(0, z) \right) dz = 0. \quad (3.71) \end{aligned}$$

Physically, this integral sum represents the net flux of mass through the region and states that the net flux must be zero. More specifically, the first integral is the difference between the flux along the North boundary and the South boundary. The second integral is the difference in flux between the East and West boundaries. The total must balance. Lacking a source or sink in the domain, this is simple conservation of mass. Numerically, (3.70) may be approximated using our finite difference scheme as

$$\begin{aligned} \sum_{i=1}^{m+2} \sum_{j=2}^{n+1} K_z \left[\frac{c_{i,j+1} - 2c_{i,j} + c_{i,j-1}}{\Delta z^2} \right] - w \left[\frac{c_{i,j} - c_{i,j-1}}{\Delta z} \right] \Delta z \Delta x \\ \sum_{j=1}^{n+2} \sum_{i=2}^{m+1} K_x \left[\frac{c_{i+1,j} - 2c_{i,j} + c_{i-1,j}}{\Delta x^2} \right] - v \left[\frac{c_{i,j} - c_{i-1,j}}{\Delta x} \right] \Delta x \Delta z = 0. \quad (3.72) \end{aligned}$$

Applying the telescoping results shown in Equations 3.67 and 3.68 and reorganizing, these double sums reduce to

$$\begin{aligned} \sum_{i=1}^{m+2} \left[K_z \left(\frac{c_{i,n+2} - c_{i,n+1}}{\Delta z} \right) - wc_{i,n+2} \right] - \left[K_z \left(\frac{c_{i,2} - c_{i,1}}{\Delta z} \right) - wc_{i,1} \right] \Delta x \\ \sum_{j=1}^{n+2} \left[K_x \frac{c_{m+2,j} - c_{m+1,j}}{\Delta x} - vc_{m+2,j} \right] - \left[K_x \frac{c_{2,j} - c_{1,j}}{\Delta x} - vc_{1,j} \right] \Delta z = 0. \quad (3.73) \end{aligned}$$

Comparison of this result with integral equation (3.71) reveals a match up to the order of the scheme. This means that to the precision of the scheme, our numeric implementation must conserve mass as well.

Model Output:

To test the numerical model output we make use of synthetic, or artificial, parameter values. This allows us to investigate the model generally without worrying about experimental results. Artificial parameter values are shown in Table 3.1. These values were selected so that each parameter was roughly on the same order, but reflect to some degree our expected relationships. That is, we in general expect $H < L$ and $w < v$, but these values are not intended to be realistic. Stream cell concentrations for these parameters are shown in Figure 3.3 plotted on a 200 by 200 grid. Results for both Dirichlet and Neumann lower boundary conditions are shown. We note immediately the strong effect of the boundaries on the stream concentration. This edge effect is due to the fact that we specify only the boundary concentration (or the derivative on the boundary in the Neumann case) and not the total flux. That is, the second sum in (3.73), which corresponds to flux at the East minus flux at the West boundary, is not zero for either the Dirichlet or Neumann case. Rather, the fluxes along the boundary balance at whatever concentration was specified by the boundary conditions. However, the sum specified by (3.73) is small for both plots, and when the grid size is doubled the value is halved. Thus, the net flux balances on the domain to first order accuracy.

Solving for E :

Specifying E as we did above in Table 3.3 and Figure 3.3 is not possible with real data as E is unknown. In fact, as has been described above, our goal is to use our model to estimate E for given measured influent and effluent concentrations. The method for doing this is simple. First, we will specify the influent concentration A , but not the effluent concentration. Rather, we will use a the Neumann condition at the downstream boundary. Leaving the value of B unspecified, we will vary E until the concentration at $x = L$ matches

Table 3.1: Artificial parameter values for testing the numeric scheme. Units dimensions are given generically in terms of length (L), time (T), and number of cells.

Parameter	Description	Value	Units
w	settling velocity	-0.5	L/T
w_d	depositional velocity	-0.5	L/T
v	stream velocity	1	L/T
H	stream depth	1	L
L	stream length	10	L
K_z	vertical turbulent diffusivity	1	L ² /T
K_x	horizontal turbulent diffusivity	1	L ² /T
A	influent cell concentration	50	cells/L ²
B	effluent cell concentration	50	cells/L ²
E	erosion rate	50	cells/(L T)

our measurement to some tolerance. However, this plan requires us to summarize the concentration at $x = L$, which will vary with z (cf. Figure 3.3b) using a Neumann condition.

Using a Neumann downstream boundary condition, the concentration at $x = L$ may be summarized as a single number by taking an average. Mathematically, we define the average concentration at $x = L$ by

$$\bar{c} = \frac{1}{H} \int_0^H c(L, z) dz \approx \frac{1}{H} \sum_{j=1}^{m+2} c_{j,n+2} \Delta z. \quad (3.74)$$

Note that this approximation of the integral is first order accurate, as is our scheme. Now, all other parameters fixed, $\bar{c} = \bar{c}(E)$ is a function of E . We can thus seek

$$E^* = \arg \min_E |\bar{c}(E) - B|. \quad (3.75)$$

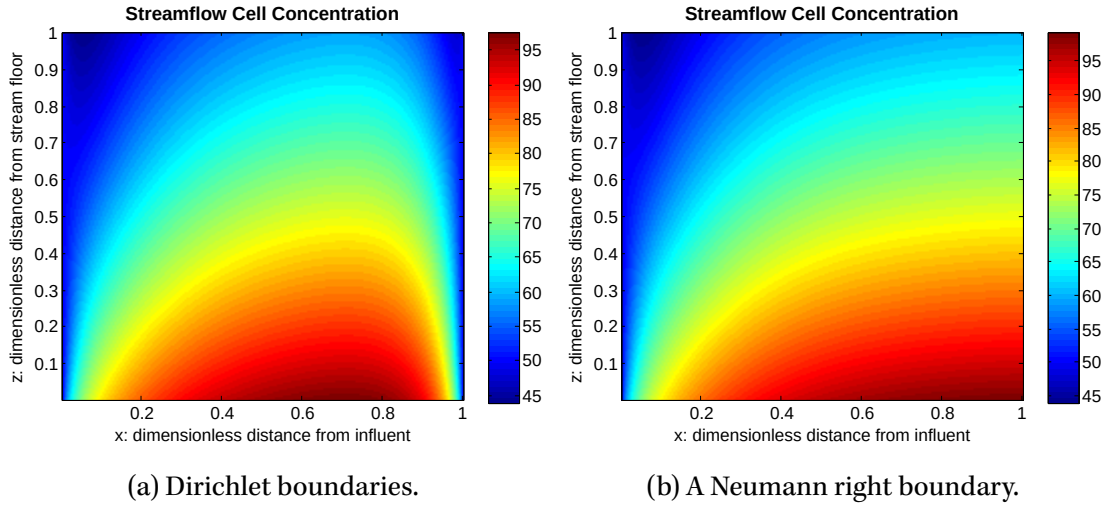


Figure 3.3: Plots of the cell concentration in the channel using the parameters given in Table 3.1

E^* is then the erosion (or adhesion) rate which results in the average downstream concentration matching the measured value of B . Computing the minimum in (3.75) is made simple using `fminunc`, one of Matlab's unconstrained optimization routines which uses an adaptive, quasi-Newton method.

We can test this scheme using the synthetic parameter values from Table 3.1 used to generate Figure 3.3b. In Figure 3.3a both boundaries were set at a concentration of $A = B = 50$ cells/L, but in Figure 3.3b only A was set to 50 cells/L. The right boundary was defined only by $\frac{\partial c(L,z)}{\partial x} = 0$. Using equation (3.74) on the solution matrix used to generate Figure 3.3b gives $\bar{c} = 78.2$ cells/L. Setting $B = 78.2$ in (3.75) yields $E^* = 50.0$, which is indeed the value used to generate the figure. A Newton-like solver such as `fminunc` requires an initial guess and can converge to a local rather than global minimum. However, in solving (3.75) `fminunc` converged quickly and uniquely for any initial guess for E between $-10,000$ and $10,000$. Above or below those values the maximum number of function evaluations must be increased, but the solver still converges to the same value.

Thus, empirically it seems that this method is quite robust with respect to the initial condition, and we use an initial value of $E = 0$ in the work that follows.

Parameterizing the Streamflow Model

A numeric steady-state implementation for the general models described in the previous chapters requires values for numerous parameters. These include values for horizontal and vertical turbulent diffusivities K_x and K_z , horizontal stream velocity v , cell settling velocity w , cell depositional velocity w_d , and cell concentrations for Dirichlet boundary conditions $A(z)$ and $B(z)$. We also need the length of the channel in question, L , as well as the average depth H over the interval of interest. Rather than make up values or search the literature for them, in this chapter these values are estimated using a combination of field measurements, calculations, and experimentation. While erosion rate E is also unknown, recall that this is not a directly measured quantity but rather the calculated model output.

Data Sample Locations:

Data used for model parameterization was collected at five locations along a major effluent channel of Mushroom Spring. These sites, named MS0, MS1, MS2, MS3, and MS4, were enumerated from the source pool and chosen to sample a decreasing temperature gradient. The physical location of these sites is shown in Figure 3.4. Spatially, these sites were approximately 0, 1.1, 3.8, 5.6, and 13.1 meters from the source pool. Water samples, from which cell counts were extracted, were collected approximately monthly over the course of a year.

Velocity data was collected on five occasions, but refinement of the velocity collection methodology resulted in only two sets of reliable data, which is described in the section below. Because sites were chosen to sample certain temperatures, not all were

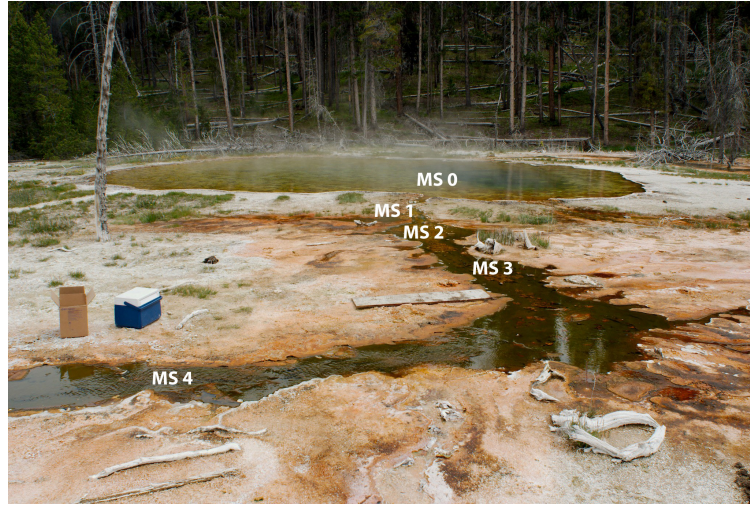


Figure 3.4: A picture of Mushroom Spring with approximate sample collection locations marked. Three additional locations were used to collect velocity data, located between MS1 and MS2, MS2 and MS3, and between MS3 and MS4.

suitable for stream velocity measurements. To compensate for this, three additional sites were selected and used only for velocity calculation. These sites are L_2 , located midway between MS1 and MS2 at approximately 2.5 meters from the source, L_4 , located between MS2 and MS3 at approximately 7.7 meters from the source, and L_6 located between MS3 and MS4 approximately 9.3 meters from the source pool. All experimental sites names and distances are summarized in Table 3.2.

Table 3.2: A summary of data collection locations and names at Mushroom Spring.

Site Name	MS0	MS1	-	MS2	-	MS3	-	MS4
Location Parameter	L_0	L_1	L_2	L_3	L_4	L_5	L_6	L_7
Approx. distance from source (m)	0	1.1	2.5	3.8	4.7	5.5	9.3	13.1

Measuring Average Stream Flow Velocity:

The stream flow velocity, v is an important model parameter. Not only does it provide the primary transport of cells in the x direction, but the value of this parameter also impacts estimates of the turbulent diffusivities as will be shown in a later section. However, in a small scale stream such as the channel of interest at Mushroom Spring, measuring stream velocity is challenging. This challenge is increased by park regulations which limit acceptable experiments to those which will not adversely impact natural sites.

In large drainage systems stream flow velocity is usually estimated using a current meter. The user wades into the flow holding the meter, or lowers it from a boat. A basic current meter is an impeller on the end of a pole. Rotations of the impeller are recorded electronically and converted into flow velocities based on the impeller's calibration. Measurements of the flow's depth and cross section allow for fluid flux estimates should such be desired [43]. In recent times, an Acoustic Doppler Current Profiler (ADCP) may be used instead of an impeller. This is a device which measures the Doppler Effect on flowing water by recording the change in frequency of reflected sound waves [44]. Unfortunately, the effluent channel at Mushroom Spring is too small for either of these methods. Current meters work on the order of meters, not centimeters, and care must be taken in Yellowstone National Park not to destroy or adversely impact natural sites.

Due to these difficulties, a simple and more primitive method was used to determine stream velocities. In this method, a floating object was introduced into the stream and progress of the float over a set distance was timed. Repeated trials allowed an average surface stream velocity to be calculated. This method was used to estimate of flow velocity at five locations in Mushroom Spring. Locations were chosen that exhibited a fairly regular channel shape and, as much as possible, uniform bed depth. Such properties facilitated the measurement of the stream's cross sectional area so that total downstream flux of



Figure 3.5: A photo of the experimental set-up used to measure stream velocities. Video was taken of the a buoyant object transiting the demarcated area. A frame-rate analysis allowed stream velocity to be calculated. In this photograph, flow is from left to right.

fluid could be computed. Selected regions were demarcated with string stretched across the channel and secured at an upper and lower location a known distance apart. Brightly colored, biodegradable floats which floated just beneath the surface of the stream were introduced into the channel above the first string, and the transit of the demarcated region by the float was filmed with a tripod-mounted digital camera. This set-up is shown in Figure 3.5. As the float left the measurement region it was captured for redeployment. The resulting movies were analyzed frame by frame. For each transit, the float's entrant and exit frame number was tabulated and the total transit frame count was calculated. This was then multiplied by the known frame rate (1/30 s) for a total transit time. Dividing the measured distance between the upper and lower strings by this time gave an average transit velocity.

Stream velocity at Mushroom Spring ranged from approximately 7 cm/s to 19 cm/s depending on location. The stream starts with a low average velocity near the source pool,

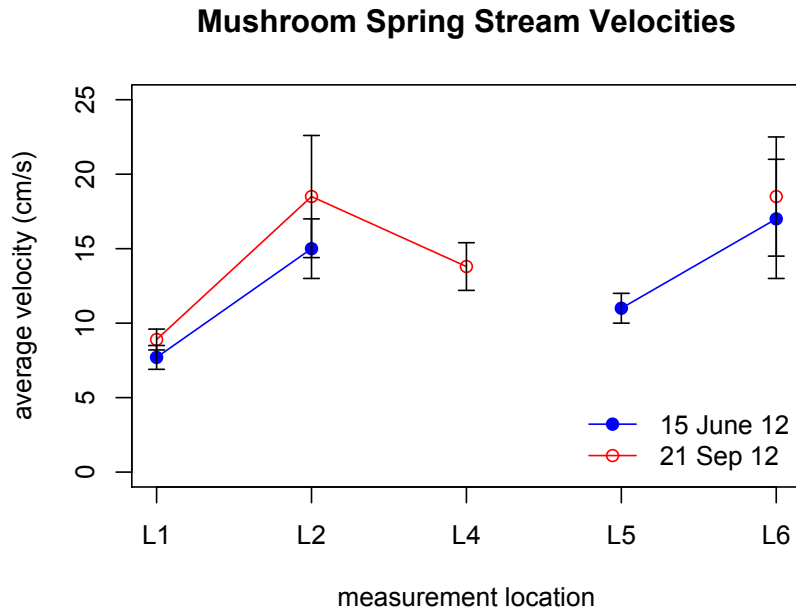


Figure 3.6: Stream velocity data collected during two trips to Mushroom Spring in 2012. Values are in cm/s and are the average velocities computed from a video frame rate analysis of a floating object. This is a graphical representation of data shown in Table 3.3. Error bars represent one standard deviation.

speeds up as it descends a small hill, then spreads out and slows down until turning a corner and narrowing again with a corresponding increase in average velocity. The overall average of measurements taken at five locations on two different dates was 13.8 cm/s with a standard deviation of 4.2 cm/s. A chart summarizing this stream-flow velocity data is shown in Figure 3.6.

At the site of each velocity measurement, careful measurements of the stream's width and depth were taken in addition to the float-transit videos. From this data we have an estimate for the depth of the stream at each location (parameter H). In addition, flux can be computed if desired by taking the product of velocity and area. For instance, using measured channel geometry data and the overall average velocity of 13.8 cm/s, the average flux of the channel is approximately 1 L/s. These data are summarized in Table 3.3. While

Table 3.3: A summary of stream geometry and velocity measurements for Mushroom Spring on two dates in 2012. These dates represent the best velocity data collected. Sample standard deviation is represented by s .

		L_1	L_2	L_4	L_5	L_6	Average
15-Jun-12:	width (cm)	19.43	18.80	-	43.64	41.71	30.89
	depth (cm)	6.43	4.25	-	2.82	1.70	3.80
	velocity (cm/s)	7.7	15.0	-	11.0	17.0	12.4
	s_{width}	2.24	2.04	-	9.53	7.85	
	s_{depth}	0.41	1.09	-	0.81	0.79	
	$s_{\text{vel.}}$	0.8	2.0	-	1.0	4.0	
21-Sep-12:	width (cm)	18.5	7.20	37.5	-	32.5	23.93
	depth (cm)	4.9	4.40	2.1	-	1.7	3.28
	velocity (cm/s)	8.9	18.5	13.8	-	18.5	14.9
	s_{width}	2.4	2.60	6.4	-	0.5	
	s_{depth}	0.7	4.40	0.6	-	0.1	
	$s_{\text{vel.}}$	0.7	4.1	1.6	-	4.0	

measurements are largely similar between dates, one exception is worth mentioning. In June, the stream was measured to be much wider at L_2 . A wide, shallow area was evident besides the deeper, main channel. In September the shallow region was no longer present.

Estimating Turbulent Diffusivities:

To estimate vertical turbulent diffusivity K_z we use a result from Fischer's textbook *Mixing in Inland and Coastal Waters* [29]. Averaging over a vertical velocity profile, Fischer shows that the vertical diffusivity can be written as

$$K_z = 0.067 H v^*, \quad (3.76)$$

where H is the channel depth and v^* the shear velocity. Here $0.067 = \kappa/6$, where the Von Karman constant κ is taken to be a universal constant with value 0.4. (Egelund gives a similar estimate with a coefficient of 0.0073 in [45]). Shear stress is given by

$$v^* = \sqrt{\frac{\tau_0}{\rho}}, \quad (3.77)$$

where τ_0 is the shear stress at the bottom of the channel and ρ is the density of the fluid [29, p. 92]. Shear velocity is computed as

$$\tau(z) = \mu \frac{dv}{dz} \quad (3.78)$$

for dynamic viscosity μ and stream velocity v . Now, estimating $\frac{dv}{dz}$ as

$$\frac{dv}{dz} \approx \frac{v}{H} \quad (3.79)$$

and combining (3.78) and (3.77) in (3.76), we can estimate vertical turbulent diffusivity as

$$K_z \approx (0.067) H \sqrt{\frac{\mu}{\rho} \cdot \frac{v}{H}}. \quad (3.80)$$

At 65°C , $\rho = 980.6 \text{ kg/m}^3$, $\mu = 4.33 \times 10^{-4}$, and K_z may be estimated as a function of v and H , parameters which we have estimates for as described above [46].

Turbulent transport in the stream-wise direction is taken to be the same as that in the transverse direction [29, p. 109]. Lacking a solid theoretical basis, diffusivities in the transverse direction (and hence stream-wise) must be estimated from experimental results. For instance, tabulating the results from 75 separate experiments done in straight, rectangular channels, Fischer gives an “approximate average” empirical result for turbu-

lent transport as

$$K_x = 0.15H\nu^* \quad (3.81)$$

[29, p. 107]. Estimating shear velocity as shown in (3.77) and (3.79) above, we can then estimate K_x as

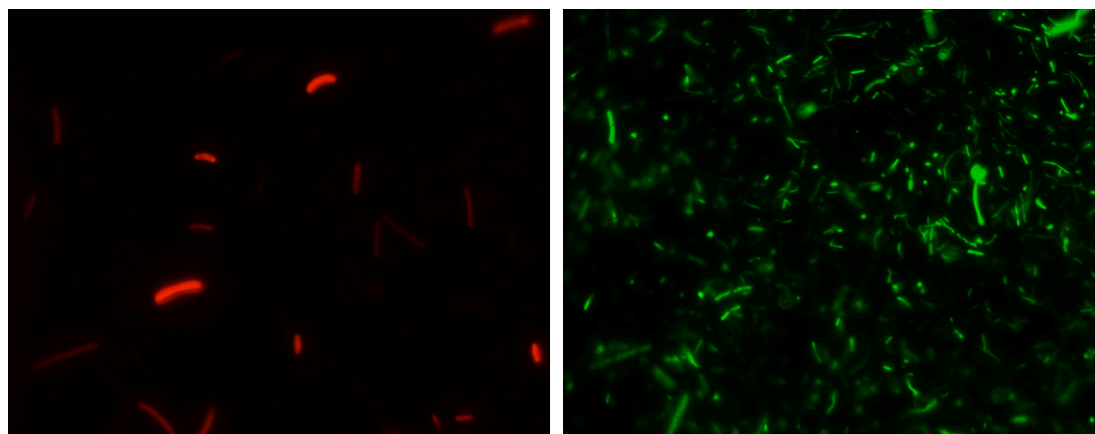
$$K_x \approx (0.15)H\sqrt{\frac{\mu}{\rho} \cdot \frac{\nu}{H}}, \quad (3.82)$$

which is once again a function of ν and H .

Potential Sources of Error. These estimates for K_z and K_x are no doubt very crude. In the case of vertical turbulent diffusivity, Fischer notes that his derivation is predicated on the channel being “very wide” with respect to the depth. In our model scenario the width of the channel may or may not satisfy this requirement. It is possible that the width of the channel impacts turbulence in a way that violates Fischer’s approximation. Beyond this, to estimate shear stress we use a very crude approximation for $\frac{dv}{dz}$. Also, in the case of horizontal turbulent diffusivity, we are using an experimental average that combines diverse results.

Measuring Boundary Values

Specifying Dirichlet boundaries requires a knowledge of cell concentrations at specified locations. To achieve this, water samples were collected at all five sites and used to obtain microscopic cell counts. To create each sample, 100 mL of channel water was forced through a 0.2 μm filter which was then immediately frozen on dry ice and stored in a -80°C freezer until analyzed. In preparation for microscopic analysis, filters were defrosted, treated with a SYBRGOLD stain, and mounted on a microscope slides. A Zeiss microscope was used to obtain images of each slide. Two different images were made for each of five randomly selected field of view on each slide. First, a rhodamine optical filter was used to image *Synechococcus* cells by exploiting their red autofluorescence,



(a) A rhodamine filtered image showing *Synechococcus* cells, which were counted manually. (b) A FITC filtered image showing all cells containing DNA. Cells in these images were counted using an automated FIJI script.

Figure 3.7: Sample micrographs of filtered samples from Mushroom Spring.

which occurs because these cells contain chlorophyll *a*. Second, a FITC filter was used to image any cells containing DNA, which was highlighted by the SYBRGOLD stain. Example images of each type are shown in Figure 3.7. Once collected, images were analyzed in the open source image analysis software FIJI [47] as described below.

Rhodamine filtered images, showing just *Synechococcus* cells in a given field of view, contained few enough cells that manual counts were practical (See Figure 3.7a). The images were imported into FIJI and processed using FIJI's cell counting macro. This macro imposes a grid over the image and registers mouse-clicks by superimposing a number at the mouse click location on the bases image and recording the total number of clicks in a table. The grid and resulting enumeration ensure that the manual count covers the complete image and guards against multiple counts for single cells. The resulting counts were exported from FIJI to a spreadsheet for further analysis.

Converting image cell counts to cells per mL is a straightforward calculation readily performed in a spreadsheet. Each image was taken at a spatial resolution of 1 pixel (px) per $0.3086\ \mu\text{m}$. Thus, the field of view (FOV) for each image of $1300\ \text{px} \times 1030\ \text{px}$ has an

area of $1.3 \times 10^5 \mu\text{m}^2$. The filter as whole, with diameter 25,000 μm , has area $4.9 \times 10^8 \mu\text{m}^2$. Dividing these two values, the filter contains 3.5×10^3 FOVs. Given a manual average count of N cells per FOV, the total number of cells on the filter is estimated to be $(3.5 \times 10^3)N$. Since each water sample was 100 mL of fluid, the average number of cells per mL is then estimated by $(3.5 \times 10^3)N/100$.

The FITC images, which show all DNA bearing cells in a given viewing field, generally contained large numbers of cells making manual counts impractical (for example, cf. Image 3.7b). Instead, a FIJI script was written to measure the total signal area in units of square pixels and applied to each image. This measurement was then converted to an estimated number of cells as described below. Though the script may over or under estimate signal area, it has the benefit of being consistent and removes a subjective element from the cell count. The script was written to apply a threshold to each image using the Li algorithm [48], and then measure the signal area using FIJI's built-in "Analyze Particles" macro. Total signal pixel counts were recorded and exported to a spreadsheet for conversion to estimated cell numbers. This process was done in batch for each set of images.

Estimating Cell Size in Terms of Pixels. In order to convert the resultant total signal area from square pixels to number of cells we must estimate the size of a cell in square pixels. This is perhaps more challenging than expected given the variety of cell morphology in each slide along with images demonstrating poor focus or contrast. To estimate average cell area 30 images were randomly selected for manual analysis, 10 each from data collected in July, August, and September of 2011. Given the large number of cells per image, the FOV was restricted to a randomly selected $300 \text{ px} \times 300 \text{ px}$ sub region to facilitate manual analysis.

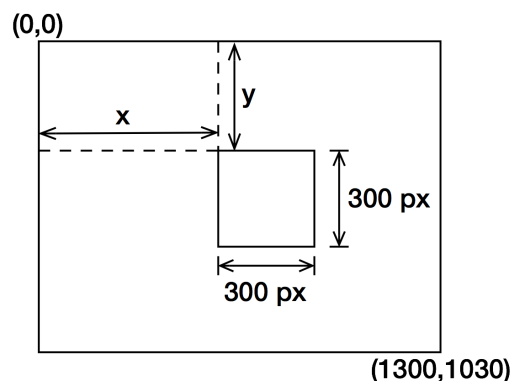


Figure 3.8: A 300 px $\times$ 300 px subregion was chosen from 30 FITC images for manual cell counts. Comparison of this this count with thresholded signal area allows as estimate for cell size in px^2 to be computed.

The random selection of a subregion was achieved by using a random number generator to select coordinates (x, y) such that $0 \leq x \leq 1000$ and $0 \leq y \leq 730$. This random coordinate is the pixel location of the upper left corner of the selected sub region. The interval's upper limits were chosen so that the 300 px $\times$ 300 px rectangle defining the subregions fits inside the 1300 px $\times$ 1030 px image. A graphic illustrating this geometry is shown in Figure 3.8. This region was selected, cropped, and a false colored scheme applied to aid manual counting by another means of another FIJI script. The script applied, a manual count was taken of each subregion using FIJI's "cell count" macro as describe previously for the rhodamine filtered images analysis.

After a manual count total was recorded for a particular sub image, another FIJI script was used to automatically measure the signal area in square pixels of the sub region. The signal area was then divided by the manual count to provide an estimate for the size of a cell in square pixels. Calculated values for cell size exhibited a wide range of values, from 102 px^2/cell to 1314 px^2/cell . Given a median value of 439 px^2/cell , large values such as 1314 px^2/cell seem anomalous. Indeed, the images from which the extremely large values

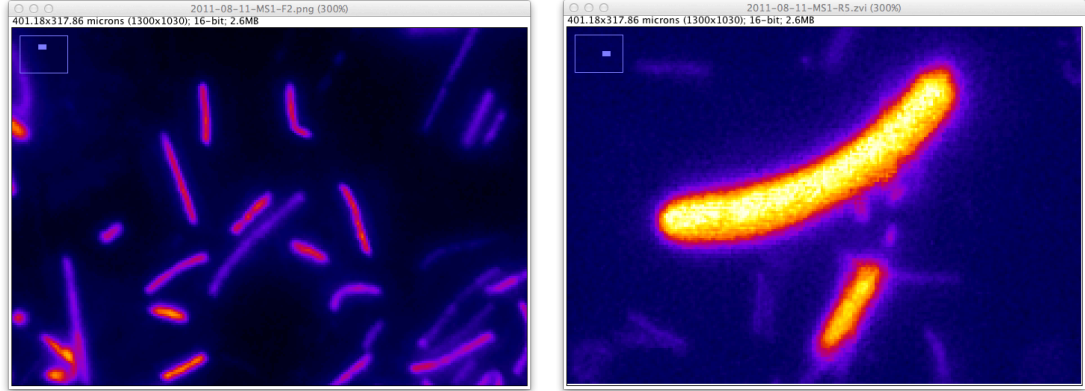
Table 3.4: Mushroom Spring cell count and error estimates from September 18, 2011.

	MS0	MS1	MS2	MS3	MS4
mean cells/mL (<i>thermus</i>)	4553	7185	10222	7148	3507
standard deviation	3240	3688	4841	3707	1726
mean Cells/mL (<i>synech.</i>)	141	1955	6082	423	300
standard deviation	105	490	3657	204	137

come exhibit poor focus. If large values are statistical anomalies they should be excluded from the mean used to compute the average size of a cell.

A common statistical test for outliers is to use the interquartile range, or IQR, to determine if a data point is anomalous. If Q_1 is the data's first quartile value, Q_2 the second, Q_3 the third, and Q_4 the fourth, then $IQR = Q_3 - Q_1$. In this method, values that fall below $Q_1 - 1.5 \times IQR$ or above $Q_3 + 1.5 \times IQR$ are considered outliers. For the data set at hand, two value are excluded as being above this threshold. The median of the resulting data is 399 px^2/cell , while the mean falls to 432 px^2/cell with a standard deviation of 228 px^2/cell . This mean value of 432 px^2/cell is used throughout while processing the FITC images as the average size of a cell in square pixels.

With an estimate for the approximate number of square pixels per cell, the total signal pixel counts can be converted to as estimated number of cells. Data collected September 18, 2011 is shown in Table 3.4. Note that estimated cell counts for both FITCH and rhodamine images show large standard deviations. The error shown there reflects only variance in filter cell counts estimates and does not reflect the additional variance in the cell size in square pixels calculation.



(a) Analysis of false color images such as this show that *Thermus* cells are, on average, 9.5 μm in length. (b) A close up of a rhodamine filtered *Synechococcus* cell. FIJI measurements of such cells yield an average length of 26.0 μm .

Figure 3.9: False color Mushroom Spring micrographs used to compute cell size.

Computing Vertical Velocities:

The settling velocity for cells in the flow was computed using Stoke's Law. Balancing drag and buoyancy allows Stokes' Law to be solved for settling velocity. For spherical particles this settling velocity, w , is given by

$$w = \frac{2(\rho_p - \rho_f)}{9\mu} g R^2, \quad (3.83)$$

where ρ_p is the density of the settling particle, ρ_f is the density of the fluid, μ is the dynamic viscosity of the fluid, g is acceleration due to gravity, and R is the radius of the settling particle [49, p. 599]. Although both *Synechococcus* and *Thermus* cells are rod shaped rather than spherical, this model can be used to provide rough estimates for cell settling velocities.

The temperature of water in the effluent channel at Mushroom Spring is about 65°C. Water at this temperature has a density of 980.59 kg/m³ and dynamic viscosity of 4.34×10^{-4} Pa s [46]. Assuming a cell density of 1.1 g/cm³ [50] and using $g = 9.81$

m/s², w can be written as a function of particle radius R . Measurement in FIJI of 60 random *Thermus* cells drawn from three random Mushroom Spring micrographs show that *Thermus* cells are on average 9.5 μm in length with a standard deviation of 2.5 μm . Using this a crude approximation of R in equation (3.83) yields an estimated settling velocity of 5.4×10^{-3} cm/s. Similarly, measurement of 60 *Synechococcus* cells in FIJI from randomly selected micrographs of Mushroom Spring samples gives an average length of 26.0 μm with a standard deviation of 9.9 μm . Again using this as a crude estimate for R gives a settling velocity of *Synechococcus* of 4.1×10^{-2} cm/s. Examples of the types of images used to estimate cell length may be seen in Figure 3.9. In all cases below, depositional velocity w_d is taken to be equal w . If not, w_d would be taken to be an arbitrary percentage of w .

Applying the Model to Field Data

The fact that the data above comes from different locations at different times makes it somewhat difficult to formulate a problem to which we may apply our model. However, closer examination reveals that we can construct four parameter sets suitable for the model code. Dividing the velocity data from Table 3.3 into two groups, we see that we have velocity data between L_0 and L_3 , as well as between L_3 and L_7 . We have cell counts from L_0 , L_3 , and L_7 (cf. Table 3.4). Thus, we can divide the stream into two segments, the upper from L_0 to L_3 and the lower from L_3 to L_7 . By averaging the channel velocity and geometry data shown in Table 3.3 across June and September for L_1 and L_2 we can approximate the stream between L_0 and L_3 by a rectangular prism with width 16 cm, length 380 cm, and depth 5 cm, with an average stream velocity of 15 cm/s. Furthermore, at each end of this segment we have cell counts for both *Thermus* and *Synechococcus* cells, meaning we can solve for E for the case of each type of cell. These cases will constitute parameter sets one and two, respectively. This data is summarized in Table 3.5. Similarly,

Table 3.5: Model parameters computed for the Mushroom Spring effluent channel between the source, L_0 , and L_3 . Stream velocity and geometry (depth and width) are averaged from Table 3.3 over June and September. Values for K_x and K_z are computed using Fischer's formulas, shown in (3.81) and (3.82), using the given H .

Parameter	Description	Value	Units
w ($r = 10 \mu\text{m}$)	settling velocity of <i>Thermus</i>	-0.0060	cm/s
w ($r = 26 \mu\text{m}$)	depositional velocity of <i>Synechococcus</i>	-0.0405	cm/s
K_z	vertical turbulent diffusivity	0.0352	cm^2/s
K_x	horizontal turbulent diffusivity	0.0789	cm^2/s
v	average stream velocity	12.5	cm/s
H	average stream depth	5.0	cm
L	stream section length	380	cm
width	average stream width	16.0	cm
A_1	influent cell concentration (<i>Thermus</i>)	72,848	cells/cm^2
B_1	effluent cell concentration (<i>Thermus</i>)	163,552	cells/cm^2
A_2	influent cell concentration (<i>Synechococcus</i>)	2,256	cells/cm^2
B_2	effluent cell concentration (<i>Synechococcus</i>)	97,312	cells/cm^2

Table 3.6: Model parameters computed for the Mushroom Spring effluent channel between L_3 and L_7 . Cell settling velocities are the same as in Table 3.5, but all other values are updated. Note that the turbulent diffusivities are different because they depend on v and H per equations (3.81) and (3.82).

Parameter	Description	Value	Units
w ($r = 10 \mu\text{m}$)	settling velocity of <i>Thermus</i>	-0.0060	cm/s
w ($r = 26 \mu\text{m}$)	depositional velocity of <i>Synech.</i>	-0.0405	cm/s
K_z	vertical turbulent diffusivity	0.0251	cm^2/s
K_x	horizontal turbulent diffusivity	0.0562	cm^2/s
v	average stream velocity	15.1	cm/s
H	average stream depth	2.1	cm
L	stream section length	930	cm
width	average stream width	38.8	cm
A_1	influent cell concentration (<i>Thermus</i>)	396,997	cells/cm^2
B_1	effluent cell concentration (<i>Thermus</i>)	136,203	cells/cm^2
A_2	influent cell concentration (<i>Synech.</i>)	236,210	cells/cm^2
B_2	effluent cell concentration (<i>Synech.</i>)	11,651	cells/cm^2

averaging the velocity and geometry data for L_4 , L_5 , and L_6 over June and September, we can approximate the lower half of the channel as a rectangle prism with width 38.8 cm, length 930 cm, and depth 2.1 cm, with an average stream velocity of 15.1 cm/s. Again, we have measurements for both types of cells allowing us to solve for two more E values. These two cases will form parameter sets three and four, respectively, and this data is summarized in Table 3.6. Note that to convert the measured cells/mL to square units (cells/cm²) we multiply by the average measured width of the stream.

Substituting these values into the model we solve for E as described in equations (3.74) and (3.75). In the first case, *Thermus* cells increase along the first segment of the stream, which suggests that E is positive as cells are eroded into the flow. Indeed, in this case $E = 1.7259 \times 10^4$ cells/(cm s). Similarly, in case 2 we see *Synechococcus* increases as well from L_0 to L_3 . For this organism $E = 3.9137 \times 10^4$ cells/(cm s). In the second segment of the stream we see that both *Thermus* and *Synechococcus* cell counts decrease between L_3 and L_7 . Thus, we expect E to be negative on this stretch. Indeed, for case 3 we find that $E = -8.2579 \times 10^3$ cells/(cm s), and for case 4 compute $E = -5.0302 \times 10^2$ cells/(cm s).

A look at the resulting concentration plots for cells in the flow, shown in Figure 3.10, raises questions about these values, however. Examination of the first two panels of Figure 3.10, which correspond to cases 1 and 2, show concentration gradients of hundreds of cells per centimeter near the bottom. This seems physically unlikely, as we might expect cells concentrations to mix better in a turbulent stream. In a related problem, the third and fourth panels of Figure 3.10 show negative concentration gradients downstream at the stream bottom. This is physically impossible and again suggests that the vertical diffusivity is too small to mix cells adequately vertically in the column.

Indeed, increasing K_z by an order of magnitude corrects both of these problems. A factor of 20, for instance, creates a much smoother vertical cell concentration gradient where all concentration values are positive. However, changing K_z also changes the

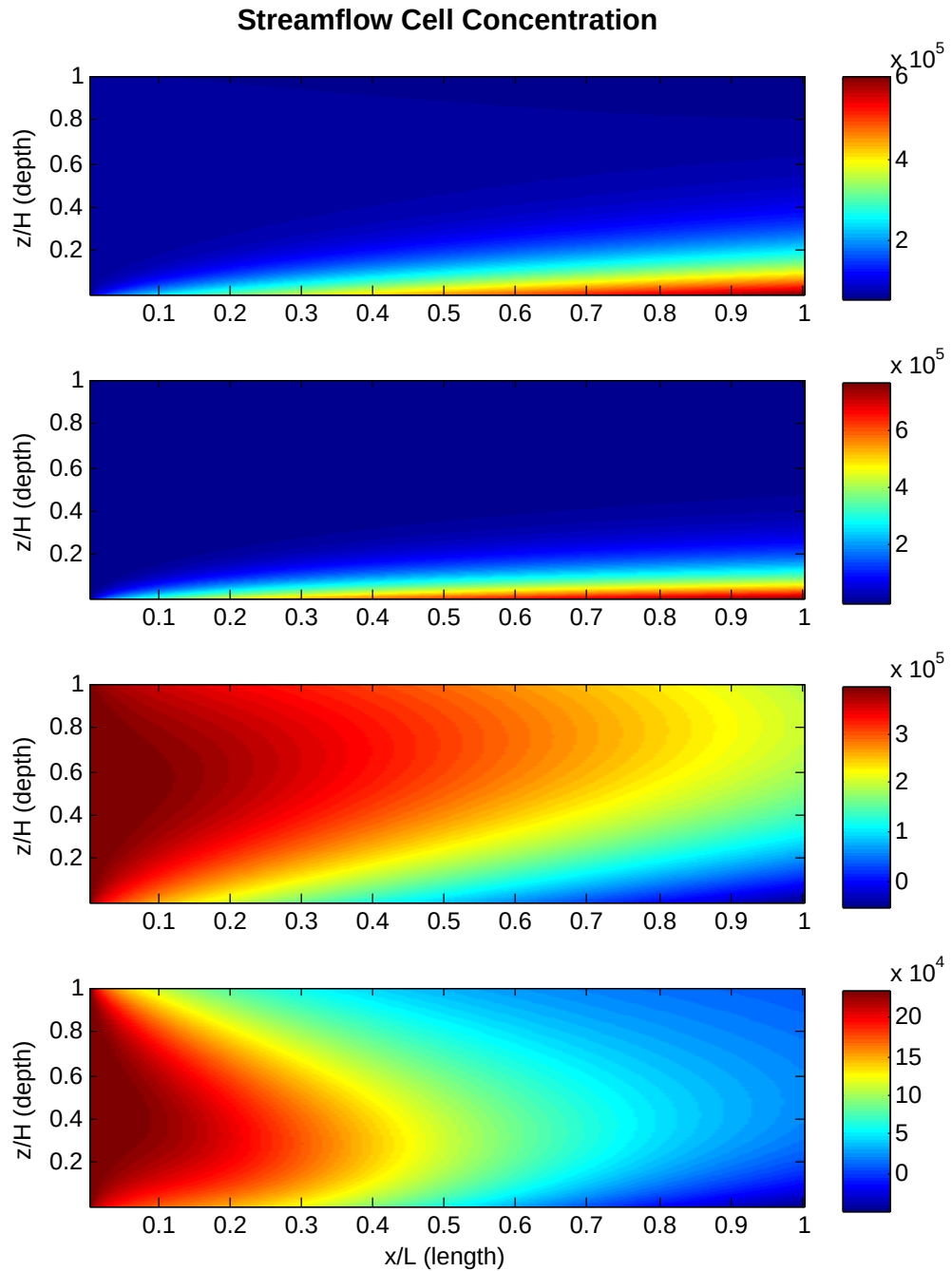


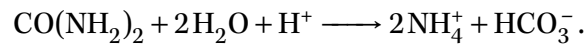
Figure 3.10: These plots show the model output using real data for (top to bottom) cases 1, 2, 3, and 4. The first panel shows the modeled concentration of *Thermus* cells between L_0 and L_3 . The second shows *Synechococcus* over the same range. The last two panels show concentrations of *Thermus* and *Synechococcus*, respectively, between L_3 and L_7 . Improbably sharp gradients and negative values suggest problems with the value used for K_z , the vertical turbulent diffusivity.

computed values of E . This means a more accurate calculation of E will require a better estimate for K_z . Here we have calculated turbulent diffusivities using Fischer's model (cf. equation (3.80)), but perhaps another model would be more appropriate, or this parameter is one which could be measured accurately in the field or otherwise estimated from data.

CHAPTER FOUR

A TUBE REACTOR MODEL: THE FORWARD PROBLEM

Biom mineralization is the process by which living organisms produce minerals. While there are multiple ways organisms engage in biom mineralization, in this chapter our interest is confined to a particular pathway to biom mineralization by microorganisms called urea hydrolysis or ureolysis. Urea hydrolysis is a chemical mechanism whereby microorganisms break down urea. This can cause an increase in the microorganism's environmental pH, which can in turn trigger precipitation of carbonate and other minerals. At circumferential neutral pH levels (pH values between 6.5 and 7.5) urea hydrolysis can be written as



Here two ammonium ions and one bicarbonate ion are formed by the hydrolysis of each urea molecule. One proton is consumed in this reaction, which causes an increase in pH. This pH increase results in the formation of carbonate ions. This increase in carbonate ion concentration leads to the precipitation of calcium carbonate when calcium ions are introduced [32].

Microbial formation of carbonate precipitates is significant in a number of applications, including medical, engineering, and agricultural/industrial contexts. In medical applications, ureolytic microorganisms can play a role in the formation of kidney stones as well as crystal encrustations on urinary tract catheters [51]. In engineering applications, ureolytic mineral formation has been used to protect building materials through mineral deposition [52] as well as reduce permeability in the subsurface [53]. In agricultural contexts urea is a major source of nitrogen in fertilizer and can contaminate groundwater [54]. In each of these scenarios, biofilms are an important source of ureolytic activity.

Critical to understanding ureolytic systems is quantitative information about ureolysis rates in biofilms. While planktonic cultures have been well studied, much less is known about ureolysis rates in biofilms. There have been studies of volume averaged rates in porous media [55,56] as well as in immobilized enzymes [57,58]. However, these studies have tended to concentrate on precipitation rates rather than kinetics. In this chapter we derive a model for ureolysis in a biofilm lined tube by combining two 1D models. We then apply this model to experimental data to estimate kinetic parameters for ureolysis in biofilms. In the model, urea consumption of the biofilm attached to the inside of the thin tube is described using a one dimensional biofilm model, which we assume is at steady-state. The kinetics of urea consumption are given by a general reaction function, which we will specialize to zero-order, first-order, and especially Michaelis-Menten cases. The output of this model is coupled through the flux term to a diffusion-advection-reaction tube reactor model. The experimental data comes from experiments run at Montana State University's Center for Biofilm Engineering and is used to parameterize the model. While experimental methods are briefly summarized here, a more detailed description is available in recent work by Connolly et al. [32].

The 1D Wall-Mounted Biofilm Model:

The 1D biofilm model is based on conservation of mass. In addition to conservation, we require two basic assumptions, namely, that properties of the biofilm change only in the direction perpendicular to the support surface and that the biofilm may be treated as a continuum. If we further assume that the biofilm is at a steady state and has a static physical profile, then the concentration of a substrate s in the biofilm depends only on the distance z from the support surface. Consider a thin differential element from depth z to $z + \Delta z$ with cross sectional area A as illustrated in Figure 4.1. Because of the steady state assumption, the flux Q of s into the biofilm at z must be balanced by the flux of substrate

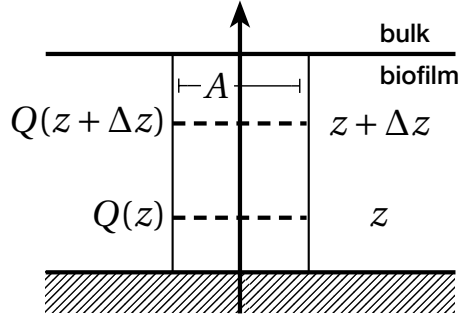


Figure 4.1: A schematic illustrating the mass balance in a differential segment of a wall mounted one-dimensional biofilm with cross-sectional area A .

out of the element at $z + \Delta z$ and by the consumption of substrate within the element by reaction $r(s)$. That is,

$$0 = AQ(z) - AQ(z + \Delta z) + Ar(s)\Delta z.$$

Dividing through by $A\Delta z$ and taking the limit as the the length of the element goes to zero this becomes

$$0 = -\frac{\partial Q}{\partial z} + r(s).$$

Applying Fick's Law, which states that flux flows from regions of high concentration to low with a magnitude proportional to the concentration gradient ($Q = -D_e \frac{ds}{dz}$) this becomes

$$0 = D_e \frac{d^2 s}{dz^2} + r(s)$$

Here we use the notation D_e to indicate the possibility that the diffusivity of urea inside the biofilm could be different than the diffusivity of urea in water, which we will indicate as D , due to the physical obstruction of polymers and cells within the biofilm [59,60]. However, we will later drop this notation and assume a single diffusion rate.

To complete our 1D biofilm model we need boundary conditions. We assume that the concentration at the top of the biofilm ($z = H$) matches that in the bulk fluid, s^0 , and

that the biofilm support surface is impermeable to the substrate. These assumptions yield a Dirichlet boundary at the top and a no-flux condition at the bottom. The model is then summarized by

$$-D_e \frac{d^2 s}{dz^2} = r(s), \quad (4.1a)$$

$$s(H) = s^0, \quad \left. \frac{ds}{dz} \right|_{z=0} = 0, \quad (4.1b)$$

The ease with which we solve this model depends entirely on how we model the reaction described by $r(s)$ inside the biofilm. For some kinetic models we can solve (4.1) analytically, but for others we must use numerical results. In all kinetic cases we are particularly interested in the flux of substrate into the top of the biofilm from the bulk fluid. This is described by

$$Q_H = -D_e \left. \frac{ds}{dz} \right|_{z=H}, \quad (4.2)$$

a quantity we will use later to link the 1D biofilm and 1D tube models.

Solutions Using Various Kinetic Models

While numerous kinetic models for reaction term $r(s)$ are possible, we limit ourselves to three fundamental models, which are illustrated in Figure 4.2. These are zero order (0-order), first order (1st-order), and Michael-Menten order (MM-order) kinetics. Zero order kinetics assume constant consumption of substrate. That is, no matter the amount of biofilm, a fixed amount of substrate is consumed. This is appropriate in situations where only a small number of molecules are in a place where they can react. In terms of biofilms, this is when the biofilm is “thin” and the substrate easily saturates the biofilm from the bulk fluid to the support surface. In this case, adding or subtracting substrate does not appreciably change the consumption rate because the saturated biofilm is already utilizing as much substrate as possible. Mathematically, we describe

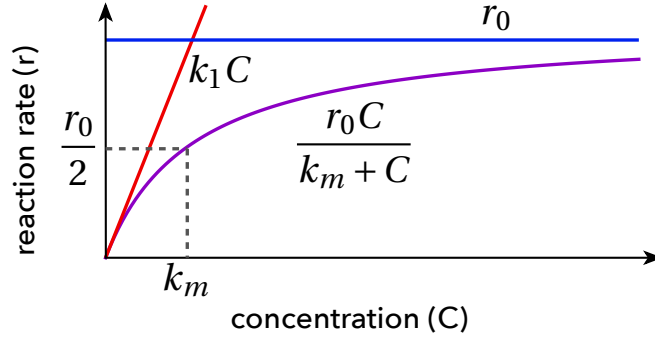


Figure 4.2: Zero-order, first-order, and Michael-Menten order kinetics plotted for substrate concentration U .

such kinetics by $r(s) = r_0$. First order kinetics are linear with respect to concentration. In this scenario, increasing substrate leads to increased consumption, and decreased substrate to decreased consumption. A 1st-order model is appropriate when substrate levels are low. For low substrate levels a change substrate concentration changes how far the substrate penetrates the biofilm and thus how much is consumed. A mathematical description is $r(s) = k_1 s$. Finally, Michaelis-Menten kinetics for intermediate concentrations, or when reaction rates go from approximately 1st-order to approximately 0-order in the same problem. The maximum rate of consumption r_0 and the half-saturation K_s completely determine MM-kinetics, which are given by

$$r(s) = \frac{r_0 s}{K_s + s}. \quad (4.3)$$

Note that for MM-kinetics, the reaction rate evaluated at the half saturation is exactly half the maximum rate, i.e. $r(K_s) = r_0/2$.

Zero Order Kinetics. For 0-order kinetics $r(s) = r_0$ and (4.1) becomes

$$-D_e \frac{d^2 s}{dz^2} = r_0, \quad (4.4a)$$

$$s(H) = s^0, \quad \left. \frac{ds}{dz} \right|_{z=0} = 0. \quad (4.4b)$$

This system is readily solved by integration, but we first nondimensionalize as an important constant emerges. A natural scale for concentration s is the bulk fluid concentration s^0 , and the most relevant length scale is the biofilm thickness, H . Set $S = \frac{s}{s^0}$ and $Z = \frac{z}{H}$. Then the system given by (4.4a) becomes

$$-\frac{d^2 S}{dZ^2} = \frac{r_0 H^2}{D_e s^0}, \quad (4.5a)$$

$$S(1) = 1, \quad \left. \frac{dS}{dZ} \right|_{Z=0} = 0. \quad (4.5b)$$

Define the right hand side of (4.5a) to be

$$-\phi^2 = \frac{r_0 H^2}{D_e s^0}. \quad (4.6)$$

A simple integration of this system yields the solution

$$S(Z) = -\frac{\phi^2}{2} (Z^2 - 1) + 1, \quad (4.7)$$

for $0 \leq Z \leq 1$. Also of interest, the flux of substrate from the bulk fluid into the biofilm is

$$Q_1 = -\frac{Ds^0}{H} \left. \frac{dS}{dZ} \right|_1 = -\frac{Ds^0}{H} \phi^2. \quad (4.8)$$

The Thiele Modulus. Note that ϕ^2 is the ratio of two time-scales: H^2/D_e is the diffusion time-scale and s^0/r_0 is the reaction time-scale. This grouping is thus a Thiele modulus, ϕ , which is the square root of the ratio of these two characteristic times

(diffusion time over reaction time). The utility of the Thiele modulus is that it measures the relative importance of diffusion and reaction in the system and may be used to quantify potential diffusion limitations in the biofilm. If the reaction time is very fast compared to the diffusion time the Thiele modulus is large. In this case, concentration at the bottom of the biofilm may be low (or zero) because substrate cannot diffuse throughout the biofilm before it is reacted, and a 0-order kinetic model would be a poor fit. On the other hand, if diffusion is very fast compared to the reaction we expect a small Thiele modulus. In this scenario it is possible that concentration will not vary much throughout the biofilm because diffusion is fast enough to keep the biofilm saturated with substrate. Here a 0-order model might be appropriate. In each of the kinetic cases the Thiele modulus emerges as an important nondimensional grouping, though the exact form varies with the kinetics.

First Order Kinetics. In the case of 1st-order kinetics $r(s) = k_1 s$ and the general model (4.1) becomes

$$D_e \frac{d^2 s}{dz^2} = k_1 s, \quad (4.9a)$$

$$s(H) = s^0, \quad \left. \frac{ds}{dz} \right|_{z=0} = 0. \quad (4.9b)$$

We again nondimensionalize by setting $S = \frac{s}{s^0}$ and $Z = \frac{z}{H}$. With these substitutions the above system becomes

$$\frac{d^2 S}{dZ^2} = \frac{k_1 H^2}{D_e} S, \quad (4.10a)$$

$$S(1) = 1, \quad \left. \frac{dS}{dZ} \right|_{Z=0} = 0. \quad (4.10b)$$

Again observe that the coefficient of S on the right hand side of (4.10a) is a ratio of diffusion time over reaction time.

$$\phi^2 = \frac{k_1 H^2}{D_e} \quad (4.11)$$

This is a first-order Thiele modulus. Using this notation for ϕ and primes to denote differentiation with respect to Z , system (4.10) may be rewritten as

$$S''(Z) - \phi^2 S = 0, \quad (4.12a)$$

$$S(1) = 1, \quad S'(0) = 0, \quad (4.12b)$$

and readily solved using basic ODE methods (say, by an integrating factor). The solution may be written as

$$S(Z) = \frac{\cosh(\phi Z)}{\cosh(\phi)}, \quad (4.13)$$

and it follows that the nondimensional interfacial flux of substrate into the biofilm is

$$Q_1 = -\frac{Ds^0}{H} \frac{dS}{dZ} \Big|_1 = -\frac{Ds^0}{H} \phi \tanh(\phi). \quad (4.14)$$

Michaelis-Menten Kinetics. The case of MM-kinetics is more difficult because it is not possible to solve for flux into the biofilm analytically. To solve for flux, a numerical method must be used. Such a method is described in a later section. For MM-kinetics, write $r(s) = \frac{r_0 s}{k_m + s}$, which results in a model given by

$$D_e \frac{d^2 s}{dz^2} = \frac{r_0 s}{k_m + s}, \quad (4.15a)$$

$$s(H) = s^0, \quad \left. \frac{ds}{dz} \right|_{z=0} = 0. \quad (4.15b)$$

While this system cannot be solved analytically, it can be nondimensionalized using the same variable transformation as in the last two cases. Substituting $S = \frac{s}{s^0}$ and $Z = \frac{z}{H}$ this system may be written as

$$\frac{d^2 S}{dZ^2} + \phi^2 g(S) = 0, \quad (4.16a)$$

$$S(1) = 1, \quad \left. \frac{dS}{dZ} \right|_{Z=0} = 0, \quad (4.16b)$$

where

$$g(S) = -\frac{\frac{k_m}{s^0} + 1}{\frac{k_m}{s^0} + S} \quad \text{and} \quad \phi^2 = \frac{H^2}{D_e} \cdot \frac{r_0}{k_m + s^0}. \quad (4.16c)$$

Once again, nondimensionalization yields a Thiele modulus, which will let us estimate the relative importance of diffusion and reaction in the biofilm. As stated above, there is no explicit formula for flux of substrate into the biofilm in this case, though in the case of thick or thin biofilms good approximations exist [61, 62]. For a general biofilm, flux will be estimated by numerically estimating

$$Q_1 = -\frac{Ds^0}{H} \left. \frac{dS}{dZ} \right|_1. \quad (4.17)$$

The Tube Reactor Model:

The 1D tube reactor model is also based on conservation of mass. Besides conservation, we assume conditions are uniform radially, allowing us to model the reactor as a long, thin tube of constant radius r extending along the x -axis. Fluid containing a specified amount of urea enters the reactor at $x = 0$, travels through the tube with fixed velocity

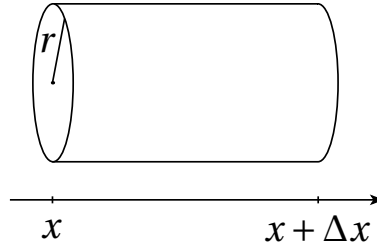


Figure 4.3: A differential segment of a the tube reactor with cross sectional area A , length Δx , and circumference $\tilde{C}$.

v , and exits at $x = L$. In addition to advective transport, we assume that urea can diffuse throughout the reactor.

Mass Balance:

Consider a short differential element spanning x to $x + \Delta x$ as shown in Figure 4.3. The segment has cross sectional area A and circumference $\tilde{C}$. Diffusive flux into the segment is given by $AQ(x)$ for diffusive flux Q , while flux due to diffusion out of the segment is $-AQ(x + \Delta x)$. Similarly, urea is brought into the segment by advection at rate $vAc(x)$ and removed at rate $-vAc(x)$. Urea is utilized by a biofilm that grows on the inner wall of the tube. The rate of utilization in general also depends on the concentration of urea available. Thus, if the utilization rate is given by $R(c)$, the amount of urea consumed is proportional to the inner surface area of the segment, that is, by $R(c)\tilde{C}\Delta x$. Putting these pieces together and assuming a steady state we have

$$0 = vAc(x) - vAc(x + \Delta x) + AQ(x) - AQ(x + \Delta x) - R(c)\tilde{C}\Delta x. \quad (4.18)$$

Divide through by $A\Delta x$ and take the limit as Δx goes to zero. Next, apply Fick's Law as we did in the previous model, that is, let $Q = -D\frac{dc}{dx}$ for urea diffusivity D . Finally, let $\delta = \tilde{C}/A = 2/r$ be the ratio between circumference and area of the tube. Then (4.18) can

be written as

$$0 = D \frac{d^2 c}{dx^2} - v \frac{dc}{dx} - \delta R(c). \quad (4.19)$$

Note that the reaction function R may depend on the height of the biofilm at point x in addition concentration c .

Boundary Conditions:

The bulk fluid enters the reactor at velocity v with concentration of urea set at c^0 , so the influent flux of urea is vc^0 . However, once inside the reactor bacteria utilize the urea, inducing a diffusive flux in addition to the advective flux. Boundary conditions at the influent ($x = 0$) end of the reactor are therefore flux balanced. That is, at the influent we specify

$$vc^0 = -D \left. \frac{dc}{dx} \right|_0 + vc(0). \quad (4.20)$$

At the effluent ($x = L$) end of the reactor we specify a no diffusion condition

$$\left. \frac{dc}{dx} \right|_L = 0. \quad (4.21)$$

Nondimensionalizing:

The system given by (4.19), (4.20), and (4.21) is readily nondimensionalized. Let $X = \frac{x}{L}$ and $C = \frac{c}{c^0}$. Then, using primes to represent differentiation with respect to X , the nondimensional tube reactor model is

$$0 = \frac{1}{\text{Pe}} C'' - C' - \frac{\delta L}{vc^0} R(c^0 C), \quad (4.22a)$$

$$1 = -\frac{1}{\text{Pe}} C'(0) + C(0), \quad C'(1) = 0. \quad (4.22b)$$

Here $Pe = \frac{\nu L}{D}$ is a Péclet number relating advective transport to diffusive transport. Typically, when Pe is small, diffusion dominates, and when Pe is large, advective transport is dominant.

The Unified Model:

To be completely specified, the tube reactor model requires information about the biofilm in the form of the reaction function $R(c)$. On the other hand, complete specification of the 1D biofilm model described in (4.1a) requires the concentration of substrate external to the biofilm (the parameter s^0), which can be provided by the tube reactor model. The key to combining these models is realizing that the utilization rate $R(c)$ is linked to the flux of substrate into the biofilm shown in equation (4.2). As substrate is consumed in the biofilm, local concentration in the biofilm is diminished, driving a Fickian flux of material from the tube reactor into the biofilm. That is, diffusion of substrate from the bulk fluid into the biofilm drives utilization in the tube reactor. Equating $R(c)$ with $Q_H(S)$ yields

$$\begin{aligned} 0 &= \frac{1}{Pe} C'' - C' - \frac{\delta L}{\nu c^0} J(C, H(X)) \\ &= \frac{1}{Pe} \frac{d^2 C}{dX^2} - \frac{dC}{dX} - \frac{\delta L}{\nu c^0} \frac{D_e s^0}{H(X)} \frac{dS}{dZ} \Big|_1. \end{aligned}$$

Writing s^0 equivalently as $s^0 = c^0 C(X)$ allows us to simplify further. In order to write the result more compactly we introduce the notation

$$\beta(X) = \delta L \frac{D_e}{\nu H(X)}. \quad (4.23)$$

This nondimensional number is the product of two nondimensional factors. The first, δL , is equal to the area of the interior surface area of the tube divided by the cross sectional

area. The second, $\frac{D_e s^0}{vH(X)}$, is the reciprocal of another Péclet number, which relates the rates of effective diffusivity to advection. However, the advective piece is here based on the length scale of biofilm height multiplied by the tube flow velocity. Note that $\beta(X)$ will increase in size if the inside area of the tube grows while the cross section remains constant, or if diffusion grows large with respect to advection. This makes physical sense: if the area of the tube walls grows then there is more biofilm and more urea usage, and if the Péclet number shrinks then the reduction of tube-wise velocity gives more time for reaction. On the other hand, $\beta(X)$ will decrease if the cross sectional area of the tube grows while the inside wall area remains fixed, or if the tube-velocity increases. Again, this makes physical sense as a lessing of wall space or increase in velocity should correspond to less biofilm and less urea usage. Using this notation for $\beta(X)$ and writing $\frac{dS}{dZ}|_1 = S_{Z(1)}$, the unified model may be written as

$$0 = \frac{1}{\text{Pe}} C'' - C' - \beta(X) C S_{Z(1)}, \quad (4.24a)$$

$$1 = -\frac{1}{\text{Pe}} C'(0) + C(0), \quad C'(1) = 0. \quad (4.24b)$$

Note that $\beta(X)$ and $S_{Z(1)}$ depend on X because the biofilm height, and hence the 1D biofilm concentration profile, change down the length of the tube.

Experimental Data:

Before describing the numerical scheme used to solve the unified problem we take a detour to describe the physical data, which will inform a final simplification of the model. This data is described in much greater detail elsewhere [32], but we summarize here in order to parameterize the model. This work was carried out by experimenters at Montana

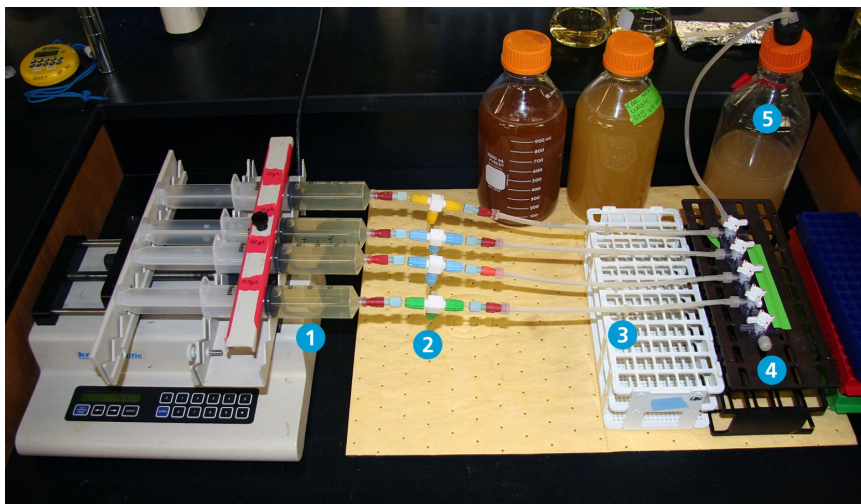


Figure 4.4: A photograph of the experimental set-up for the tube reactor experiment. Here 4 reactors are being run in parallel. Each consists of (1) a syringe pump, (2) sampling port, (3) silicone tube, and (4) valve, and flows into a (5) waste media container.

State University's Center for Biofilm Engineering, principally James Connolly and Adam Rothman, working under the direction of Robin Gerlach.

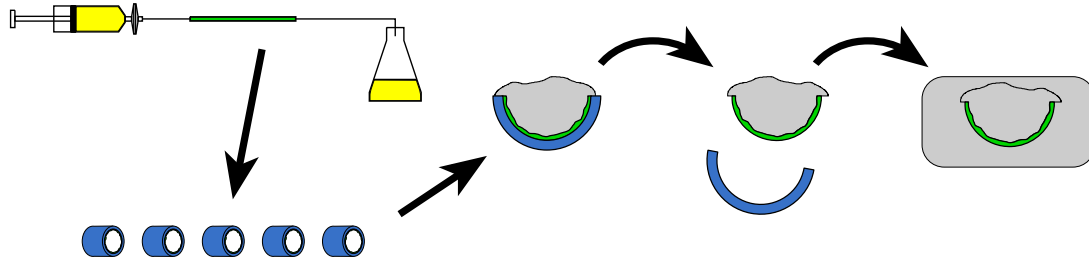
To mimic biofilm growing in small pore-spaces, cultures of a biofilm containing a green fluorescent protein gene (*gfp*) were grown in 10 cm long silicone tubes with an interior diameter of 0.8 mm. The microorganism was *Escherichia coli* MJK2 [63], and a *gfp* strain was selected to aid in imaging and biofilm quantification as will be described shortly. After the tubes were inoculated, syringe pumps pushed sterile media containing urea in concentrations ranging from 0.1 g/L to 15 g/L through the tubing at a rate of 1.0 mL/hr for as little as 10 days, or for more than two months, depending on the experiment. Flow was continuous except for short periods when the syringe pumps were exchanged. Sample ports above the tubing allowed influent samples to be taken for analysis, while effluent samples were taken by simply disconnecting the the downstream end of the tube and filling a vial. This set up is illustrated in Figure 4.4.

Table 4.1: A summary of parameters used in the unified tube reactor model. Note that c^0 and H are given as a range of values

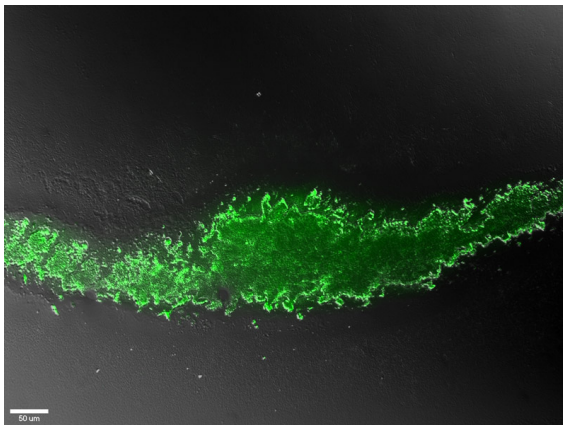
Name:	value:	units:	description:	source:
r	0.8	mm	inner radius of tube reactor	measurement
δ	2.5	1/mm	ratio of circumf. to area	calculated
L	1000	mm	length of reactor	measurement
D	4.932	mm ² /hr	diffusivity of urea in water	(cite Louise1952)
Q	1000	mm ³ /hr	fluid flux through reactor	measurement
ν	497.4	mm/hr	average fluid velocity	calculated as $Q/\pi r^2$
Pe	10084	1	Peclet number	calculated as $\nu L/D$
c^0	0.5, 5, 10, 15	g/L	influent urea calculation	measurement
$H(X)$	0–0.35	mm	biofilm thickness	measurement

The tube reactor experiments described above give many parameters for the unified model directly. These are shown in Table 4.1. More than that, however, dissection of the reactors provided biofilm thickness data. This information allows us to connect the tube and biofilm models by computing the critical parameter $\beta(X)$ as shown in equation (4.23) (and hence equation (4.24a)). To find biofilm thickness, each 10 cm tube was cut into five 2 cm sections. Each section was cut in half lengthwise, filled with cryoembedding medium, and frozen on dry ice. Once frozen, the silicon tube was peeled off the frozen medium, which was frozen into a larger cutting medium mold for cryosectioning. These frozen samples were cut in 5 μm cross sections and mounted on microscope slides. Each tube segment was used to create 5 cross sections for analysis. This process is illustrated in Figure 4.5a.

Once mounted on slides, each cross section was imaged under a microscope. The green fluorescent protein produced by the bacteria allowed clear micrographs to be taken



(a) The tube reactor is cut into sections. Each section is cut in half and filled with cutting medium, which is frozen. A thin cross section is then imaged using a microscope.



(b) An overlay of a transmission (visible light) and fluorescent image showing a biofilm cross section.



(c) A thresholded biofilm cross section.

Figure 4.5: This figure illustrates the reactor cryosection method used to obtain biofilm thickness data and gives an example of the resulting cross sectional fluorescent micrograph and a thresholded image. The white area on the thresholded images is quantified as a measurement of biofilm area and used to estimate biofilm height.

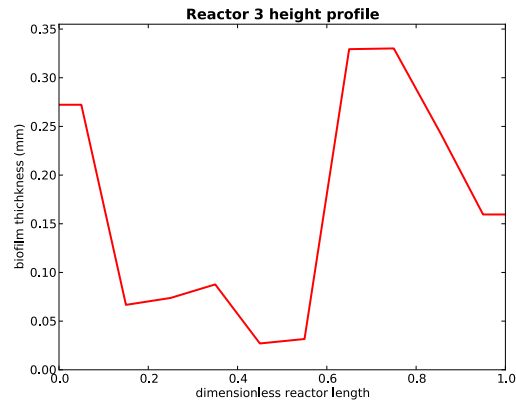


Figure 4.6: An example plot of a height profile extracted from cryosection data. In this plot the x -axis has been nondimensionalized by dividing by the tube length, 10 cm. Note that this image was taken from an experimental run where 10 height profiles were measured instead of 5.

using epifluorescence microscopy. These fluorescent images were then analyzed in the open source software FIJI [47]. FIJI was used to threshold images using the automatic triangle method [64]. See Figure 4.5 for example images. Once thresholded, the average biofilm thickness (parameter H) could be determined by dividing the arc length of the tube section by the thresholded area of the fluorescent image. Replicate values were tabulated and averaged to estimate height values for each tube segment in each tube reactor. Using linear interpolation to combine values from different sections of the tube allowed the construction of average height profiles for biofilms across all tube. An example of such a profile is shown in Figure 4.6.

In addition to biofilm height data, the reactor experiments allowed measurement of influent and effluent concentrations of urea. By measuring what went into the reactor, approximating how much biofilm was in the reactor using height data, and measuring the resulting effluent concentration of urea, we obtained data sets of inputs and outputs that can be used in the mathematical model to solve for kinetic parameters. We denote influent data as c^0 . Effluent values are denoted c_L . Using this notation, the normalized

effluent value will be written $C_L = c_L/c^0$. Before applying this data, however, we describe a numerical scheme for solving a simplified version of the unified problem

Solving the Unified Model

The experimental data shows that the Péclet number for the tube reactors is on the order of 10^4 (cf. Table 4.1). Thus the $1/\text{Pe}$ terms in (4.24) are very small. This suggests that diffusion is far less important to the transport of urea than advection. Omitting these small terms, the unified model can be simplified to

$$C' = \beta(X)CS_{Z(1)}, \quad (4.25a)$$

$$C(0) = 1. \quad (4.25b)$$

The removal of the second order term reduces the order of the unified model, which means that both boundary conditions in (4.24b) are not needed. We chose to retain the influent boundary condition because it contains model data in the form of $C(0)$. To solve this simplified system we need to know the value of $S_{Z(1)}(X)$ at a given location X in the channel. To get this information we turn our attention to solving the 1D biofilm problem.

Solving the 1D Biofilm Problem:

Recall from the discussion of the unified problem that our interest in the 1D biofilm problem focuses on computing the flux of urea into the biofilm at the fluid-biofilm interface at a particular value of X . For the case of zero or first order kinetics this is done as the flux is known. Using the result of (4.8), for the zero order problem

$$\beta CS_{Z(1)} = \beta C\phi^2 = \delta L \frac{D}{\nu H} C \left(\frac{r_0 H^2}{D c^0 C} \right) = \delta L \frac{r_0 H}{\nu c^0}. \quad (4.26)$$

where we use $D_e = D$, a simplification we will use from this point forward. Note that we also suppress the dependence of β and H (and thus ϕ) on X in the rest of this section for notational simplicity. That is, the right hand side of (4.25a) may be written explicitly in terms of the 0-order rate r_0 . Similarly, the flux into the biofilm for the 1st-order problem is known. Combining the result shown in (4.14) with (4.25a), we write

$$\beta CS_{Z(1)} = \beta C \phi \tanh(\phi), \quad (4.27)$$

where $\phi^2 = \frac{k_1 H^2}{D_e}$ is the Thiele modulus given in (4.11). Again, we have an explicit representation of the right hand side of (4.25a), which only depends on the unknown kinetic parameter.

For Michaelis-Menten kinetics the flux must be computed numerically. Originally, a custom solver using Newton's Method was written in Python for this purpose. Once the concentration profile was found in the biofilm, the derivative at the surface was numerically computed using a finite difference approximation. However, this code was slow and did not always converge for extreme parameter choices. Since the key kinetic parameters are unknown, this was a real problem. For these reasons, a better method was implemented using Matlab's `bvp4c` solver. This solver is able to integrate a system of ODEs subject to two-point boundary value conditions, and uses an adaptive mesh and error control based on computed residuals. In addition to greatly speeding up computational time, the solver can return an approximation of the derivative as well as the solution itself. That is, the flux can be returned directly to the calling function without need to store the concentration profile of urea in the biofilm, which is not needed in the unified problem.

Solving the Tube Reactor Model:

Returning to the flow-wise tube problem, (4.25) cannot be solved explicitly in the case of general kinetics. However, the simplified unified model (4.25) can be readily solved numerically. Using a first-order upwind approximation for the derivative, (4.25a) can be written as

$$\frac{C_{i+1} - C_i}{\Delta X} - \beta_i [S_{Z(1)}]_i C_i = 0.$$

Solving for C_{i+1} results in

$$C_{i+1} = (\beta_i [S_{Z(1)}]_i \Delta X + 1) C_i. \quad (4.28)$$

Now, discretizing the nondimensional domain into m evenly sized pieces, and given values for the kinetic parameters, we begin with $i = 1$ and iterate (4.28) up to $i = m$. At each grid point β_i must be evaluated using the height profile of the biofilm, and the flux $[S_{Z(1)}]_i$ of substrate into the biofilm must be computed. Note that the only concentration value available from experimental data is the effluent concentration C_m , which we denote c_L . Of course, in general the kinetic parameter values (such as r_0 , k_m , or k_1) are unknown and the model cannot simply be run in this iterative value.

While the unified model cannot be solved explicitly for general kinetics because of the dependence of β_i and $[S_{Z(1)}]_i$ on the perpendicular biofilm problem, the case of 0-order kinetics can be solved directly. Integrating (4.25a) from 0 to X and applying the boundary condition from (4.25b) means

$$\int_0^X C'(\hat{X}) d\hat{X} = \int_0^X \delta L \frac{r_0 H(\hat{X})}{\nu c^0} d\hat{X} \quad (4.29)$$

may be written as

$$c^0(C(X) - C(0)) = \frac{r_0 \delta}{\nu} \int_0^X H(\hat{X}) L d\hat{X}. \quad (4.30)$$

This can of course be solved for $C(X)$. Writing the solution in dimensional variables, (4.30) can be solved as

$$c(x) = c^0 + \frac{r_0 \delta}{\nu} \int_0^x H(\hat{x}) d\hat{x}. \quad (4.31)$$

Thus, the concentration of substrate at any point in the tube is readily computed in the case of 0-order kinetics.

As stated above, in general kinetic parameters are not known or easy to solve for, and deriving a method for doing so is the topic of the rest of this chapter and the next. However, the 0-order case is once again an exception to this. First of all, solving for the 0-order rate does not even require the above model. Rather, all that is needed is knowledge of the mass of substrate consumed per volume of biofilm per unit time. Symbolically, this could be written as

$$r_0 = \frac{\Delta c Q}{V_{\text{bio}}}, \quad (4.32)$$

where c is the concentration of substrate, Q the system flux, and V_{bio} the volume of the biofilm. In the case of our reactor geometry we can write

$$V_{\text{bio}} = \pi \int_0^L r^2 - (r - H(x))^2 dx = 2\pi r \int_0^L H(x) dx - \pi \int_0^L H(x)^2 dx, \quad (4.33)$$

where r is the radius of the tube. The estimate for r_0 found by combining (4.32) and (4.33) is closely approximated for thin biofilms by the solution to the 0-order problem shown in (4.31). Evaluate this equation at $x = L$ and substitute $\delta = 2\pi r / (\pi r^2)$ and $\nu = Q / (\pi r^2)$. Then, solving for r_0 yields

$$r_0 = \frac{(c_L - c^0) Q}{2\pi r \int_0^L H(x) dx}. \quad (4.34)$$

This is approximately (4.32), where the volume of the biofilm is somewhat crudely estimated.

A First Attempt at Finding MM-kinetics

This section describes the initial attempt to find Michaelis-Menten kinetic values using the unified tube reactor model and numerical methods described above. This work utilizes data sets from two early experiments which will be described in this section. The attempt ultimately failed in that we were unable to resolve parameter estimates with confidence levels, but the attempt was important as it pointed to the need for a more flexible and sophisticated method of parameter estimation. These results also informed test problem parameter choices when testing later methods. These more sophisticated methods will be described in the next chapter.

While MM-kinetic values for urealytic *E. coli* biofilms are unknown, pure enzymatic work suggested a k_m value somewhere between 0.17 and 0.21 g/L [65]. From this previous work it was estimated that k_m for the *E. coli* MJK2 biofilms was close to 0.5 g/L. Thus, a much higher influent concentration of urea, say 10 g/L, would create an environment for the organism where its kinetics would be well approximated by 0-order kinetics. With these considerations in mind, two experiments were run over the period of several months. The first was designed to estimate r_0 using (4.32). Knowing r_0 , a second set of experimental values would allow (4.35) to be solved for k_m .

The first experiment used an influent value of 10 g/L. Four tubes survived to a steady-state. For each reactor a biofilm profile was created using the cryosection technique previously described. Profiles for these four tubes are shown in Figure 4.7. Once a profile was constructed, the total volume of each biofilm could be estimated by computing

$$V_{\text{bio}} = \pi \int_0^L r^2 - (r - H(x))^2 dx,$$

Table 4.2: Summary values for the r_0 experiment. Influent media had a urea concentration of 10 g/L, but the measured value was always less. This could be due to possible contamination by bacteria above the sample port, or continuing reactions happening in the vial between sampling and testing. Here the standard deviation, s , is the standard deviation of the r_0 column.

	Influent	Effluent	Biofilm	
	Average	Average	Volume	r_0
Tube	(g/L)	(g/L)	(mm ³)	(g/L hr)
1	8.04	4.77	51.83	63.67
2	9.03	6.13	55.57	52.18
3	8.80	5.99	56.83	49.27
4	8.62	6.27	40.00	60.00
mean				56.28
s				0.65

where $H(x)$ is the interpolated piecewise linear function of biofilm thickness values and r the radius of the reactor. Note that since the biofilm thickness was measured only at 5 interior points, the first and last data points were repeated to give values at $x = 0$ and $x = L$. With biofilm volume estimates obtained, a value for r_0 is readily calculated from (4.32). Average influent and effluent values as well as estimates for biofilm volume and the resulting r_0 calculation for each of the four reactors appear in Table 4.2. The average of the r_0 values computed by this method was 56.3 g/(L hr) with a standard deviation of $s = 0.65$ g/(L hr). Generally this estimate of r_0 was rounded to 60 g/L when used in the work that follows.

Given an estimate for r_0 , a second experiment was run to estimate k_m . Six tubes were grown for over two months at an influent urea concentration of 0.5 g/L in order to create a data set that could be used to solve for k_m . Four tubes survived the entire growth and the

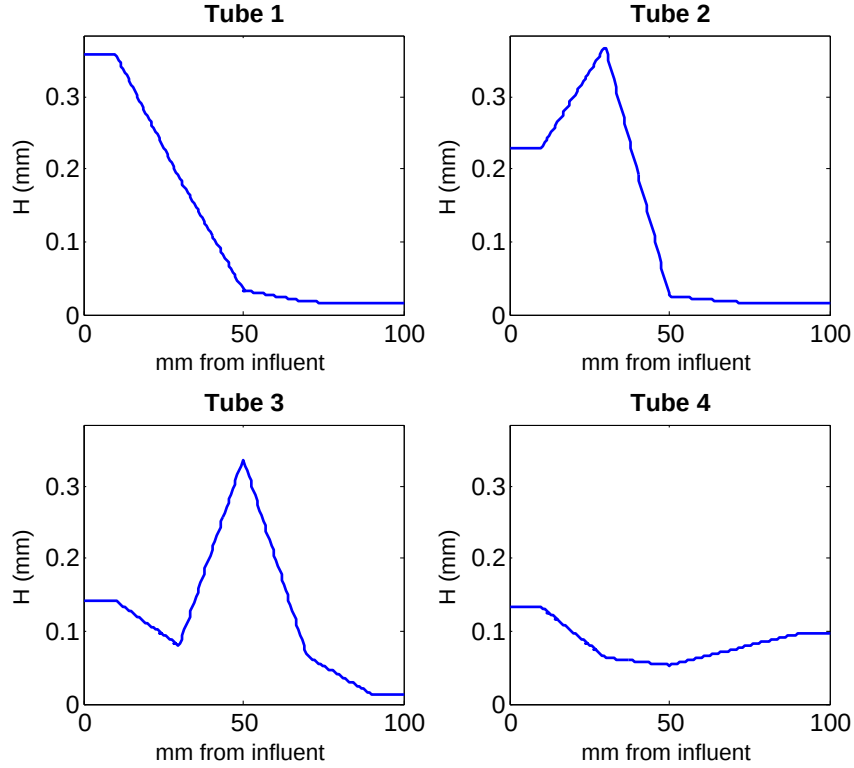


Figure 4.7: Biofilm profiles for the surviving four reactors run at 10 g/L or urea. This data was used to estimate r_0 .

dissection process to steady state. The profiles for these biofilms are shown in Figure 4.8. Now, with experimental data that gives a measured effluent value c_L for a given measured influent value c^0 and r_0 set, a simple strategy to find k_m is to vary k_m until the difference between $c^0 C_L$ and c_L is minimized. This is easily done using minimization routines such as Matlab's `fminsearch`, `fminbd`, and `fminunc`, or even a nonlinear solver like `fsolve`. Using `fsolve` with only one unknown parameter it is easy to solve

$$f(k_m) = |c^0 C_L - c_L| = 0 \quad (4.35)$$

numerically for k_m . Of course, the solver required an initial guess for k_m to which it was insensitive.

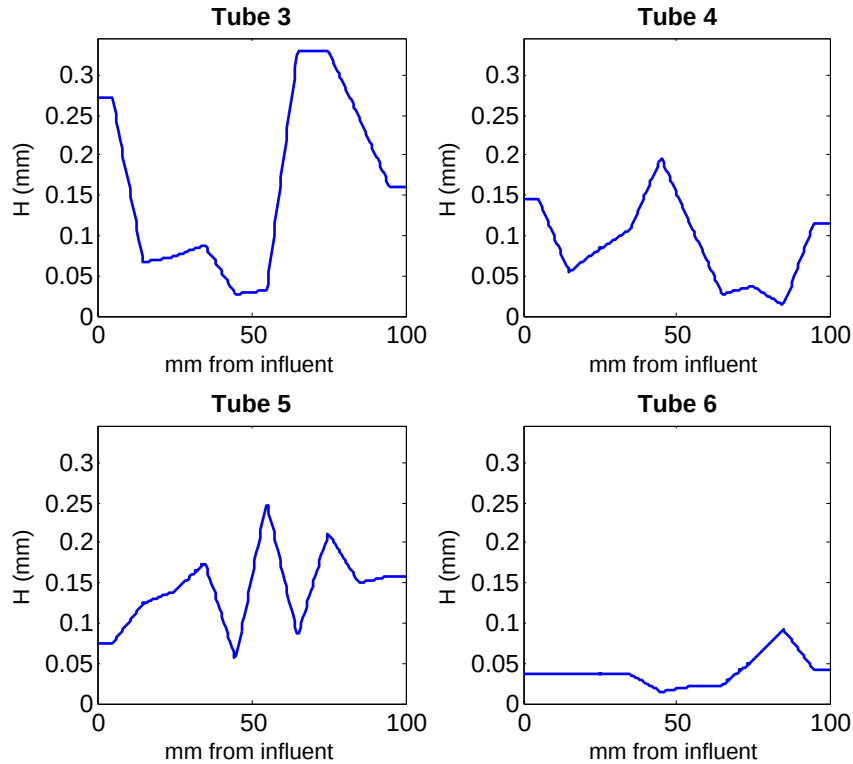


Figure 4.8: Biofilm profiles for the surviving four reactors run at 0.5 g/L or urea. This data was used to estimate k_m in conjunction with an estimate of r_0 from the previous experiment.

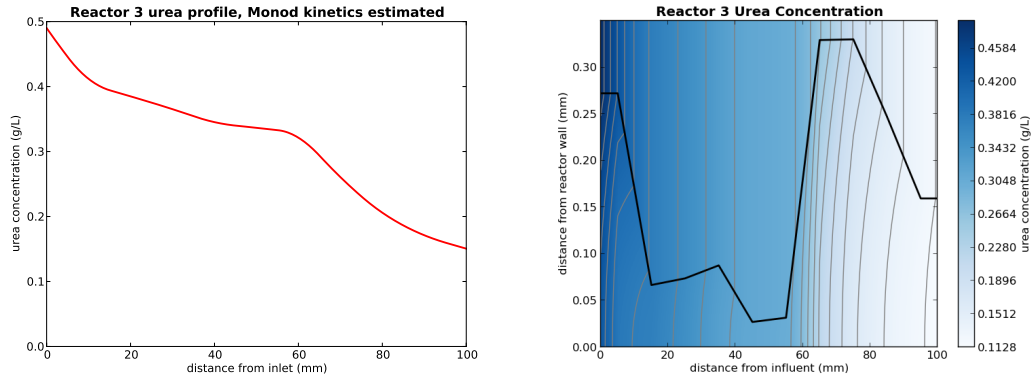
Results for this procedure are shown by tube for the data in Table 4.3. The average model value for k_m was 2.44 g/L. The large value for the standard deviation, s , yields an approximate 95% confidence interval of 0 to 5 g/L, however, so this value is not very certain. In addition, data from Tube 6 appears to be anomalous. The measured influent and effluent values are on par with the other tubes, but the estimated k_m value is much smaller. Examination of the biofilm height profile for Tube 6 showed that the biomass was much lower than the other three tubes. Because the effluent values were measured to be approximately the same, it seems likely that a large detachment event occurred just prior to sampling, yielding a k_m value incongruent with the measured biomass. If this value is excluded from the data set, the remaining three values average to an estimated k_m value of

Table 4.3: Summary values for the second experiment. Here we solve for k_m by fixing r_0 at 60 g/(L hr), the value determined by the previous experiment. Tube 6 shows an anomalous result, perhaps due to a large detachment event shortly before the final sampling and dissection. Here s is the standard deviation of the k_m estimates.

Tube	Influent	Effluent	k_m
	Average	Average	
	(g/L)	(g/L)	(g/L)
3	0.49	0.16	3.90
4	0.49	0.17	2.20
5	0.49	0.13	2.90
6	0.50	0.16	0.75
Average			2.44
s			1.33

3 g/L with $s = 0.85$ g/L. As more sophisticated methods were developed and tested, values of $r_0 = 60$ g/L and $k_m = 3$ g/L were often used to create test data.

With an estimate for r_0 and k_m , we can easily solve (4.25) for urea values in each reactor. Example plots are shown in Figure 4.9. The obvious problem with this methodology, however, is that we are fitting a two parameter model with what amounts to two data points. With two data points, we can of course solve for each parameter. The question becomes are these values correct? At least three (or more) data points are needed to determine this. Adding more experiments, while clearly necessary, raises additional questions, however. What if the experiments, unbeknownst to the experimenter, are run slightly differently? More troubling yet, what if the concentration assumed to yield a zero order rate is actually incorrect? Above we used 10 g/L to determine r_0 , but what if 15 g/L is the actual concentration to use? Such a mistake is definitely possible. In



(a) Urea values in the bulk fluid as a function of position in the tube. (b) Using results from the 1D biofilm problem we can solve show values of urea in the biofilm as well as in the bulk fluid. The dark black line indicate the biofilm surface.

Figure 4.9: Given r_0 and k_m urea values throughout the reactors are readily computed using the unified tube reactor model.

the cases described above, pure enzyme kinetics were used to estimate a value for k_m around which to design an experiment. The model values, however, were roughly 10 times larger. Since the concentration estimated as necessary to produce r_0 was based on this enzyme value, perhaps it is too small as well. Finally, the above method takes a casual approach to uncertainty quantification, which in the cases described above consists of simply taking the standard deviation of the parameter estimates, which are fit individually on a tube-by-tube basis. With the possibility of multiple measurement errors between replicates as well as inter experimental errors, it is unlikely that the final confidence levels expressed by a simple standard deviation calculation of a small number of trials sufficiently captures experimental uncertainty. In fact, another experiment was run and several of these problems were encountered. The data did not at all agree with the previous estimates of r_0 and k_m , was sampled differently by a new experimenter, and cast doubt on the appropriate maximum urea concentration.

The difficulties raised above point to a need for a more sophisticated approach to estimating r_0 and k_m , as well as the need for more experimental data. Such a method should ideally use the unified model formulated here to look at the total data from an experiment, or group of experiments, and estimate both parameters simultaneously. It should not make *a priori* assumptions about the values of the kinetic parameters, and it should provide more robust error/uncertainty quantification. These considerations in mind, a third round of experiments was conducted to gather better data. Before we can discuss this data, however, we require a more sophisticated statistical modeling approach which is developed in the next chapter.

CHAPTER FIVE

A TUBE REACTOR MODEL: THE INVERSE PROBLEM

The process of calculating causal factors from a set of observations is called an inverse problem. To specify an inverse problem requires a mathematical model of the system under study, which can calculate the value of the observations given a complete knowledge of the inputs. This is called the “direct” or “forward” problem [66]. Such a forward problem linking the experimental observations and input parameters was described in a previous chapter in the form of the unified tube reactor biofilm model. The experiments described there result in data which we can observe, effluent concentrations, based on parameter values which we cannot observe, the kinetic parameters r_0 , k_m , or k_1 . In the unified model we saw that given parameter values for r_0 and k_m , the urea effluent value can be readily computed. In addition, given an estimate for either r_0 or k_m , we could solve for the other, unknown parameter using a simple fitting routine. The problem with this approach is that in actuality neither parameter value is known and we may not even know the “best” kinetic model to use. This difficulty motivates our pursuit of an inverse problem formulation.

Inverse problems are common in fields such as image processing or geophysics, but also arise in applications involving model fitting [67]. It is this application which interests us in the context of the unified tube model. In this model, we have data in the form of influent and effluent concentrations for each tube reactor, and a model (forward problem) which connects the two. The model, however, depends on kinetic parameters which we do not know. Our goal in deriving an inverse problem formulation is to fit kinetic parameters to the data using the forward model, and obtain a level of confidence for that fit. This will be done taking into account all experimental results simultaneously. In addition, we seek an inverse problem formulation where we can easily change the kinetic model and test the

fit of the data to multiple models if desired. The present chapter begins with a completely naive approach to the inverse problem which seeks to minimize a cost function, and then develops a more sophisticated and flexible approach using a Markov Chain Monte Carlo (MCMC) method.

A Naive Approach to the Inverse Problem

The simplest and most naive approach to inverse modeling is to formulate a cost function comparing the difference between model output and experimental values and then seek to minimize this function with respect to model inputs (e.g. r_0 , k_m , or k_1). When the cost function is $\|\cdot\|_2^2$, then this is a nonlinear least squares (NLS) formulation [68, p. 12]. The description of this approach is facilitated by introducing some new notation. Denote the vector of experimentally measured effluent concentrations by $\mathbf{u}_L$. Let the calculated effluent concentration of the forward model be written as $\hat{u}_L = g(\theta)$. Then the vector $\mathbf{g}(\theta) = \hat{\mathbf{u}}_L^\theta$ denotes a vector of calculated effluent values. These hatted quantities indicate what are, in the parlance of statistics, known as “estimators,” as they are model estimates for the (generally unknown) true values. In these functions θ could be a vector or scalar quantity depending on the choice of kinetics, which are summarized in Table 5.1. Note that these naming choices represent a change in notation from the last chapter, where we called the effluent concentration c_L . In this chapter we will use c to refer to the cost function. With these notational choices described, we can now define the cost function $c(\theta)$ by

$$c(\theta) = \|\mathbf{u}_L - \hat{\mathbf{u}}_L^\theta\|_2^2 = \sum_{k=1}^n ([u_L]_k - g_k(\theta))^2. \quad (5.1)$$

As stated above, in this approach we seek an optimal value of θ , denoted θ_{opt} , such that

$$\theta_{\text{opt}} = \operatorname{argmin} c(\theta). \quad (5.2)$$

Table 5.1: Parameter θ could be a scalar or a vector depending on model input choices. Besides basic kinetics, we may assume that the data precision, λ , is known or unknown.

Kinetics	θ
0-order	r_0
1st-order	k_1
MM-order	(r_0, k_m)
MM-order with λ	(r_0, k_m, λ)

To test the NLS approach to the inverse problem we create a set of synthetic data so that we can test the method output against what we know about the data. The physical values used to generate this artificial data were picked to be similar to measurements of data from the first two experiments described in the last chapter so as to create realistic effluent values. The first step to creating the synthetic data was to compute a known set of “true” data using the forward problem and chosen parameters. That is, given a biofilm profile with a realistic height, an influent urea concentration, and known r_0 and k_m values, an effluent concentration was computed using the unified model which was considered to be “true”. We denote this value by $g(\theta) = \hat{u}_L^\theta$. This was done for multiple influent concentrations, which allows us to create a vector of “true” values $\mathbf{g}(\theta) = \hat{\mathbf{u}}_L^\theta$. Next, virtual tube effluent values were created by adding a known quantity of random normal noise each of these “true” values. This takes the form

$$\begin{aligned}
 u_L &= \hat{u}_L^\theta + \varepsilon, \\
 &= g(\theta) + \varepsilon, \text{ where } \varepsilon \sim N(0, \sigma^2),
 \end{aligned}
 \tag{5.3}$$

for each u_L in $\mathbf{u}_L$. This created a set of noisy “jittered” data. The test of the inverse method was to see if the method could use this jittered data to find parameter values close to the

true values. This was a simple process where various combinations of synthetic values can be used. To illustrate the outcome of this method, however, a particular example was chosen and is described below.

Synthetic Cost Example 1

For this first example, data was generated using a flat biofilm with a uniform thickness of 0.1 mm and with the urea influent concentration set at $u^0 = 1 \times 10^{-3}$ mg/mm³. For the purposes of the simulation, $\theta = [r_0, k_m]$ with $r_0 = 100 \times 10^{-3}$ mg/(mm³ h) and $k_m = 10 \times 10^{-3}$ mg/mm³ were used. These parameter values resulted in an modeled effluent concentration of $u_L = 0.6291 \times 10^{-3}$ mg/mm³. Taking this to be the “true” value for the simulation, normally distributed zero-centered noise with variance $\sigma^2 = 10^{-8}$ was added to this value to create eight simulated experimental values ($\mathbf{u}_L = (0.7154, 0.6611, 0.4984, 0.5858, 0.6634, 0.9870, 0.9061, 0.4942) \times 10^{-3}$ mg/mm³). Given this synthetic data, a script utilizing Matlab’s `lsqnonlin` routine was used to minimize (5.1) and to find θ_{opt} .

Minimizing the least squares cost function requires providing an initial estimate θ_0 to the solver. It quickly became clear that for this problem the solver is sensitive to the initial guess. Starting with different values for θ_0 results in different values for θ_{opt} , as recorded in Table 5.2. This is a well known characteristic of NLS, but normally the issue is convergence to multiple, distinct local extrema. However, this is not the case here. Substituting each θ_{opt} back into the model results in the same value for u_L , which suggests that the cost-space contains a “valley” of minima. Indeed, plotting k_m against r_0 for each value of θ_{opt} in Table 5.2 we see these points are in a straight line with a slope of approximately 0.13 hours as shown in Figure 5.1.

In hindsight the reasons for these non-unique minima are clear and illustrate a real difficulty of creating an inverse model for the forward problem under consideration here. If $u^0 \ll k_m$ then Michaelis-Menten kinetics are well approximated by linear kinetics. That

Table 5.2: Different initial starting values, θ_0 , yield different values for θ_{opt} . However, evaluating the model at θ_{opt} in each case yields the same value for u_L .

θ_0	$\theta_{\text{opt}} = (r_0, k_m)$	$u_L \times 10^{-3} \text{ (mg/mm}^3\text{)}$	$k_m / r_0 \text{ (hr)}$
(0.06, 0.020)	(0.063565, 0.007711)	0.689	0.1213
(0.08, 0.030)	(0.086151, 0.010747)	0.689	0.1247
(0.04, 0.040)	(0.069534, 0.008512)	0.689	0.1224
(0.10, 0.010)	(0.099713, 0.012570)	0.689	0.1260
(0.12, 0.005)	(0.119253, 0.015197)	0.689	0.1274

is,

$$r(u) = \frac{r_0 u}{k_m + u} \approx \frac{r_0}{k_m} u = k_1 u. \quad (5.4)$$

Similarly, for $u^0 \gg k_m$

$$r(u) = \frac{r_0 u}{k_m + u} \approx \frac{r_0 u}{u} = r_0.$$

In the above example, $u^0 = 1 \times 10^{-3}$ is an order of magnitude smaller than the chosen k_m , which is $1 \times 10^{-2} \text{ mg/mm}^3$. The true effluent value, $u_L = 0.6291 \times 10^{-3}$, is smaller yet. Thus, urea concentration in the simulated reactor are always less than the half-saturation k_m . The addition of biofilm further decreases urea values in the tube, and thus the model's Michaelis-Menten kinetics are well approximated by first order kinetics where the first order rate is the ratio of k_m to r_0 . Indeed, this is demonstrated by the final column of Table 5.2, where the first order rate is approximately 0.12 hours – a good match for the regression slope of 0.13 hours shown in Figure 5.1. The difficulty here is that of course for real data we do not know the true value of k_m , and thus cannot exploit this feature to solve for r_0 .

As will be shown in the next example, this unique minimum problem can to some degree be mitigated by using data that brackets the possible range of k_m values. That

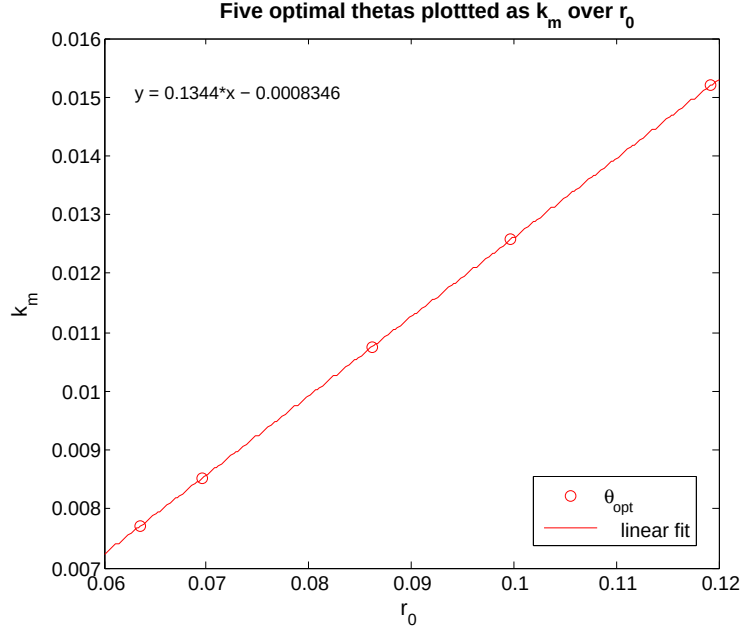


Figure 5.1: Plotting r_0 against k_m using the θ_{opt} values in Table 5.2 shows that the solver is finding the floor of a cost valley. Here $R^2 = 0.999999$.

is, minimizing the least squares cost function uniquely is more successful when we use data which contains u_L values which are both below and above the actual value of k_m . Of course, if we have absolutely no idea what k_m should be doing so may still prove impossible. However, if prior knowledge or lab data suggests that k_m is bounded between particular values this may be possible. This is illustrated in the following example using a second set of synthetic data.

Synthetic Cost Example 2

Data was once again generated using a flat biofilm with a uniform thickness of 0.1 mm. Rather than a fixed influent concentration, however, five values were used and influent urea concentration were set at $\mathbf{u}^0 = (0.5, 5, 10, 15, 20) \times 10^{-3} \text{ mg/mm}^3$. Running the model with $\theta = [r_0, k_m]$, for $r_0 = 50 \times 10^{-3} \text{ mg}/(\text{mm}^3 \text{ h})$, and $k_m = 5 \times 10^{-3} \text{ mg/mm}^3$ resulted in effluent values as show in column 2 of Table 5.3. Note that these values are

both lower (0.31×10^{-3} and 3.8×10^{-3} mg/mm³) and higher (8.4×10^{-3} , 13.1×10^{-3} , and 18.0×10^{-3} mg/mm³) than k_m . As in the previous example, normally distributed zero-mean noise with variance $\sigma^2 = 10^{-8}$ was added to model output values to create simulated experimental values.

In this example, three data points were created for each computed effluent concentration to simulate an experiment where three reactors were run at each influent concentration. These 15 values can be used individually in (5.1), or can be averaged for each influent value into five representative values. This would represent scenarios where data are used individually or aggregated into mean results by influent concentration.

This data set furnishes three scenarios for inverse problems. In the first case, we may assume that experimental measurements are measured without error and test if the minimization of (5.1) yields the correct argument. This should be regarded as completely basic: if the solver cannot solve the direct inverse problem with no noise we have little hope it will be able to cope with the addition of noise. This done, the second case is to test the model using the averaged noisy data. We will denote the components of this data set by $\overline{u_L} + \varepsilon$, where the over-bar represents the arithmetic mean and ε is given by (5.3). This calculation is also computationally cheaper than using the complete data set, as the minimization problem is done on a vector of length five rather than 15. Finally, we will investigate whether or not the minimization process is successful with the complete data set of 15 data points.

The results of all three scenarios are shown in Table 5.4. In each case, the cost minimization solver was able to find a minimum for (5.1). When using the u_L values directly with no added noise the solver found returned the value of θ used to generate the data, i.e. $\theta = \theta_{\text{opt}}$. When noise was added but the replicates were averaged before solving the solver also converged, but no longer to the exact value of the original θ , which is to be expected as the minimum has been shifted by the noise. This was the case as well when

Table 5.3: Synthetic data for the second cost experiment. The five influent values are both less than (in the case of 0.5 and 5×10^{-3} mg/mm³) and greater than k_m . The cost minimization process can be tested on all 15 noisy values in column three, or on the averaged noisy values in column 4.

u^0	$\hat{u}_L$	$\hat{u}_L + \varepsilon$	$\overline{\hat{u}_L + \varepsilon}$
(All entries have units $\times 10^{-3}$ mg/mm ³)			
0.5	0.3145907	0.3096715	0.3238754
		0.3116112	
		0.3503434	
5	3.826704	4.004383	3.738201
		3.597290	
		3.612930	
10	8.425695	8.365007	8.372572
		8.484470	
		8.268239	
15	13.14499	13.24277	13.29502
		13.35193	
		13.29037	
20	18.00990	17.66760	17.87289
		17.98945	
		17.96161	

Table 5.4: Results of Synthetic Experiment 2 using each of the possible data sets from Table 5.3. Note that in each case the solver converges to a value near the value of θ used to generate the data, $r_0 = 50 \times 10^{-3} \text{ mg}/(\text{mm}^3 \text{ h})$ and $k_m = 5 \times 10^{-3} \text{ mg}/\text{mm}^3$.

Data Set:	$\hat{u}_L$	$\hat{u}_L + \varepsilon$	$\overline{\hat{u}_L + \varepsilon}$
θ_0	$\theta_{\text{opt}} = (r_0, k_m)$	$\theta_{\text{opt}} = (r_0, k_m)$	$\theta_{\text{opt}} = (r_0, k_m)$
(40, 4.0)	(49.99999, 4.999999)	(49.23341, 4.503543)	(49.23281, 4.503343)
(40, 9.0)	(49.99999, 4.999999)	(49.22955, 4.502352)	(49.21993, 4.500636)
(50, 5.0)		(49.23419, 4.503755)	(49.23410, 4.503747)
(60, 8.0)	(50.00000, 5.000000)	(49.22976, 4.502418)	(49.23734, 4.504672)
(90, 1.0)	(49.99999, 4.999999)	(49.23281, 4.503345)	(49.21891, 4.500166)

(All entries have units of $\times 10^{-3} \text{ mg}/\text{mm}^3$ or $\times 10^{-3} \text{ mg}/(\text{h mm}^3)$)

all 15 data points were used. In this case the same altered value of θ_{opt} was found as in the averaged case: $\theta_{\text{opt}} = (49.2 \times 1/h, 4.5) \times 10^{-3} \text{ mg}/\text{mm}^3$.

We can verify that the values in Table 5.4 are indeed minima by numerically computing the Hessian and Jacobian matrices of the cost function given in (5.1) at θ_{opt} . Let $\theta_1 = r_0$ and $\theta_2 = k_m$. Then the Jacobian of c , J_c , is defined by

$$J_c = \begin{bmatrix} \frac{\partial c}{\partial \theta_1} & \frac{\partial c}{\partial \theta_2} \end{bmatrix} \quad (5.5)$$

and the Hessian of c , H_c , by

$$H_c = \begin{bmatrix} \frac{\partial^2 c}{\partial \theta_1^2} & \frac{\partial^2 c}{\partial \theta_1 \partial \theta_2} \\ \frac{\partial^2 c}{\partial \theta_1 \partial \theta_2} & \frac{\partial^2 c}{\partial \theta_2^2} \end{bmatrix} \quad (5.6)$$

Recall that if θ^* is a local minimizer of $c(\theta)$, then $J_c(\theta^*) = 0$. Further, if H_c exists in an open neighborhood of θ^* and $H_c(\theta^*)$ is positive definite (has positive eigenvalues) then θ^* is a strict local minimizer and $c(\theta)$ has a minimum at θ^* [68, p. 35].

In the case of the exact data (the second column of Table 5.3), evaluating Equations (5.5) and (5.6) at $\theta^* = (50.0, 5.0) \times 10^{-3}$ yields a Jacobian on the order of 10^{-35} and a Hessian with positive eigenvalues of 8.78×10^{-4} and 9.11×10^{-2} . So, θ^* is clearly a minimum. When using the five averaged noisy values at $\theta^* = (49.2, 4.50) \times 10^{-3}$ the Jacobian is still zero with components on the order of 10^{-8} (the order of the variance), and the eigenvalues of $H_c(\theta_{\text{opt}})$ are both still positive at 2.57×10^{-3} and 7.52×10^{-2} . The final case of using all 15 of the noisy data is very similar to this result. For $\theta^* = (49.2, 4.50) \times 10^{-3}$ the Jacobian values are on the order of 4×10^{-8} , and the Hessian eigenvalues are 6.76×10^{-3} and 2.16×10^{-1} . An example plot of the cost function illustrating such a minimum is illustrated in Figure 5.2, which was generated for the second case of averaged noisy data. The other two cases yield similar images and are not presented here.

To summarize the results of this second experiment, if the span of experimental influent conditions contains k_m , then the minimization of the cost function (5.1) exists and can be numerically evaluated. This method contains flaws, however. First, the minima can be difficult to find numerically and is sensitive to the initial guess. Because the minimum exists in a valley which is steep in one direction and shallow in the other, the solver may find the valley but not converge to the minimum in the valley. In fact, different Matlab minimization routines have different levels of success locating these minima, especially with a poor initial guess. In our experience `lsqnonlin` was the most robust for this problem and showed the most insensitivity to the initial guess. This is perhaps not surprising, as `lsqnonlin` is specifically designed to solve nonlinear least squares problems of the form $\min_x \|f(x)\|_2^2$. Second, (and perhaps more importantly) using simple cost minimization via NLS we can only assign symmetric confidence intervals around the

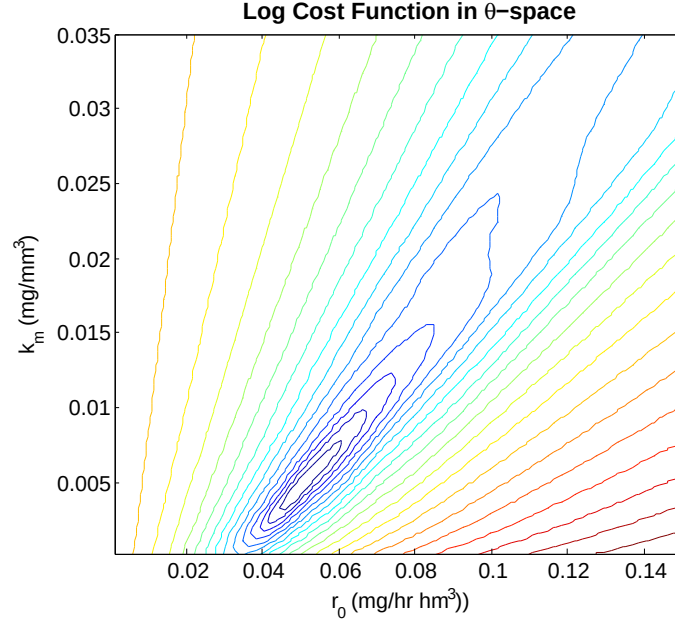


Figure 5.2: A plot of the cost function values given by (5.1) in $(r_0 \times k_m)$ space very near to θ_{opt} . Cost contours surround the minimum that occurs at $\theta_{\text{opt}} = (49.2, 4.50) \times 10^{-3}$, and demonstrate a lack of symmetry of the cost space about the minimum.

NLS estimate θ_{opt} to estimate the true value of θ . NLS works by locally approximating the nonlinear model $g(\theta)$ by its Jacobian $J_g = J_g(\theta)$. That is, NLS linearizes g as

$$g(\theta) \approx J_g \theta + \varepsilon.$$

This means that

$$\theta_{\text{opt}} \sim N(\theta, \hat{\sigma}^2 (J_g^T J_g)^{-1}) \quad (5.7)$$

where we use “ $\sim$ ” to indicate “approximately distributed by” and where

$$\hat{\sigma}^2 = \frac{1}{n-1} c(\theta), \quad (5.8)$$

for cost function $c(\theta)$ defined as in (5.1) [69, p. 146]. However, we do not necessarily expect that θ_{opt} will be normally distributed. If the θ_{opt} distribution is not normal, but asymmetric as suggested by Figure 5.2, then (5.7) would give us a symmetric confidence interval when such is truly not appropriate. Thus, a more sophisticated method which does not depend on the initial guess θ_0 and which gives good interval estimates for θ is necessary. Before developing that method, we pause to discuss the shape of the cost space and the disparity of Hessian eigenvalue sizes.

The Shape of the Cost Space:

While all of the Hessian eigenvalues in the above example are positive, they are also quite small. This is due to the units in which the problem is expressed. However, after adjusting for units there is still a clear disparity between the size of eigenvalues in each pair. In each case, the larger eigenvalue is one to two orders of magnitude larger than the smaller. This means that locally near θ_{opt} the cost function changes concavity rapidly in one direction, and more slowly in another. Call the larger eigenvalue λ_1 and its corresponding eigenvector $v_1 = [\nu_{11}, \nu_{12}]$. Similarly, denote the smaller eigenvalue λ_2 and its eigenvector $v_2 = [\nu_{21}, \nu_{22}]$. The direction of rapid change in c near θ_{opt} corresponds to the direction of v_1 , which we define to be the slope of dominant eigenvalue, defined by $\alpha_{v_1} = \nu_{12}/\nu_{11}$. In the same way, the direction of slower change corresponds to that of v_2 , which we call the slope of the minor eigenvalue, which is defined as $\alpha_{v_2} = \nu_{21}/\nu_{22}$. This disparity is seen in Figure 5.2, where the minimum contour is stretched diagonally in the direction of α_{v_2} .

The direction given by α_{v_2} is related to the ratio $\alpha_{\text{opt}} = k_m^{\text{opt}}/r_0^{\text{opt}}$, where $\theta_{\text{opt}} = (r_0^{\text{opt}}, k_m^{\text{opt}})$. This may be seen graphically by comparing various values of α_{opt} to the slope of the minor eigenvector, which is done in Figure 5.3. In this figure, changes in the value of α_{opt} correspond directly to a change in α_{v_2} .

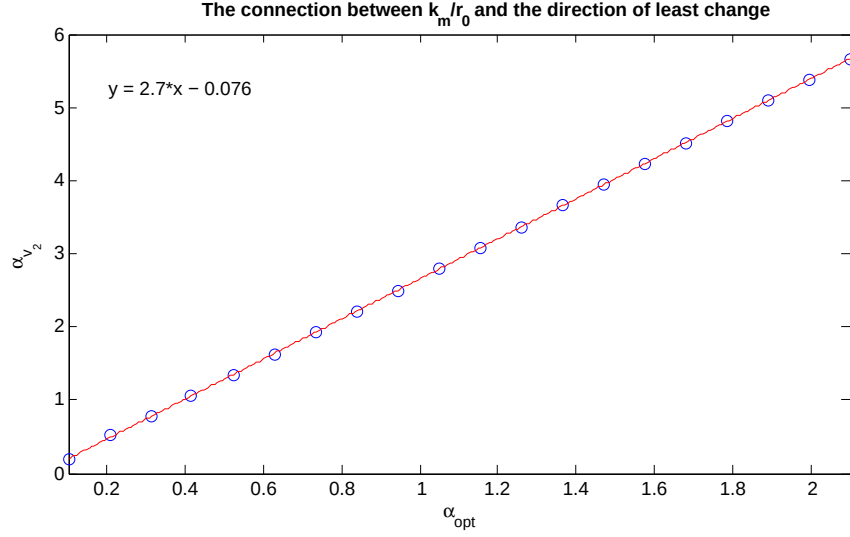


Figure 5.3: A plot illustrating the relationship between $\alpha_{\text{opt}} = k_m^{\text{opt}}/r_0^{\text{opt}}$ and the slope of the minor eigenvector. As α changes so does the eigenvector slope, which is visible as the diagonal orientation elliptic contour in Figure 5.2. For this regression $R^2 = 0.99997$.

The cost function shown in (5.1) is readily plotted over a range of r_0 and k_m values for a given set of experimental values, synthetic or otherwise. Cost function plots can be computationally expensive depending on the grid resolution and the number of elements in $\mathbf{u}_L$, but the visual representation they provide of the cost space can give us insight as has already been seen in Figure 5.2. However, a contour plot like the one shown in Figure 5.2 interpolates numeric data, and is thus in general less desirable than an exact plot of model output made using a utility like Matlab's `imagesc`. Plots produced by this method yield additional insight into the cost space. For instance, plots such as those shown in Figure 5.4 illustrate the problem caused by increasing levels of noise, that is, increasing σ^2 in (5.3), in finding the cost minimum. As Figure 5.4 shows, as more noise is added to the synthetic data the cost minimum becomes shallower and consequently harder to locate numerically. Another feature of the cost space visible in plots is the slight upward bend of the “valley” when viewed left to right. This feature can be somewhat subtle

but is visible in Figure 5.4A with careful study. The blue elliptically shaped minimum is at a slightly lower angle than the greenish valley to the upper left.

The overall shape of the cost plots, as well as the effect of larger noise and the slight bend, are best explained by considering what happens to model values, and hence cost values, as r_0 and k_m singly and together grow arbitrarily large. To determine this it is helpful to consider the factored form for MM-kinetics given by,

$$r(u) = \frac{r_0}{k_m} \left(\frac{u}{1 + \frac{u}{k_m}} \right). \quad (5.9)$$

In addition, dividing the domain of r_0 and k_m up into two pieces as shown in Figure 5.5 helps with the analysis of (5.9). Finally, we must keep in mind that for a given tube the urea concentration is less than or equal to the influent concentration. That is, $0 \leq c \leq c^0$ for all r_0 and k_m .

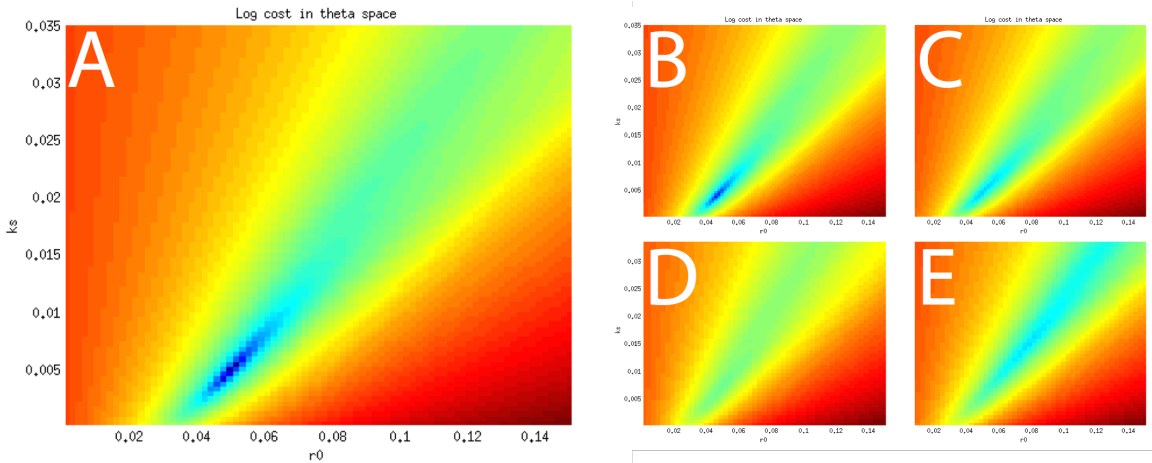


Figure 5.4: These cost space plots for synthetic data show the effect of adding error to the system. Here cool colors indicate small values and warm colors high values. Here $\log(c(\theta))$ is plotted rather than $c(\theta)$ to show more gradation from low to high. Image (A) shows the cost space with no error. The other four plots show the effects of increasing error: In (B) $\sigma = 1 \times 10^{-4}$, (C) $\sigma = 2 \times 10^{-4}$, (D) $\sigma = 3.7 \times 10^{-4}$, and (E) $\sigma = 5 \times 10^{-4}$. Units for σ are all mg/mm^3 . Note that as the error increases the cost “valley” decreases in depth until finally, in E, deepening while shifting up and to the right.

Now, consider quadrant I in Figure 5.5. Here r_0 and k_m are both relatively the same size, that is, within a few orders of magnitude of one another. In this quadrant the cost function (5.1) returns a value with a minimum at the location of θ_{opt} that is proportional to the sample variance of the synthetic data generated by (5.3). This follows from (5.1) and (5.3) as

$$c(\theta_{\text{opt}}) = \sum_{k=1}^n (u_k^L - g_k(\theta_{\text{opt}}))^2 = (n-1)\hat{\sigma}^2. \quad (5.10)$$

In quadrant II, k_m stays relatively small but r_0 is large. In this case, the model returns a value of $\hat{u}_L^\theta = 0$ as the influent concentration is completely exhausted by the organism. This is seen in (5.9), where for large r_0 and finite k_m the reaction is completely dominated by r_0 . This case corresponds to microbes who are voracious and quickly consume all available substrate. Consequently, in this region the cost function converges to $\|\mathbf{u}_L\|_2^2$. In quadrant IV, the case where k_m grows but r_0 stays fixed, $r(u)$ in (5.9) converges to 0 and thus the model output is just $\hat{u}_L^\theta = u^0$. This means that the cost function approaches the difference between the influent concentration and the data, $\|\mathbf{u}_L - u^0\|_2^2$. Physically, this quadrant corresponds to a metabolically slow organism and in the limit, one which does not consume substrate. The final case is found in quadrant III. Here both r_0 and k_m are growing. When both parameters grow proportionally by $k_m = \alpha r_0$, (5.9) becomes

$$r(u) = \frac{1}{\alpha} \left(\frac{u}{1 + \frac{u}{\alpha r_0}} \right) \rightarrow \alpha^{-1} u, \text{ as } k_m \rightarrow \infty.$$

Since u is bounded $r(u)$ approaches a constant. Correspondingly, the cost function also approaches a constant. Physically this corresponds to a range of parameter values where 1st-order kinetics well approximate what is happening in the organism.

Now we can see why the cost valley becomes more shallow until finally shifting, as seen in Figure 5.4. As the variance grows, the depth of the minimum in quadrant I

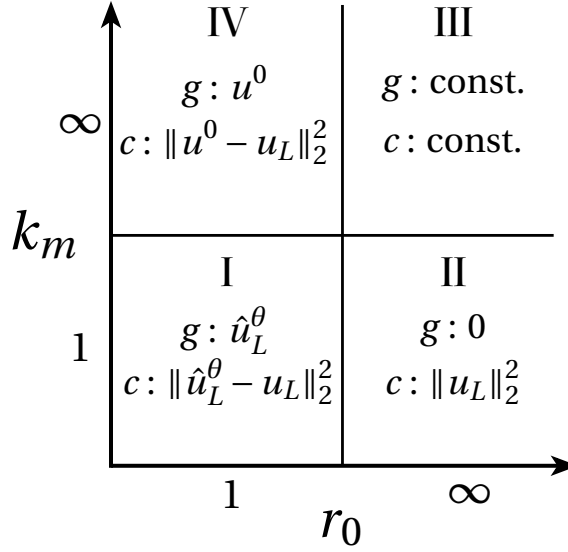


Figure 5.5: Dividing the parameter space into quadrants aids in understanding the behavior of the cost space. A flat “valley” develops in the cost space along the line $\alpha = k_m/r_0$ when the constant in quadrant III approaches the order of the values in quadrant I. Here g represents the model output, and c the output of the cost function.

decreases. This is seen in (5.10): as σ^2 grows so does the $c(\theta_{\text{opt}})$. For some level of σ^2 the constant value reached in quadrant III will be less than or equal to $(n-1)\hat{\sigma}^2$. When this happens we no longer have a unique minimum in quadrant I, but a “trench” or “valley” of local critical points oriented in a direction related to the ratio of k_m and r_0 . This movement of the minimum will have important consequences for the statistical model described later in this chapter. The bend in the cost valley is harder to analyze, but we hypothesize it comes from the shift from MM-order kinetics in the first quadrant of Figure 5.5 to 1st-order kinetics in third quadrant for a fixed σ^2 .

A First Bayesian Approach

A more careful description of the inverse problem requires that we have some understanding of how experimental errors occur. That is, where does ε in (5.3) really come from? The experimental process yields error in five significant places. First, the method

does not allow accurate measurement of biofilm at the influent and effluent end of the tubes. Second, we linearly interpolate biofilm thickness between measured points and this may not be an accurate approximation. Third, biofilm thickness may not be constant around the cross section of the tube but we assume that it is. Fourth, biofilm detachment events have the potential to cause a mismatch between measured effluent concentrations and biomass estimation. Finally, another potential source of error is the assumption that kinetics are uniform throughout the biofilm. Possible differences in cell density, metabolic activity, or enzyme production from one part of the biofilm to another would also result in inaccurate rate estimation.

The first three sources of error arise because of our inability to measure from the entire reactor simultaneously and in three dimensions. These are thus measurement errors, which we assume are random errors rather than systematic errors. We therefore model the cumulative effect of these errors as normally distributed. The last two sources of error are modeling errors that we do not take into account directly. The possibility of an error due to detachment is not quantifiable here and is thus excluded from the model. However, the effect of this error is readily understood. A detachment event before measurement would result in an overestimation of kinetic rates because of an underestimation in biomass as compared to measured effluent values. The possibility that kinetics are not uniform is also not explicitly modeled here, but if kinetics are regarded as macroscopic averages of microscopic random differences in the biofilm, then this error is also normally distributed [70, p. 16].

Defining the Inverse Problem

The inverse problem is defined by two main assumptions. First, we assume that the error incurred by the model is normal as well as independent and identically distributed

(iid). That is, we assume that

$$\mathbf{u}_L = \mathbf{g}(\theta) + \boldsymbol{\varepsilon}, \quad (5.11)$$

where $\boldsymbol{\varepsilon} \sim N_n(0, \sigma^2 I)$. This is a common assumption when considering modeling and measurement errors [69, p. 155]. Intrinsic in this form is the assumption that each of the random errors previously described are cumulatively summarized by a single parameter, $\boldsymbol{\varepsilon}$, where $\boldsymbol{\varepsilon}$ is a vector containing a component error ε_i corresponding to each $(u_L^\theta)_i$ generated by $\mathbf{g}(\theta)$. The second assumption is that the values of θ are not correlated to influent urea concentrations. We base this assumption on the supposition that the kinetic properties of the microbe in question are constant with respect to urea concentration. That is, that the parameters of interest represent physical properties of the organism that are invariant with respect to model environmental conditions. It is possible that kinetic properties do vary with environmental factors and are not fixed, but such a scenario is not taken into account by this model.

Given these assumptions it follows that $\mathbf{u}_L$, given θ , is normally distributed with mean $\mathbf{g}(\theta)$ and standard deviation σ . That is, defining the precision, λ , by $\lambda = 1/\sigma^2$ for convenience,

$$\mathbf{u}_L \mid \theta, \lambda \sim N_n(\mathbf{g}(\theta), \lambda^{-1} I) = P(\mathbf{u}_L \mid \theta, \lambda). \quad (5.12)$$

Here the likelihood function N_n is the multivariate normal (MVN) of dimension n , where n is the length of $\mathbf{u}_L$. Note that in the work that immediately follows we assume that the variance σ^2 is known, though later this assumption will be dropped.

Likelihood Distribution. We can write the MVN likelihood function explicitly as

$$P(u_L \mid \theta, \lambda) = \sqrt{\frac{\lambda}{(2\pi)^n}} \exp\left(-\frac{\lambda}{2} \|\mathbf{u}_L - \mathbf{g}(\theta)\|_2^2\right), \quad (5.13)$$

where we again assume for now that λ is known. Note that $\|\mathbf{u}_L - \mathbf{g}(\theta)\|_2^2$ is the cost function $c(\theta)$ shown in (5.1). This equation says that, given a value of θ , we can calculate the probability that an effluent concentration falls within a particular range. Note that this is the opposite of what we want when working with real data because we do not know the value of θ . Rather, we would like to know the posterior distribution $P(\theta | u_L)$. Bayes' formula is the key to getting what we want because it relates the likelihood distribution to the posterior distribution. Bayes' formula states that

$$P(\theta | u_L, \lambda) \propto P(u_L | \theta, \lambda) P_0(\theta), \quad (5.14)$$

where P_0 is the prior distribution which contains whatever knowledge we already have regarding θ [69, p. 100]. For the work that follows, we will use a uniform prior where we assume only that we have upper and lower bounds for the parameters of interest. That is, that we know the range in which the true parameters must fall. We will also focus on MM-kinetics, though the results generalize to other kinetics. Let $I_{r_0} = [r_0^{\min}, r_0^{\max}]$ and $I_{k_m} = [k_m^{\min}, k_m^{\max}]$ be the support for r_0 and k_m , respectively. Then, using absolute values to denote the length of the interval, we can write the prior as

$$P_0(\theta) = \begin{cases} \frac{1}{|I_{r_0}| |I_{k_m}|} & \text{if } \theta \in I_{r_0} \times I_{k_m}, \\ 0 & \text{otherwise.} \end{cases} \quad (5.15)$$

Posterior Distribution. With a known likelihood function and prior distribution we can write the posterior distribution explicitly using Bayes' Theorem (cf, for example [69, p.

156] as

$$P(\theta | u_L) = \frac{P(u_L | \theta)P_0(\theta)}{P(u_L)} = \begin{cases} \frac{P(u_L | \theta)P_0(\theta)}{\int P(u_L | \theta)P_0(\theta) d\theta}, & \text{if } \theta \in I_{r_0} \times I_{k_m}, \\ 0 & \text{otherwise.} \end{cases} \quad (5.16)$$

Note that the problem at hand has only two dimensions whose support is explicitly given by the prior as I_{r_0} and I_{k_m} . Thus, we can discretize $I_{r_0} \times I_{k_m}$ into an $n \times n$ grid and, after evaluating the likelihood function at every grid point, simply approximate the integral in the denominator of (5.16) using a numeric scheme such as Matlab's trapz, which is second order accurate in Δr_0 and Δk_m . This allows us to easily compute values for $P(\theta | u_L)$ and plot or summarize them as is described below.

Summarizing the Posterior. Once the posterior probability distribution is known, “best” fit parameter values can be computed. There is no single way to estimate a best fit from the posterior. Rather, there are several plausible options [71, p. 37].

1. The maximum a posteriori (MAP) estimate given by

$$\hat{\theta}_{\text{MAP}} = \underset{\theta}{\operatorname{argmax}} P(\theta | u_L). \quad (5.17)$$

For the given Bayesian description this is θ_{opt} from (5.2).

2. The median of the marginal posterior distributions. Assuming $\theta = (r_0, k_m)$, the marginal posteriors are given by

$$P(r_0) = \int_{I_{k_m}} P(r_0, k_m | u_L) dk_m, \quad (5.18)$$

and

$$P(k_m) = \int_{I_{r_0}} P(r_0, k_m | u_L) dr_0. \quad (5.19)$$

From these we can compute the median values m_{r_0} and m_{k_m} that satisfy

$$0.5 = \int_{r_0^{\min}}^{m_{r_0}} P(r_0) dr_0,$$

and

$$0.5 = \int_{k_m^{\min}}^{m_{k_m}} P(k_m) dk_m.$$

3. The mean of the marginal posteriors, which are computed by

$$\bar{r}_0 = \int_{I_{r_0}} r_0 P(r_0) dr_0,$$

and

$$\bar{k}_m = \int_{I_{k_m}} k_m P(k_m) dk_m,$$

where $P(r_0)$ and $P(k_m)$ are computed as shown in (5.18) and (5.19).

4. The MAP of the marginal posteriors. That is,

$$(\hat{r}_0)_{\text{MAP}} = \operatorname{argmax}_{r_0} P(r_0), \quad (5.20)$$

and

$$(\hat{k}_m)_{\text{MAP}} = \operatorname{argmax}_{k_m} P(k_m). \quad (5.21)$$

Note that these might be easier to find than the posterior MAP itself described in (5.17).

The above list is by no means exhaustive. Once the posterior distribution is known many statistics can be computed using the distribution or its marginal distributions. An example of an additional important summary is the “credible interval.” The $(1 - p) \times 100\%$ credible interval is defined to be the interval which has a $(1 - p) \times 100\%$ chance of containing the value of the parameter [69, p. 100]. This can be computed numerically from the posterior marginal distributions shown in (5.18) and (5.19). The lower bound of a $(1 - p\%)$ credible interval for r_0 is found by solving for $(r_0)_{p1}$ in

$$\frac{p}{2} = \int_{r_0^{\min}}^{(r_0)_{p1}} P(r_0) dr_0.$$

The upper limit is found by solving for $(r_0)_{p2}$ in

$$1 - \frac{p}{2} = \int_{r_0^{\min}}^{(r_0)_{p2}} P(r_0) dr_0.$$

Upper and lower credible intervals for k_m are found analogously.

Synthetic Cost Example 3

We once again turn to synthetic data to test the Bayesian inverse problem formulation described above. As before, the synthetic data was generated using a flat, 0.1 mm thick biofilm. Influent values of $\mathbf{u}^0 = (0.5, 5, 10, 15, 20) \times 10^{-3} \text{ mg/mm}^3$ were used, and the value of $\theta = [r_0, k_m]$, was set to $r_0 = 50 \times 10^{-3} \text{ mg}/(\text{mm}^3 \text{ h})$, and $k_m = 10 \times 10^{-3} \text{ mg/mm}^3$. Three replicates were created for each influent concentration by adding noise with a standard deviation of $\sigma = 1 \times 10^{-4}$. These were averaged together to create a representative effluent for each influent concentration. These synthetic data are shown in Table 5.5. The additional information needed to differentiate this method from the last is knowledge about the prior for θ . From personal communication with collaborators about plausible ranges of r_0 and k_m , it was decided that a reasonable range was $0.1 \times 10^{-2} \leq r_0 \leq 15 \times 10^{-2}$

Table 5.5: Synthetic data for the third cost experiment. Influent concentrations again span both sides of k_m . Here we give only the averaged effluent values for three replicates created with noise set at $\sigma = 10^{-4}$ and omit the replicates themselves.

u^0	$\hat{u}_L$	$\overline{\hat{u}_L + \varepsilon}$
(units are $\times 10^{-3}$ mg/mm ³)		
0.5	0.393248	0.348996
5	4.254824	4.177644
10	8.784139	8.815491
15	13.522679	13.454171
20	18.348212	18.340452

mg/(mm³ hr) and $0.25 \times 10^{-3} \leq k_s \leq 35 \times 10^{-3}$ mg/mm³. These intervals represent I_{r_0} and I_{k_m} , respectively, and this information furnishes a uniform prior using (5.15).

Results for this example from applying the Bayesian inverse approach are shown in Figure 5.6 and Table 5.6. Comparison of these plots with the earlier log cost plots shown in Figure 5.4 shows that the long valley that resulted from the linearization of kinetics in quadrant III of Figure 5.5 now manifests in the posterior space as a long ridge of elevated densities after the exponentiation in (5.13) and scaling in (5.16). This feature causes the marginal distributions to be right skewed, which in turn results in the marginal posterior mean and median estimates for r_0 and k_m being above the true values. It is possible to sharpen the posterior peak and reduce the right-skewedness of the marginal posteriors by reducing the amount of noise in the model. In an experiment, this could be done by adding additional tubes at each concentration. Just as reducing noise made the cost space minimum in Figure 5.4 clearer, reducing noise sharpens and raises the peak of the posterior space. This can be seen in the magnifying effect of λ on the exponentiated

Table 5.6: Various estimates for r_0 and k_m , which are easily derived from $P(\theta | u)$. Note that the marginal mean and medians are to the right of the true values due to the right skew of the marginal distributions.

	$r_0 \times 10^{-3}$	$k_m \times 10^{-3}$
	mg/(mm ³ hr)	mg/mm ³
True value	50	10
Marginal MAP	50	8.8
95% credible interval	[41, 93]	[5.2, 30]
Marginal posterior mean	58	13
Marginal posterior median	56	12

cost function at the heart of the likelihood function shown in (5.13), and is illustrated in Figure 5.7.

Shortcomings of This Approach

Realization of the powerful effect of the precision on the model drives the final phase of our statistical model development. In short, λ is a powerful parameter which also depends on experimental data and whose true value is unknown (recall that λ is the reciprocal of variance). Up to this point, we have treated λ as given, but for real data we do not know the true variance of the data. Because our ability to resolve a MAP and the width of our credible intervals for r_0 and k_m depend so strongly on our estimate for λ , it is prudent to try to estimate λ from data. Beyond this, we also hope to apply this method to real data, where the true variance is not known.

However, adding a parameter to the model will necessitate a change in approach. So far we have relied on a brute force calculation of the posterior distribution, which involves computing an expensive cost function (5.1) and then (5.13) on a two-dimensional grid of values over the entire domain for r_0 and k_s . This process is slow because first, the cost

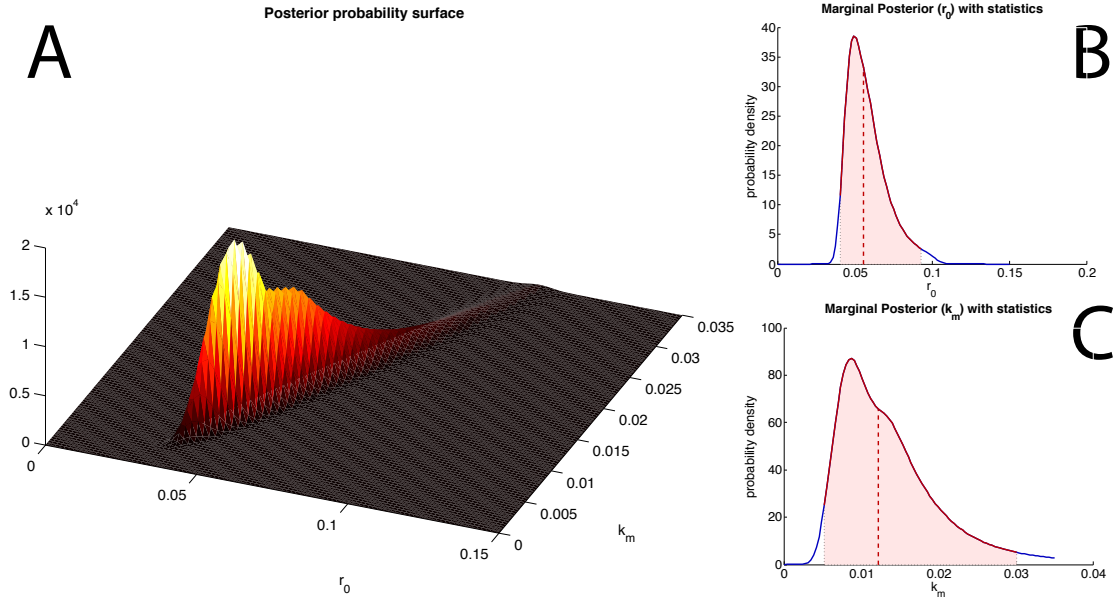


Figure 5.6: These plots summarize the output of the third synthetic cost example. Image (A) shows a 3D representation of the posterior distribution. Figures (B) and (C) show the r_0 and k_m marginal distributions, respectively. The shaded pink areas in (B) and (C) are the 95% credible intervals for r_0 and k_m , respectively. The vertical dashed line shows the median value for each marginal distribution. These statistics are summarized in Table 5.6.

function is expensive to evaluate over the grid as it involves hundreds of ODE solves per tube, and second, many cost values must be computed even though they correspond to low probability combinations that have little effect on the marginal posteriors. Adding another dimension in λ would compound these problems by requiring computations over a three-dimensional grid describing the domains of r_0 , k_m , and λ . In addition, while we can experimentally determine a reasonable range of r_0 and k_m values, we have less information about what the true precision for real data should be, and the computation is too expensive to iterate over a grid in 3D over large ranges of possible λ values. The solution to these difficulties is to change the method by which we calculate the posterior. In the next section we turn from an explicit calculation over a 2D grid of parameters to a Markov Chain Monte Carlo (MCMC) method.

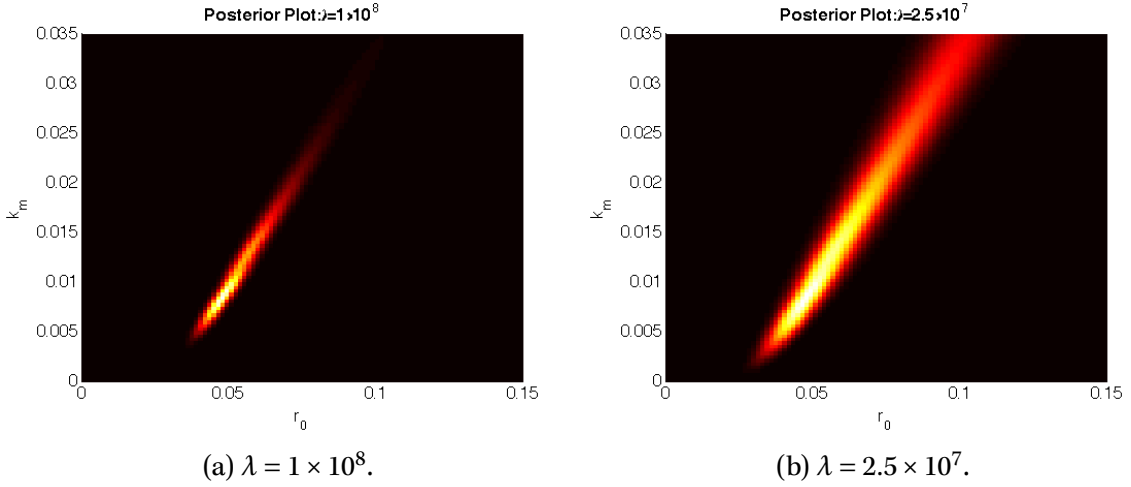


Figure 5.7: Changing the value of the precision, $\lambda = 1/\sigma^2$, has a large effect on the posterior. In particular, greater precision reduces the “ridge” or “tail” that causes the marginal distributions to be markedly right-skew. These plots were created using data from Example 3.

A Second Bayesian Approach: MCMC

In this section we keep much of the machinery from the last, but reverse the previous assumption that the variance of the data is known. The inverse problem is still given by (5.12) and (5.15), but now we now seek $P(\theta, \lambda \mid u_L)$ rather than $P(\theta \mid u_L, \lambda)$. Proceeding as before by Bayes’ formula,

$$P(\theta \mid u_L, \lambda) \propto P(u_L \mid \theta, \lambda) P(\theta, \lambda) = P(u_L \mid \theta, \lambda) P_0(\theta) P_0(\lambda). \quad (5.22)$$

Here, the factorization of the prior follows because we assume that the kinetic parameters and experimental precision are independent of each other. The likelihood function $P(u_L \mid \theta, \lambda)$ and prior $P_0(\theta)$ are the same as those shown in (5.13) and (5.15). What we need is a definition of $P_0(\lambda)$, the prior for λ . If $g(\theta)$ were linear, then the scaled sample variance

follows a χ -squared distribution [69, p. 74], [71, p. 76]. That is,

$$\lambda s^2 \nu \sim \chi_\nu^2,$$

where s^2 is the sample variance with degrees of freedom $\nu = n - 1$, where n is one less than the number of sampled effluent concentrations. This suggests a χ -squared distribution for our non-linear problem [69, p. 163]. Thus, the prior distribution for λ is

$$P_0(\lambda; \nu) = \begin{cases} \frac{2^{-\nu/2}}{\Gamma(\nu/2)} (\lambda s^2 \nu)^{\nu/2-1} e^{-\lambda s^2 \nu/2} & \text{if } \lambda > 0, \\ 0 & \text{otherwise.} \end{cases} \quad (5.23)$$

The Metropolis Algorithm

To estimate $P(\theta, \lambda \mid u_L)$, we do not compute the posterior given by (5.22) directly, but draw samples from $P(\theta, \lambda \mid u_L)$ using a Markov Chain Monte Carlo (MCMC) method. These methods work by constructing a Markov chain of samples whose stationary distribution is the posterior density function. By evaluating realizations of this chain iteratively, a picture of the posterior density is built for the parameter values based on the observed data as the chain converges to that stationary distribution [69, p. 159], [71, p. 287]. There are many ways to do this with various levels of sophistication. We use one of the earliest and simplest methods, the Metropolis Algorithm, which was first described in 1953 [72]. There are various modern descriptions of this algorithm, see for instance Gelman or Smith [71, p. 289], [69, p. 160].

Informally, the method works as follows. First, we pick a starting vector of parameter values. Next, we use a “proposal” distribution to generate a new proposed set of values for those parameters. Compare the posterior density of the proposed values to that of the previous values. If the proposed values increase the posterior density, then keep the

new set of parameters and repeat the process. If not, then with some probability keep the proposed set of values anyway, otherwise discard them and keep the previous values. As this process is iterated, the effect is to always accept steps that increase the posterior density but only occasionally accept steps which decrease it [71, p. 290]. Over many iterations, the iterates will converge to the posterior density function. Initially during the “burn in period,” however, the iterates may be far from the the posterior density and must be discarded from the final analysis.

A formal description of the Metropolis algorithm is aided by unifying the notation for the parameters of interest. Let $q = (\theta, \lambda)$ denote the vector of parameters. For example, in the case of MM-kinetics we have $q_1 = r_0$, $q_2 = k_m$, and $q_3 = \lambda$. In the case of 1st-order kinetics, q would be defined by $q_1 = k_1$, and $q_2 = \lambda$. Then we proceed as follows.

1. Pick a starting vector q^0 . This can be done by picking an experimentally likely value, a value derived from a previous estimate, or by selecting a random point in the parameter space. In the work that follows we use a random starting point. Selecting $\delta \sim U(0, 1) \times U(0, 1) \times U(0, 1)$, where U is the uniform distribution, we set q^0 by

$$\begin{aligned} q_1^0 &= (r_0^{\max} - r_0^{\min})\delta_1 + r_0^{\min}, \\ q_2^0 &= (k_m^{\max} - k_m^{\min})\delta_2 + k_m^{\min}, \\ q_3^0 &= 10^{-8}\delta_2. \end{aligned}$$

Here r_0 and k_m extreme values are the same as those used in (5.15), and 10^{-8} is used for q_3 because this is the order of observed experimental variance.

2. Loop for $t = 1, \dots, T$:

- (a) Sample a proposal q^* using a proposal distribution $J_t(q)$. This distribution must be symmetric in the sense that $J_t(q_a | q_b) = J_t(q_b | q_a)$ for all q_a, q_b , and

t . This is not a general requirement, but the simplifying characteristic that makes this algorithm “Metropolis.” In the work that follows we will use the uniform distribution $U([-1, 1])$, which is trivially symmetric. Selecting a $\delta \sim U(-1, 1) \times U(-1, 1) \times U(-1, 1)$, we set

$$q_i^* = q_i^{t-1} + \delta_i z_i,$$

for $i = 1, 2, 3$, and where $\mathbf{z}$ is a vector of tuning parameters. More is said about the values of $\mathbf{z}$ in the next section.

(b) Calculate the “acceptance criteria” (or “importance ratio”)

$$r = \frac{P(q^* | u)}{P(q^{t-1} | u)}. \quad (5.24)$$

(c) Draw $R \sim U([0, 1])$. Set

$$q^t = \begin{cases} q^* & \text{if } R < r, \\ q^{t-1} & \text{if } r \leq R. \end{cases} \quad (5.25)$$

This step utilizes “rejection sampling” [70, pp. 103–105].

The main work in the Metropolis algorithm occurs in the calculation of the importance ratio r . In our application this is done using Bayes’ theorem and the independence of the priors to write

$$r = \frac{P(q^* | u_L)}{P(q^{t-1} | u_L)} = \frac{P(u_L | q^*)}{P(u_L | q^{t-1})} \cdot \frac{P_0(q_1^*, q_2^*) P_0(q_3^*)}{P_0(q_1^{t-1}, q_2^{t-1}) P_0(q_3^{t-1})}$$

Since $P_0(q_1^*, q_2^*) = P_0(q_1^{t-1}, q_2^{t-1}) = P_0(\theta)$ is constant on its support as shown in (5.15), this equation simplifies to

$$r = \begin{cases} \frac{P(u_L | q^*) P_0(q_3^*)}{P(u_L | q^{t-1}) P_0(q_3^{t-1})} & \text{if } (q_1^*, q_2^*) \in I_{r_0} \times I_{k_m}, \\ 0 & \text{otherwise.} \end{cases} \quad (5.26)$$

Substituting the likelihood function (5.13) and the prior (5.23) into (5.26) and simplifying yields

$$r = \frac{(q_3^*)^{\frac{\nu-1}{2}}}{(q_3^{t-1})^{\frac{\nu-1}{2}}} \exp \left[-\frac{q_3^*}{2} (\|\mathbf{u}_L - \mathbf{g}(q_1^*, q_2^*)\|_2^2 - 1) + \frac{q_3^{t-1}}{2} (\|\mathbf{u}_L - \mathbf{g}(q_1^{t-1}, q_2^{t-1})\|_2^2 - 1) \right], \quad (5.27)$$

if $(q_1^*, q_2^*) \in I_{r_0} \times I_{k_m}$, and $r = 0$ otherwise. Note that by saving the results of the previous iteration we need only make one expensive calculation to compute r , namely, the evaluation of the forward model $\mathbf{g}(q_1^*, q_2^*)$. To decrease numeric error due to extreme values resulting from the exponential, we compute $\log(r)$ rather than r and exponentiate the result.

Difficulties Using the Metropolis Algorithm. A number of problems may be encountered using an MCMC scheme such as the Metropolis algorithm. First, it is unclear how many iterations T are “enough.” An insufficient number of iterations may result in a chain $\{q^1, q^2, \dots, q^T\}$ that grossly misrepresents the target posterior distribution. Said another way, early iterations are strongly influenced by the starting guess q^0 , and the algorithm must run long enough for q^t to converge in distribution to the desired target. Second, the proposal distribution requires tuning so that the percentage of “accepted” proposals is neither too large nor too small. A high acceptance rate means that the algorithm is

progressing too conservatively and insufficiently exploring the parameter space. On the other hand, too low of an acceptance rate means that the chain is inefficient and the time needed to converge to the target distribution may be unacceptably long. What constitutes a “good” acceptance rate? Estimates of “good” acceptance rates vary among experts. Values range from 0.23 for a multidimensional problem to 0.44 for a 1D problem [71, p. 307] or some value between 0.2 and 0.3 [70, p. 174].

The solutions to these two problems are relatively straightforward. Because early iterations in the chain are likely poor estimates of the target posterior, a “burn-in” period is needed where initial samples in the chain are discarded. How much of the chain should be discarded depends on the application. It could be a few thousand iterates, or more conservatively, the first half of every chain as advocated by Gelman [71, p. 295]. Tuning the proposal distribution was done by trial and error, though adaptive [73] or other more sophisticated methods exist [74]. An experimentally significant change in $q_1 = r_1$ would be on the order of 10^{-2} , while a significant change in $q_2 = k_m$ would be on the order of 10^{-3} . Similarly, since we estimate $q_3 = \lambda$ to be on the order of 10^8 , a change of 10^7 would be significant. Using these as starting guesses for how much to perturb our proposal distribution $U([-1, 1])$, we can run 100 iterations several times and empirically test the acceptance rate. Adjusting these sensitivities, we can manipulate the acceptance rate to whatever level is desired. At the end of this process we used

$$z_1 = 5 \times 10^{-2},$$

$$z_2 = 5 \times 10^{-3},$$

$$z_3 = 5 \times 10^7,$$

for the vector of tuning parameters $\mathbf{z}$.

A third, more difficult problem with MCMC is that the within-chain correlation may be high. Since each draw q^t depends on the previous draw q^{t-1} , consecutive samples are not independent. This is precisely what makes the chain “Markov”, as the future step q^{t+1} depends only on the present step q^t . However, as the chain lengthens, samples far enough apart in the chain will be uncorrelated. The distance between independent samples is called the “autocorrelation length.” A short autocorrelation is desirable as this often indicates fast convergence [69, p. 170]. Autocorrelation for q_j is defined as

$$R(k) = \frac{\sum_{i=1}^{T-k} (q_j^i - \bar{q}_j)(q_j^{i+k} - \bar{q}_j)}{\sum_{i=1}^n (q_j^i - \bar{q}_j)^2} = \frac{\text{cov}(q_j^i, q_j^{i+k})}{\text{var}(q_j^i)} \quad (5.28)$$

between components in the chain which are k iterations apart [69, p. 170], [70, p. 177]. The integrated autocorrelation time (IACT) is a single-number summary of this autocorrelation. It may be written as

$$\tau_{\text{int}} = 1 + 2 \sum_{k=1}^{\infty} R(k),$$

for the autocorrelation defined in (5.28) [67, p. 175], [75]. Rather than implement these computations manually, however, we used code written by Wolff [75, 76] to find the normalized correlation time and the integrated autocorrelation time, τ_{int} . Once we have the IACT, we can compute the number of effective samples as

$$n_{\text{eff}} = \frac{T}{\tau_{\text{int}}},$$

where T is the length of the chain in question. Then, in assessing the convergence of a chain we look at indicators such as whether or not a zero autocorrelation has been reached, the magnitude of τ_{int} , and the size of n_{eff} .

Table 5.7: A summary of MCMC results for Example 4.

	$r_0 \times 10^{-3}$	$k_m \times 10^{-3}$	$\lambda \times 10^8$
	mg/(mm ³ hr)	mg/mm ³	(mg/mm ³) ²
True value	50	10	1.0
Marginal MAP	49	8.5	1.0
Marginal posterior median	53	11	1.1
95% credible intervals	[41, 80]	[5.8, 24]	[0.34, 2.9]
τ_{int}	208	216	216

Synthetic Cost Example 4

To illustrate the Metropolis scheme, we once again turn to synthetic data. In this case, we use the same data described in Example 3 and summarized in Table 5.5, for which three noisy replicates were made for each influent concentration and averaged to create the data set. Recall that this data was produced using a 0.1 mm thick flat biofilm with influent concentrations set at $\mathbf{u}^0 = (0.5, 5, 10, 15, 20) \times 10^{-3}$ mg/mm³. The data was generated using true parameter values of $r_0 = 50 \times 10^{-3}$ mg/(mm³ h), $k_m = 10 \times 10^{-3}$ mg/mm³, and $\lambda = 1 \times 10^8$ (mg/mm³)². In order to evaluate the prior in (5.23) for λ we need an estimate of the sample variance s^2 . To calculate s^2 we use the Satterthwaite equation to pool the variance across all influent concentrations. That is, given that the number k of replicates for each N influent concentrations is the same,

$$s^2 = \frac{1}{Nk} \sum_{i=1}^{i=N} s_i^2, \quad (5.29)$$

where each s_i^2 is the variance of the k tubes run at the same u^0 . For this synthetic data, this value is $s^2 = 2.5 \times 10^{-9}$, which corresponds to a sample standard deviation of $s = 5 \times 10^{-5}$.

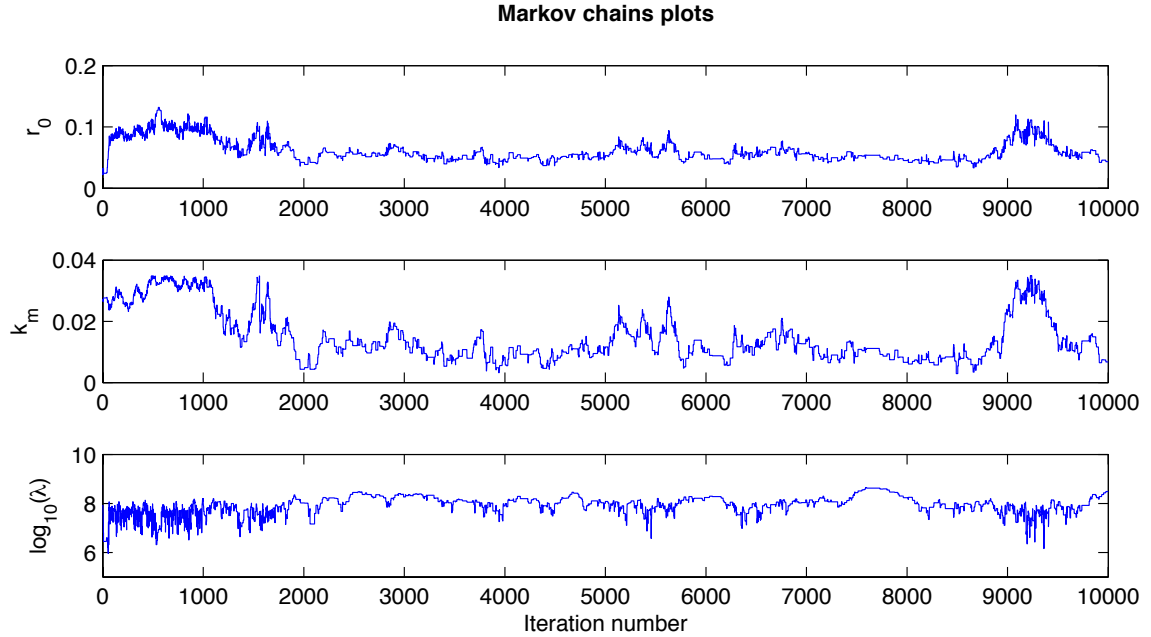


Figure 5.8: These plots of the Markov chains for all three parameters of interest in Example 4 are crucial in determining the burn-in period. Inspection of these plots put the burn-in period at about 2,500 iterations.

Results for a $T = 10,000$ iteration chain with a random start are shown in Table 5.7 and in Figures 5.8, 5.9 and 5.10. First, we use Figure 5.8 to examine the plot of the r_0 , k_m , and λ chains. Inspection of Figure 5.8 shows that the burn-in period is approximately 2,500 iterations. At that point all three panels of Figure 5.8 show that the chain begins to oscillate around a fairly constant interval. The chains do wander away from this interval roughly between iterations 8,500 and 9,500, but return quickly to the previous steady value. Figure 5.9 gives a different view of the burn-in period. In the left panel we see the r_0 and k_m chains plotted over the cost plot from Example 3, while in the right panel we see the same plot starting from iteration 2,500. Removing the initial iterations shows samples from the posterior distribution.

Taking the burn-in length to be 2,500 iterations, we next create the histograms shown in Figure 5.10 using the remaining 7,500 simulation results. Inspecting these, we

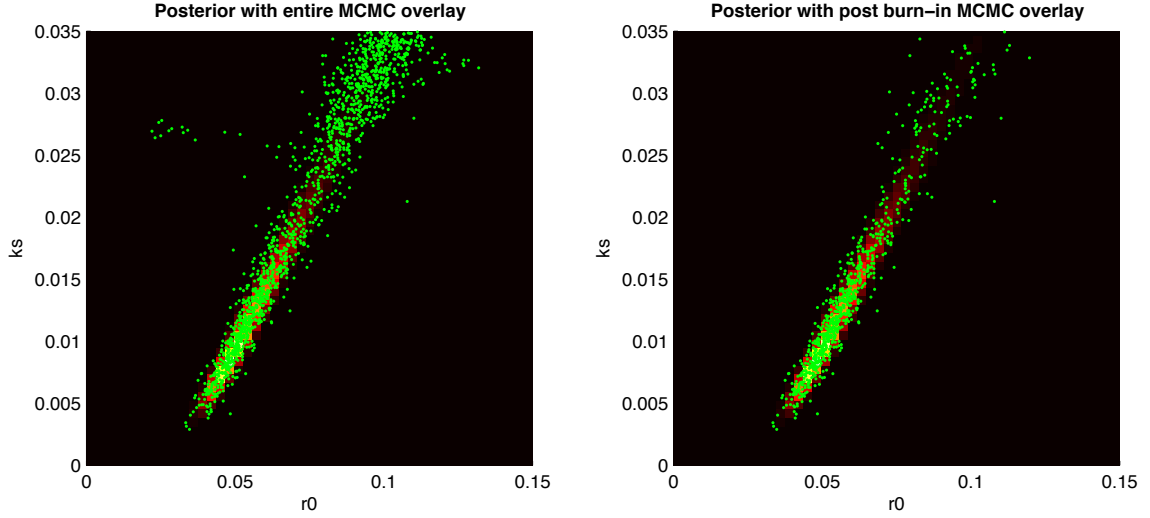


Figure 5.9: The left panel shows the complete MCMC chain for example 4. The r_0 and k_m iterates are plotted as green dots overlaying the cost space plot from Example 3. The random starting point of the algorithm is visible near the top left of the left panel. The right panel shows the chain after the 2,500 burn-in values are dropped.

see that each chain clusters around the known true value of the parameter, though the distributions are again right-skewed as we saw in Example 3 for the case of r_0 and k_m , where λ was assumed known (cf. Figure 5.6). From these histogram approximations of the marginal distributions we can simply calculate the 95% credible intervals, which are shown along with the MAPs and marginal medians in Table 5.7. The credible intervals clearly contain the true parameter values. As a final check, we calculate Wolff's autocorrelation on post burn-in chains. In each case, the autocorrelation reaches zero and yields a integrated autocorrelation time (τ_{int}) of approximately 213. This suggests the chain of 7,500 iterations has the statistical power of approximately $7,500/213 = 35$ independent samples.

The Effect of Noise

In Example 2 (cf. Table 5.3) we saw the powerful effect of error on the depth and discernibility of the cost minimum. This was illustrated in Figure 5.4. In our discussion

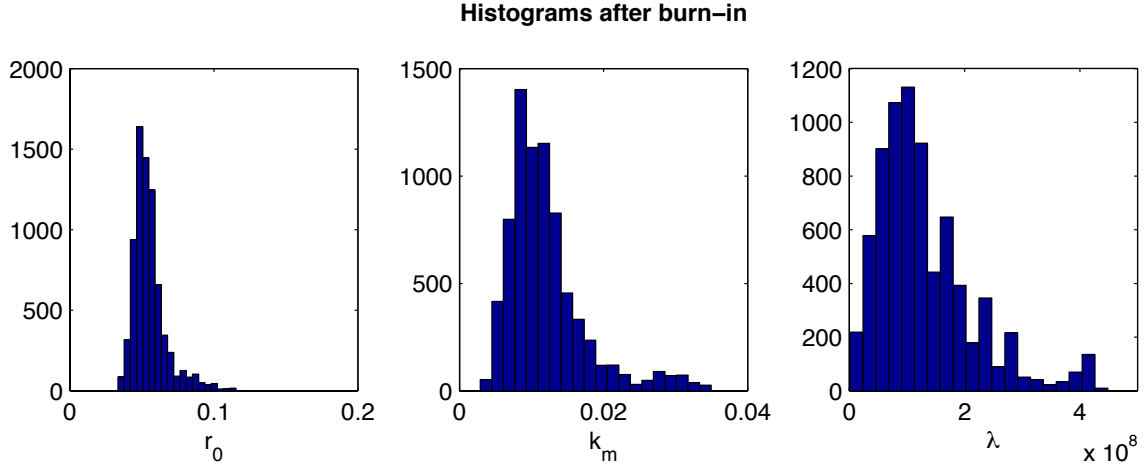


Figure 5.10: Histograms for each parameter from the Markov chains in Example 4 after the exclusion of the burn-in values.

of the shape of the cost space we saw that the long valley was due to the convergence of the model in the r_0/k_m direction to a constant along the valley's floor. When the variance is much less than this constant, the minimum is distinct from the rest of the cost space. On the other hand, a high variance leads to a diminishing of the minimum and its eventual shift in the r_0/k_m direction (cf Figure 5.4E). This sensitivity to error manifests as a ridge in the plots of the posterior, as shown in Figure 5.7. Here, exponentiation turned the minimum into a maximum, and the “valley” into a “ridge”, but the addition of noise blurs the distinction between the ridge and the peak, and elongates the ridge in the r_0/k_m direction. This follows from the same analysis summarized in Figure 5.5. Using the same parameter space divisions as in Figure 5.5, we see that the posterior density is close to 0 in quadrants II and IV, but in quadrants I and III depends on the value of λ . High precision, that is, low variance, causes the peak in quadrant I to be tall compared to quadrant III values. Lack of precision means the distinction between the peak and the ridge becomes difficult to distinguish as posterior density in the ridge increases.

It should come as no surprise then, that the number of iterations to convergence of the MCMC scheme illustrated in Example 4 depends heavily on the variance, $\sigma^2 = 1/\lambda^2$. If

the variance is too large, then the chains will not converge in distribution to the posterior. A unique MAP may not exist, or if the MAP does exist may not have much more density than the ridge values. Because of this, Markov chains will have very long integrated autocorrelation times. By way of illustration, applying MCMC to a noisy example with $\lambda = 2 \times 10^7$ generated a chain with a τ_{int} length of over 12,000, meaning an hours-long computation to generate a chain of 10,000 samples did not converge to the posterior. The Metropolis algorithm will converge to the posterior eventually, however [77]. It is possible that in this example the chain never advanced beyond the the burn-in period. Thus, in the presence of excessive noise the MCMC method is too computationally intense to be practical.

There are a number of possible solutions to this dilemma. The list below is ordered from the simplest to the most complex solution.

1. Decrease experimental noise by increasing the number of replicates. That is, add more tubes to the experiment. In the examples above, we have created data by averaging replicate tubes. The variance of those mean data is equal to the variance of the replicates divided by the number of replicates. A large variance can thus be reduced by additional replicates. Ten times more replicates would give an order of magnitude smaller variance. Of course, running 30 replicates for each concentration in the lab rather than three may well be impractical.
2. Change the kinetic model. It is possible that the chosen kinetics are not appropriate for the experimental data. First order, zero order, or other kinetic choices may lead to better convergence. Or, it is possible that for particular kinetics the unified model leads to an overdetermined problem with unidentifiable parameters and is not the best way to model this system.

3. The statistical model may need to be changed, for example by changing the prior for r_0 , k_m , and/or λ . In the method described above, we use an uninformative prior for both r_0 and k_m . We know that a ridge will form in the direction of r_0/k_m in quadrant III (cf. Figure 5.5) for noisy data. We may then wish to weigh the scheme toward the lower left end of that ridge. For instance, a linear prior making values near $r_0^{\min}$ and $k_m^{\min}$ say, twice as likely as the values near $r_0^{\max}$ and $k_m^{\max}$ could be used. In general, the addition of any additional information about r_0 and k_m will help with convergence.
4. Change the proposal distribution in the Metropolis implementation. In the scheme above we use a uniform distribution to randomly choose the proposal values for each of our parameters. There are many more sophisticated ways to choose a proposal, and many more modern (and complicated) algorithms beyond Metropolis' 1953 scheme. For example, Haario et al. derive a Gaussian adaptive proposal whose mean and covariance depend on the whole history of accepted steps. This removes the necessity of manually tuning the proposal distribution [73]. The "t-walk" method by Christen and Fox utilizes specialized proposals for moving through the parameter space including a very large step taken with some low probability to speed convergence [74].

Synthetic Cost Example 5

A final synthetic example is included here to show that in the case of thin biofilms and 1st-order kinetics, the "naive" NLS approach to the inverse problem outlined in the beginning of this chapter works very well. Comparable results follow from building $P(k_1, \lambda \mid u_L)$ using the MCMC scheme as well. Since both NLS and MCMC give similar results, MCMC is probably "over-kill" for this scenario as it is much more expensive to run. However, the success of MCMC in this situation demonstrates the flexibility of the

Table 5.8: This synthetic data was generated using $k_1 = 15 \text{ hr}^{-1}$, a uniform biofilm height of 0.02 mm, and normal noise with $\sigma = 2 \times 10^{-4}$. Note that two values are created for each influent concentration.

u^0	u_L	u_L
0.5	0.3657788	0.3179259
5	4.309207	4.256669
10	8.527836	8.770893
15	12.82251	12.98115

(All entries have units $\times 10^{-3} \text{ mg/mm}^3$)

approach using different kinetic models. That is, the MCMC method has the advantage that after changing the kinetic parameters we can just start building a chain by sampling from the posterior regardless of whether or not NLS converges well.

New kinetics dictate the need for a new synthetic data set. Synthetic data for this example was generated using a flat biofilm of height 0.02 mm. This is nearly an order of magnitude thinner than the preceding examples, and close to the values obtained in an actual experiment involving multiple tubes at multiple influent concentrations. Two replicates each for each influent concentration $u^0 = (0.5, 5, 10, 15) \times 10^{-3} \text{ mg/mm}^3$ were created using equation (5.3) with $\sigma = 2 \times 10^{-4}$ and $k_1 = 15 \text{ hr}^{-1}$. Because the unified model with 1st-order kinetics does not require an ODE solve at each tube grid point, this scenario runs much faster than the model with MM-order kinetics. Consequently, we do not average the replicates into representative means to decrease the size of the data set, but use all eight values directly in the inverse problem. This data is shown in Table 5.8, and a plot of the cost function showing the difference between this synthetic data and 1st-order model outputs computed over a range of k_1 values is shown in Figure 5.11.

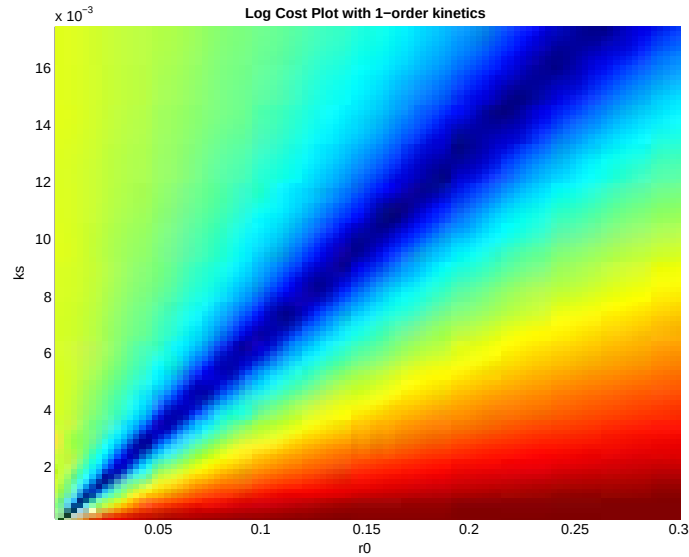


Figure 5.11: A plot of the cost function over a range of r_0 and k_m values for Example 5. This plot was created using 1st-order kinetics, where $k_1 = r_0/k_m$.

We minimize the NLS problem using Matlab's `nlinfit` routine from a random starting guess for k_1 . The solver converges quickly and robustly for a wide range of starting values to $k_1 = 14.9 \text{ hr}^{-1}$, with standard deviation given by (5.8) as $s = 0.42 \times 10^{-3} \text{ mg/mm}^3$. This is very near the true value of $k_1 = 15.0$. Evaluating the Jacobian at $k_1 = 14.9$, and presuming $\hat{k}_1$ is normally distributed (cf. (5.7)), we calculate the 95% confidence interval for k_1 to be $[13.9, 15.9]$.

The MCMC scheme works in this scenario as well, and yields entirely comparable results. A chain of length 30,000 was computed in 15 seconds (compared to the NLS solver, which took about 0.8 seconds). The burn-in period is approximately 12,500 iterations, as may be seen by Figure 5.12. The marginal MAP and median posterior estimates are $k_1 = 14.9 \text{ hr}^{-1}$, which agree exactly with the NLS estimate, and the 95% credible intervals for k_1 are very similar as well as may be seen in Table 5.9. Looking at the histogram for k_1 in Figure 5.13 shows that distribution of k_1 values is well approximated by a normal

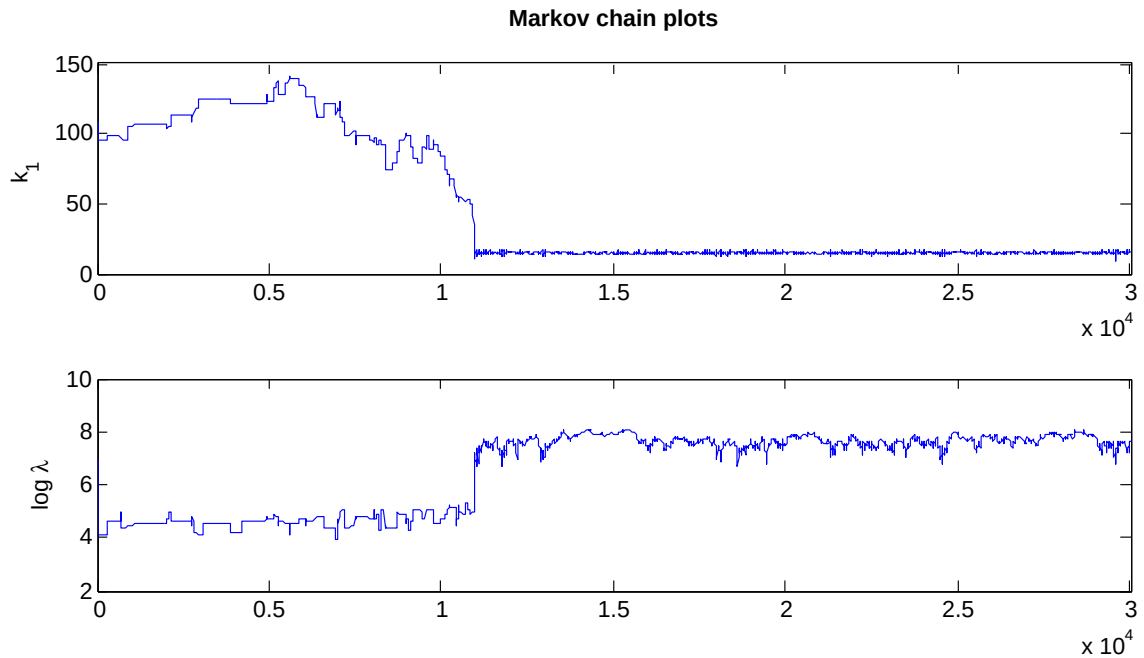


Figure 5.12: These plots of the Markov chains for all k_1 and λ show a clear burn-in period of about 10,000 iterations for Example 5. We take the burn-in length to be 12,500 just to be certain.

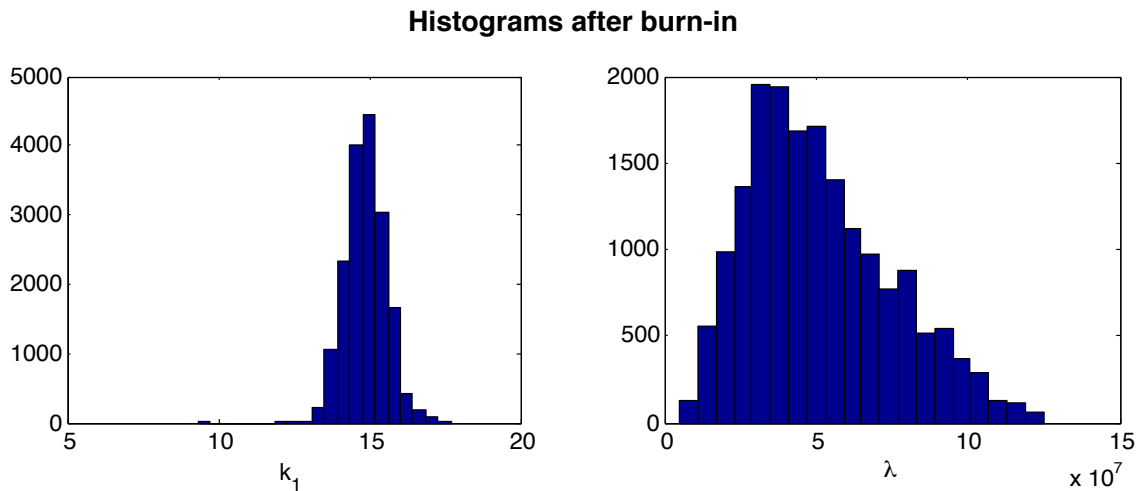


Figure 5.13: MCMC histograms for k_1 and λ from Example 5 after the exclusion of burn-in values. Note that the distribution of k_1 values looks quite normal. This illustrates why the NLS and MCMC confidence intervals for k_1 are so similar.

Table 5.9: A summary of NLS and MCMC results for Example 5. Parameter k_1 is well approximated by the marginal statistics of both schemes, and the 95% credible intervals are comparable as well.

	k_1 (hr ⁻¹)	$\lambda \times 10^6$ (mm ³ /mg) ²
True value	15	25
NLS estimate	14.9	110
NLS 95% confidence interval	[13.9, 15.9]	NA
MCMC Marginal MAP estimate	14.9	32
MCMC Marginal posterior median	14.9	51
MCMC 95% credible interval	[13.7, 16.1]	[18, 95]

distribution. Thus, the normal approximation that NLS uses to estimate k_1 and its confidence intervals (cf. (5.7)) agrees closely with the actual posterior for k_1 .

Model Results Using Real Data

We close this chapter by applying our model to a real data set. At the end of the previous chapter, two experimental data sets containing real experimental data were described. In the first, the tube reactors were run at a target influent concentration of 10 g/L of urea. As was described, this experiment was designed to naively estimate r_0 . A second experiment where all the reactors were run at a target influent concentration of 0.5 g/L was later run in an attempt to find k_m by coupling the second data set with the first. Recall that shortcomings of that methodology, discussed at the end of Chapter 4, motivated the inverse problem formulation of this chapter. In the present section, this final data set is the first where multiple tubes were run at multiple target influent concentrations. In Example 2 we showed that this spread of influent concentrations was

Table 5.10: Summary influent and effluent urea concentration data from the final tube reactor experiment. Influent and effluent values were computed by averaging three samples taken just before the reactors were stopped for sectioning.

Tube #	Target u^0	u^0	u_L
1	0.5	0.702192	0.253874
2	5	7.713749	6.534032
3	10	8.725820	8.033678
4	15	13.19321	12.40295
6	5	4.369748	2.783953
7	10	8.453768	7.132574
8	15	13.26853	12.14999
9	0.5	0.632783	0.434043

(Units are $\times 10^{-3}$ mg/mm³)

necessary to isolate r_0 and k_m . This final data set consisted initially of twelve tube reactors, three at each target influent concentration of 0.5, 5, 10, and 15 g/L of urea. Eight of these tubes were deemed to have reached steady state and are used here. The surviving influent and effluent concentration data from these reactors are shown in Table 5.10. The height profiles, measured using the cryosectioning technique described in the last chapter, are shown in Figure 5.14. These data are described in more detail in Connolly et al. [32], along with MM- and 1st-order kinetic parameter estimates derived using a COMSOL Multiphysics version 4.3a model.

Michaelis-Menten kinetics:

Using this data, we first try to solve for MM-kinetics using the techniques described in Example 4. First, we estimate the sample variance $s^2 = 4.69 \times 10^{-8}$ using the Satterth-

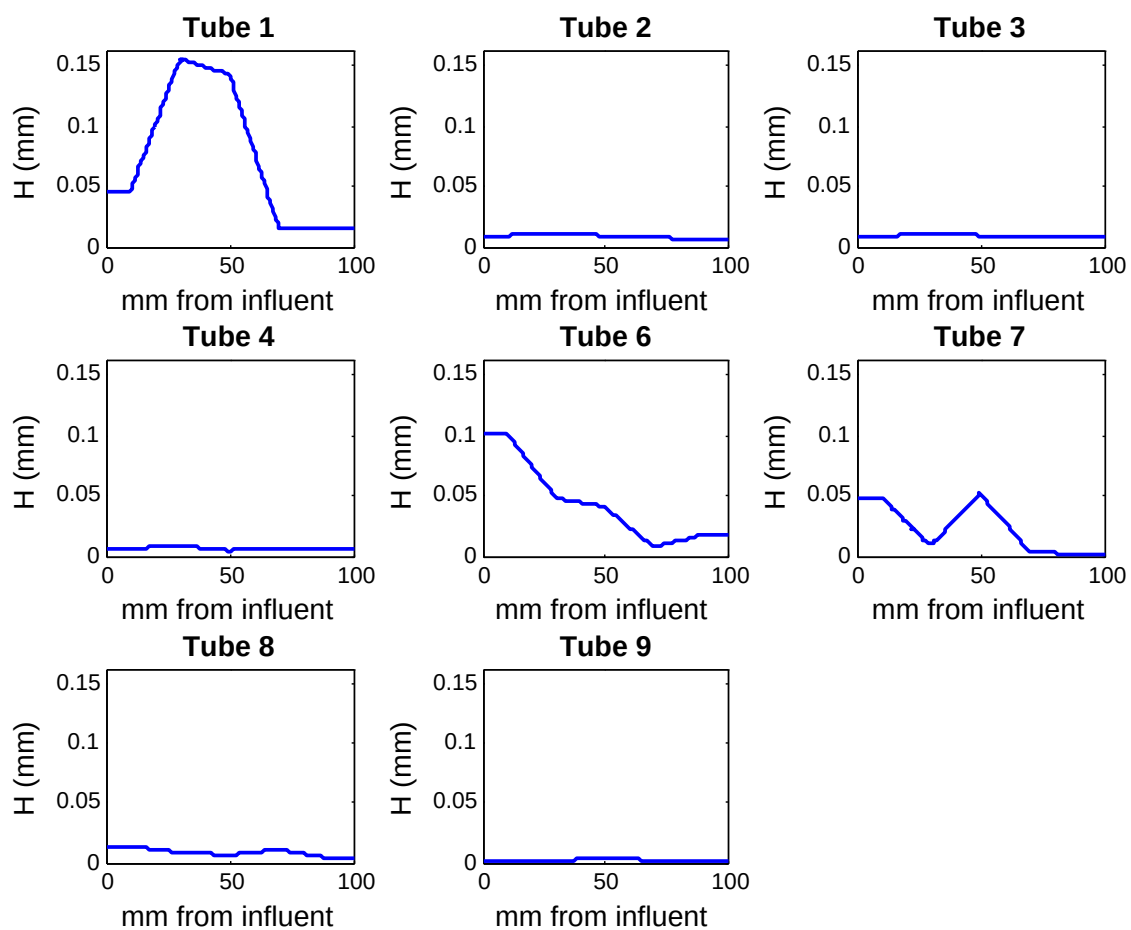


Figure 5.14: Biofilm height profiles from each of the eight reactors which survived the entire growth, sampling, and cryosectioning process. The average biofilm thickness across all tubes and all measurements was 0.0220 mm with a standard deviation of 0.0327 mm.

waite formula shown in (5.29). Next, influent and effluent concentration vectors were set to the values shown in the third and fourth columns of Table 5.10. A random q^0 was chosen and the MCMC scheme previously described was applied on a domain of $I_{r_0} \times I_{k_m} \times I_\lambda$ where,

$$I_{r_0} = [0.001, 1.8] \text{ mg}/(\text{mm}^3 \text{ hr})$$

$$I_{k_m} = [0.006, 0.06] \text{ mg}/\text{mm}^3$$

$$I_\lambda = (0, \infty) (\text{mg}/\text{mm}^3)^{-2}.$$

The chain was run to a length of 100,000 iterations, which are shown in Figure 5.15, with a 19.5% acceptance rate. Focusing on the chains in Figure 5.15 individually, it would appear that despite the length of these chains, none of the chains converged to the posterior distribution except possibly the chain for λ . However, examination of the autocorrelation time paints a different story. Figure 5.16 shows a plot of the autocorrelation time shown in equation 5.28. Note that the autocorrelation time goes to zero at a distance of about 2,500 samples. This suggests the chains have converged. Looking back at Figure 5.15, we see that after something like 3,000 iterations the plots for r_0 and k_m are correlated. Thus, it is possible that this data is under determined and r_0 and k_m are not individually determinable. In the next section we will pursue this line of inquiry.

Ignoring the possibility of non identifiability or non convergence, we can take Gelman's advice, discard the first half of the each chain, and histogram the retained 50,000 iterates as shown in Figure 5.17. From these we can estimate each parameter using the posterior median and MAP as previously described. These results are shown in Table 5.11.

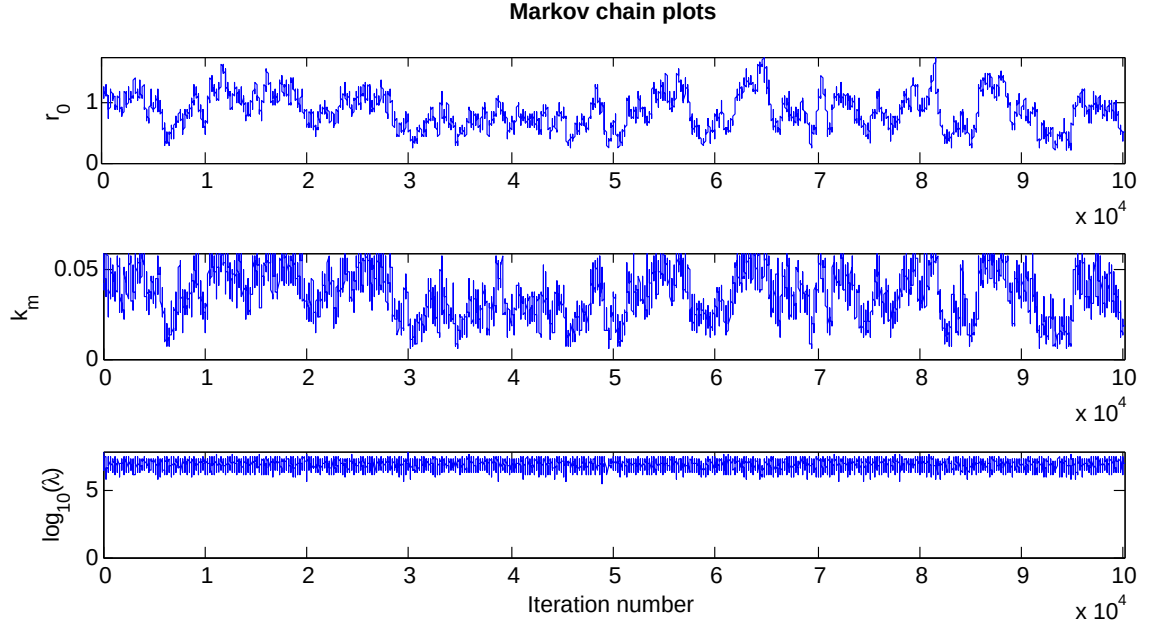


Figure 5.15: These chains show no real indication of a burn-in period in the first two panels, though in the third λ very quickly jumps into the range of 10^5 .

First Order Kinetics:

The correlation between the first and second panel of Figure 5.15 suggest that the inverse problem may be under determined. The key to reducing the number of parameters in the system is shown in equation 5.4. Rather than look for r_0 and k_m individually, we can search for a 1st-order kinetic parameter which encapsulates the ratio between r_0 and k_m . In Example 5 we showed that the NLS and MCMC methods give very similar results in the case of thin biofilms, as the posterior for k_1 is well approximated by a normal distribution. That is the case using real data as well. Results for 1st-order parameter estimation from these data is shown in Table 5.12.

As was the case with Example 5, there is very good agreement here between the NLS and MCMC results. While NLS returns $k_1 = 19.1 \text{ hr}^{-1}$, the MCMC posterior median is $k_1 = 19.2 \text{ hr}^{-1}$. Similar, the 95% confidence intervals from NLS is (14.3, 24.0), which compares favorably with the MCMC 95% credible interval of (14.7, 24.0). We consider these

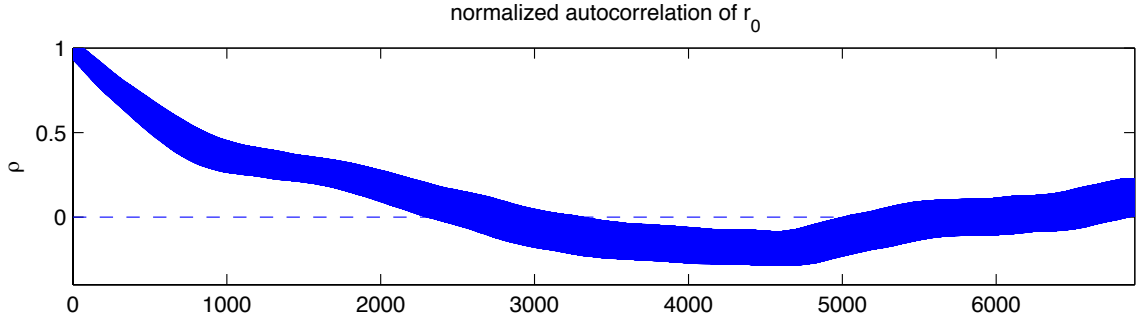


Figure 5.16: A plot of the autocorrelation function for parameter r_0 for the real data using MM-kinetics. Note that the autocorrelation goes to zero after approximately 2,500 samples. That is, samples about 2,500 apart are statistically independent of one another. The plot for k_m is very similar and is thus not included.

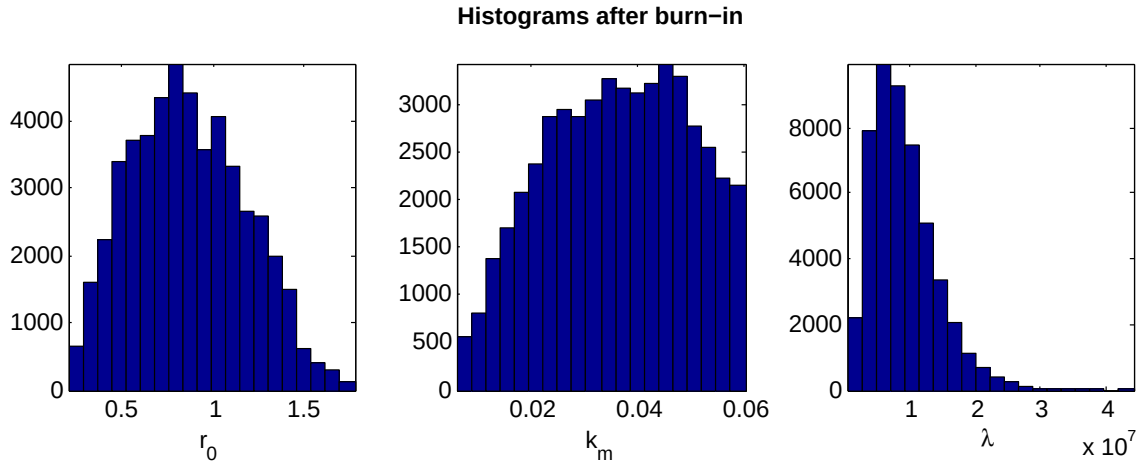


Figure 5.17: The histograms for r_0 and k_m echo the wide range of values shown in Figure 5.15 by being quite wide.

results much more robust than the MCMC MM-kinetic estimates shown in Table 5.11. While convergence of the chains in the MM-kinetic example is dubious as witnessed by Figure 5.15 and IACT values of $\tau_{\text{int}} \approx 800$, in this 1st-order case the chains visibly converge (cf. Figure 5.18) and exhibit an IACT of $\tau_{\text{int}} \approx 4$. Despite the problems with the MM-kinetic chains, it is telling that the ratio of r_0 and k_m values in the MM-case are reasonably close to the 1st-order estimates. Using the MAP values from Table 5.11 we find $r_0/k_m = 0.80/0.045 = 17.8$. Similarly, using the posterior median values from Table 5.11

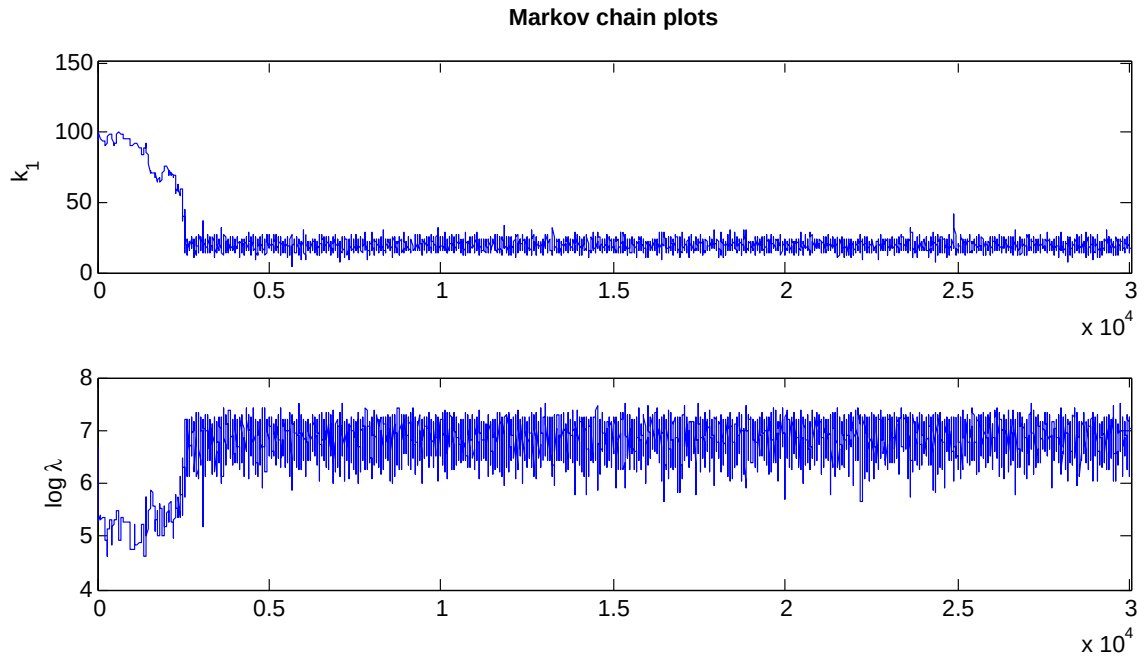


Figure 5.18: These plots of the Markov chains for all k_1 and λ show a clear burn-in period of about 3,000 iterations. We take the burn-in length to be 5,000 just to be certain.

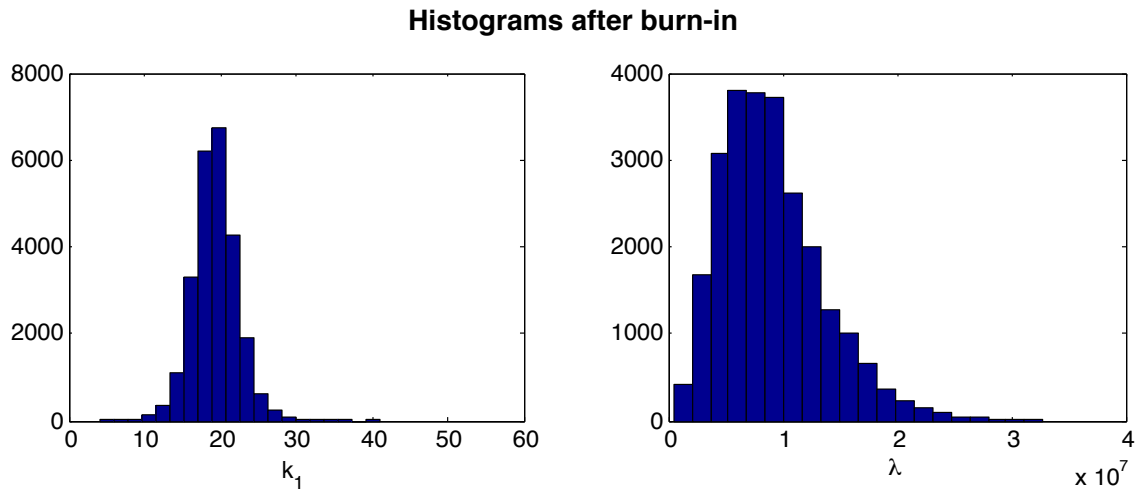


Figure 5.19: MCMC histograms for k_1 and λ for real data after the exclusion of burn-in values. We again note that the distribution of k_1 values looks quite normal, while the λ histogram has a χ^2 -distribution shape.

Table 5.11: Parameter estimates using MCMC with MM-kinetics on the data shown in Table 5.10 and Figure 5.14. Results from Connolly et. al are given for a comparison as these are the only similar estimates available.

	r_0	k_m	$\sigma = \sqrt{(1/\lambda)}$
MAP	0.80	0.045	0.42
Posterior median	0.84	0.037	0.35
95% credible interval	(0.38, 1.4)	(0.014, 0.057)	(0.23, 0.62)
Connolly estimate (cite)	0.96	0.033	na
τ_{int}	825	691	7

(Units are $\times 10^{-3}$ mg/mm³)

we have $r_0/k_m = 0.84/0.037 = 22.7$. Both of these value are in the MCMC 95% credible intervals from Table 5.12. It could be that in the presence of sufficient noise r_0 and k_m are unidentifiable and that the inverse problem can only determine the ratio of these two parameters.

Table 5.12: A summary of 1st-order kinetic parameter estimates for the real data. Here NLS results are compared to MCMC results. Again, comparison with Connolly et al. is made as these are the only similar estimates available.

	k_1 (hr ⁻¹)	$\sigma = \sqrt{(1/\lambda)}$ (10 ⁻³ mg/mm ³)
NLS estimate	19.1	0.27
NLS 95% confidence interval	(14.3, 24.0)	na
MCMC Marginal MAP estimate	19.8	0.3
MCMC Marginal posterior median	19.2	0.34
MCMC 95% credible interval	(14.7, 24.0)	(0.24, 0.57)
τ_{int}	5.0	3.8
Connolly estimate	23.2	na
Connolly 95% confidence interval	(17, 29.4)	na

CHAPTER SIX

CONCLUSIONS

Biofilms are nearly ubiquitous in natural and artificial systems. As a consequence, many natural and artificial systems involve water flowing over a microbial mat. Despite advances in imaging and experimental sophistication, mathematical models continue to play an important role in studying how material is transported in biofilm-laden systems. In this dissertation we have derived two low dimensional models which describe biofilm streamflow systems. In the first, the model describes how particulates such as cells transfer from the flow to the mat and back. This model was parameterized and tested using data from an effluent stream at Mushroom Spring in Yellowstone National Park. The second model examined the transfer of dissolved material, urea, in the context of a tube reactor experiment. After formulation of the model, an inverse problem formulation was used to quantify model error and estimate parameters of interest.

Mushroom Spring Streamflow Models

In the first half of this dissertation we made use of an erosion term in the lower boundary condition to quantify the transfer of particles to and from the microbial mat. First, we used a 1D model to examine various possible ways to incorporate such an erosion term. Here we found that some formulations lead to nonunique solutions. Others result in erosion rates completely specified by the influent cell concentration and stream velocity. We settled on a flux balanced form as the most informative type of boundary condition, and used that condition in the remainder of our modeling.

Given the limited utility of a 1D model, we next concentrated on developing a 2D streamflow model. This model focused on the depth and length of the channel but ignored the width. After deriving this model from a mass balance, we went on to show that

under our assumptions a steady state solution exists. Solving this steady state system was done numerically using a first order accurate finite difference scheme. In order to solve for the erosion rate term, we specified a Neumann downstream boundary condition and compared the average cell concentration along this boundary with a known target concentration. This allowed us to solve for the erosion rate. However, to apply this model in the field required a large number of parameters. We described at some length experimental methods for estimating cell concentration from field data, and used this data to parameterize the model. Unfortunately, the realities of collecting field data kept us from achieving information at great spatial resolution. Combining all the experiments allowed us to divide the stream into two segments, in which were able to use the model to estimate cell erosion and adhesion rates.

While we were able to estimate erosion and adhesion rates using field data (i.e. compute parameter E), we have some doubt that these estimates are accurate. While the field data is of course noisy, the difficulty stems from the size of the vertical turbulent diffusivity constant, K_z . Plots of the cell concentration in the flow seem to indicate that our estimate of K_z is too small. Of course, changing the value of K_z changes the estimate of the erosion rate, casting doubts on the accuracy of the values which we calculated. This suggests a ready source of possible future work. A better understanding of the role of K_z in estimating E would be useful. Besides this, a more suitable model for K_z could be an improvement. Fischer's model made a number of assumptions which are perhaps not appropriate to our case. Aside from using a better model for K_z , it may be possible to estimate turbulence parameters using field experiments.

The Unified Tube Reactor

In the second half of this dissertation we built a unified tube reactor model comprising two coupled 1-dimensional models to describe the consumption of substrate by a

biofilm growing in a tube or in a long, narrow pore-space. This unified model is flexible with respect to the kinetics used in the biofilm, and is able to make use of biofilm height profile data from experiments. When naive attempts at parameter estimation using experimental data failed, we developed an “inverse problem” formulation which allowed us to look at data from many reactors simultaneously. This also allowed us to account for model and experimental uncertainty explicitly in the form of a statistical likelihood function. When nonlinear least squares was not able to solve the inverse problem, we developed a Markov Chain Monte Carlo method which could. This MCMC formulation had the additional benefit of allowing us to apply previous knowledge about parameter ranges in the form of prior distributions. We also had the flexibility to decide whether to model experimental variance as a known or unknown quantity.

Along the way we learned four lessons of importance to experimenters. First, we learned that we can only expect to resolve Michaelis-Menten kinetic parameters from an inverse problem formulation when we consider data that brackets a range of effluent concentrations that includes the half-saturation, k_m . Given that the value for k_m may not be known, it behooves the experimenter to include as wide a range of influent concentrations as possible. A second lesson learned is that NLS is not necessarily appropriate for such a problem. Not only might the solution heavily depend on the initial parameter estimates used, but the posterior probability distribution is not necessarily symmetric. If this is the case, then the confidence interval returned by NLS may be misleading. A third lesson is that for resolving MM-kinetic parameters, low levels of noise are critical. The sample variance will decrease proportionally to the number of tubes that are used. Our recommendation is thus that experimenters seeking to determine kinetics using this model use as many tube reactors as is physically practical. A final point is that, in the case of Michaelis-Menten kinetics and in the presence of noise, the inverse problem may more readily determine first order kinetics (approximated as the ratio between r_0 and k_m)

than the parameters themselves. Even when both parameters do resolve, the slope in the direction of r_0/k_m is an important feature of the cost space.

The unified tube reactor model is also relatively simple with respect to physics as compared to other modeling efforts such as those by Connolly et al. However, the application of the model to real data yielded similar results [32]. The lesson here seems to be that in situations characterized by a large amount of noise, a computationally simple model may compete well with a more detailed physical model.

Of course, no modeling project is ever completely done, and this work has several possible natural extensions. First, it would be useful to quantify how much noise is “too much” to resolve MM-kinetics. In particular, how does the magnitude of this noise compare to model parameters such as r_0 ? In a related vein, can “large” values of noise be overcome by a greater range of influent concentrations, particularly farther along the MM-kinetic curve where concentrations are high? Adding tubes will reduce variance, but perhaps a wider range of influent concentrations would allow an experimenter to reduce the number of tubes needed. Finally, we have only explored zero-, first-, and Michaelis-Menten kinetics, but this model may give insight into other kinetic models as well.

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