Modeling biofilm antimicrobial resistance
by Michael Grant Dodds

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Chemical Engineering
Montana State University
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Abstract:

The goal of this work was to develop and test a computer model capable of integrating mechanisms of biofilm resistance to disinfection by antimicrobial agents. Resistance mechanisms considered included retarded penetration due to a stoichiometric reaction between the antimicrobial agent and biomass, retarded penetration due to a catalytic reaction between the antimicrobial agent and the biomass, and the existence of a fraction of the cells in the biofilm in a resistant phenotype.

Mathematical models of these processes were derived and translated to a computer model, MATLAB. A formal sensitivity analysis resulted in the statement that the parameters used in these models were necessary to capture a hypothetical data set, but that, in some situations, fewer parameters were sufficient. The model predicted the effect of physiological changes in a subpopulation of the biofilm and demonstrated that even a small resistant subpopulation could potentially foil effective disinfection of that biofilm. The models also predicted that the physical mechanism employed by the antimicrobial agent had a profound interaction with the manner in which that agent was applied to the biofilm. An analysis of the penetrative capacity of an agent showed this parameter was critical in the success of disinfection of a mature biofilm. Seven sets of fitted experimental data revealed the model to be flexible and adept at capturing the effects seen over a wide range of antimicrobial agents and biofilm species. Moreover, the physical system describing the antimicrobial challenge often was telling in deciding which model best fit the data.

While a specific mechanism mentioned above might dominate the resistance of a biofilm to a challenge, care should be taken before eliminating the others. The physical system surrounding the challenge and the qualitative features of the data often dictated an elimination of one or more models. These models also allow for a better measurement of an agent's true efficacy on a biofilm. It was also possible with these models to extrapolate from a disinfection experiment to another with dissimilar conditions.

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**MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana**

November, 1998

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ACKNOWLEDGMENTS

It is my belief that this single page, of all the pages herein, was the most difficult to write. Too many people are responsible, directly or indirectly, for the place, the time, and the purpose.

In all fairness, I owe a debt to my advisor, Phil Stewart, for the patience and wisdom necessary to see this work come to fruition. My committee members, Bonnie Tyler and Tim McDermott, also are owed their due. Despite the fact my research drastically changed in nature through the course of my time here, my committee continued to provide valuable help and insight. Sara Sanderson, a former graduate student at the Center for Biofilm Engineering, through her thesis, is also responsible for the inception of the ideas herein. Lest I forget, the Center for Biofilm Engineering students, faculty, and staff have made my time here an experience I will treasure.

My parents, Heather and Brian, provided the direction and caring it took to steer me to this place I find myself today. My friends, especially Jonathan and Alan, showed me much later in life that sometimes direction is unwanted, and that the wind will take you where it will.

I dedicate this work to my brother, Russell Brian Dodds, who, to my greatest delight, may be the brighter of us two.

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NOMENCLATURE

Symbol	Definition	Unit
C	Concentration of biocide agent	$\mu\text{g}_b\text{-cm}^{-3}$
C_b	Concentration of biocide agent in the bulk fluid	$\mu\text{g}_b\text{-cm}^{-3}$
D_e	Effective diffusion coefficient for biocide in biofilm	$\text{cm}^2\text{-sec}^{-1}$
Y_{xb}	Stoichometric conversion factor of biomass by biocide	$\mu\text{g}_x\text{-}\mu\text{g}_b^{-1}$
k_{dis}	Disinfection rate constant	$\mu\text{g}_b^{-1}\text{-cm}^3\text{-sec}^{-1}$
k_o	Zero-order reaction rate constant	$\mu\text{g}_b\text{-cm}^{-3}\text{-sec}^{-1}$
t_{pen}	Time needed to fully penetrate biofilm with biocide	sec
t_{dose}	Total time biocide applied to biofilm	sec
ϵ_s	Fraction of the biofilm with same physiology as planktonic counterpart	
X	Viable biomass density in biofilm after disinfection	$\mu\text{g}\text{-cm}^{-3}$
X_o	Viable biomass density in biofilm before disinfection	$\mu\text{g}\text{-cm}^{-3}$
z	Spatial coordinate, or depth, into the biofilm	cm
L_f	Total thickness of the biofilm	cm

p	Physiology parameter that describes the reduction of biocide disinfection efficacy on the resistant subpopulation of the biofilm	
ϕ	Thiele modulus, which indicates presence of transport limitations	
τ	Ratio of dose time to penetration time	
ψ	Combined dose parameter	
ζ	Unit-less spatial parameter	
a	Penetration depth into the biofilm	cm
t	Time	sec

ABSTRACT

The goal of this work was to develop and test a computer model capable of integrating mechanisms of biofilm resistance to disinfection by antimicrobial agents. Resistance mechanisms considered included retarded penetration due to a stoichiometric reaction between the antimicrobial agent and biomass, retarded penetration due to a catalytic reaction between the antimicrobial agent and the biomass, and the existence of a fraction of the cells in the biofilm in a resistant phenotype.

Mathematical models of these processes were derived and translated to a computer model, MATLAB. A formal sensitivity analysis resulted in the statement that the parameters used in these models were necessary to capture a hypothetical data set, but that, in some situations, fewer parameters were sufficient. The model predicted the effect of physiological changes in a subpopulation of the biofilm and demonstrated that even a small resistant subpopulation could potentially foil effective disinfection of that biofilm. The models also predicted that the physical mechanism employed by the antimicrobial agent had a profound interaction with the manner in which that agent was applied to the biofilm. An analysis of the penetrative capacity of an agent showed this parameter was critical in the success of disinfection of a mature biofilm. Seven sets of fitted experimental data revealed the model to be flexible and adept at capturing the effects seen over a wide range of antimicrobial agents and biofilm species. Moreover, the physical system describing the antimicrobial challenge often was telling in deciding which model best fit the data.

While a specific mechanism mentioned above might dominate the resistance of a biofilm to a challenge, care should be taken before eliminating the others. The physical system surrounding the challenge and the qualitative features of the data often dictated an elimination of one or more models. These models also allow for a better measurement of an agent's true efficacy on a biofilm. It was also possible with these models to extrapolate from a disinfection experiment to another with dissimilar conditions.

BACKGROUND

Biofilms, layers of attached microorganisms attached to a surface, are a ubiquitous part of aquatic systems. Although biofilms can play important and useful roles in many settings, much of the effort to manage biofilms has focused on removing or controlling unwanted biofilm. Biofilms foul, corrode, and degrade many industrial products (Costerton, et al. 1987; Notermans 1994; Lynch, et al. 1988). The negative impact of biofilms extends beyond measurement in dollars, as many diseases and infections are now recognized as being mediated by biofilms (Hoyle, et al. 1991). Research examining the efficacy of antimicrobial agents has resounded with the finding that many if not all antimicrobial agents currently in use are less effective against biofilms than they are against freely suspended microorganisms. If biofilms are ubiquitous in aquatic environments, then serious effort should be put forward to screen every method currently considered as effective against biofilms (Hoyle, et al. 1991; Jouenne, et al. 1994; Ntsama-Essomba, et al. 1997). This is a daunting task. As this information is collected, it must be combined with fundamental research into the mechanisms of biofilm resistance, yielding, it is hoped, improved

and novel methods that are effective against biofilms. Without mechanistic information, the best situation that can be envisioned are correlations that suggest ways to modify existing antimicrobial treatments.

The goal of this was to develop and test a computer model capable of integrating resistance mechanisms of a biofilm to disinfection by antimicrobial agents. In developing this model, it was important to review the current chemical-based strategies used to control a biofilm. Beyond this, it was important to appreciate the possible mechanisms that protect a biofilm from chemical challenges. Finally, the existing models of biofilm disinfection should be inspected for strengths and weaknesses.

Chemical Strategies for Disinfection of a Biofilm

The methods in place for removing and controlling a biofilm are not limited to chemical means. Indeed, mechanical methods are commonly used, often in conjunction with chemical treatments. In many situations, however, mechanical access to a site colonized by a biofilm is impractical, or the identification and location of that site is impossible. In these situations, a water-borne chemical agent is preferred. For the purposes of

this work, chemical agents will be categorized as either non-specific or specific agents. A non-specific agent is usually widely reactive (e.g. an oxidizer) and is not intended to attack a particular microbial population. Arguably, the active site of disinfection of many of these compounds can be traced and characterized, but the important defining factor in this division is that a non-specific agent will also interact with a wide range of other chemical entities. The other chemical category, specific agents, are those agents that are targeted or specifically tailored to interact with a specific microbial process (e.g. an antibiotic) with the intent of disrupting or modifying the outcome of that process.

Non-Specific or Reactive Agents

Many of the antimicrobial products in use fall into the category of non-specific agents. Oxidizing antimicrobials, such as chlorine and chloramines, are used in the drinking-water industry in great volumes, in an effort to control the biofilm colonization that inevitably occurs in those systems (Geldreich 1989). Other agents, such as quaternary ammonia compounds (QACs) and medically relevant antiseptics (e.g. biguanides)

should be considered as part of this category. Each of these agents, though used to impede microbes, also can have deleterious effects on the system around them, including induced corrosion, poor water quality, and halogenated bi-products (Booth, et al. 1995; Judd, et al. 1995; LeChevallier, et al. 1988). The use of these agents in a natural aqueous environment can be considered "messy," in that the agent can and will react with other entities besides the desired ones. Halogenated antimicrobial compounds are known to interact with metals, exacerbating corrosion (LeChevallier, et al. 1993). They may also interact with humics and other organic compounds, producing halogenated organics that are considered to pose health risks (Judd, et al. 1995). These agents are applied into the bulk fluid of the aqueous system, either continuously or periodically. It is expected that they will interact with the other components endogenous to the water environment before they reach the biofilm, participating in the kinds of reactions mentioned above. Within a biofilm, these unwanted reactions are amplified due to the increased density of components. The nature of these reactions will be explored in more detail later.

Specific or Targeted Agents

Targeted compounds are used most frequently in clinical situations, as the non-specific compounds mentioned previously are dangerous by their very nature to the health of the patients. Here, antibiotics and other chemotherapeutic agents come to the fore. These agents are designed to interact with a specific target within the microbe, without detrimental interactions in the host tissues. A dosage amount is prescribed for the agents, usually given in terms of minimum inhibitory concentration (MIC). The very unit of measurement lends insight in the drawbacks of these compounds: these agents are tested for the minimum dosage that inhibits microbiological function. The tacit statement is that these agents should be used at these levels, or the same kinds of health threats the non-specific agents present become a possibility. Moreover, this also implies that the delivery of less than the MIC will lead to an unsatisfactory treatment of the microbiological community (Schentag 1998). These agents are used most frequently as a treatment to existing clinical situations, though they can be used in a prophylactic manner in some

instances. In a biofilm, the MICs for which these agents are prescribed may become an issue, which will be examined below.

Biofilm Resistance to Disinfection

Many instances of partial or complete failure of traditional antimicrobial treatments, for both classes of compounds, appear in the literature (Anwar, et al. 1992; Gander 1996; Takeo, et al. 1994; Stickler, et al. 1991). Hypotheses have been put forward and tested, many with interesting implications. It is generally acknowledged that biofilms enjoy an increased resistance to most, if not all, antimicrobial efforts (LeChevallier, et al. 1988; Jones, et al. 1989; Hoyle, et al. 1991). The mechanisms for such resistance are still being elucidated, and it is likely that they will be complex and fundamental in nature. At first-pass, these mechanisms as they stand can be lumped into two categories: constitutive and adaptive.

Constitutive Resistance Factors

Constitutive resistance factors are inherent to the biofilm mode of bacterial systems, meaning that simply by assuming a biofilm phenotype, the microbes gain these resistance mechanisms. Efforts to unveil the “biofilm phenotype” of many microbes have shown that the protein expression, size, metabolism, and membrane proteins, to name but a few, all change when a microbe exists in a biofilm (Costerton, et al. 1995). It could be said that the microbe is “reinvented” in a biofilm. This distinct phenotype, along with the transport issues that arise with a biofilm, may go far to explain a biofilm’s resistance to antimicrobial agents.

Delivery of an Antimicrobial Agent The delivery of an antimicrobial agent to a biofilm is generally hampered by the physical environment within and without a biofilm. A biofilm is usually attached to a surface surrounded by a bulk fluid. The very geometry of this situation hampers transport of the agent, as the surface to which the biofilm is anchored is generally not permeable to the agent. Beyond this, transport into a cluster of biofilm cells is often diffusion-limited, meaning the agent is not convectively swept

into contact with the microbes; rather it must drift towards the microbes, driven by concentration gradients (Stewart 1994; Xu, et al. 1996). This phenomena, prevalent in other aquatic systems, is known as external mass transfer resistance, and tends to decrease the concentration of the antimicrobial agent at its surface. In the case of non-specific antimicrobials, reaction with the abiotic components, chiefly trapped particles, corrosion products, and exopolysaccharide, can reduce the amount of agent delivered to the microbes (Srinivasan 1994; Chen, et al. 1996). The last item, the polymer that serves to bind the biofilm together, also is known to react and bind classes of antibiotics (Anwar, et al. 1992). Once each of these obstacles has been overcome by the antimicrobial agent, reaction with the microbe itself serves to further deplete the supply of agent reaching the biofilm (Chesney, et al. 1996; Lynch, et al. 1998). Studies focusing on reactive, non-specific agents and their concentration profiles within a biofilm have shown that the transport of the agent into the biofilm cannot keep up with the reaction and neutralization within the film (Xu, et al. 1996; Chen, et al. 1996). It is important to note here again that this mechanism is not actively sought-out by the biofilm, as nutrient transport into the biofilm is also hampered.

Phenotype of a Biofilm Much of the physiology within a biofilm is still unelucidated. The variety of changes undergone by a microbe to adapt to its new environment is staggering. Sigma factors and stationary-phase protein expression seems to indicate that an individual microbe within the biofilm is relatively quiescent compared to its planktonic counterpart (Costerton, et al. 1995). This is very important when dealing with an antibiotic as a agent, as antibiotics generally function more effectively on a cell with a high metabolism, as it is the metabolism mechanism that is often targeted by these compounds (Anwar, et al. 1992). Studies examining the effect of biofilm age on the efficacy of both reactive and targeted agents have shown that as the biofilm ages, its resistance increases in general (Anwar, et al. 1992; Brown, et al. 1988). The transport issues discussed above, while protecting a biofilm from environmental stress, also diminish the availability of nutrients. This triggers quiescence in a cell, causing cell size and permeability to drop, while stress response gene expression increases (Anwar, et al. 1992; Brown, et al. 1988; Costerton, et al. 1995). The size and permeability of a cell changing comes as no shock, but the up expression of gene products designed to ward off the effects of environmental and metabolic stressors is somewhat

puzzling. These stress response genes seemed to be tied to alternate sigma factors that are expressed in stationary phase and under conditions of stress (Dempsey 1991; Lynch, et al. 1998). Membrane protein complements also shift, and some of these stress response products are expressed on the outer membrane and into the surrounding environment (Anwar, et al. 1992; Hoyle, et al. 1991). These physiological responses, while not necessarily intended to protect a biofilm member from an antimicrobial agent, are none the less effective against a wide range of agents.

Density Studies examining the relationship between antimicrobial activity and cell density within the biofilm show that this parameter is paramount in determining the outcome of the treatment (Brown, et al. 1993; Srinivasan 1994). This alone, when taking into consideration the fact that biofilm cell density is often several orders of magnitude higher than the densest planktonic suspension, is reason to question the traditional definition of an effective treatment. An antibiotic's MIC, in particular, is determined by testing against a standard density of cells.

Adaptive Responses

Adaptation to the environment around it determines the fate of a microbe. Biofilm microbes, like their planktonic counterparts, have similar mechanisms. The constitutive resistance mechanisms previously described are static features, or change slowly over time. Microbes also have the capacity to mount active responses to an environmental threat.

Stress Response Genes. Perhaps paramount in a biofilm's ability to resist an antimicrobial attack lies in a host of genetic systems collectively known as stress response genes. Two regulation systems that have been identified in *E. coli* as being triggered by oxidative stress are *oxyR* and *soxR*, whose gene products enzymatically convert hydrogen peroxide and superoxide, respectively, to non-lethal compounds, chiefly water and oxygen (Dempsey 1991; Lynch, et al. 1998). These genes are essential for any microbe undergoing oxidative metabolism, as reactive oxygen species are inevitable bi-products of that process. Other agents that are not specifically targeted by these gene products may still have some indirect enzymatic interactions (Dukan, et al. 1996c; Chapman, et al. 1993; Imlay, et al. 1988). Studies have shown that the lack of these genes greatly

reduces the survival rate of otherwise normal microbes (Chesney, et al. 1996; Dukan, et al. 1996a; Dukan, et al. 1996b; Imlay, et al. 1988). In theory, these stress response products are no more effective in a biofilm than in a planktonic suspension, but the constitutive mechanisms elucidated previously may have a synergistic effect.

Selection of Resistant Phenotypes A biofilm that survives a challenge by an antimicrobial agent is likely to be repopulated by the most successful members of that biofilm. Studies examining repeated dosing of a biofilm with the same agent have shown that the surviving biofilm, when allowed to re-grow, becomes more resistant to future attacks (Sanderson, et al. 1997; Stanley, et al. 1989). This phenomenon, originally noticed with the use of oxidative compounds, has now been shown to be an issue in medical situations, especially with burn victims (Bryan 1982). Indeed, industry concerns often rotate the type of biocide used on a biofilm to avoid this problem (Leder 1989; Cloete, et al. 1992).

Cooperation Within the Biofilm Recently, studies have shown that the effects of pheromone-like compounds are incredibly effective within a biofilm (Stickler, et al. 1998; Davies, et al. 1998). Likely, the increased

proximity and density of microbes within the biofilm facilitates this phenomenon. It is known that some pathogenic species use this kind of quorum sensing in their progression to a disease state: at first doing little more than reproducing until some critical population count occurs, then as one expressing virulence factors (Greenberg 1997; Winson, et al. 1995). Conceivably, a member of the biofilm community near the out surfaces could signal an antimicrobial attack through these kinds of pheromones, preparing the members further within the biofilm for the challenge.

Modeling Disinfection in a Biofilm

Biofilm modeling to date has largely been focused on growth, nutrient profiles, cell distribution, composition, diffusion effects, and other fundamental concepts. These kinds of models continue to be revised as more fundamental research is completed. With the complexities noted above, it comes as no surprise that models produced can often mire in complexity. Often, a subset of mechanisms is considered. While this may

seem grossly reductionist, these models make up for their simplicity with their utility.

Reactive Agents

Modeling the effects of reactive agents on biofilms has largely been restricted to incorporating the delivery problems associated with the physical structure of the biofilm (Chen, et al. 1996; Stewart, et al. 1996; Xu, et al. 1996). The concepts previously ascribed to the constitutive resistance of biofilms to the delivery of an adequate amount of antimicrobial agent have been incorporated into mass transport equations, and the results have been satisfactory, albeit incompletely so. This kind of model system goes far in representing the qualitative results seen in studies focusing on reactive antimicrobial agents. With these systems in hand, suggestions on how to alter traditional antimicrobial treatments have been possible (Stewart, et al. 1997). Reactive antimicrobial agents have long been selected for their ability to kill planktonic suspensions of microbes. Modeling of the effects of these agents on a biofilm has led to serious speculation on the appropriateness of this selection criteria. With

a biofilm system, the thickness of a biofilm and the penetrative ability of an agent seem to be the important factors in determining the success or failure of a treatment. It is these kinds of predictions that show the utility of modeling studies.

Targeted Agents

The medical field has examined the use of targeted agents on biofilms, and several model systems have been suggested (Anwar, et al. 1992; Dibdin 1992; Nichols 1994). Again, out of these studies, the traditional methods of selecting targeted agents fall short. The factors shown to dominate the outcome of a treatment are biofilm age and exopolysaccharide content. Just as reactive agents have been shown to interact with the abiotic polymer surrounding the microbes within, certain classes of antibiotics have been shown to interact in an analogous manner with the extracellular polymers. Again, this results in retarded delivery of the agent to the microbes within the biofilm.

MODEL

The full models derived here integrate a conceptual model of physiologically heterogeneous biofilm with one of two possible transport models.

Conceptual Model

The conceptual models considered in this project can be divided into three parts. The first portion of the conceptual model defines the physical parameters of the biofilm. The second and third are two alternative concepts dealing with how the antimicrobial agent reacts within the biofilm. It is intended with these two transport models to encompass the classes of antimicrobials discussed previously: non-specific and targeted. The bulk fluid in every case below is assumed to be well mixed, with a constant concentration of antimicrobial agent available.

Biofilm Conceptual Model

A one-dimensional model of a biofilm is assumed, which can be pictured as a uniformly thick slab resting on an inert surface. The total cell density is considered to be evenly distributed. No account for voids and abiotic components is made. The major deviation between this model and a traditional "slab" biofilm is that the population phenotype is divided into two categories: resistant and susceptible. The intent is to provide for a phenotypic variance described previously as a potential mechanism for biofilm resistance to antimicrobial treatments. The susceptible population, whose fraction of the population is denoted by ϵ_s on Figure 1, is just as susceptible as their planktonic counterparts to a chemical attack. The resistant cell fraction, denoted by ϵ_r on Figure 1, enjoys a reduced susceptibility.

Figure 1 - Conceptual diagram of biofilm

Stoichiometric Transport Submodel

A mathematically tractable set of equations describing the concentration of the antimicrobial agent is desired. It is assumed that the biofilm has a finite amount of neutralizing capacity, which will be depleted stoichiometrically as the antimicrobial agent penetrates the biofilm. This neutralizing capacity is a function of all the components within the biofilm

and is not necessarily confined to the biotic portion of the biofilm. In the case of a reactive, non-specific reactive agent, it can be assumed that the agent will react with the biofilm constituents and be depleted at a much faster rate than characteristic disinfection rate. The agent must deplete all the neutralizing capacity in a layer of biofilm before proceeding.

Figure 2 - Conceptual model of stoichiometric penetration submodel

Moreover, the agent will continue to react with the dead microbes until all reactive sites have been depleted. The biocide must, quite literally, “burn”

its way through the biofilm. As illustrated in Figure 2, as time passes, the penetration profile into the biofilm moves closer to the substratum. The mathematical form for the profile is assumed to be linear, as experimental efforts to measure reactive agent concentration within a biofilm have shown this feature (Chen, 1996).

Another assumption must be made for the case when the agent reaches the substratum. With little left to impede its progress, it is assumed that it rapidly rises to the bulk concentration throughout the biofilm. This represents a break-through, or saturation, situation.

Catalytic Transport Submodel

On the other end of the spectrum, it is expected that some agents will be more specific and only interact with the microbiological fraction of the biofilm. In this case, penetration is only retarded if the agent is acted upon by an enzymatic processes. Reaction-diffusion theory is called on to balance the flux of the agent into the biofilm with the enzymatic process. In this manner, a decaying profile is set up very rapidly within the film and progresses no further. The penetration depth can be calculated by use of

the Thiele modulus, a dimensionless parameter that compares the reaction rate and diffusion rate of the agent. As the ratio of these rates decrease, the penetration into the film increases, as shown in Figure 3. Unlike the stoichiometric model outlined above, the catalytic model encompasses the possibility that the antimicrobial agent will never fully penetrate the biofilm. If the Thiele modulus is greater than one, the agent will never reach the substratum, thereby leaving a fraction of the biofilm unchallenged.

Figure 3 - Conceptual model of catalytic penetration submodel

Derivation of Full Stoichiometric Model

The derivation of the full stoichiometric model begins with underlying assumptions. First, the planktonic disinfection model, well established in the literature, is assumed valid. The normal disinfection rate coefficient, k_{dis} , is stated as dependent on the depth into the biofilm. In the outer susceptible portion of the biofilm, the disinfection rate coefficient is that of a suspended planktonic culture. In the deeper resistant zone of the biofilm, it is defined as a fraction of the suspended culture disinfection rate coefficient. That fraction, denoted by p here, is smaller than one. Second, it is assumed that the antimicrobial agent will react with the biofilm constituents at a constant rate. This reaction is considered rapid, relative to the characteristic disinfection rate, and will draw the agent concentration to zero. Once the biomass has been reacted by the agent, it is unable to further neutralize any more biocide. Live and dead cells have the same capacity to react with the agent. Third, the cell distribution

within the biofilm is assumed to be homogenous and remain constant throughout the time span of interest in the disinfection process.

The governing description of disinfection is of the form:

$$\frac{dX}{dt} = -k_{dis}(z) \cdot X(t) \cdot C(z,t) \quad (1)$$

C varies in z and t . A rapid reaction rate leads to a linear concentration profile of C in z :

$$C(z,t) = \begin{cases} 0 & 0 \leq t \leq t_1 \\ C_b - C_b \cdot \frac{z}{a} & t_1 \leq t \leq t_{pen} \quad 0 \leq z \leq L_f \\ C_b & t_{pen} \leq t \leq \infty \end{cases} \quad (2)$$

The boundary conditions are expressed in the form of t , while the equation is in z . A conversion in the equation to time dependence is required. The penetration depth of the biocide, a , at a given time is defined as the depth into the film where the concentration of the biocide falls to zero, and can be found from a matching flux equation:

$$\frac{D_e \cdot C_b}{a} = \frac{X_b}{Y_{xb}} \cdot \frac{da}{dt}$$

The left-hand term describes the flux of antimicrobial agent through the layer of biomass in which the neutralizing capacity of the biomass has

been depleted. The right-hand term describes the rate of advance of the penetration front. When integrated once, with the initial condition $z=0$ at $t=0$, this yields:

$$\frac{a^2}{2} = \frac{D_e \cdot C_b \cdot Y_{xs}}{X_b} \cdot t$$

This is rearranged to give two very useful equations that describe the time needed to penetrate to a point within the biofilm:

$$a = \left(\frac{2 \cdot D_e \cdot C_b \cdot Y_{xs}}{X_b} \cdot t \right)^{1/2} \quad (3) \quad \text{and} \quad t = \frac{a^2 \cdot X_b}{2 \cdot D_e \cdot C_b \cdot Y_{xs}} \quad (4)$$

Returning to (1), integrating the case where $t \leq t_{\text{pen}}$, which includes the first two boundary conditions in (2):

$$\ln \left(\frac{X}{X_o} \right) = -k_{dis} \cdot \int_0^{t_1} 0 \cdot dt - k \cdot C_b \cdot \int_{t_1}^{t_{dose}} dt + k_{dis} \cdot C_b \cdot \int_{t_1}^{t_{dose}} \frac{z}{a} \cdot dt$$

Solving for the penetration depth, a , by substituting (3) into the integral, and drawing the constants derived from that outside the integral:

$$\ln \left(\frac{X}{X_o} \right) = -k_{dis} \cdot C_b \cdot (t_{dose} - t_1) + k_{dis} \cdot C_b \cdot \left(\frac{X_b}{2 \cdot D_e \cdot C_b \cdot Y_{xs}} \right)^{1/2} \cdot z \cdot \int_{t_1}^{t_{dose}} t^{-1/2} \cdot dt$$

The final unknown time parameter, t_l , can be found with the substitution of (4):

$$\ln\left(\frac{X}{X_o}\right) = -k_{dis} \cdot C_b \cdot \left(t_{dose} - \frac{z^2 \cdot X_b}{2 \cdot C_b \cdot D_e \cdot Y_{xs}} \right) + \dots$$

$$k_{dis} \cdot C_b \cdot \left(\frac{X_b}{2 \cdot D_e \cdot C_b \cdot Y_{xs}} \right)^{1/2} \cdot z \cdot 2 \cdot \left(t_{dose}^{1/2} - \left(\frac{z^2 \cdot X_b}{2 \cdot C_b \cdot D_e \cdot Y_{xs}} \right)^{1/2} \right) \quad (5)$$

To simplify this unwieldy expression, some grouping of constants will prove useful:

$$\psi \equiv k_{dis} \cdot C_b \cdot t_{dose} \quad \zeta \equiv \frac{z}{L_f} \quad \tau \equiv \frac{t_{dose}}{t_{pen}} = \frac{2 \cdot D_e \cdot C_b \cdot Y_{xs}}{L_f^2 \cdot X_b}$$

Which then transforms (5) into a nice form:

$$\ln\left(\frac{X}{X_o}\right) = -\psi + \frac{\psi}{\tau} \cdot \zeta^2 + \frac{2 \cdot \psi}{\sqrt{\tau}} \cdot \zeta - \frac{2 \cdot \psi}{\tau} \cdot \zeta^2$$

$$\ln\left(\frac{X}{X_o}\right) = -\psi \cdot \left(\tau^{-1} \cdot \zeta^2 - 2 \cdot \tau^{-1/2} \cdot \zeta + 1 \right)$$

$$\ln\left(\frac{X}{X_o}\right) = -\psi \cdot \left(\tau^{-1/2} \cdot \zeta - 1 \right)^2 \quad (6)$$

Before integration of (6) with respect to the spatial variable, ζ , can proceed, it is important to recognize that the "constant", ψ , is a function of ζ , as k_{dis} varies in ζ . This complicates the integration, the solution to be split into

cases. Additionally, several boundaries will hinge on the penetration depth into the biofilm, a , which was solved for using (3) previously. Rather than expand a into the bulky form as seen before, it is advantageous to use an alternative form of τ :

$$\frac{a}{L_f} = \left(\frac{2 \cdot D_e \cdot C_b \cdot Y_{xs}}{X_b \cdot L_f^2} \cdot t \right)^{1/2} = \tau^{1/2}$$

The cases for (6) then are:

$$\ln\left(\frac{X}{X_o}\right) = \begin{cases} 0 & 1 \geq \zeta \geq \tau^{1/2} \\ -\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^p & \tau^{1/2} \geq \zeta \geq 0 \end{cases} \quad \begin{matrix} \varepsilon_s \geq \tau^{1/2} \geq 0 \text{ and } t_{dose} \leq t_{pen} \\ 1 \geq \tau^{1/2} > \varepsilon_s \text{ and } t_{dose} \leq t_{pen} \end{matrix}$$

$$\ln\left(\frac{X}{X_o}\right) = \begin{cases} 0 & 1 \geq \zeta \geq \tau^{1/2} \\ -p \cdot \psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^p & \tau^{1/2} \geq \zeta \geq \varepsilon_s \\ -\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^p & \varepsilon_s \geq \zeta \geq 0 \end{cases}$$

Two new constants have been introduced into our system of equations.

The first, ε_s , is defined as the fraction of the biofilm cells that are as susceptible to a biocide as is a planktonic cell. The second, p , represents the inherent physiological resistance of a microorganism in a biofilm state and is applied to the disinfection parameter, k_{dis} , reducing the biocide's killing rate.

Integration of the system of equations with respect to the dimensionless spatial parameter, ζ , over the entire depth of the biofilm generates an average survival fraction. Rather than localize the results of these equations to a certain depth within the biofilm, it is preferable to look at the survival fraction of the entire biofilm. So, the average survival fraction is defined as:

$$\int_0^1 \frac{X}{X_0} \cdot d\zeta = \begin{cases} \int_{\tau^{1/2}}^1 \exp(0) \cdot d\zeta + \int_0^{\tau^{1/2}} \exp\left(-\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta & \epsilon_s \geq \tau^{1/2} \geq 0 \text{ and } t_{dose} \leq t_{pen} \\ \int_{\tau^{1/2}}^1 \exp(0) \cdot d\zeta + \int_{\epsilon_s}^{\tau^{1/2}} \exp\left(-p \cdot \psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta + \dots & 1 \geq \tau^{1/2} > \epsilon_s \text{ and } t_{dose} \leq t_{pen} \\ \int_0^{\epsilon_s} \exp\left(-\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta & \end{cases}$$

Some of the parts of this solution can be solved analytically, even though the forms above in general have no closed form solutions, through the use of variable substitutions, and the subsequent use of a convenient definition of the error function (Gradshteyn, et al. 1984):

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = \int_{\tau^{1/2}}^1 \exp(0) \cdot d\zeta + \int_0^{\tau^{1/2}} \exp\left(-\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta \quad \varepsilon_s \geq \tau^{1/2} \geq 0$$

$$\text{let } y = \psi^{1/2} \cdot (\tau^{-1/2} \cdot \zeta - 1)$$

$$dy = \psi^{1/2} \cdot \tau^{-1/2} \cdot d\zeta$$

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = 1 - \tau^{1/2} + \frac{1}{\psi^{1/2} \cdot \tau^{-1/2}} \cdot \int_{-\psi^{1/2}}^0 \exp(-y^2) \cdot dy$$

$$\text{with the identity } \frac{\sqrt{\pi}}{2} \cdot \text{erf}(z) \equiv \int_0^z \exp(-t^2) \cdot dt$$

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = 1 - \tau^{1/2} + \frac{\sqrt{\pi}}{2 \cdot \psi^{1/2} \cdot \tau^{-1/2}} \cdot \text{erf}(\psi^{1/2})$$

Though this particular solution seems very good, it is important to note that the error function, *erf*, approaches a value of one as the argument increases. So, if the argument, $\psi^{1/2}$, is greater than 2, the *erf* term is approximately equal to one. If this solution were applied to an integral with two non-zero bounds, the following form would be produced:

$$\int_a^b \exp(-y^2) \cdot dy = \int_0^b \exp(-y^2) \cdot dy - \int_0^a \exp(-y^2) \cdot dy = \frac{\sqrt{\pi}}{2} \cdot [\text{erf}(b) - \text{erf}(a)] \approx \frac{\sqrt{\pi}}{2} \cdot [1 - 1] \quad (7)$$

Again, this will occur with sufficiently large values of a and b . Through the variable transformation, the term $\psi^{1/2}$ will be included, which is inevitably

large. Recalling the definition of ψ , as t_{dose} increases, so will ψ . Therefore, substitution into (7) for a and b with a form containing $\psi^{1/2}$ will inevitably result in zero as a solution.

So, a numerical solution for the integrals should be used. Examining plots of the integrand reveals that the function is smooth and continuous, so many numerical solutions are available.

To examine the case where $t_{dose} > t_{pen}$, (2) must be solved for all three conditions, again going through all the machinations previously described:

$$\ln\left(\frac{X}{X_o}\right) = -k_{dis} \cdot \int_0^{t_1} 0 \cdot dt - k \cdot C_b \cdot \int_{t_1}^{t_{pen}} dt + k_{dis} \cdot C_b \cdot \int_{t_1}^{t_{pen}} \frac{z}{a} \cdot dt - k_{dis} \cdot C_b \cdot \int_{t_{pen}}^{t_{dose}} dt$$

$$\ln\left(\frac{X}{X_o}\right) = \begin{cases} -\frac{\Psi}{\tau} \cdot (\zeta^2 - 2 \cdot \zeta + \tau) & \epsilon_s \geq \zeta \geq 0 \quad \text{and} \quad t_{dose} \leq t_{pen} \\ -\frac{p \cdot \Psi}{\tau} \cdot (\zeta^2 - 2 \cdot \zeta + \tau) & 1 \geq \zeta > \epsilon_s \quad \text{and} \quad t_{dose} \leq t_{pen} \end{cases}$$

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = \int_0^{\epsilon_s} \exp\left(-\frac{\Psi}{\tau} \cdot (\zeta^2 - 2 \cdot \zeta + \tau)\right) \cdot d\zeta + \int_{\epsilon_s}^1 \exp\left(-\frac{p \cdot \Psi}{\tau} \cdot (\zeta^2 - 2 \cdot \zeta + \tau)\right) \cdot d\zeta$$

Once the solutions are combined, the final solution is:

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = \begin{cases} \int_{\tau^{1/2}}^1 \exp(0) \cdot d\zeta + \int_0^{\tau^{1/2}} \exp\left(-\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta & \varepsilon_s \geq \tau^{1/2} \geq 0 \quad \text{and} \quad t_{dose} \leq t_{pen} \\ \int_{\tau^{1/2}}^1 \exp(0) \cdot d\zeta + \int_{\varepsilon_s}^{\tau^{1/2}} \exp\left(-p \cdot \psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta + \int_0^{\varepsilon_s} \exp\left(-\psi \cdot (\tau^{-1/2} \cdot \zeta - 1)^2\right) \cdot d\zeta & 1 \geq \tau^{1/2} > \varepsilon_s \quad \text{and} \quad t_{dose} \leq t_{pen} \\ \int_0^{\varepsilon_s} \exp\left(-\frac{\psi}{\tau} \cdot (\zeta^2 - 2 \cdot \zeta + \tau)\right) \cdot d\zeta + \int_{\varepsilon_s}^1 \exp\left(-\frac{p \cdot \psi}{\tau} \cdot (\zeta^2 - 2 \cdot \zeta + \tau)\right) \cdot d\zeta & t_{dose} > t_{pen} \end{cases}$$

Q.E.D.

Derivation of Full Catalytic Model

The derivation of the full catalytic model begins with underlying assumptions. First, the planktonic disinfection model, well established in the literature, is assumed valid. The normal disinfection rate coefficient, k_{dis} , is stated as dependent on the depth into the biofilm. In the outer susceptible portion of the biofilm, the disinfection rate coefficient is that of a suspended planktonic culture. In the deeper resistant zone of the biofilm, it is defined as a fraction of the suspended culture disinfection rate coefficient. That fraction, denoted by p here, is smaller than one. Second, it is assumed that the antimicrobial agent will be reacted by an enzymatic process within the biofilm, and that this reaction is zero-order. This reaction is considered rapid relative to the characteristic disinfection

rate, and will draw the agent concentration to zero. Third, the cell distribution within the biofilm is assumed to be homogenous and remain constant throughout the time span of interest in the disinfection process.

The governing differential equation for a steady-state diffusion and reaction when the reaction follows zero-order kinetics is:

$$D_e \cdot \frac{d^2 C}{dz^2} = -k_o$$

Which, through the use of the following definitions:

$$u \equiv \frac{C}{C_b} \quad \zeta \equiv \frac{z}{L_f} \quad \phi \equiv \frac{k_o \cdot L_f^2}{2 \cdot D_e \cdot C_b} \quad (1)$$

becomes:

$$\frac{d^2 u}{d\zeta^2} = 2 \cdot \phi^2 \quad (2)$$

It is unknown what kind of penetration profiles to expect. Therefore, the differential equation must be integrated with two sets of boundary conditions:

$$\begin{cases} 1) u = 1 \text{ at } \zeta = 0 \\ 2) u = 0 \text{ at } \zeta = \frac{a}{L_f} \end{cases} \quad \text{when the biofilm is not fully penetrated.}$$

$$\begin{cases} 1) u = 1 \text{ at } \zeta = 0 \\ 2) \frac{du}{d\zeta} = 0 \text{ at } \zeta = 1 \end{cases} \quad \text{when the biofilm is fully penetrated.}$$

Where the penetration depth, a , is defined as the depth into the biofilm at which the antimicrobial agent concentration is zero.

Integrating (2) twice with respect to the spatial variable, ζ , using the boundary conditions above, produces the solutions:

$$\begin{cases} u = 0 & \frac{a}{L_f} < \zeta \leq 1 \\ u = \phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1 & 0 < \zeta \leq \frac{a}{L_f} \end{cases} \quad \text{when the biofilm is not fully penetrated.}$$

$$u = \phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1 \quad \text{when the biofilm is fully penetrated.}$$

$$\text{where } a \equiv \left(\frac{2 \cdot D_e \cdot C_b}{k_o} \right)^{1/2}$$

Once these parameters have been found, the basic planktonic disinfection model must be coupled with the predicted concentration profiles.

$$\frac{dX}{dt} = -k_{dis}(z) \cdot C(z, t) \cdot X(t)$$

This can now be transformed and integrated, using the substitutions in (1)

to arrive at:

$$\ln\left(\frac{X}{X_o}\right) = -\psi \cdot u(\zeta) \quad (3)$$

where, $\psi \equiv k_{dis} \cdot t_{dose} \cdot C_b$

Now appropriate substitutions to (3) with the solutions to $u(\zeta)$ should be made. Before this step can be carried out, it is important to realize that the "constant", ψ , is a function of ζ , as k_{dis} varies in ζ . This complicates the substitution, requiring that the solution be split into cases. Additionally, several boundaries will hinge on the peneration depth into the biofilm, a .

$$\ln\left(\frac{X}{X_o}\right) = \begin{cases} \begin{cases} -\psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1] & 0 \leq \zeta \leq \frac{a}{L_f} \\ 0 & \frac{a}{L_f} \leq \zeta \leq 1 \end{cases} & 0 \leq \frac{a}{L_f} \leq \epsilon_s \text{ and } \left(\frac{a}{L_f} < 1 \text{ or } \phi \geq 1 \right) \\ \begin{cases} -\psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1] & 0 \leq \zeta \leq \epsilon_s \\ -p \cdot \psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1] & \epsilon_s < \zeta < \frac{a}{L_f} \\ 0 & \frac{a}{L_f} \leq \zeta < 1 \end{cases} & \epsilon_s < \frac{a}{L_f} < 1 \text{ and } \left(\frac{a}{L_f} < 1 \text{ or } \phi \geq 1 \right) \\ \begin{cases} -\psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi^2 \cdot \zeta + 1] & 0 \leq \zeta \leq \epsilon_s \text{ and } \left(\frac{a}{L_f} = 1 \text{ or } \phi < 1 \right) \\ -p \cdot \psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi^2 \cdot \zeta + 1] & \epsilon_s \leq \zeta \leq 1 \text{ and } \left(\frac{a}{L_f} = 1 \text{ or } \phi < 1 \right) \end{cases} & \end{cases}$$

From this solution integration with respect to the spatial parameter, ζ , over the entire depth of the biofilm generates an average survival fraction:

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = \begin{cases} \int_{a/L_f}^1 \exp(0) \cdot d\zeta + \int_0^{a/L_f} \exp(-\psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1]) \cdot d\zeta & 0 \leq \frac{a}{L_f} \leq \varepsilon_s \text{ and } \left(\frac{a}{L_f} < 1 \text{ or } \phi \geq 1 \right) \\ \int_{a/L_f}^1 \exp(0) \cdot d\zeta + \int_{\varepsilon_s}^{a/L_f} \exp(-p \cdot \psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1]) \cdot d\zeta + \dots & \varepsilon_s < \frac{a}{L_f} < 1 \text{ and } \left(\frac{a}{L_f} < 1 \text{ or } \phi \geq 1 \right) \\ \int_0^{\varepsilon_s} \exp(-\psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1]) \cdot d\zeta & \\ \int_{\varepsilon_s}^1 \exp(-p \cdot \psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1]) \cdot d\zeta + \int_0^{\varepsilon_s} \exp(-\psi \cdot [\phi^2 \cdot \zeta^2 - 2 \cdot \phi \cdot \zeta + 1]) \cdot d\zeta & \frac{a}{L_f} = 1 \text{ or } \phi < 1 \end{cases}$$

Q.E.D.

Summary of Models

Two models have been developed that incorporate transport and physiological resistance mechanisms. From the full models, three other alternate submodels are possible. The first and second are reductions of the full stoichiometric and full catalytic models, and lack the physiological resistance mechanism described by ε_s and p . Functionally, these two parameters are set to unity. The two models, when transport phenomena

are removed, devolve into a case where physiology alone drives the biofilm's resistance to antimicrobial agents. As each model is simplified, the degrees of freedom (DOF) available are reduced, as depicted in Table 1.

Table 1 - Summary of Model DOF and Parameters Used

Model	DOF	Parameters	Explanation
Full Stoichiometric	4	$k_{dis}, t_{pen}, \epsilon_s, p$	Combined stoichiometric transport model and physiological resistance model
Full Catalytic	4	$k_{dis}, \phi, \epsilon_s, p$	Combined catalytic transport model and physiological resistance model
Physiology	3	k_{dis}, ϵ_s, p	Physiological resistance model
Transport Stoichiometric	2	k_{dis}, t_{pen}	Stoichiometric transport model
Transport Catalytic	2	k_{dis}, ϕ	Catalytic transport model

The physiology model, while not rigorously established here, has an analytical solution. As with the other models, it is of the general form of two decaying exponentials that are functions of ϵ_s , p , k_{dis} , C_b , and t_{dose} :

$$\int_0^1 \frac{X}{X_o} \cdot d\zeta = \varepsilon_s \cdot \exp(-\psi) + (1 - \varepsilon_s) \cdot \exp(-p \cdot \psi)$$

where $\psi \equiv k_{dis} \cdot C_b \cdot t_{dose}$

Model Solutions

The mathematical solutions were transformed into computer routines compatible with MATLAB, a general-use mathematical computer software package. Appendix D holds the bulk of this code. Where numerical integration was required, the trapezoidal rule was invoked. Again, examination of a plot of the integrands of the numerical solutions revealed a smooth and continuous function. The implementation of the trapezoidal rule used allowed for variable-width slices of the function, so greater precision in curved portions of the function was possible. An analysis of the accuracy of the trapezoidal rule approximation of the numerical integrals revealed that at least one hundred slices of the function were required, and greater than a thousand produced no appreciable benefit

(not shown). In most instances, a thousand discrete slices of the function was used as the default.

Experimental Data Fitting

Seven sets of experimental data were examined and fitted using the mathematical models previously described. A constrained minimization technique was used to establish the values for the parameters used in each permutation of the models. This was necessary because, as previously described, an analytical solution to the models was not generally possible. Generally, the analysis followed three steps. First, the user defined the data set, bulk fluid concentrations, and as many other parameters as possible given the available information (available in Appendix C). Second, the remaining parameters were constrained, or given maximum and minimum values, and initial guesses for the parameters were made. Third, a general fitting routine, Mainfit.m (available in Appendix D) was called to run each of the five models. The constrained minimization routine used, constr.m, was from the MATLAB Optimization Toolbox (version 5.2), and is copyrighted. Briefly, the minimization routine attempted to find the

parameter values within the ranges specified that minimized the sum of the squared residual error between the actual data and the predicted data, based on the parameters. This was an iterative process, and as the solutions to most of the models were of a numerical nature, computationally expensive. There existed the additional problem of local minima, a phenomenon where a model displayed a minimum at certain parameter values that was not the best minimum available. Accordingly, the initial guesses provided to the minimization routine were crucial to the success of this analysis.

When possible, independent measurements were used for as many parameters as possible. In most cases, the disinfection rate constant, bulk antimicrobial agent concentration, and dose times were the only parameters that could be selected using independent measurements. This leads to several degrees of freedom in many of the models. Obviously, as the degree of freedom value for a model increases, the likelihood of finding a "good" fit increases. To answer this problem, the Akaike Information Criterion (AIC) was invoked to evaluate the degrees of freedom

(DOF) and sum of the squared residual error (RSS) generated from fitting a number of experimental data points (n) (Venables, et al. 1994).

$$AIC = n \cdot \ln(RSS/n) + 2 \cdot DOF$$

As the AIC decreased, the model was deemed a better fit.

RESULTS

The statistical validation of this model is first presented. A formal sensitivity analysis of the parameters used in each penetration model was performed. The purpose of this was to verify that each parameter was necessary for the model to accurately describe the quantitative effects seen in an antimicrobial attack on a biofilm

The models previously described have value in predicting the qualitative and quantitative effects of each parameter. Moreover, the models when viewed graphically describe the kinds of regimes seen in disinfection of a biofilm. In the next section, the essential predictions are examined. The similarities and differences in these predictions are also presented.

Finally, seven sets of published disinfection data are examined, and the results are presented in the final section of this chapter. A variety of different antimicrobial agents are inspected, and both penetration submodels are tested.

A mathematical software package, MATLAB, was used to implement the models previously described (The MathWorks 1998).

Formal Sensitivity Analysis

It is important when testing a model to show that all the parameters are necessary. A formal sensitivity analysis is presented here to establish the necessity of each parameter in both penetration submodels. A statistical analysis software package, MINITAB, was used to carry out this analysis (Minitab 1998), the full results for which can be found in Appendix A. For each penetration submodel, a range of each parameter was selected, and the response to all the possible combinations of these parameters was calculated. The values chosen within the range of each parameter were chosen factorially, except in the case of the sensitive fraction parameter. The response, in the case of this model, is the predicted survival fraction of the microbes within the biofilm. This data was then fitted with the purely empirical polynomial function shown below, and a degree of error was produced based on the sum of the squared residual error.

$$Em(p_1 \dots p_N) = \sum_{i=1}^N a_i \cdot p_i^2 + \sum_{i=1, j=1}^N a_{i,j} \cdot p_i \cdot p_j + \sum_{i=1}^N a_i \cdot p_i$$

Each parameter was then dropped from this empirical model, and the error was recalculated. If the loss of one of the parameters does effect the

quality of the fit, then the model is considered sensitive to that parameter. However, if the model shows no reduction in quality of fit, then that parameter becomes suspect. Below, each submodel is examined, and the sensitivity of the response is examined by way of a cumulative percentage error plot, where the absolute values of the residuals generated by the empirical model are traced.

Stoichiometric Model Sensitivity Analysis

The values used in this sensitivity analysis are shown in Table 2. Each value used was selected factorially from a reasonable range, meaning the mean of the ends of the range was used. The exception was the sensitive fraction parameter, which was selected on a logarithmic scale.

Table 2 - Parameter Selection for Stoichiometric Sensitivity Analysis

Parameter	Values Used
t_{dose} [time]	19000,9300,600
k_{dis} [conc.-time] ⁻¹	1,0.5,0.00001
t_{pen} [time]	20000,10000,1
ϵ_s []	1,0.99,0.1
p []	0.1,0.05,0.00001

An effort was made to select dose times that reflected the penetration times used. In addition, the physiological resistance parameter was selected to roughly mirror the disinfection rate constant. This was done with the idea in mind that the penetration model operates in three distinct regimes. The first occurs when the dose time is much smaller than the penetration time. This equates to a heavily transport-limited situation, where the antimicrobial has insufficient time to penetrate the biofilm well. The second occurs when the dose time is nearly equivalent to the penetration time. This means that the antimicrobial has penetrated in the biofilm but has not yet saturated it. The third situation arises when the dose time is much larger than the penetration time, which results in a near-bulk antimicrobial concentration within the film. In each of these cases, the parameter sensitivity changes.

Dose Time Less than Penetration Time Figure 4 depicts the sensitivity of the empirical model to the loss of each parameter. Two parameters seem critical: the disinfection rate constant and the penetration time. The lack of the other terms in the empirical model does not affect the quality of fit.

Figure 4 - Sensitivity of stoichiometric empirical model when $t_{\text{dose}} < t_{\text{pen}}$

Dose Time Equivalent to Penetration Time Figure 5 shows that the disinfection rate constant is paramount in the quality of fit for the empirical model. However, the penetration time is less important. The physiological resistance and sensitive fraction parameters become important to the goodness of fit.

Figure 5 - Sensitivity of stoichiometric empirical model when $t_{\text{dose}} \sim t_{\text{pen}}$

Dose Time Greater than Penetration Time Figure 6 summarizes the parameters important to the empirical model in this case. The model has become insensitive to the penetration time, while the physiological resistance and sensitive fraction parameters are influential. The disinfection rate constant remains key to the quality of fit of the empirical model.

Figure 6 - Sensitivity of stoichiometric empirical model when $t_{dose} > t_{pen}$

Summary Of Parameter Sensitivity Table 3 summarizes the relative importance of each parameter for the cases above. The interpretation of these results will be discussed later in this work.

Table 3 - Sensitivity Summary for Stoichiometric Empirical Model

Parameter	$t_{dose} < t_{pen}$	$t_{dose} \sim t_{pen}$	$t_{dose} > t_{pen}$
k_{dis}	moderate	high	high
t_{pen}	moderate	low	none
ϵ_s	none	moderate	moderate
p	none	moderate	moderate

Catalytic Sensitivity Analysis

The values used in this sensitivity analysis are shown in Table 4. Each value used was selected factorially from a reasonable range. As previously, the exception is the sensitive fraction parameter, which was selected on a logarithmic scale.

Table 4 - Parameter Selection for Catalytic Sensitivity Analysis

Parameter	Values Used
t_{dose} [time]	500,255,10
k_{dis} [conc.-time] ⁻¹	0.01,0.005,0.00001
ϕ []	2,1,0.5
ϵ_s []	1,0.99,0.1
p []	0.1,0.05,0.00001

Again, the physiological resistance parameter was selected to roughly mirror the disinfection rate constant. The Thiele modulus range was selected to represent three distinct cases. The first, where the Thiele modulus is larger than one, describes a situation where the antimicrobial agent penetrates at a slower rate than it is consumed within the biofilm. This leads to poor penetration and subsequent lack of effect on the biofilm. The second, where the Thiele modulus is equal to one, occurs in a

condition of equality between the penetration rate and consumption rate. This means that the antimicrobial agent has a barely detectable concentration level at the base of the biofilm. The third case, where the Thiele modulus is less than one, depicts a situation where the antimicrobial agent freely penetrates the biofilm. Here, the biofilm is not saturated but will have some detectable level of antimicrobial agent throughout.

Thiele Modulus Less Than One Figure 7 depicts the sensitivity of the catalytic empirical model to each of the parameters. As before, k_{dis} is an important parameter, while the Thiele modulus is moderate in importance. The lack of the physiological resistance and sensitive fraction parameters in the empirical model does little to adversely effect the quality of fit, and can be considered of low and moderate importance in this transport regime, respectively.

Figure 7 - Sensitivity of catalytic empirical model when $\phi < 1$

Thiele Modulus Equal To One Figure 8 shows the effect of a balanced reaction rate and diffusion rate of the antimicrobial agent, indicated by the Thiele modulus equaling one. Here, the disinfection rate constant subsides from its greater importance in a less mass-transport resistance system. The Thiele modulus itself becomes an important parameter. Again, the two physiology parameters remain of little importance.

Figure 8 - Sensitivity of catalytic empirical model when $\phi = 1$

Thiele Modulus Greater than One Figure 9 illustrates the case where mass-transport resistance, indicated by the Thiele modulus, is the driving parameter. Again, the physiology parameters remain of moderate to low importance.

Summary Of Parameter Sensitivity Table 5 summarizes the relative importance of each parameter for the cases above. The interpretation of these results will be discussed later in this work.

Figure 9 - Sensitivity of catalytic empirical model when $\phi > 1$

Table 5 - Sensitivity Summary For Catalytic Empirical Model

Parameter	$\phi < 1$	$\phi = 1$	$\phi > 1$
k_{dis}	high	moderate	moderate
ϕ	moderate	moderate	high
ϵ_s	moderate	moderate	moderate
p	moderate	moderate	moderate

Model Predictions

The predictive capabilities of a model are important and, with this model, serve to suggest modifications and strategies to better disinfect a biofilm with traditional antimicrobial agents. Many predictions are possible with this model, but three essential ideas are presented here. The first idea revolves around the biofilm physiological state. The biofilm thickness, density, sensitive fraction, and physiological resistance are all parameters that require attention. Previous studies have already shown that biofilm thickness and density are of paramount importance (Brown, et al. 1993; Srinivasan, et al. 1995). The remaining parameters, sensitive fraction (ϵ_s) and physiological resistance (p), are inspected for their effects on the outcome of an antimicrobial challenge. The second idea focuses on dosing strategy. A great deal of the effort involved with disinfecting a biofilm is related to the question of how much antimicrobial agent to apply and for how long. The effect of the dosing concentration is, therefore, key, and its effects on both penetration models are illustrated below. The third concept is central to the properties of the antimicrobial agent within a biofilm. Previously, it was suggested that the antimicrobial agent's

penetrative ability might be critical to the outcome of a biofilm challenge. Again, each penetration submodel is examined for the effect of this property. The implications of these findings are discussed in a later section of this work. The full numerical values used for these predictions can be found in Appendix B.

Biofilm Physiology

In the absence of mass-transport resistance, the inherent resistance of the biofilm determines its fate under an antimicrobial assault. Figure 10 illustrates the importance of the sensitive fraction parameter, ϵ_s , on the survival fraction of the biofilm. As this parameter increases, more of the biofilm is inactivated by the agent. The other fraction of the biofilm population enjoys a much slower disinfection rate.

Figure 10 - Effect of sensitive fraction (ϵ_s) in the absence of mass transport resistance

The physiological resistance parameter, p , is also fundamental in understanding the survival of a biofilm under challenge by an antimicrobial agent. Figure 11 depicts the effects of this parameter on the outcome of an antimicrobial attack. In the absence of this parameter, the resistant fraction of the population reacts identically to the challenge. As this parameter increases, the resistant fraction enjoys a slower disinfection rate.

Figure 11 - Effect of physiological resistance (p) in the absence of mass transport resistance

Dose Concentration

It is intuitively obvious that simply raising the dose concentration without lowering the contact time of the agent leads to a better disinfection of a biofilm, as this insures a greater delivery of the agent. What is desired is to examine the effect of varying the dose concentration while maintaining a constant dose amount. Using the simple formula below, the

concentration (C_b) is varied and the time (t_{dose}) is varied inversely, yielding a constant dose amount.

$$\text{dose amount} = \text{dose concentration} \times \text{dose time [=]} \text{mass}$$

Figure 12 - Effect of bulk concentration on stoichiometric penetration model in the absence of physiology

Figure 12 shows the effect of this variation on the stoichiometric penetration model, in the absence of a resistant subpopulation within the biofilm. It is predicted, by this model, that the dose concentration has no effect on the disinfection of a biofilm, as long as the dose amount remains constant. Put simply, this model predicts that the antimicrobial agent's

concentration can be doubled with its contact time halved, and the same disinfection profile will be observed.

Figure 13 - Effect of bulk concentration on catalytic penetration model in the absence of physiology

In contrast, the effect of the dose concentration on the catalytic penetration model, depicted in Figure 13, is dramatic. It is predicted that the dose concentration must reach a threshold level before full penetration of the biofilm can occur.

Antimicrobial Penetration

Previously, it was argued that the penetrative ability of the antimicrobial agent would play an important role in the outcome of the challenge to a biofilm. Traditionally, agents have been selected based on their cost, disinfection rate constant, and other parameters.

The penetration of an agent under the stoichiometric penetration model is governed by several parameters. Here, the conversion constant between biocide and biomass, Y_{xb} , is considered. Keeping in mind that a more penetrative agent may be less effective at inactivating microbes, a simple ratio was used to ensure a level of fairness in this analysis. This ensures that as the conversion constant increases, the disinfection rate coefficient decreases.

$$Y_{xb,1} \cdot k_{dis,1} = Y_{xb,2} \cdot k_{dis,2}$$

Figure 14 - Effect of biocide penetration on stoichiometric model in the absence of physiology

Figure 14 illustrates the effect of selecting different conversion constants on the outcome of a challenge under the stoichiometric penetration model. Clearly, the larger this parameter, the more effective the antimicrobial challenge will be, even at the expense of lowering the disinfection rate coefficient proportionally in the process.

The penetration of an antimicrobial agent under the catalytic penetration model is affected by numerous parameters. Here, the zero-

order reaction rate constant, k_o , is inspected. Again, a ratio of the disinfection rate constant and reaction rate constant is used, in an attempt to make a fair analysis. This ensures that, as the zero-order reaction rate goes down, the disinfection rate coefficient follows.

$$\frac{k_{o,1}}{k_{dis,1}} = \frac{k_{o,2}}{k_{dis,2}}$$

Figure 15 shows the effect of varying the reaction rate constant on the outcome of a antimicrobial challenge under the catalytic penetration model. As with the stoichiometric model, the trend is clear. Improving the penetration of the agent, in this case by lowering the reactivity of the agent within the biofilm, is predicted to be quite effective against a biofilm.

Figure 15 - Effect of biocide penetration on catalytic model in the absence of physiology

Experimental data

In some cases, the nature of the antimicrobial agent used precludes the selection of a model based on physical improbability. In each case examined below, a summary table is provided listing the parameter values derived from the constrained minimization. If a parameter is not used for

this model, the notation “N/A” is used to indicate this. Additionally, models of note are displayed in a figure. These figures show the average survival fraction (log reduction) plotted versus the dose time. When multiple concentrations are considered, the dose time is multiplied by the dose concentration. For the purposes of consistency, full models are uniformly displayed as a solid line, a physiology model is displayed as a dotted line, and a transport-alone model is displayed as a dashed line. For a full description of the data sets and parameters used and estimated, consult Appendix C.

Tobramycin Challenge on a *Pseudomonas aeruginosa* Biofilm

The data used in this analysis comes from Anwar et al. and represents an antimicrobial challenge to an aged *P. aeruginosa* biofilm isolated from a C-F patient’s lung, with tobramycin (20 mg/L) used as the antimicrobial agent (Anwar, et al. 1989a; Anwar, et al. 1989b). The first data set, shown in Figure 16, is a disinfection time series over five hours on a two-day-old

biofilm, grown in a chemostat under iron limitations. The physiology, full catalytic, and catalytic transport models are shown.

Figure 16 - Survival fraction of young *P. aeruginosa* biofilm treated with tobramycin. Log survival fraction (Anwar et al., 1998a&b) is plotted versus time (*). Models shown are full catalytic (solid), physiology (dotted), and catalytic transport (dashed).

Table 6 summarizes the parameter values used with all models and the resulting AIC values. Clearly, the full catalytic model ranks the best by the AIC method.

Table 6 – Results For Tobramycin Challenge to Young *P. aeruginosa*

Model	AIC	K_{dis}	t_{pen}	ϕ	ε_s	p
Full Cat.	-36.72	1.18E-04	N/A	0.922	0.98824	1.00E-04
Physiology	-24.56	3.36E-05	N/A	N/A	0.97426	6.72E-02
Full Stoich.	-22.56	3.36E-05	1.02	N/A	0.97425	6.73E-02
Trans. Cat.	-12.48	1.18E-03	N/A	0.998	N/A	N/A
Trans. Stoich.	3.04	1.55E-05	1	N/A	N/A	N/A

Figure 17 - Survival fraction of old *P. aeruginosa* biofilm treated with tobramycin.. Log survival fraction (*) (Anwar et al., 1998a&b) is plotted versus time. Models shown are full catalytic (solid), physiology (dotted), and catalytic transport (dashed).

In the same publications, the authors also looked at the effect of aging a biofilm on the disinfection of the biofilm. Because of this, another data set, that of a seven-day-old biofilm was available. Figure 17 shows this new time series. The authors noted that a ten-fold increase in tobramycin concentration was required to achieve even a ten-fold less disinfection of the older biofilm. With this in mind, all the parameters selected for the previous data set were passed to this data set, and the normally loose constraints allowed for the parameters were tightened. The exception to this was the sensitive fraction parameter, which was allowed wider latitude. Table 7 shows the values used to fit each model, though only the full catalytic, physiology, and catalytic transport models are plotted on Figure 17. The AIC values in Table 7 show the catalytic transport model to be superior.

Table 7 - Results For Tobramycin Challenge to Old *P. aeruginosa*

Model	AIC	k_{dis}	t_{pen}	ϕ	ϵ_s	p
Trans. Cat.	-31.87	5.00E-06	N/A	0.986	N/A	N/A
Full Cat.	-30.09	9.54E-05	N/A	0.978	0.35107	4.27E-02
Physiology	-28.42	1.54E-05	N/A	N/A	0.47865	2.65E-02
Full Stoich.	-26.42	1.54E-05	1	N/A	0.47865	2.65E-02
Trans. Stoich.	-12.18	6.56E-07	1	N/A	N/A	N/A

Hydrogen Peroxide Challenge on a *Pseudomonas aeruginosa* Biofilm

The data used in this analysis is unpublished work by Jim Elkins, a Master's degree candidate in the Microbiology department at MSU, and represents a hydrogen peroxide challenge (50mM) to a *P. aeruginosa* grown on an inclined plane under drip-flow conditions (Elkins 1998). The thickness of the biofilm was estimated at 114 μ m, and an attempt was made to estimate the Thiele modulus, which can be found in Appendix C. The Thiele modulus estimation ranged from 1.92 to 4.98. The difference between these two values lies in the assumption of an external mass-transfer layer, which increases the Thiele modulus accordingly. This external mass-transfer layer, also known as a "stagnant layer," lies above

the biofilm and lacks convective transport. Accordingly, the hydrogen peroxide concentration is dampened at the biofilm surface, and a reduced delivery of the agent is expected. Planktonic disinfection data provided an estimate of the disinfection rate coefficient, and a mean value of $2.68\text{E-}2$ (mM-sec^{-1}) was selected, though this value was allowed to vary by 10%. Again, these calculations are described in detail in Appendix C. With the proper constraints in place, another constrained minimization was attempted, and Table 8 summarizes the values obtained from the fit of each model to the data. The predicted average survival fraction with error bars using the two Thiele modulus bounds is shown in Figure 18, and the value obtained from an error minimization predicts an intermediate curve, also shown. The AIC values therein indicate the physiology model is an appropriate choice for best fit. Figure 19 shows the mean data values from three sets of data, and the full catalytic, physiology, and catalytic transport models.

Table 8 - Results for Peroxide Challenge to *P. aeruginosa*

Model	AIC	k_{dis}	t_{pen}	ϕ	ϵ_s	p
Physiology	-15.47	2.95E-02	N/A	N/A	0.25674	1.00E-07
Trans. Cat.	-14.51	2.95E-02	N/A	2.27	N/A	N/A
Full Stoich.	-13.47	2.95E-02	1	N/A	0.25675	1.00E-07
Full Cat.	-11.09	2.95E-02	N/A	1.92	0.28323	5.83E-03
Trans. Stoich.	-8.22	3.35E-03	1.02	N/A	N/A	N/A

Figure 18 - Survival fraction of *P. aeruginosa* biofilm treated with hydrogen peroxide. Log survival fraction data (Elkins) plotted versus time (squares) with upper and lower values as error (plus). ϕ bounds are shown (solid), and the ϕ minimizing residual error is shown (solid/dotted)

Figure 19 - Survival fraction of *P. aeruginosa* biofilm treated with hydrogen peroxide. Log survival fraction data (Elkins) plotted versus time (*) with fitted models: full catalytic (solid), physiology (dotted), and catalytic transport (dashed)

Chlorhexidine Gluconate Challenge on a MRSA *Staphylococcus aureus* Biofilm

The data presented in this analysis comes from Oie et al. and represents a chlorhexidine gluconate (0.1, 0.5, and 1.0 %wt) challenge to methicillin-

resistant *Staphylococcus aureus* grown on silicone disks for twenty-four hours (Oie, et al. 1996). The species were a mix of clinical isolates. While the authors did not report the thickness of the biofilms, it can be assumed, based on the brief incubation time, that the biofilm was no more than a few cell layers thick. Table 9 summarizes the results from a constrained minimization. The AIC values for each model indicate the physiology model is the best fit to the data. Figure 20 depicts the survival data and two model curves: physiology and stoichiometric transport.

Table 9 - Results For Chlorhexidine Challenge to MRSA *S. aureus*

Model	AIC	k_{dis}	T_{pen}	ϕ	ϵ_s	p
Physiology	2.78	3.08E-02	N/A	N/A	0.99367	2.60E-02
Full Stoich.	4.78	3.10E-02	2.07	N/A	0.99367	2.59E-02
Full Cat.	4.78	3.07E-02	N/A	0.063	0.99368	2.62E-02
Trans. Cat.	11.78	1.00E+00	N/A	0.999	N/A	N/A
Trans. Stoich.	29.45	3.12E-03	1	N/A	N/A	N/A

Figure 20 - Survival fraction of *S. aureus* biofilm treated chlorhexidine gluconate. Log survival fraction data (Oie, et al. 1996) plotted versus time (*) with fitted models: physiology (dotted) and stoichiometric transport (dashed)

Benzalkonium Chloride Challenge on a *Listeria monocytogenes* Biofilm

The data fitted to the models comes from Frank et al. and is of a benzalkonium chloride (BAC) challenge to a *L. monocytogenes* biofilm grown on glass slides for fourteen days (Frank, et al. 1990). The authors noted between 10^6 and 10^7 cfu/cm² on these glass slides. Three different

concentration challenges were given to the biofilm: 100ppm, 400ppm, 800ppm. Hence, the survival fraction data shown on Figure 21 has been plotted versus the dose concentration times the dose time, which in effect is the dose amount. The graph includes two model curves: stoichiometric transport and physiology. Table 10 reviews the values used for each model. Again, the AIC values point to the physiology model as the best fit for this data.

Table 10 - Results for BAC Challenge to *L. monocytogenes*

Model	AIC	k_{dis}	t_{pen}	ϕ	ϵ_s	p
Physiology	25.66	2.29E-06	N/A	N/A	0.99894	2.25E-03
Full Stoich.	27.65	2.36E-06	100	N/A	0.99894	2.19E-03
Full Cat.	27.66	2.28E-06	N/A	0.001	0.99894	2.26E-03
Trans. Stoich.	69.44	2.00E-08	40.2	N/A	N/A	N/A
Trans. Cat.	71.13	1.59E-08	N/A	0.001	N/A	N/A

Figure 21 - Survival fraction of *L. monocytogenes* biofilm treated with benzalkonium chloride. Log survival fraction data (Frank, et al. 1990) plotted versus time (*) with fitted models: physiology (dotted) and stoichiometric transport (dashed)

Monochloramine Challenge on a *Pseudomonas aeruginosa* Biofilm

The data examined here is from Rohini Srinivasan, a Master's degree graduate in the Chemical Engineering department at MSU, and illustrates a monochloramine (4 mg/L) challenge to a *P. aeruginosa* biofilm grown to an initial aerial density of 1.1×10^8 cfu/cm² on stainless steel slides (Srinivasan

1994). This represents a substantial biofilm, and it is expected that mass transport will be an issue in this antimicrobial treatment. Figure 22 shows the survival fraction data and three models: full stoichiometric, physiology, and stoichiometric transport. Again, a summary of the values used in each model is provided in Table 11. The full stoichiometric model enjoys a good AIC value and should be regarded as the best fit for this data.

Table 11 - Results for Monochloramine Challenge to *P. aeruginosa*

Model	AIC	k_{dis}	t_{pen}	ϕ	ϵ_s	p
Full Stoich.	-19.03	1.93E-03	551	N/A	0.95018	1.55E-01
Physiology	-14.12	8.21E-04	N/A	N/A	0.91337	3.57E-01
Full Cat.	-12.11	8.23E-04	N/A	0.069	0.91328	3.58E-01
Trans. Cat.	-11.17	4.98E-03	N/A	0.97	N/A	N/A
Trans. Stoich.	2.06	4.19E-04	1	N/A	N/A	N/A

Figure 22 - Survival fraction of *P. aeruginosa* biofilm treated with monochloramine. Log survival fraction data (Srinivasan 1994) plotted versus time (*) with fitted models: full stoichiometric (solid), physiology (dotted), and stoichiometric transport (dashed)

Sodium Hypochlorite Challenge on an artificial *Pseudomonas* *aeruginosa* Biofilm

The data used here was obtained from Kate Grobe, a Master's degree candidate in the Chemical Engineering department at MSU, and represents three data sets of a sodium hypochlorite (~10 mg/L) challenge to an alginate-entrapped *P. aeruginosa* biofilm (Grobe 1998). Care was taken to

ensure the cells within the alginate beads were physiologically adjusted to their environment. The author noted an average cell density of 6.75×10^9 cfu/mL. The beads were large (~2mm), so transport phenomena are again predicted to be a factor in this treatment, as other investigators have reported similar findings (Chen, et al. 1996). Figure 23 shows the survival fraction, again plotted against dose amount, and the three models of interest: full stoichiometric, physiology, and stoichiometric transport. Table 12 summarizes the values used in each model case. The AIC values show that the full stoichiometric model is marginally better than the stoichiometric model at fitting this data.

Table 12 - Results for Hypochlorite Challenge to *P. aeruginosa*

Model	AIC	k_{dis}	t_{pen}	ϕ	ϵ_s	p
Full Stoich.	37.34	1.94E-01	5.80E+04	N/A	0.92398	1.00E-04
Trans. Stoich.	37.51	2.27E-05	5.80E+04	N/A	N/A	N/A
Trans. Cat.	44.48	9.75E-06	N/A	0.132	N/A	N/A
Physiology	46.48	7.97E-02	N/A	N/A	0.001	1.21E-04
Full Cat.	48.48	8.16E-02	N/A	0.033	0.001	1.18E-04

Figure 23 - Survival fraction of *P. aeruginosa* artificial biofilm treated with sodium hypochlorite. Log survival fraction data (Grobe) plotted versus time (*) with fitted models: full stoichiometric (solid), physiology (dotted), and stoichiometric transport (dashed)

Summary

From the preceding analysis, it is clear that no one model fits every set of data. With the incorporation of the AIC into this analysis, and the subsequent penalization of the full models compared to the other reduced models, it appears that the reduced models may provide adequate utility in fitting some data sets. Judging by the AIC values, a trend develops when

examining several sets of dissimilar data. If a transport-only model (stoichiometric and catalytic transport) appears to be in the best few fits to the data, then that transport mechanism is likely involved in that experiment. If the physiology-only model appears similarly, then physiological resistance is indicated. If both processes are involved, then one of the full models will appear in the best few fits, only edging out the reduced models if the fit it provides is clearly superior. If the transport-only models or the physiology-only model appear to have the worst AIC values, then those mechanisms are suspect. Likewise, the full models that combine those concepts become suspect.

DISCUSSION

The results described previously require some interpretation, especially when considering the fits to experimental data. The sensitivity analysis performed showed the necessity of each of the hypothesized. However, it became clear that less than the necessary number of parameters could be sufficient in some cases. Here, the concepts of sufficient and necessary will be examined. The predictions offered by the models should be discussed, as they provide insight into the qualitative shapes of disinfection data later seen in the experimental section. Finally, the experimental data will be examined closely.

Sensitivity Analysis

A formal sensitivity analysis of the derived models established the necessity of each of the model parameters. While much of the data used in this analysis was previously shown, Appendix A holds the MINITAB programming macro and the survival fractions of the hypothesized data set. From Appendix A, a re-creation of the MINITAB analysis is possible.

The following discussion addresses the question of necessary parameters versus sufficient parameters, as well as drawing conclusions to indicate the physical conditions required to consider each model.

Full Stoichiometric Model

When the dose time is less than the penetration time, transport phenomena dominate the sensitivity analysis. As the dose time approaches the penetration time, the penetration time becomes increasingly unimportant. At the saturation point, the penetration time becomes insignificant in predicting the survival fraction within the biofilm. In this situation, the disinfection rate coefficient is still important, and the two physiology parameters become important. As shown in Table 1, these three parameters are necessary to describe a purely physiological model.

The time scale of the disinfection process determines the importance of these two submodels (stoichiometric transport and physiology). Given a dose time that is less than the penetration time, transport phenomena will dominate the disinfection process. On a longer time scale, the agent will eventually penetrate and inactivate all but the resistant subpopulation.

The stoichiometric model can be stated as being sensitive to each parameter used in it, but each parameter is made necessary by the physical circumstances surrounding the antimicrobial treatment. In other words, each parameter is necessary, but fewer may be sufficient.

Full Catalytic Model

Because of a rapidly established concentration profile, the time scale of the antimicrobial agent becomes less of an issue than in the previous model. However, the issue of transport limitation remains. In this model, the extent of transport limitation is embodied in the Thiele modulus.

When this model is transport-limited, the Thiele modulus is the most important parameter. Inversely, if mass transport is not a factor, the model becomes most sensitive to the disinfection rate coefficient. In all but the most mass-transport-limited systems, penetration into the resistant subpopulation is likely. Hence, the two physiology parameters are always of some consideration, at least given the conditions used in Table 4. Those values could be modified to bring out two different regimes, as in the case of the previous model. However, to do so is to invite the question

of manipulating the sensitivity analysis to a certain end. Rather than undertaking this somewhat dubious method, the same arguments concerning the regimes in which this model operates should be considered. If the system is predominantly mass-transfer-limited, then the disinfection rate coefficient and Thiele modulus will likely be the drivers in the sensitivity of the model. In other words, the full catalytic model could be reduced to the model described as "Catalytic Transport" in Table 1. Likewise, if the system is not transport-limited, the Physiology model becomes adequate.

Again, the catalytic model is sensitive to each of the parameters, though the physical situation of the antimicrobial treatment may dictate fewer are necessary.

Model Predictions

While not intuitively obvious, the few predictions made in this work provide much of the framework needed to understand the complexities that follow with the fitted experimental data.

Biofilm Physiology

In the situations described above where mass transport is not a dominant or even evident factor in a biofilm's resistance to an antimicrobial challenge, the two physiology parameters along with the disinfection rate provide a simple, yet effective, method in modeling the survival of a biofilm.

Another notable result to come from this prediction is that of a "true" disinfection rate coefficient, p^*k_{dis} , against a biofilm. Measured on planktonic cells, the disinfection rate coefficient is a gross overestimation of the effectiveness of an agent on a mature biofilm. The Physiology model provides a clear and simple method for evaluating the true efficacy of an antimicrobial agent on a biofilm. As the number is typically one to four orders of magnitude (shown later in Table 13) less than that on a planktonic counterpart, it is small wonder why biofilms seem so hardy and resist antimicrobial agents so well. In the discussion on the experimental data that follows, this "true" disinfection rate coefficient will be established for the agents.

Dose Concentration

The two predictions made with these models show that the actual mechanism of interaction between a biofilm and an agent is critical. In this case, the stoichiometric model predicts an insensitivity to dose concentration, providing the dose amount is constant. Were an agent to actually enjoy this mechanism, the amount of agent used to treat a biofilm could be drastically lowered. Unfortunately, this has not been borne out in practice. While this behavior cannot be predicted by the stoichiometric transport model, it can be captured by the catalytic model.

In the case of the catalytic transport model, the dose concentration determines penetration depth into the biofilm. Even if the dose amount is kept constant, the inactivation of a biofilm is always more advanced when a higher concentration is used, even for a shorter time.

Considering the fact that these two models were designed to cover a variety of chemical agents, it is not surprising that they differ in their predictions in this matter.

Agent Penetration

The two transport models seem to agree in the matter of agent penetration. In the case of the stoichiometric transport model, the biocide-to-biomass conversion constant, if improved, predicts a greatly increased efficacy in penetrating the biofilm. The catalytic transport model, where the penetration is mediated by the zero-order enzymatic reaction rate, predicts a similar trend if this parameter is decreased. In both of these models, the disinfection of a biofilm is dominated by the antimicrobial agent's ability to penetrate the biofilm. Therefore, it seems natural that an agent which better penetrates the biofilm will have greater impact.

What is not obvious, and for that reason expressly explored in the results, is that an agent that suffers a reduction in the disinfection rate coefficient will still prove superior. While the conclusion that an agent which penetrates a biofilm better will better disinfect that biofilm seems obvious, the historical selection of an antimicrobial agent only takes into account the disinfection rate coefficient. Based on these predictions, it is

evident that a balance between disinfection and penetration should be struck for an agent to be effective on a biofilm.

In summary, the two models predict that an agent which better penetrates the biofilm, either through a higher agent/biomass conversion constant or through a lower degradative enzymatic rate, will better inactivate that biofilm.

Experimental Data

Short of attempting to measure and verify the physical reality of the parameters used in this model system, the best way of establishing the credibility of this model is to attempt to use it to describe the qualitative and quantitative behavior seen in actual antimicrobial challenges to a biofilm.

Tobramycin Challenge on a *Pseudomonas aeruginosa* Biofilm

As stated before, the polysaccharide matrix of a biofilm is a known binding agent of aminoglycosides. Moreover, there is some evidence that an enzymatic pathway is available in pseudomonads that can degrade these classes of agents (Bryan, 1982). Admittedly, this is only evidenced so far in long-term burn wounds and urinary tract infections. The resistance mechanism there is plasmid and transposon mediated, and strains carrying degradative enzymes are selected by chronic use of aminoglycosides in topical solutions. However, given the chronic nature of a C-F lung infection and that repeated use of antibiotic therapy is likely, the idea is worth consideration. The physical situation suggests that the predominant transport model will be catalytic in nature. The nature of mucoid pseudomonads, particularly this species, also suggests that many physiological changes occur in the transition to a biofilm state. Therefore, the idea that physiological resistance may be a factor in this experimental system should also be considered a likelihood.

Table 6 clearly shows that the full catalytic model is superior, in terms of AIC value, to any other model attempted. However, the Thiele modulus

required by this model to fit the data is approximately one. Recall that a Thiele modulus of one is equivalent to a situation where the agent has a barely detectable concentration at the biofilm/substratum interface. When the Thiele modulus is just under one, the agent has a slight concentration level at the substratum. Such a physical circumstance seems highly improbable. Indeed, examining the pure catalytic transport model reveals an unimpressive fit. The second-best AIC number can be attributed to the physiology model. However, the physiology model, if examined in the limit as the dose time goes to infinity, cannot fit the asymptotic approach to some maximal kill that seems to be evidenced by the data. The third candidate, the full stoichiometric model, cannot fit the qualitative data trend adequately.

Without further data to examine, a conclusive statement regarding the "best" model for this data cannot be made. However, the full catalytic model and the physiology model are the strongest.

In the same experimental system, but with an older biofilm (Table 7), a role for the catalytic transport model is implied by the ranking of the models. The first item in the table, the catalytic transport model, barely hedges the full catalytic model, indicating that transport phenomena are

becoming increasingly dominant. The third-ranked physiology model remains a strong candidate.

The two data sets, when considered together, imply a great deal about the kind of interaction tobramycin has with a biofilm of this species. In the case of the two-day-old biofilm, it seems clear that physiology alone is enough to accurately describe the survival of the film. Moreover, the model predicts that 97.4% of the biofilm is in a sensitive state, thus easily disinfected. As the biofilm ages and grows thicker, some transport limitation should be expected. This is played out in the case of the seven-day-old biofilm, as both variations of the catalytic come to the fore.

The selection of parameters for each data set illustrates the utility of this modeling system. Recall that the parameters gained from the two-day-old biofilm were passed to the model of the fourteen-day-old biofilm. Examination of the disinfection rate coefficient and physiological resistance factor shows that these values changed less than an order of magnitude. The susceptible fraction parameter, however, decreased from 97.4% to 35-45%. This makes sense, as this implies that, as a biofilm grows and ages, more of the population enters this resistant phenotype. With several of these data sets, it becomes possible to track the migration

of the population within the biofilm to this resistant state. The utility of this is profound, as an agent can be screened against a growing biofilm to determine when the agent becomes inadequate against the biofilm.

Researchers have often noted that a biofilm must be suppressed before it matures. This modeling system offers a way to collect data and determine that point in time.

Hydrogen Peroxide Challenge on a *Pseudomonas aeruginosa* Biofilm

Hydrogen peroxide is known to be enzymatically converted to oxygen and water by several enzymes, collectively known as peroxidases or catalases. The biofilm thickness, and the abundance of catalase in a *Pseudomonas spp.* Biofilm, make it very likely that the catalytic transport model will be in evidence in this system. The age of the biofilm, and the thickness, also make it likely that a physiology gradient might exist. However, the penetration depth into the film, given the relatively high value for the calculated Thiele modulus range, indicates that it is unlikely to even have a measurable peroxide concentration in the resistant fraction of the biofilm.

The physiology model, judging by the AIC value from Table 8, is nominally a better choice for fitting this data. However, the physiological resistance parameter is very high. In fact, the value for this parameter is at the maximum value used for the constraints of this parameter.

Accordingly, this model must be judged suspect for that reason alone.

The next best fit, again using the AIC as a guideline, is the catalytic transport model. Independent calculations show this to be very likely. The third selection, the full stoichiometric model, should be discarded because, as previously suggested, a catalytic transport system is predicted for this antimicrobial agent. The fourth model, the full catalytic model, is a combination of the first two models and falls short only due to the extra degree of freedom penalized for by the AIC system.

Considering the physical situation and the independent calculations made, the catalytic transport model shows the best fit to this data. It is important to note that, in Figure 19, the error in measurement (not shown, though shown in Figure 18) makes all three models plotted reasonable candidates. Examining the vertical axis shows that the measured reduction in survival is very small, and even minor error in the data gives rise to a large variance, as seen in Figure 18. Were the time scale for this

experiment extended, and an asymptotic approach to a maximal kill shown, the two catalytic models would then become the only possible choices, and the physiology model could be discarded for that reason.

Chlorhexidine Gluconate Challenge on a MRSA
Staphylococcus aureus Biofilm

The physical situation in this data set clearly paves the way for the physiology model to best describe this data. With only twenty-four hours of biofilm growth, the expected thickness of this biofilm is at most a few cells. Therefore, transport limitation is unlikely.

The physiology model is ranked as the best model, based on the low AIC value in Table 9. Figure 20 shows that the data is fitted well by this model. It is important to note that the sensitive fraction, which is greater than 99%, is quickly killed by the agent. The remaining fraction of 1%, however, is more than two orders of magnitude more resistant to the agent, and the eradication of these microbes remains difficult. The stoichiometric transport model is also shown in the figure, as the chemistry involved would indicate this a possibility. However, this model shows a clear failure to qualitatively fit the data presented. The full stoichiometric model, a

combination of the physiology and this failed transport model, only succeeds due to the physiology model incorporated within it. Examination of Table 9 reveals that the penetration time for the full stoichiometric model is set so low that the transport aspect of the model is negligible.

Benzalkonium Chloride Challenge on a
Listeria monocytogenes Biofilm

The biofilm grown for these experiments was relatively old (14 days), but not particularly thick (10^7 - 10^8 cfu/cm²). The chemistry involved in a quaternary ammonia compound indicates that the stoichiometric transport model is preferred, as no specific enzymatic pathway exists to degrade QACs.

As with the previous data set, Table 10 shows the physiology model to be ranked superior by the AIC. Neither full model gains benefit by the incorporation of a transport model, evidenced by Figure 21. Clearly, the physiology model is sufficient to model the processes involved in this antimicrobial treatment.

This data set and the analysis of it bear striking resemblance to the previous data set. The purpose of the inclusion of this set, while appearing redundant, is to illustrate the point that this modeling effort can be used on a wide variety of antimicrobial agents. BAC, a quaternary ammonia compound, bears little resemblance to a biguanide such as chlorhexidine, either in structure or in function. While the mechanisms are dissimilar, the idea that they operate under a simplified physical system, in this case the physiology model, is profound and represents the fundamental utility of this modeling effort.

Monochloramine Challenge on a *Pseudomonas aeruginosa* Biofilm

The high density of the biofilm in this data set lends credence to the incorporation of a transport model, particularly the stoichiometric given the chemistry surrounding monochloramine. Additionally, the species used in this experiment is known to be a good biofilm former, and it is likely an adaptation to a biofilm phenotype will be seen. Correspondingly, the physiology model will be considered a possibility.

Table 11 shows a clear advantage to the full stoichiometric model. Stoichiometric transport alone is clearly insufficient to capture the data (Figure 22). The physiology model, a close second, provides an adequate fit, while missing the initial stages of transport resistance.

This data set is the first clear example of the utility of a four-parameter model. As seen in Figure 22, the full model allows for two slope changes during the disinfection. The first can be seen at the saturation point, when the dose time approaches the penetration time. The second is located at the point at which the sensitive fraction is nearly eliminated and the resistant subpopulation remains. While the physiology model describes the data in the long run, it underestimates the sensitive fraction and overestimates the physiological resistance parameter. Were this experiment used to estimate the true disinfection rate against a mature biofilm, the use of the physiological resistance parameter from the physiological model would lead to an incorrect result regarding the true disinfection rate.

From this, it should be noted that the selection of a model for the purposes of using the values gleaned from an analysis can be critical. As evidenced from this data set, the choice between two models, while

appearing arbitrary, has great bearing on the parameter values extracted. Again, *a priori* knowledge of the underlying chemistry and the physical situation surrounding the disinfection should be clearly understood and used as the primary selection guide in choosing an appropriate model. The AIC should be seen as a “tie-breaker” between two equally likely models, not as the primary selection method.

Sodium Hypochlorite Challenge on
an artificial *Pseudomonas*
aeruginosa Biofilm

The thickness of the artificial biofilm beads in this experiment, coupled with previous findings that indicate chlorine is transport limited in this system, lead to the assertion that the stoichiometric transport model will be in evidence in this data set. In addition, the thickness and density of the cells within the beads suggests that a physiology gradient may also be a factor.

The shape of the disinfection curve in this case clearly implies a stoichiometric transport limitation (Figure 23). Table 12 reveals that the full stoichiometric model edges out the stoichiometric model, in terms of

the AIC value. The physiology model offers little to this data set (Figure 23). However, the combination of the stoichiometric transport and physiology model into the full stoichiometric model again shows the utility of a four-parameter model. The full model captures, in part, the initial dip in the survival fraction at the onset of the treatment. Nearly 90% of the biofilm is immediately inactivated.

This data set, in particular, questions the assumption of a uniform cell distribution within the biofilm. Nutrient limitations alone would suggest that the bulk fluid interface to the biofilm would support more microbial activity. Therefore, the population distribution would be skewed toward the outside of the biofilm. This would result, in the case of transport resistance, in a quick disinfection of the denser outside of the biofilm, then a retarded disinfection of the more deeply embedded remainder.

The decision between the full stoichiometric and transport model is somewhat arbitrary. The physical situation surrounding the data set suggests both stoichiometric transport and physiology are factors in this experiment. The success, at least in part, of the full model in capturing the initial dip in the survival fraction lends more weight to this model. Additionally, the AIC values for each, while close, indicate the full model is

superior, even at the cost of eight AIC points versus the four AIC point penalty applied to the transport model. With these factors in mind, the full stoichiometric model is held superior for this data set.

Summary of Experimental Data Fitting

While it is not possible to conclude a rigorous method to determine the best model to fit a data set, summarizing the findings above may lend insight.

Table 13 - Summary of Best Model Fits to Experimental Data Sets

Data Set	Physical Features	Best Model Fit	<i>P</i>
Young <i>P. aeruginosa</i> with tobramycin	Thin biofilm (3E7cfu/cm ²), catalytic	Physiology	6.72E-2
Old <i>P. aeruginosa</i> with tobramycin	Thick biofilm (2E9cfu/cm ²), catalytic	Full Cat.	4.27E-2
<i>P. aeruginosa</i> with peroxide	Thick film (114μm), transport resistance (1.91 < ϕ < 4.98), catalytic	Trans. Cat	N/A
<i>S. aureus</i> with chlorhexidine	Thin film (24 hr incubation)	Physiology	2.60E-2
<i>L. monocytogenes</i> with BAC	Thin film (1E7cfu/cm ²)	Physiology	2.25E-3
<i>P. aeruginosa</i> with	Thick film (1.1E8cfu/cm ²),	Full Stoich.	1.55E-1

monochloramine	stoichiometric		
<i>P. aeruginosa</i> with hypochlorite	Thick beads (2mm dia), high cell density (6.75E9cfu/mL), stoichiometric	Full Stoich.	1.00E-4

Two conclusions can be drawn from the summary. First, the physical conditions of an experiment indicate which models are likely to prove best at fitting the data. Second, the actual disinfection rate coefficient against the resistant subpopulation of the biofilm is drastically reduced. From the table, the disinfection rate coefficient is one to four orders of magnitude less than predicted against a susceptible or planktonic population.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions are based on the formal sensitivity analysis of the models, predictions made by the models, and experimental data fitted by these models:

Fitting these models to experimental data showed multiple resistance mechanisms are possible. These mechanisms included a physiologically resistant subpopulation of the biofilm, stoichiometric reaction of the antimicrobial agent leading to retarded penetration, and catalytic reaction of the antimicrobial agent leading to retarded penetration.

These models provided a framework for quantitating the difference between the disinfection rate in a biofilm and a planktonic suspension. This difference was found to be one to four orders of magnitude. This demonstrates the need to measure the disinfection rate of an antimicrobial agent on the resistant subpopulation of a biofilm rather than on a planktonic solution.

The models were proven flexible enough to be used on a variety of antimicrobial agents, biofilm species, and experimental methods. The

antimicrobial agent classes examined included biguanides, halogens, quaternary ammonia compounds, and aminoglycosides. The microbe species were comprised of several *Pseudomonas* spp., *Listeria monocytogenes*, and *Staphylococcus aureus*. The experimental systems considered covered chemostats, batch reactors, drip-flow chambers, and artificial biofilm beads. The materials used as a substratum for biofilm growth included stainless steel, glass, and silicone.

The predictions made by these models indicate that if a transport limitation is evidenced in a biofilm, it is important to overcome that limitation, even at the expense of the disinfecting ability of the agent. In the case of an agent that is retarded by a catalytic reaction, this limitation can be overcome by increasing the dose concentration, even at the expense of reducing the dose time. Conversely, an agent that is retarded by a stoichiometric reaction receives no additional benefit from this strategy.

The following suggestions are offered to advance the validation, utility, and predictive capacity of the models presented in this work:

The initial cell density should be made to be dependent on the depth into the biofilm. This would allow for a population gradient within the biofilm, which has been noted experimentally.

Data sets should be produced, showing the survival fraction of the biofilm over a series of time points. Care should be taken to sample initial time points rapidly, as the measurement of a small transport retardation is only evident at these times. Multiple data sets with different dose concentrations would be ideal. Additionally, a well-designed experimental system would allow for the independent measurements of a number of parameters, thus reducing the uncertainty involved with numerous degrees of freedom.

Some consideration to the mechanism of interaction between a biofilm and an antimicrobial agent should be given. In the case of specific agents, that interaction is generally known, but side reactions with unexpected components within the biofilm should be explored. This knowledge would

then allow a definite selection of either the catalytic or stoichiometric transport system, again reducing the uncertainty surrounding data fitting.

The concept of striking a balance between the penetration and disinfection qualities of an antimicrobial agent should be explored. If it is borne out by experimental evidence that an agent with a high penetration potential and a low disinfection rate is superior to a traditional agent against a biofilm, the effects of this finding would be wide reaching.

The resistant subpopulation within a biofilm is the true target of an antimicrobial challenge. This work has shown the removal of the sensitive fraction within the film to be generally easy, and that it is the resistant fraction that proves to be the hardship. This suggests research to track the migration of the sensitive population to this resistant state over time. Presumably, the resistant fraction of a biofilm is a function of the agent used on the biofilm. Knowing this migration trend for a variety of agents could suggest which agents are better suited for the removal of a biofilm at a certain point in this migration.

The catalytic transport models currently use zero-order reaction kinetics as their basis. Some consideration to improving this assumption should be given. Incorporating concentration-dependent kinetics may provide better verisimilitude.

Currently, the disinfection rate coefficient is assumed to be independent of antimicrobial agent concentration. As the agent concentration approaches zero, this is certainly not the case. Some experimental data collected independently of this work has shown the potential for concentration dependence in this parameter, especially at high concentrations. Thus, care should be taken when examining a wide range of agent concentrations lumped as a single data set.

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APPENDICES

APPENDICES

- A. MINITAB Sensitivity Analysis
- B. Prediction Parameters
- C. Published Data Sets and Parameter Estimations
- D. MATLAB Code

APPENDIX A

MINITAB Sensitivity Analysis

The formal sensitivity analysis, as stated formerly, used a statistical analysis software package, MINITAB. Macro files eased the repetition of this process and allowed for quick changes to parameter values. Within this appendix are the two macros used, sens_s.mtb and sens_c.mtb, and the worksheets generated by them. Appendix D holds the MATLAB code used to generate the data shown in the worksheets below.

Stoichiometric Model

Echo Sens_s.mtb

Note Given three response columns (C1-C3), and four parameter columns (C4-C7),
generates the rest of the analysis

Note Assume we've pasted the cells.

Note Make our other columns for regression purposes.

Note Squares first.

Name C8 = 'k^2'

Let 'k^2' = k**2

Name C9 = 'tpen^2'

Let 'tpen^2' = tpen**2

Name C10 = 'es^2'

Let 'es^2' = es**2

Name C11 = 'p^2'

Let 'p^2' = p**2

Note Then the cross products.

Name C12 = 'k*tpen'

```

Let 'k*tpen' = k * tpen
Name C13 = 'k*es'
Let 'k*es' = k * es
Name C14 = 'k*p'
Let 'k*p' = k*p
Name C15 = 'tpen*es'
Let 'tpen*es' = tpen*es
Name C16 = 'tpen*p'
Let 'tpen*p' = tpen*p
Name C17 = 'es*p'
Let 'es*p' = es*p

```

Note Need an indices to plot our ordered residuals.

```

Name K1 = 'len'
Name C18 = 'I'
N C1 'len'
Set 'I'
  1( 1 : 'len' / 1 )1
End.
Let 'I' = I*100/'len'

```

Note Ok, so lets do our first regression on C1

```
Name C19 = 'Fit R1:All' K2 = 'R1:All'
```

```
Regress C1 14 'k'-es*p';
```

```
Fits 'Fit R1:All';
```

```
MSE 'R1:All';
```

```
Constant;
```

```
Brief 0.
```

```
Name C20 = 'Fit R1:-k' K3 = 'R1:-kdis'
```

```
Regress C1 9 'tpen'-p' 'tpen^2'-p^2' 'tpen*es'-es*p';
```

```
Fits 'Fit R1:-k';
```

```
MSE 'R1:-kdis';
```

```
Constant;
```

```
Brief 0.
```

```
Name C21 = 'Fit R1:-tpen' K4 = 'R1:-tpen'
```

```
Regress C1 9 'k' 'es'-'k^2' 'es^2' 'p^2' 'k*es' 'k*p' 'es*p';
```

```
Fits 'Fit R1:-tpen';
```

```
MSE 'R1:-tpen';
```

```
Constant;
```

```
Brief 0.
```

```
Name C22 = 'Fit R1:-es' K5 = 'R1:-es'
```

```
Regress C1 9 'k' 'tpen' 'p'-'tpen^2' 'p^2' 'k*tpen' 'k*p' 'tpen*p';
```

```
Fits 'Fit R1:-es';
```

```
MSE 'R1:-es';
```

```
Constant;
```

Brief 0.

Name C23 = 'Fit R1:-p' K6 = 'R1:-p'

Regress C1 9 'k'-'es' 'k^2'-'es^2' 'k*tpen' 'k*es' 'tpen*es';

Fits 'Fit R1:-p';

MSE 'R1:-p';

Constant;

Brief 0.

Note Regression's done, so lets calculate Abs Res and order

Name C24 = 'ar R1:All'

Let 'ar R1:All' = ABSO(C1-'Fit R1:All')

Sort 'ar R1:All' 'ar R1:All';

By 'ar R1:All'.

Name C25 = 'ar R1:-k'

Let 'ar R1:-k' = ABSO(C1-'Fit R1:-k')

Sort 'ar R1:-k' 'ar R1:-k';

By 'ar R1:-k'.

Name C26 = 'ar R1:-tpen'

Let 'ar R1:-tpen' = ABSO(C1-'Fit R1:-tpen')

Sort 'ar R1:-tpen' 'ar R1:-tpen';

By 'ar R1:-tpen'.

Name C27 = 'ar R1:-es'

Let 'ar R1:-es' = ABSO(C1-'Fit R1:-es')

Sort 'ar R1:-es' 'ar R1:-es';

By 'ar R1:-es'.

Name C28 = 'ar R1:-p'

Let 'ar R1:-p' = ABSO(C1-'Fit R1:-p')

Sort 'ar R1:-p' 'ar R1:-p';

By 'ar R1:-p'.

Note Now, we need a plot of the residuals

Layout;

Text .65 .50 "All";

Text .65 .45 "w/o Kdis";

Text .65 .40 "w/o tpen";

Text .65 .35 "w/o Es";

Text .65 .30 "w/o p";

Line .55 .52 .62 .52;

Type 1;

Size 1.5;

Line .55 .47 .62 .47;

Type 2;

Size 1.5;

Line .55 .42 .62 .42;

Type 3;

```

    Size 1.5;
Line .55 .37 .62 .37;
    Type 4;
    Size 1.5;
Line .55 .32 .62 .32;
    Type 5;
    Size 1.5;
Line .54 .29 .75 .29;
    Size 1.5;
Line .75 .29 .75 .55;
    Size 1.5;
Line .75 .55 .54 .55;
    Size 1.5;
Line .54 .55 .54 .29;
    Size 1.5.
Plot 'I*'ar R1:All' 'I*'ar R1:-k' 'I*'ar R1:-tpen' 'I*' &
    'ar R1:-es' 'I*'ar R1:-p';
Connect;
    Type 1 2 3 4 5;
    Size 1.8 1.8 1.8 1.8 1.8;
Title "Error plots for case where tdose < tpen";
Legend 0 1 0 1;
Overlay;
ScFrame;
ScAnnotation;
Axis 1;
    Label "|Obs-Pred|";
    TSize 2.0;
Axis 2;
    Label "Culmulative %";
    TSize 2.0.
EndLayout.

```

Note Ok, so lets do our second regression on C2

Name C29 = 'Fit R2:All' K7 = 'R2:All'

Regress C2 14 'k'-'es*p';

Fits 'Fit R2:All';

MSE 'R2:All';

Constant;

Brief 0.

Name C30 = 'Fit R2:-k' K8 = 'R2:-kdis'

Regress C2 9 'tpen'-'p' 'tpen^2'-'p^2' 'tpen*es'-'es*p';

Fits 'Fit R2:-k';

MSE 'R2:-kdis';

Constant;

Brief 0.

Name C31 = 'Fit R2:-tpen' K9 = 'R2:-tpen'

Regress C2 9 'k' 'es'-'k^2' 'es^2' 'p^2' 'k*es' 'k*p' 'es*p';

Fits 'Fit R2:-tpen';

MSE 'R2:-tpen';

Constant;

Brief 0.

Name C32 = 'Fit R2:-es' K10 = 'R2:-es'

Regress C2 9 'k' 'tpen' 'p'-'tpen^2' 'p^2' 'k*tpen' 'k*p' 'tpen*p';

Fits 'Fit R2:-es';

MSE 'R2:-es';

Constant;

Brief 0.

Name C33 = 'Fit R2:-p' K11 = 'R2:-p'

Regress C2 9 'k' 'es' 'k^2'-'es^2' 'k*tpen' 'k*es' 'tpen*es';

Fits 'Fit R2:-p';

MSE 'R2:-p';

Constant;

Brief 0.

Note Regression's done, so lets calculate Abs Res and order

Name C34 = 'ar R2:All'

Let 'ar R2:All' = ABSO(C2-'Fit R2:All')

Sort 'ar R2:All' 'ar R2:All';

By 'ar R2:All'.

Name C35 = 'ar R2:-k'

Let 'ar R2:-k' = ABSO(C2-'Fit R2:-k')

Sort 'ar R2:-k' 'ar R2:-k';

By 'ar R2:-k'.

Name C36 = 'ar R2:-tpen'

Let 'ar R2:-tpen' = ABSO(C2-'Fit R2:-tpen')

Sort 'ar R2:-tpen' 'ar R2:-tpen';

By 'ar R2:-tpen'.

Name C37 = 'ar R2:-es'

Let 'ar R2:-es' = ABSO(C2-'Fit R2:-es')

Sort 'ar R2:-es' 'ar R2:-es';

By 'ar R2:-es'.

Name C38 = 'ar R2:-p'

Let 'ar R2:-p' = ABSO(C2-'Fit R2:-p')

Sort 'ar R2:-p' 'ar R2:-p';

By 'ar R2:-p'.

Note Now, we need a plot of the residuals

Layout;

Text .65 .50 "All";


```

Text .65 .45 "w/o Kdis";
Text .65 .40 "w/o tpen";
Text .65 .35 "w/o Es";
Text .65 .30 "w/o p";
Line .55 .52 .62 .52;
  Type 1;
  Size 1.5;
Line .55 .47 .62 .47;
  Type 2;
  Size 1.5;
Line .55 .42 .62 .42;
  Type 3;
  Size 1.5;
Line .55 .37 .62 .37;
  Type 4;
  Size 1.5;
Line .55 .32 .62 .32;
  Type 5;
  Size 1.5;
Line .54 .29 .75 .29;
  Size 1.5;
Line .75 .29 .75 .55;
  Size 1.5;
Line .75 .55 .54 .55;
  Size 1.5;
Line .54 .55 .54 .29;
  Size 1.5;
Plot 'I'*'ar R2:All' 'I'*'ar R2:-k' 'I'*'ar R2:-tpen' 'I'* &
  'ar R2:-es' 'I'*'ar R2:-p';
Connect;
  Type 1 2 3 4 5;
  Size 1.8 1.8 1.8 1.8 1.8;
Title "Error plots for case where tdose ~ tpen";
Legend 0 1 0 1;
Overlay;
ScFrame;
ScAnnotation;
Axis 1;
  Label "|Obs-Pred|";
  TSize 2.0;
Axis 2;
  Label "Culmulative %";
  TSize 2.0;
EndLayout.

```

Note Ok, so lets do our third regression on C3

Name C39 = 'Fit R3:All' K12 = 'R3:All'

Regress C3 14 'k'-'es*p';

Fits 'Fit R3:All';

MSE 'R3:All';

Constant;

Brief 0.

Name C40 = 'Fit R3:-k' K13 = 'R3:-kdis'

Regress C3 9 'tpen'-'p' 'tpen^2'-'p^2' 'tpen*es'-'es*p';

Fits 'Fit R3:-k';

MSE 'R3:-kdis';

Constant;

Brief 0.

Name C41 = 'Fit R3:-tpen' K14 = 'R3:-tpen'

Regress C3 9 'k' 'es'-'k^2' 'es^2' 'p^2' 'k*es' 'k*p' 'es*p';

Fits 'Fit R3:-tpen';

MSE 'R3:-tpen';

Constant;

Brief 0.

Name C42 = 'Fit R3:-es' K15 = 'R3:-es'

Regress C3 9 'k' 'tpen' 'p'-'tpen^2' 'p^2' 'k*tpen' 'k*p' 'tpen*p';

Fits 'Fit R3:-es';

MSE 'R3:-es';

Constant;

Brief 0.

Name C43 = 'Fit R3:-p' K16 = 'R3:-p'

Regress C3 9 'k'-'es' 'k^2'-'es^2' 'k*tpen' 'k*es' 'tpen*es';

Fits 'Fit R3:-p';

MSE 'R3:-p';

Constant;

Brief 0.

Note Regression's done, so lets calculate Abs Res and order

Name C44 = 'ar R3:All'

Let 'ar R3:All' = ABSO(C3-'Fit R3:All')

Sort 'ar R3:All' 'ar R3:All';

By 'ar R3:All'.

Name C45 = 'ar R3:-k'

Let 'ar R3:-k' = ABSO(C3-'Fit R3:-k')

Sort 'ar R3:-k' 'ar R3:-k';

By 'ar R3:-k'.

Name C46 = 'ar R3:-tpen'

Let 'ar R3:-tpen' = ABSO(C3-'Fit R3:-tpen')

Sort 'ar R3:-tpen' 'ar R3:-tpen';

By 'ar R3:-tpen'.

```

Name C47 = 'ar R3:-es'
Let 'ar R3:-es' = ABSO(C3-'Fit R3:-es')
Sort 'ar R3:-es' 'ar R3:-es';
  By 'ar R3:-es'.
Name C48 = 'ar R3:-p'
Let 'ar R3:-p' = ABSO(C3-'Fit R3:-p')
Sort 'ar R3:-p' 'ar R3:-p';
  By 'ar R3:-p'.

```

Note Now, we need a plot of the residuals

Layout;

```

Text .65 .50 "All";
Text .65 .45 "w/o Kdis";
Text .65 .40 "w/o tpen";
Text .65 .35 "w/o Es";
Text .65 .30 "w/o p";
Line .55 .52 .62 .52;
  Type 1;
  Size 1.5;
Line .55 .47 .62 .47;
  Type 2;
  Size 1.5;
Line .55 .42 .62 .42;
  Type 3;
  Size 1.5;
Line .55 .37 .62 .37;
  Type 4;
  Size 1.5;
Line .55 .32 .62 .32;
  Type 5;
  Size 1.5;
Line .54 .29 .75 .29;
  Size 1.5;
Line .75 .29 .75 .55;
  Size 1.5;
Line .75 .55 .54 .55;
  Size 1.5;
Line .54 .55 .54 .29;
  Size 1.5.
Plot 'I'*'ar R3:All' 'I'*'ar R3:-k' 'I'*'ar R3:-tpen' 'I'* &
  'ar R3:-es' 'I'*'ar R3:-p';
Connect;
  Type 1 2 3 4 5;
  Size 1.8 1.8 1.8 1.8 1.8;
Title "Error plots for case where tdose > tpen";

```

```

Legend 0 1 0 1;
Overlay;
ScFrame;
ScAnnotation;
Axis 1;
  Label "|Obs-Pred|";
  TSize 2.0;
Axis 2;
  Label "Culmulative %";
  TSize 2.0.
EndLayout.

```

Note Copy MSE's to next to last column

Name C49 = 'MSEs'

Copy k2-k16 'MSEs'

Note The End.

The responses (R1-R3) generated from the parameter values are below.

Responses			Parameters			
$t_{dose} < t_{pen}$	$t_{dose} \sim t_{pen}$	$t_{dose} > t_{pen}$	k_{dis}	t_{pen}	ϵ_s	p
9.94E-01	9.11E-01	8.35E-01	1.00E-05	1.00E-00	1.00E-00	1.00E-05
9.94E-01	9.11E-01	8.35E-01	1.00E-05	1.00E-00	1.00E-00	5.00E-02
9.94E-01	9.11E-01	8.35E-01	1.00E-05	1.00E-00	1.00E-00	1.00E-01
9.94E-01	9.12E-01	8.37E-01	1.00E-05	1.00E-00	9.90E-01	1.00E-05
9.94E-01	9.12E-01	8.37E-01	1.00E-05	1.00E-00	9.90E-01	5.00E-02
9.94E-01	9.12E-01	8.37E-01	1.00E-05	1.00E-00	9.90E-01	1.00E-01
9.99E-01	9.91E-01	9.84E-01	1.00E-05	1.00E-00	1.00E-01	1.00E-05
9.99E-01	9.87E-01	9.75E-01	1.00E-05	1.00E-00	1.00E-01	5.00E-02
9.99E-01	9.83E-01	9.67E-01	1.00E-05	1.00E-00	1.00E-01	1.00E-01
10.00E-01	9.71E-01	8.93E-01	1.00E-05	1.00E+04	1.00E-00	1.00E-05
10.00E-01	9.71E-01	8.93E-01	1.00E-05	1.00E+04	1.00E-00	5.00E-02
10.00E-01	9.71E-01	8.93E-01	1.00E-05	1.00E+04	1.00E-00	1.00E-01
10.00E-01	9.71E-01	8.94E-01	1.00E-05	1.00E+04	9.90E-01	1.00E-05
10.00E-01	9.71E-01	8.94E-01	1.00E-05	1.00E+04	9.90E-01	5.00E-02
10.00E-01	9.71E-01	8.94E-01	1.00E-05	1.00E+04	9.90E-01	1.00E-01
10.00E-01	9.92E-01	9.84E-01	1.00E-05	1.00E+04	1.00E-01	1.00E-05
10.00E-01	9.91E-01	9.80E-01	1.00E-05	1.00E+04	1.00E-01	5.00E-02
10.00E-01	9.90E-01	9.75E-01	1.00E-05	1.00E+04	1.00E-01	1.00E-01
10.00E-01	9.79E-01	9.46E-01	1.00E-05	2.00E+04	1.00E-00	1.00E-05
10.00E-01	9.79E-01	9.46E-01	1.00E-05	2.00E+04	1.00E-00	5.00E-02

10.00E-01	9.79E-01	9.46E-01	1.00E-05	2.00E+04	1.00E-00	1.00E-01
10.00E-01	9.79E-01	9.46E-01	1.00E-05	2.00E+04	9.90E-01	1.00E-05
10.00E-01	9.79E-01	9.46E-01	1.00E-05	2.00E+04	9.90E-01	5.00E-02
10.00E-01	9.79E-01	9.46E-01	1.00E-05	2.00E+04	9.90E-01	1.00E-01
10.00E-01	9.92E-01	9.85E-01	1.00E-05	2.00E+04	1.00E-01	1.00E-05
10.00E-01	9.92E-01	9.83E-01	1.00E-05	2.00E+04	1.00E-01	5.00E-02
10.00E-01	9.91E-01	9.81E-01	1.00E-05	2.00E+04	1.00E-01	1.00E-01
7.24E-131	2.22E-16	2.22E-16	5.00E-01	1.00E-00	1.00E-00	1.00E-05
7.24E-131	2.22E-16	2.22E-16	5.00E-01	1.00E-00	1.00E-00	5.00E-02
7.24E-131	2.22E-16	2.22E-16	5.00E-01	1.00E-00	1.00E-00	1.00E-01
9.97E-03	9.55E-03	9.14E-03	5.00E-01	1.00E-00	9.90E-01	1.00E-05
3.13E-09	1.06E-103	3.60E-198	5.00E-01	1.00E-00	9.90E-01	5.00E-02
9.83E-16	1.18E-204	2.22E-16	5.00E-01	1.00E-00	9.90E-01	1.00E-01
8.97E-01	8.59E-01	8.23E-01	5.00E-01	1.00E-00	1.00E-01	1.00E-05
2.80E-07	9.50E-102	3.22E-196	5.00E-01	1.00E-00	1.00E-01	5.00E-02
8.73E-14	1.05E-202	2.22E-16	5.00E-01	1.00E-00	1.00E-01	1.00E-01
7.68E-01	4.82E-02	2.22E-16	5.00E-01	1.00E+04	1.00E-00	1.00E-05
7.68E-01	4.82E-02	2.22E-16	5.00E-01	1.00E+04	1.00E-00	5.00E-02
7.68E-01	4.82E-02	2.22E-16	5.00E-01	1.00E+04	1.00E-00	1.00E-01
7.68E-01	4.82E-02	9.61E-03	5.00E-01	1.00E+04	9.90E-01	1.00E-05
7.68E-01	4.82E-02	1.36E-89	5.00E-01	1.00E+04	9.90E-01	5.00E-02
7.68E-01	4.82E-02	1.92E-176	5.00E-01	1.00E+04	9.90E-01	1.00E-01
9.00E-01	8.89E-01	8.53E-01	5.00E-01	1.00E+04	1.00E-01	1.00E-05
8.11E-01	9.17E-02	7.68E-89	5.00E-01	1.00E+04	1.00E-01	5.00E-02
7.95E-01	7.53E-02	7.75E-176	5.00E-01	1.00E+04	1.00E-01	1.00E-01
8.36E-01	3.27E-01	6.02E-02	5.00E-01	2.00E+04	1.00E-00	1.00E-05
8.36E-01	3.27E-01	6.02E-02	5.00E-01	2.00E+04	1.00E-00	5.00E-02
8.36E-01	3.27E-01	6.02E-02	5.00E-01	2.00E+04	1.00E-00	1.00E-01
8.36E-01	3.27E-01	6.02E-02	5.00E-01	2.00E+04	9.90E-01	1.00E-05
8.36E-01	3.27E-01	6.02E-02	5.00E-01	2.00E+04	9.90E-01	5.00E-02
8.36E-01	3.27E-01	6.02E-02	5.00E-01	2.00E+04	9.90E-01	1.00E-01
9.00E-01	8.94E-01	8.80E-01	5.00E-01	2.00E+04	1.00E-01	1.00E-05
8.66E-01	3.58E-01	9.09E-02	5.00E-01	2.00E+04	1.00E-01	5.00E-02
8.55E-01	3.46E-01	7.93E-02	5.00E-01	2.00E+04	1.00E-01	1.00E-01
5.38E-261	2.22E-16	2.22E-16	1.00E-00	1.00E-00	1.00E-00	1.00E-05
5.38E-261	2.22E-16	2.22E-16	1.00E-00	1.00E-00	1.00E-00	5.00E-02
5.38E-261	2.22E-16	2.22E-16	1.00E-00	1.00E-00	1.00E-00	1.00E-01
9.94E-03	9.11E-03	8.35E-03	1.00E-00	1.00E-00	9.90E-01	1.00E-05
9.81E-16	1.13E-204	2.22E-16	1.00E-00	1.00E-00	9.90E-01	5.00E-02
9.68E-29	2.22E-16	2.22E-16	1.00E-00	1.00E-00	9.90E-01	1.00E-01
8.95E-01	8.20E-01	7.52E-01	1.00E-00	1.00E-00	1.00E-01	1.00E-05
8.71E-14	1.01E-202	2.22E-16	1.00E-00	1.00E-00	1.00E-01	5.00E-02
8.48E-27	2.22E-16	2.22E-16	1.00E-00	1.00E-00	1.00E-01	1.00E-01
7.64E-01	4.45E-02	2.22E-16	1.00E-00	1.00E+04	1.00E-00	1.00E-05

7.64E-01	4.45E-02	2.22E-16	1.00E-00	1.00E+04	1.00E-00	5.00E-02
7.64E-01	4.45E-02	2.22E-16	1.00E-00	1.00E+04	1.00E-00	1.00E-01
7.64E-01	4.45E-02	9.23E-03	1.00E-00	1.00E+04	9.90E-01	1.00E-05
7.64E-01	4.45E-02	1.86E-176	1.00E-00	1.00E+04	9.90E-01	5.00E-02
7.64E-01	4.45E-02	2.22E-16	1.00E-00	1.00E+04	9.90E-01	1.00E-01
9.00E-01	8.79E-01	8.09E-01	1.00E-00	1.00E+04	1.00E-01	1.00E-05
7.95E-01	7.53E-02	7.48E-176	1.00E-00	1.00E+04	1.00E-01	5.00E-02
7.83E-01	6.37E-02	2.22E-16	1.00E-00	1.00E+04	1.00E-01	1.00E-01
8.33E-01	3.24E-01	5.76E-02	1.00E-00	2.00E+04	1.00E-00	1.00E-05
8.33E-01	3.24E-01	5.76E-02	1.00E-00	2.00E+04	1.00E-00	5.00E-02
8.33E-01	3.24E-01	5.76E-02	1.00E-00	2.00E+04	1.00E-00	1.00E-01
8.33E-01	3.24E-01	5.76E-02	1.00E-00	2.00E+04	9.90E-01	1.00E-05
8.33E-01	3.24E-01	5.76E-02	1.00E-00	2.00E+04	9.90E-01	5.00E-02
8.33E-01	3.24E-01	5.76E-02	1.00E-00	2.00E+04	9.90E-01	1.00E-01
9.00E-01	8.87E-01	8.61E-01	1.00E-00	2.00E+04	1.00E-01	1.00E-05
8.55E-01	3.46E-01	7.93E-02	1.00E-00	2.00E+04	1.00E-01	5.00E-02
8.47E-01	3.38E-01	7.11E-02	1.00E-00	2.00E+04	1.00E-01	1.00E-01

Catalytic Model

Echo Sens_s.mtb

Note Given three response columns (C1-C3), and four parameter columns (C4-C7),
generates the rest of the analysis

Note Assume we've pasted the cells.

Note Make our other columns for regression purposes.

Note Squares first.

Name C8 = 'k^2'

Let 'k^2' = k**2

Name C9 = 'thiele^2'

Let 'thiele^2' = thiele**2

Name C10 = 'es^2'

Let 'es^2' = es**2

Name C11 = 'p^2'

```

Let 'p^2' = p**2
Note Then the cross products.
Name C12 = 'k*thiele'
Let 'k*thiele' = k * thiele
Name C13 = 'k*es'
Let 'k*es' = k * es
Name C14 = 'k*p'
Let 'k*p' = k*p
Name C15 = 'thiele*es'
Let 'thiele*es' = thiele*es
Name C16 = 'thiele*p'
Let 'thiele*p' = thiele*p
Name C17 = 'es*p'
Let 'es*p' = es*p

```

Note Need an indices to plot our ordered residuals.

```

Name K1 = 'len'
Name C18 = 'I'
N C1 'len'
Set 'I'
  1( 1 : 'len' / 1 )1
End.
Let 'I' = I*100/'len'

```

Note Ok, so lets do our first regression on C1

```

Name C19 = 'Fit R1:All' K2 = 'R1:All'

```

```

Regress C1 14 'k'-'es*p';

```

```

  Fits 'Fit R1:All';

```

```

  MSE 'R1:All';

```

```

  Constant;

```

```

  Brief 0.

```

```

Name C20 = 'Fit R1:-k' K3 = 'R1:-kdis'

```

```

Regress C1 9 'thiele'-'p' 'thiele^2'-'p^2' 'thiele*es'-'es*p';

```

```

  Fits 'Fit R1:-k';

```

```

  MSE 'R1:-kdis';

```

```

  Constant;

```

```

  Brief 0.

```

```

Name C21 = 'Fit R1:-thiele' K4 = 'R1:-thiele'

```

```

Regress C1 9 'k' 'es'-'k^2' 'es^2' 'p^2' 'k*es' 'k*p' 'es*p';

```

```

  Fits 'Fit R1:-thiele';

```

```

  MSE 'R1:-thiele';

```

```

  Constant;

```

```

  Brief 0.

```

```

Name C22 = 'Fit R1:-es' K5 = 'R1:-es'

```

```

Regress C1 9 'k' 'thiele' 'p'-'thiele^2' 'p^2' 'k*thiele' 'k*p' 'thiele*p';

```

```

Fits 'Fit R1:-es';
MSE 'R1:-es';
Constant;
Brief 0.
Name C23 = 'Fit R1:-p' K6 = 'R1:-p'
Regress C1 9 'k'-es' 'k^2'-es^2' 'k*thiele' 'k*es' 'thiele*es';
Fits 'Fit R1:-p';
MSE 'R1:-p';
Constant;
Brief 0.

```

Note Regression's done, so lets calculate Abs Res and order

```

Name C24 = 'ar R1:All'
Let 'ar R1:All' = ABSO(C1-'Fit R1:All')
Sort 'ar R1:All' 'ar R1:All';
By 'ar R1:All'.
Name C25 = 'ar R1:-k'
Let 'ar R1:-k' = ABSO(C1-'Fit R1:-k')
Sort 'ar R1:-k' 'ar R1:-k';
By 'ar R1:-k'.
Name C26 = 'ar R1:-thiele'
Let 'ar R1:-thiele' = ABSO(C1-'Fit R1:-thiele')
Sort 'ar R1:-thiele' 'ar R1:-thiele';
By 'ar R1:-thiele'.
Name C27 = 'ar R1:-es'
Let 'ar R1:-es' = ABSO(C1-'Fit R1:-es')
Sort 'ar R1:-es' 'ar R1:-es';
By 'ar R1:-es'.
Name C28 = 'ar R1:-p'
Let 'ar R1:-p' = ABSO(C1-'Fit R1:-p')
Sort 'ar R1:-p' 'ar R1:-p';
By 'ar R1:-p'.

```

Note Now, we need a plot of the residuals

```

Layout;
Text .65 .50 "All";
Text .65 .45 "w/o Kdis";
Text .65 .40 "w/o thiele";
Text .65 .35 "w/o Es";
Text .65 .30 "w/o p";
Line .55 .52 .62 .52;
Type 1;
Size 1.5;
Line .55 .47 .62 .47;
Type 2;

```



```

Size 1.5;
Line .55 .42 .62 .42;
Type 3;
Size 1.5;
Line .55 .37 .62 .37;
Type 4;
Size 1.5;
Line .55 .32 .62 .32;
Type 5;
Size 1.5;
Line .54 .29 .75 .29;
Size 1.5;
Line .75 .29 .75 .55;
Size 1.5;
Line .75 .55 .54 .55;
Size 1.5;
Line .54 .55 .54 .29;
Size 1.5;
Plot 'I'*'ar R1:All' 'I'*'ar R1:-k' 'I'*'ar R1:-thiele' 'I'* &
'ar R1:-es' 'I'*'ar R1:-p';
Connect;
Type 1 2 3 4 5;
Size 1.8 1.8 1.8 1.8 1.8;
Title "Error plots for case where thiele < 1";
Legend 0 1 0 1;
Overlay;
ScFrame;
ScAnnotation;
Axis 1;
Label "|Obs-Pred|";
TSize 2.0;
Axis 2;
Label "Culmulative %";
TSize 2.0;
EndLayout.

Note Ok, so lets do our second regression on C2
Name C29 = 'Fit R2:All' K7 = 'R2:All'
Regress C2 14 'k'-'es*p';
Fits 'Fit.R2:All';
MSE 'R2:All';
Constant;
Brief 0.
Name C30 = 'Fit R2:-k' K8 = 'R2:-kdis'
Regress C2 9 'thiele'-'p' 'thiele^2'-'p^2' 'thiele*es'-'es*p';

```

```

Fits 'Fit R2:-k';
MSE 'R2:-kdis';
Constant;
Brief 0.
Name C31 = 'Fit R2:-thiele' K9 = 'R2:-thiele'
Regress C2 9 'k' 'es'-'k^2' 'es^2' 'p^2' 'k*es' 'k*p' 'es*p';
Fits 'Fit R2:-thiele';
MSE 'R2:-thiele';
Constant;
Brief 0.
Name C32 = 'Fit R2:-es' K10 = 'R2:-es'
Regress C2 9 'k' 'thiele' 'p'-'thiele^2' 'p^2' 'k*thiele' 'k*p' 'thiele*p';
Fits 'Fit R2:-es';
MSE 'R2:-es';
Constant;
Brief 0.
Name C33 = 'Fit R2:-p' K11 = 'R2:-p'
Regress C2 9 'k'-'es' 'k^2'-'es^2' 'k*thiele' 'k*es' 'thiele*es';
Fits 'Fit R2:-p';
MSE 'R2:-p';
Constant;
Brief 0.

```

Note Regression's done, so lets calculate Abs Res and order

```

Name C34 = 'ar R2:All'
Let 'ar R2:All' = ABSO(C2-'Fit R2:All')
Sort 'ar R2:All' 'ar R2:All';
By 'ar R2:All'.
Name C35 = 'ar R2:-k'
Let 'ar R2:-k' = ABSO(C2-'Fit R2:-k')
Sort 'ar R2:-k' 'ar R2:-k';
By 'ar R2:-k'.
Name C36 = 'ar R2:-thiele'
Let 'ar R2:-thiele' = ABSO(C2-'Fit R2:-thiele')
Sort 'ar R2:-thiele' 'ar R2:-thiele';
By 'ar R2:-thiele'.
Name C37 = 'ar R2:-es'
Let 'ar R2:-es' = ABSO(C2-'Fit R2:-es')
Sort 'ar R2:-es' 'ar R2:-es';
By 'ar R2:-es'.
Name C38 = 'ar R2:-p'
Let 'ar R2:-p' = ABSO(C2-'Fit R2:-p')
Sort 'ar R2:-p' 'ar R2:-p';
By 'ar R2:-p'.

```

Note Now, we need a plot of the residuals

Layout;

Text .65 .50 "All";

Text .65 .45 "w/o Kdis";

Text .65 .40 "w/o thiele";

Text .65 .35 "w/o Es";

Text .65 .30 "w/o p";

Line .55 .52 .62 .52;

Type 1;

Size 1.5;

Line .55 .47 .62 .47;

Type 2;

Size 1.5;

Line .55 .42 .62 .42;

Type 3;

Size 1.5;

Line .55 .37 .62 .37;

Type 4;

Size 1.5;

Line .55 .32 .62 .32;

Type 5;

Size 1.5;

Line .54 .29 .75 .29;

Size 1.5;

Line .75 .29 .75 .55;

Size 1.5;

Line .75 .55 .54 .55;

Size 1.5;

Line .54 .55 .54 .29;

Size 1.5.

Plot 'I'*'ar R2:All' 'I'*'ar R2:-k' 'I'*'ar R2:-thiele' 'I'* &
'ar R2:-es' 'I'*'ar R2:-p';

Connect;

Type 1 2 3 4 5;

Size 1.8 1.8 1.8 1.8 1.8;

Title "Error plots for case where thiele = 1";

Legend 0 1 0 1;

Overlay;

ScFrame;

ScAnnotation;

Axis 1;

Label "|Obs-Pred|";

TSize 2.0;

Axis 2;

Label "Culmulative %";

TSize 2.0.
EndLayout.

Note Ok, so lets do our third regression on C3

Name C39 = 'Fit R3:All' K12 = 'R3:All'

Regress C3 14 'k'-'es*p';

Fits 'Fit R3:All';

MSE 'R3:All';

Constant;

Brief 0.

Name C40 = 'Fit R3:-k' K13 = 'R3:-kdis'

Regress C3 9 'thiele'-'p' 'thiele^2'-'p^2' 'thiele*es'-'es*p';

Fits 'Fit R3:-k';

MSE 'R3:-kdis';

Constant;

Brief 0.

Name C41 = 'Fit R3:-thiele' K14 = 'R3:-thiele'

Regress C3 9 'k'-'es'-'k^2'-'es^2'-'p^2'-'k*es'-'k*p'-'es*p';

Fits 'Fit R3:-thiele';

MSE 'R3:-thiele';

Constant;

Brief 0.

Name C42 = 'Fit R3:-es' K15 = 'R3:-es'

Regress C3 9 'k'-'thiele'-'p'-'thiele^2'-'p^2'-'k*thiele'-'k*p'-'thiele*p';

Fits 'Fit R3:-es';

MSE 'R3:-es';

Constant;

Brief 0.

Name C43 = 'Fit R3:-p' K16 = 'R3:-p'

Regress C3 9 'k'-'es'-'k^2'-'es^2'-'k*thiele'-'k*es'-'thiele*es';

Fits 'Fit R3:-p';

MSE 'R3:-p';

Constant;

Brief 0.

Note Regression's done, so lets calculate Abs Res and order

Name C44 = 'ar R3:All'

Let 'ar R3:All' = ABSO(C3-'Fit R3:All')

Sort 'ar R3:All' 'ar R3:All';

By 'ar R3:All'.

Name C45 = 'ar R3:-k'

Let 'ar R3:-k' = ABSO(C3-'Fit R3:-k')

Sort 'ar R3:-k' 'ar R3:-k';

By 'ar R3:-k'.

Name C46 = 'ar R3:-thiele'

```

Let 'ar R3:-thiele' = ABSO(C3-'Fit R3:-thiele')
Sort 'ar R3:-thiele' 'ar R3:-thiele';
  By 'ar R3:-thiele'.
Name C47 = 'ar R3:-es'
Let 'ar R3:-es' = ABSO(C3-'Fit R3:-es')
Sort 'ar R3:-es' 'ar R3:-es';
  By 'ar R3:-es'.
Name C48 = 'ar R3:-p'
Let 'ar R3:-p' = ABSO(C3-'Fit R3:-p')
Sort 'ar R3:-p' 'ar R3:-p';
  By 'ar R3:-p'.

```

Note Now, we need a plot of the residuals
Layout;

```

Text .65 .50 "All";
Text .65 .45 "w/o Kdis";
Text .65 .40 "w/o thiele";
Text .65 .35 "w/o Es";
Text .65 .30 "w/o p";
Line .55 .52 .62 .52;
  Type 1;
  Size 1.5;
Line .55 .47 .62 .47;
  Type 2;
  Size 1.5;
Line .55 .42 .62 .42;
  Type 3;
  Size 1.5;
Line .55 .37 .62 .37;
  Type 4;
  Size 1.5;
Line .55 .32 .62 .32;
  Type 5;
  Size 1.5;
Line .54 .29 .75 .29;
  Size 1.5;
Line .75 .29 .75 .55;
  Size 1.5;
Line .75 .55 .54 .55;
  Size 1.5;
Line .54 .55 .54 .29;
  Size 1.5.
Plot 'I'*'ar R3:All' 'I'*'ar R3:-k' 'I'*'ar R3:-thiele' 'I'* &
  'ar R3:-es' 'I'*'ar R3:-p';
Connect;

```

```

Type 1 2 3 4 5;
Size 1.8 1.8 1.8 1.8 1.8;
Title "Error plots for case where thiele > 1";
Legend 0 1 0 1;
Overlay;
ScFrame;
ScAnnotation;
Axis 1;
  Label "|Obs-Pred|";
  TSize 2.0;
Axis 2;
  Label "Culmulative %";
  TSize 2.0.
EndLayout.

```

Note Copy MSE's to next to last column
 Name C49 = 'MSEs'
 Copy k2-k16 'MSEs'

Note The End.

The responses (R1-R3) and parameters used by this macro to generate the sensitivity analysis are below.

Responses			Parameters			
$\phi > 1$	$\phi = 1$	$\phi < 1$	k_{dlk}	ϕ	ϵ_{σ}	ρ
9.67E-01	4.29E-01	1.91E-01	1.00E-05	5.00E-01	1.00E-00	1.00E-05
9.67E-01	4.29E-01	1.91E-01	1.00E-05	5.00E-01	1.00E-00	5.00E-02
9.67E-01	4.29E-01	1.91E-01	1.00E-05	5.00E-01	1.00E-00	1.00E-01
9.68E-01	4.34E-01	1.99E-01	1.00E-05	5.00E-01	9.90E-01	1.00E-05
9.68E-01	4.34E-01	1.98E-01	1.00E-05	5.00E-01	9.90E-01	5.00E-02
9.67E-01	4.33E-01	1.97E-01	1.00E-05	5.00E-01	9.90E-01	1.00E-01
9.96E-01	9.37E-01	9.14E-01	1.00E-05	5.00E-01	1.00E-01	1.00E-05
9.95E-01	9.00E-01	8.44E-01	1.00E-05	5.00E-01	1.00E-01	5.00E-02
9.93E-01	8.65E-01	7.79E-01	1.00E-05	5.00E-01	1.00E-01	1.00E-01
9.87E-01	7.43E-01	5.98E-01	1.00E-05	1.00E-00	1.00E-00	1.00E-05
9.87E-01	7.43E-01	5.98E-01	1.00E-05	1.00E-00	1.00E-00	5.00E-02
9.87E-01	7.43E-01	5.98E-01	1.00E-05	1.00E-00	1.00E-00	1.00E-01
9.87E-01	7.43E-01	5.98E-01	1.00E-05	1.00E-00	9.90E-01	1.00E-05
9.87E-01	7.43E-01	5.98E-01	1.00E-05	1.00E-00	9.90E-01	5.00E-02

9.87E-01	7.43E-01	5.98E-01	1.00E-05	1.00E-00	9.90E-01	1.00E-01
9.96E-01	9.40E-01	9.17E-01	1.00E-05	1.00E-00	1.00E-01	1.00E-05
9.96E-01	9.28E-01	8.93E-01	1.00E-05	1.00E-00	1.00E-01	5.00E-02
9.95E-01	9.16E-01	8.70E-01	1.00E-05	1.00E-00	1.00E-01	1.00E-01
9.93E-01	8.72E-01	7.99E-01	1.00E-05	2.00E-00	1.00E-00	1.00E-05
9.93E-01	8.72E-01	7.99E-01	1.00E-05	2.00E-00	1.00E-00	5.00E-02
9.93E-01	8.72E-01	7.99E-01	1.00E-05	2.00E-00	1.00E-00	1.00E-01
9.93E-01	8.72E-01	7.99E-01	1.00E-05	2.00E-00	9.90E-01	1.00E-05
9.93E-01	8.72E-01	7.99E-01	1.00E-05	2.00E-00	9.90E-01	5.00E-02
9.93E-01	8.72E-01	7.99E-01	1.00E-05	2.00E-00	9.90E-01	1.00E-01
9.97E-01	9.44E-01	9.20E-01	1.00E-05	2.00E-00	1.00E-01	1.00E-05
9.97E-01	9.40E-01	9.12E-01	1.00E-05	2.00E-00	1.00E-01	5.00E-02
9.96E-01	9.35E-01	9.04E-01	1.00E-05	2.00E-00	1.00E-01	1.00E-01
1.19E-07	4.08E-168	2.22E-16	5.01E-03	5.00E-01	1.00E-00	1.00E-05
1.19E-07	4.08E-168	2.22E-16	5.01E-03	5.00E-01	1.00E-00	5.00E-02
1.19E-07	4.08E-168	2.22E-16	5.01E-03	5.00E-01	1.00E-00	1.00E-01
10.00E-03	9.96E-03	9.93E-03	5.01E-03	5.00E-01	9.90E-01	1.00E-05
4.72E-03	4.84E-11	4.96E-19	5.01E-03	5.00E-01	9.90E-01	5.00E-02
2.23E-03	2.35E-19	2.48E-35	5.01E-03	5.00E-01	9.90E-01	1.00E-01
9.00E-01	8.96E-01	8.93E-01	5.01E-03	5.00E-01	1.00E-01	1.00E-05
3.98E-01	1.70E-09	1.24E-17	5.01E-03	5.00E-01	1.00E-01	5.00E-02
1.76E-01	5.84E-18	4.40E-34	5.01E-03	5.00E-01	1.00E-01	1.00E-01
1.98E-01	3.92E-02	2.80E-02	5.01E-03	1.00E-00	1.00E-00	1.00E-05
1.98E-01	3.92E-02	2.80E-02	5.01E-03	1.00E-00	1.00E-00	5.00E-02
1.98E-01	3.92E-02	2.80E-02	5.01E-03	1.00E-00	1.00E-00	1.00E-01
1.98E-01	3.95E-02	2.85E-02	5.01E-03	1.00E-00	9.90E-01	1.00E-05
1.98E-01	3.95E-02	2.85E-02	5.01E-03	1.00E-00	9.90E-01	5.00E-02
1.98E-01	3.95E-02	2.85E-02	5.01E-03	1.00E-00	9.90E-01	1.00E-01
9.00E-01	8.99E-01	8.98E-01	5.01E-03	1.00E-00	1.00E-01	1.00E-05
7.06E-01	1.75E-01	1.25E-01	5.01E-03	1.00E-00	1.00E-01	5.00E-02
5.81E-01	1.24E-01	8.86E-02	5.01E-03	1.00E-00	1.00E-01	1.00E-01
5.99E-01	5.20E-01	5.14E-01	5.01E-03	2.00E-00	1.00E-00	1.00E-05
5.99E-01	5.20E-01	5.14E-01	5.01E-03	2.00E-00	1.00E-00	5.00E-02
5.99E-01	5.20E-01	5.14E-01	5.01E-03	2.00E-00	1.00E-00	1.00E-01
5.99E-01	5.20E-01	5.14E-01	5.01E-03	2.00E-00	9.90E-01	1.00E-05
5.99E-01	5.20E-01	5.14E-01	5.01E-03	2.00E-00	9.90E-01	5.00E-02
5.99E-01	5.20E-01	5.14E-01	5.01E-03	2.00E-00	9.90E-01	1.00E-01
9.00E-01	9.00E-01	8.99E-01	5.01E-03	2.00E-00	1.00E-01	1.00E-05
8.29E-01	5.88E-01	5.63E-01	5.01E-03	2.00E-00	1.00E-01	5.00E-02
7.79E-01	5.62E-01	5.44E-01	5.01E-03	2.00E-00	1.00E-01	1.00E-01
2.62E-14	2.22E-16	2.22E-16	1.00E-02	5.00E-01	1.00E-00	1.00E-05
2.62E-14	2.22E-16	2.22E-16	1.00E-02	5.00E-01	1.00E-00	5.00E-02
2.62E-14	2.22E-16	2.22E-16	1.00E-02	5.00E-01	1.00E-00	1.00E-01
10.00E-03	9.92E-03	9.85E-03	1.00E-02	5.00E-01	9.90E-01	1.00E-05

2.23E-03	2.43E-19	2.66E-35	1.00E-02	5.00E-01	9.90E-01	5.00E-02
4.98E-04	5.97E-36	7.16E-68	1.00E-02	5.00E-01	9.90E-01	1.00E-01
9.00E-01	8.93E-01	8.85E-01	1.00E-02	5.00E-01	1.00E-01	1.00E-05
1.77E-01	6.04E-18	4.71E-34	1.00E-02	5.00E-01	1.00E-01	5.00E-02
3.52E-02	1.05E-34	8.99E-67	1.00E-02	5.00E-01	1.00E-01	1.00E-01
1.40E-01	2.77E-02	1.98E-02	1.00E-02	1.00E-00	1.00E-00	1.00E-05
1.40E-01	2.77E-02	1.98E-02	1.00E-02	1.00E-00	1.00E-00	5.00E-02
1.40E-01	2.77E-02	1.98E-02	1.00E-02	1.00E-00	1.00E-00	1.00E-01
1.40E-01	2.82E-02	2.07E-02	1.00E-02	1.00E-00	9.90E-01	1.00E-05
1.40E-01	2.82E-02	2.07E-02	1.00E-02	1.00E-00	9.90E-01	5.00E-02
1.40E-01	2.82E-02	2.07E-02	1.00E-02	1.00E-00	9.90E-01	1.00E-01
9.00E-01	8.98E-01	8.95E-01	1.00E-02	1.00E-00	1.00E-01	1.00E-05
5.82E-01	1.24E-01	8.86E-02	1.00E-02	1.00E-00	1.00E-01	5.00E-02
4.38E-01	8.78E-02	6.27E-02	1.00E-02	1.00E-00	1.00E-01	1.00E-01
5.70E-01	5.14E-01	5.10E-01	1.00E-02	2.00E-00	1.00E-00	1.00E-05
5.70E-01	5.14E-01	5.10E-01	1.00E-02	2.00E-00	1.00E-00	5.00E-02
5.70E-01	5.14E-01	5.10E-01	1.00E-02	2.00E-00	1.00E-00	1.00E-01
5.70E-01	5.14E-01	5.10E-01	1.00E-02	2.00E-00	9.90E-01	1.00E-05
5.70E-01	5.14E-01	5.10E-01	1.00E-02	2.00E-00	9.90E-01	5.00E-02
5.70E-01	5.14E-01	5.10E-01	1.00E-02	2.00E-00	9.90E-01	1.00E-01
9.00E-01	8.99E-01	8.98E-01	1.00E-02	2.00E-00	1.00E-01	1.00E-05
7.79E-01	5.62E-01	5.44E-01	1.00E-02	2.00E-00	1.00E-01	5.00E-02
7.16E-01	5.44E-01	5.31E-01	1.00E-02	2.00E-00	1.00E-01	1.00E-01

APPENDIX B

Prediction Parameters

The predictions used in this work were generated using MATLAB. Each follows a set format. First, two routines, svardefs.m and cvardefs.m, set default values for the parameters. Second, the parameter of interest is varied, and plotting and saving options are set. Third, the predictive routine, a nested iterative system, generates the predicted survival fraction given the above parameters and options.

Default Parameter Values

```
% svardefs.m
%
% sets up defaults for Stoichiometric system.
```

```
De = 1e-5;
Yxb = 50;
Xb = 1e4;
p = 0.01;
Cb = 1;
Lf = 1e-2;
Kdis = 1;
tdose = 10000;
Es = 0.90;
```

```
res = 1000; % resolution of argapp.m
```

```
% cvardefs.m
```

```
%
% sets up defaults for Catalytic system.
De = 1e-5;
Xb = 1e4;
p = 0.01;
Cb = 400;
Lf = 1e-2;
Kdis = 0.001;
tdose = 10000;
Es = 0.90;
Krxn = 100;

res = 1000; % resolution of argapp.m
```

Prediction Code

```
% pred.m
%
% predicts the effects of varying parametrs
Grph_xlabel = 'Kdis*Cb*tdose [ ]';
Grph_ylabel = 'Log10(X/Xo)';

setglobal % for gendata.m

figdir

ASFgrph(Grph_num,Grph_xlabel,Grph_ylabel,Grph_title);

fprintf('%s\n\n',Grph_title);

% Make sure we account for numerous values of these...
l = length(Krxn)+length(Cb)+length(Yxb)+length(De)+length(Xb)+length(Lf)-5;
tpen = zeros(1,l);
thiele = zeros(1,l);
tdosed = zeros(1,2);
l = 0;

for Krxn1 = 1:length(Krxn)
for Cb1 = 1:length(Cb)
for Yxb1 = 1:length(Yxb)
for De1 = 1:length(De)
for Xb1 = 1:length(Xb)
```

```

for Lfl = 1:length(Lf)

    l = l + 1;
    tpen(l) = Lf(Lfl)^2*Xb(Xbl)/(2*De(Del)*Cb(Cbl)*Yxb(Yxbl));
    thiele(l) = sqrt(Krxn(Krxnl)*Lf(Lfl)^2/(2*De(Del)*Cb(Cbl)));

    if tdosemax == 0
        tdosed(l,:)=[0.0001 tpen(l)*1.5];
    else
        tpen(l) = tdosemax;
        tdosed(l,:)=[0.001 tdosemax];
    end

    for Kdisl = 1:length(Kdis)
        for pl = 1:length(p)
            for EsI = 1:length(Es)

                if eval(printwhen)

                    %kdis=Params(1); tpen=Params(2); thiele=Params(3); es=Params(4); p=Params(5);
                    switch model
                        case 1, Params = [Kdis(Kdisl) tpen(l) n_thiele Es(EsI) p(pl)];
                        case 4, Params = [Kdis(Kdisl) tpen(l) n_thiele n_es n_p];
                        case 3, Params = [Kdis(Kdisl) n_tpen n_thiele Es(EsI) p(pl)];
                        case 2, Params = [Kdis(Kdisl) n_tpen thiele(l) Es(EsI) p(pl)];
                        case 5, Params = [Kdis(Kdisl) n_tpen thiele(l) n_es n_p];
                    end

                    fprintf('Generating data for 0-%3.0f seconds,\n',max(tdosed(l,:)));
                    fprintf(' with Kdis: %3.3g Tpen: %3.3g Thiele: %3.3g Es: %3.3g p: %3.3g\n',Params);

                    [tdosec,ASFc] = gendata(Params,Cb(Cbl),tdosed(l,:),model);
                    plot(tdosec.*(Cb(Cbl)*Kdis(Kdisl)),log10(ASFc),'-','LineWidth',2); hold on;

                end

            end
        end
    end
end
end
end
end
end
end
end
end

```

```

end

fprintf('Done.\n');

if ASFmin ~= 0
    ylim([ASFmin 0]);
    fprintf('Rescaled Y-Axis to %g.\n',ASFmin);
end
if PsiMax ~= 0
    xlim([0 PsiMax]);
    fprintf('Rescaled X-Axis to %g.\n',PsiMax);
else
    xlim([0 max(tdosed(:,2))]);
end

if length(fname)
    % kill xaxis formatting
    set(gca,'xtickmode','manual');
    set(gca,'xtick',[]);

    fname=[figuredir fname];
    eval(['print -dmfile ' fname]);
    fprintf('Graph written to %s.\n',fname);
end

zoom on;
fprintf('You can zoom with mouse (see "help zoom" for details).\n\n');

```

Prediction Scenarios Used in This Thesis

```

% pred_es.m
%
% predicts the effect of a changing sensitive fraction on physiology model

% what would we like our graph to look like?
Grph_num = 5;
Grph_title = 'Effect of Sensitive Fraction Parameter In the Abscense of Transport
Resistance';

cvardefs % default parameters
svardefs % put the ones you want to use _last_

```

```

Es = [.9 .99 .999]; % vary this
tdosemax = 50; % set this to manually scale graph, otherwise 0 for autoscale to 1.5*tpen
ASFmin = -5; %set this to scale the y-axis manually, otherwise 0 for autoscale
PsiMax = 0; % set this to scale the x-axis manually, otherwise, 0
fname = 'p_es'; % set this to write out plot, or " for no save.
printwhen = '1'; %set this '1' for each, or a conditional for when to print

```

```

model = 3; % 1-5

```

```

pred

```

```

% pred_p.m

```

```

%

```

```

% predicts the effect of a changing physiological resistance on physiology model

```

```

% what would we like our graph to look like?

```

```

Grph_num = 4;

```

```

Grph_title = 'Effect of Physiology Parameter In the Abscense of Transport Resistance';

```

```

cvardefs % default parameters

```

```

svardefs % put the ones you want to use _last_

```

```

p = [p p*10 p*100]; % vary this

```

```

tdosemax = 100; % set this to manually scale graph, otherwise 0 for autoscale to
1.5*tpen

```

```

ASFmin = -5; %set this to scale the y-axis manually, otherwise 0 for autoscale

```

```

PsiMax = 0; % set this to scale the x-axis manually, otherwise, 0

```

```

fname = 'p_p'; % set this to write out plot, or " for no save.

```

```

printwhen = '1'; %set this '1' for each, or a conditional for when to print

```

```

model = 3; % 1-5

```

```

pred

```

```

% pred_cb_s.m

```

```

%

```

```

% predicts the effect of a changing bulk concentration on the stoichiometric model

```

```

% what would we like our graph to look like?

```

```

Grph_num = 6;

```

```

Grph_title = 'Effect of Bulk Concentration on Stoichiometric Transport';

```

```

cvardefs % default parameters

```

```

svardefs % put the ones you want to use _last_

```

```

Cb = [Cb/2 Cb Cb*2]; % vary this
tdosemax = 0; % set this to manually scale graph, otherwise 0 for autoscale to 1.5*tpen
ASFmin = -5; %set this to scale the y-axis manually, otherwise 0 for autoscale
PsiMax = 0; % set this to scale the x-axis manually, otherwise, 0
fname = 'p_cb_s'; % set this to write out plot, or '' for no save.
printwhen = '1'; %set this '1' for each, or a conditional for when to print

```

```

model = 4; % 1-5

```

```

pred

```

```

% pred_cb_c.m
%
% predicts the effect of a changing bulk concentration on the catalytic model

```

```

% what would we like our graph to look like?
Grph_num = 7;
Grph_title = 'Effect of Bulk Concentration on Catalytic Transport';

```

```

svardefs % put the ones you want to use _last_
cvardefs % default parameters

```

```

Cb = [Cb/2 Cb Cb*2]; % vary this
tdosemax = 150; % set this to manually scale tdose maximum, otherwise 0 for autoscale
to 1.5*tpen
ASFmin = -3; %set this to scale the y-axis manually, otherwise 0 for autoscale
PsiMax = tdosemax/5; % set this to scale the x-axis manually, otherwise, 0
fname = 'p_cb_c'; % set this to write out plot, or '' for no save.
printwhen = '1'; %set this '1' for each, or a conditional for when to print

```

```

model = 5; % 1-5

```

```

pred

```

```

% pred_prop_s.m
%
%
% predicts the effect of a changing biocide properties on stoichiometric model

```

```

% what would we like our graph to look like?
Grph_num = 8;
Grph_title = 'Effect of Biocide Properties on Stoichiometric Transport';

```

```

cvardefs % default parameters

```

```
svardefs % put the ones you want to use _last_
```

```
Yxb = [Yxb/2 Yxb Yxb*2]; % vary this
```

```
Kdis = [Kdis*2 Kdis Kdis/2];
```

```
tdosemax = 0; % set this to manually scale graph, otherwise 0 for autoscale to 1.5*tpen
```

```
ASFmin = -5; %set this to scale the y-axis manually, otherwise 0 for autoscale
```

```
PsiMax = 0; % set this to scale the x-axis manually, otherwise, 0
```

```
fname = 'p_prop_s'; % set this to write out plot, or '' for no save.
```

```
printwhen = 'Yxb1 == Kdis1'; %set this '1' for each, or a conditional for when to print
```

```
model = 4; % 1-5
```

```
pred
```

```
% pred_prop_c.m
```

```
%
```

```
% predicts the effect of a changing biocide properties on catalytic model
```

```
% what would we like our graph to look like?
```

```
Grph_num = 9;
```

```
Grph_title = 'Effect of Biocide Properties on Catalytic Transport';
```

```
svardefs % put the ones you want to use _last_
```

```
cvardefs % default parameters
```

```
Krxn = [Krxn/2 Krxn Krxn*2]; % vary this
```

```
Kdis = [Kdis/2 Kdis Kdis*2];
```

```
tdosemax = 150; % set this to manually scale graph, otherwise 0 for autoscale to  
1.5*tpen
```

```
ASFmin = -5; %set this to scale the y-axis manually, otherwise 0 for autoscale
```

```
PsiMax = tdosemax/2.5; % set this to scale the x-axis manually, otherwise, 0
```

```
fname = 'p_prop_c'; % set this to write out plot, or '' for no save.
```

```
printwhen = 'Krxn1 == Kdis1'; %set this '1' for each, or a conditional for when to print
```

```
model = 5; % 1-5
```

```
pred
```


APPENDIX C

Published Data Sets and Parameter Estimations

The MATLAB code used to fit published data seen in the previous sections is included in this section. Where independent measurement or estimation was possible, that information is shown here.

Tobramycin Challenge on a *Pseudomonas aeruginosa* Biofilm

Two data sets pulled from these publications were analyzed. The first, as stated previously, describes an antimicrobial challenge to a two-day-old biofilm. The second is of a similar system but for two parameters: the biofilm is seven-days-old and the antibiotic concentration was increased ten-fold. The MATLAB code is shown below.

```
% fit_toby.m
%
% from (Anwar, H., Biesen, T. et.al., 1989) and (Anwar, H., Dasgupta, M. et. al., 1989)

% Data gleaned from the experiment
Cb = 20; % want this in mg/L
tdosed = (60*60).*[0.001 1 2 3 4 5]; % want this in seconds
ASFd=[100 10 3 1.5 1.3 1.2]./100;

% what would we like our graph to look like?
```

```
Grph_num = 51;
Grph_title = 'Tobramycin Disinfection of Young P.aeruginosa Biofilm';
Grph_xlabel = 'tdose [sec]';
Grph_ylabel = 'Log10(X/Xo)';
```

```
% Parameters required for a minimization:
```

```
% Ranges appropriate for our experiment to model
```

```
% (leave these alone unless you have some thoughts as to the values these should be
```

```
kr = [1e-2 1e-8]; % range of kdis
```

```
tpenr = [1 100]; % range of tpen
```

```
esr = [.999 0.96]; % range of es
```

```
pr = [1 1e-4]; % range of p
```

```
thieler = [0.001 2]; % range of thiele mod.
```

```
% Hmm, how to describe this parameter... Ok, it's the number of guesstimates the
% fitting routine will take to try to find good initial guesses for the constrained
% minimization. The number of guesses is calculated by iguess^4 for the stoichometroc
% model, and iguess^3 for the catalytic. So this should be '3' if you've a clear
% idea of what the ranges should be, and maybe '5' if you've got really broad ranges,
% and '0' if you just want to skip the finding initial gueses, and just use the
% means of the ranges. Alternately, you can uncomment the line below and pick your
% initial guesses: (make sure iguess is '0'). As an aside, choosing iguess to be
% '10', and then making the ranges wide might be seem a good starting point, but the
% existance of local minima will stymie this approach. Vi
```

```
% InitGuess = 0; % this for the means of the ranges
```

```
% User-specified InitGuess is the way to go, with a 6x5 array
```

```
% Columns are: [k tpen theiele es p];
```

```
% Rows are: [Stoich_Full; Stoich_Trans; Stoich_Phys; Cat_Full; Cat_Trans; Cat_Phys]
```

```
InitGuess = [...
```

```
3.4362e-05 1.02 0 0.98096 3.753e-02;...
```

```
1.5080e-05 1 0 1 1.000e+00;...
```

```
3.4382e-05 1 0 0.98081 3.820e-02;...
```

```
1.3044e-04 0 0.933 0.98793 4.908e-03;...
```

```
4.8315e-04 0 0.994 1 1.000e+00;...
```

```
3.4370e-05 0 0.001 0.98087 3.788e-02;...
```

```
];
```

```
iguess = 0;
```

```
conversion % needed for new system
```

```
% Finally, call our fitting routines
```

```
Mainfit
```

```

%handfit
% Parameters and other info stored in Results, which is defined [6 7]
% Each row contains [Error num_its kdis tpen thiele es p], though not all will be filled
%   num_its -> number of iteration loops the constrained minimization
% A string array, Rstring, contains a formatted string of the above information.
% Each row represents one method of data fitting, in this order:
%   [Stoich_Full; Stoich_Trans; Stoich_Phys; Cat_Full; Cat_Trans; Cat_Phys]

% fit_tobo.m
%
% from (Anwar, H., Biesen, T. et.al., 1989) and (Anwar, H., Dasgupta, M. et. al., 1989)

% Data gleaned from the experiment
Cb = 200; % want this in mg/L
tdosed =(60*60).*[0.001 1 2 3 4 5]; % want this in seconds
ASFd=[100 40 30 20 15 13]./100;

% what would we like our graph to look like?
Grph_num = 52;
Grph_title = 'Tobramycin Disinfection of Old P.aeruginosa Biofilm';
Grph_xlabel = 'tdose [sec]';
Grph_ylabel = 'Log10(X/Xo)';

% Parameters required for a minimization:
% Ranges appropriate for our experiment to model
% (leave these alone unless you have some thoughts as to the values these should be
kr = [1e-2 1e-8]; % range of kdis
tpenr = [1 100]; % range of tpen
esr = [.01 0.7]; % range of es
pr = [1 1e-4]; % range of p
thieler = [0.001 2]; % range of thiele mod.

% (ommitted)

InitGuess = [...
1.500e-005 1 0.00000 0.47969 3.000e-002;...
6.5875e-07 1 0 1 1.000e+00;...
1.500e-005 0 0.00000 0.47969 3.000e-002;...
2.1067e-04 0 0.986 0.3183 2.342e-02;...
4.9796e-06 0 0.985 1 1.000e+00;...
1.2374e-04 0 0.001 0.46535 3.256e-03;...
];

iguess = 0;

```

conversion % needed for new system

% Finally, call our fitting routine

Mainfit

% or, run handfit

%handfit

Hydrogen Peroxide Challenge on a *Pseudomonas aeruginosa* Biofilm

An independent estimation of the Thiele modulus for this data set was possible. That data, and the MATLAB code used to fit the remaining parameters, are below. The aqueous diffusion coefficient for hydrogen peroxide was obtained from the literature (van Stroe-Biezen, et al. 1993). The diffusion coefficient for oxygen in water was taken from the empirical formula (Han, et al. 1996):

$$D_{O_2,T} = -4.410 + \frac{773.8}{T} - \left(\frac{506.4}{T} \right)^2 \quad \text{where } T \text{ is in Kelvin}$$

The external mass transfer resistance coefficient for oxygen was obtained in the literature (Siegrist, et al. 1987). Together, it was possible to then estimate the external mass transfer resistance coefficient for hydrogen peroxide by:

$$k_{HP,25C} = k_{O_2,20C} \cdot \frac{D_{HP,25C}}{D_{O_2,20C}}$$

With these values in hand, and those from the experimental system, it was possible to estimate a high and low value for the Thiele modulus. The low value assumes no external mass transfer resistance, while the high value takes that into account.

krxn:	8.33E-03	[=]	mM/mgP-sec
Xb:	3.8		mgP/cm ³
kzero	3.17E-02	=krxn*Xb	mM/cm ³ -sec
Lf:	1.14E-02		cm
Daq	1.40E-05		cm ² /sec
Dratio:	0.8		
De:	1.12E-05	=Dratio*Daq	cm ² /sec
Cb:	5.00E-02		mM/cm ³
thiele:	1.916505	This represents "best case" for thiele	
km:	1.70E-03		cm/sec
a:	4.95E-01	=kzero*Lf/Km*sqrt(2 *De/kzero/Lf ²)	(mM/cm ³) ²
Cs+a*sqrt(Cs)-Cb=	0.00E+01	should be 0.	
Cs:	7.40E-03		
min Cs:	5.00E-08	Cb / 1e6	
check:	0.00E+01		
thiele:	4.982533	This represents "worst case" for thiele	

Given these estimates, the range of the Thiele modulus could be constrained. The planktonic counterpart to this biofilm challenge produced an estimate of the disinfection rate constant.

Disinfection Rate Constant Regression on Wild Type Planktonic Data

Cb= 0.05 [=] mM/cm³

Time (sec)

Time*Cb

0	0
1200	60
2400	120
3600	180

CFUs/mL

log₁₀(X/X₀)

Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
2.00E+07	2.60E+07	4.10E+07	0.00	0.00	0.00
3.90E+06	5.10E+05	3.00E+05	-0.71	-1.71	-2.14
9.20E+05	2.00E+03	2.50E+02	-1.34	-4.11	-5.21
1.70E+04	1.10E+03	1.50E+01	-3.07	-4.37	-6.44

Kdis for W.T. [mM-sec]⁻¹ R²

Set 1	1.4995E-02	0.925
Set 2	2.7448E-02	0.923
Set 3	3.7947E-02	0.923
Mean	2.6797E-02	

Finally, the two MATLAB programs incorporating the survival rate data for the biofilm and each of these estimates are shown below. The first, `fit_per.m`, takes the predicted disinfection rate coefficient, upper and lower Thiele modulus, and experimental data, and then runs each of the five models with these values, allowing for 10% deviation in the predicted values. The second, `pred_per.m`, uses the Thiele modulus calculations above to establish an upper and lower bound to the Thiele modulus and shows the predicted survival fraction curve to adequately describe the experimental data.

```
% fit_per.m
%
% from unpublished data (Elkins, 1998)

% Data gleaned from the experiment
Cb = 0.05; % mM/cm^3
tdosed = [0.001 1200 2400 3600];
PerSurv1=[100.0 55.3 62.1 98.4];
PerSurv2=[100.0 70.6 98.3 93.4];
PerSurv3=[100.0 90.0 58.3 54.8];
ASFd1=PerSurv1./max(PerSurv1);
ASFd2=PerSurv2./max(PerSurv2);
ASFd3=PerSurv3./max(PerSurv3);
ASFd=mean([ASFd1; ASFd2; ASFd3;]);

% what would we like our graph to look like?
Grph_num = 99;
Grph_title = 'Fit to Jims H2O2 Data';
Grph_xlabel = 'tdose [sec]';
Grph_ylabel = 'Log10(X/Xo)';

% Parameters required for a minimization:
% Ranges appropriate for our experiment to model
```

% (leave these alone unless you have some thoughts as to the values these should be

Kdis=[2.6797e-002];

Kdis=[Kdis*0.9 Kdis*1.1];

kr = [min(Kdis) max(Kdis)]; % range of kdis

tpenr = [1 100]; % range of tpen

esr = [1 .1]; % range of es

pr = [1 1e-7]; % range of p

thiele = [1.916505805 4.982533997];

thieler = [min(thiele) max(thiele)]; % range of thiele mod.

% (omitted)

InitGuess = [...

2.6797e-02 1 0 .25 1e-4;...

2.6797e-02 1 0 1 1;...

2.6797e-02 1 0 .25 1e-4;...

2.6797e-02 0 2.2 .25 1e-4;...

2.6797e-02 0 2.2 1 1;...

2.6797e-02 0 0.001 .25 1e-4;...

];

iguess = 0;

conversion % needed for new system

% Finally, call our fitting routines

Mainfit

%handfit

% pred_per.m

%

% two prediction curves based on estimated Thiele values

tic

ASFgrph(99,'tdose [sec]','Log(ASF)','Fit to Jims H2O2 Data');

Xb = 3.8;

Cb = 0.05;

Lf = 0.0114;

Es = 1;

p = 1;

Krxn = 3.17e-2;

res = 100; % resolution of argapp.m

```

Kdis=[1.4995E-02 2.7448E-02 3.7947E-02];
tdosed =[0.001 1200 2400 3600];
PerSurv1=[100.0 55.3 62.1 98.4];
PerSurv2=[100.0 70.6 98.3 93.4];
PerSurv3=[100.0 90.0 58.3 54.8];
ASFd1=PerSurv1./max(PerSurv1);
ASFd2=PerSurv2./max(PerSurv2);
ASFd3=PerSurv3./max(PerSurv3);
ASFd=mean([ASFd1; ASFd2; ASFd3;]);

%plot(tdosed,log10(ASFd1),'*'); hold on;
%plot(tdosed,log10(ASFd2),'o'); hold on;
%plot(tdosed,log10(ASFd3),'x'); hold on;
%Kdis=sum(Kdis)/3;
plot(tdosed,log10(ASFd),'s'); hold on;
Kdis=mean(Kdis);

[tdosec,ASFp]=gendata([Kdis 0 2.2 Es p],Cb,tdosed,5);
plot(tdosec,log10(ASFp),'-'); hold on;

%legend('Data','Predicted',3);
%legend('Set 1','Set 2','Set 3');

s={'-' '-o' '-x'};

axis([0 max(tdosed)*1.1 min(log10([ASFd1 ASFd2 ASFd3])) 0]);

divs = 20;

tdose=0.0001:max(tdosed)*1.1/(divs-1):max(tdosed)*1.1;
thiele = [1.916505805 4.982533997];
pen = 1./thiele;
%thiele = sqrt(Krxn*Lf^2/(2*De))
%pen = sqrt(2*De/Krxn/Lf^2)

ASFc=ones(length(Kdis)*length(thiele),length(tdose));
l = 0;

for Kdl = 1:length(Kdis)
for Thl = 1:length(thiele)
l = l + 1;
for Tdl = 1:length(tdose)

ASFc(l,Tdl)=ASFcat(Kdis(Kdl)*tdose(Tdl)*Cb,thiele(Thl),pen(Thl),p,Es,res);

```

```

end
plot(tdose,log10(ASFc(l,:)),char(s(Kdl)), 'LineWidth',2); hold on;
%Kdl,Thl,log10(ASFc(l,:));
end
end

for l=1:length(tdosed)
    plot([tdosed(l) tdosed(l)],...
        log10([min([ASFd1(l) ASFd2(l) ASFd3(l)]) max([ASFd1(l) ASFd2(l)
        ASFd3(l)])]),'-+');
    hold on;
end

toc

```

Chlorhexidine Gluconate Challenge on a MRSA *Staphylococcus aureus* Biofilm

Only the MATLAB code is shown for three antimicrobial agent concentrations.

```

% fit_chex.m
%
% from (Oie et.al., 1996)

% Data gleaned from the experiment
ASFd01=(10.^[5.56 4.78 3.55 3.62])./10^5.56;
Cb01 = 0.1;
ASFd05=(10.^[5.56 2.99 2.18 2.41])./10^5.56;
Cb05 = 0.5;
ASFd10=(10.^[5.56 3.93 2.86 2.28])./10^5.56;
Cb10 = 1.0;

ASFd=[ASFd01 ASFd05 ASFd10];
tdosed =60.*[0.001 10 30 60];
tdosed =[tdosed.*Cb01 tdosed.*Cb05 tdosed.*Cb10]; % Well, this isn't really tdosed.
Cb=max([Cb01 Cb05 Cb10]);

% what would we like our graph to look like?

```

```

Grph_num = 21;
Grph_title = 'Chlorhexidine Disinfection of S.aureus Biofilm';
Grph_xlabel = 'tdose*Cb [%wt-sec]';
Grph_ylabel = 'Log10(X/Xo)';

```

```

% Parameters required for a minimisation:
% Ranges appropriate for our experiment to model
% (leave these alone unless you have some thoughts as to the values these should be
kr = [1 1e-6]; % range of kdis
tpenr = [1 1000]; % range of tpen
esr = [1.0 0.8]; % range of es
pr = [1 1e-5]; % range of p
thieler = [0.01 10]; % range of thiele mod.

```

```

% (omitted)

```

```

InitGuess = [...
3.2745e-02 5.99 0 0.99243 2.270e-02;...
3.4555e-03 1 0 1 1.000e+00;...
3.0828e-02 1 0 0.99339 2.284e-02;...
3.0827e-02 0 0.135 0.99361 2.293e-02;...
3.4542e-03 0 0.0104 1 1.000e+00;...
3.0457e-02 0 0.001 0.99361 2.280e-02;...
];

```

```

iguess = 0;

```

```

conversion % needed for new system

```

```

% Finally, call our fitting routines
Mainfit
%handfit

```

Benzalkonium Chloride Challenge on a *Listeria monocytogenes* Biofilm

Again, only the MATLAB code for three different agent concentrations is shown here..

```

% fit_bac.m
%
% from (Frank et.al., 1990)

IASFd100=[0 -2.3 -3.1 -3.1 -2.4 -3.5 -4.4];
Cb100 = 100;
IASFd400=[0 -1.7 -3.2 -4.0 -3.7 -4.3 -5.7];
Cb400 = 400;
IASFd800=[0 -2.1 -3.1 -3.3 -3.4 -4.0 -4.05];
Cb800 = 800;

IASFd=[IASFd100 IASFd400 IASFd800];
ASFd=10.^IASFd;
tdosed =60.*[0.001 0.5 4 8 12 16 20];
tdosed=[Cb100.*tdosed Cb400.*tdosed Cb800.*tdosed];
Cb=max([Cb100 Cb400 Cb800]);

% what would we like our graph to look like?
Grph_num = 31;
Grph_title = 'BAC Disinfection of L.monocytogenes Biofilm';
Grph_xlabel = 'tdose*Cb [ppm-sec]';
Grph_ylabel = 'Log10(X/Xo)';

% Parameters required for a minimixation:
% Ranges appropriate for our experiment to model
% (leave these alone unless you have some thoughts as to the values these should be
kr = [1 1e-11]; % range of kdis
tpenr = [1 5000]; % range of tpen
thieler = [0.001 10]; % range of thiele mod.
esr = [1.0 0.1]; % range of es
pr = [1 1e-6]; % range of p

% (omitted)

InitGuess = [...
2.3350e-06    100     0    0.99916 1.290e-03;...
2.2428e-06     1     0     1    1.000e+00;...
2.2831e-06     1     0    0.99915 1.503e-03;...
2.3314e-06     0    0.176 0.99915 1.544e-03;...
1.1900e-07     0    0.01     1    1.000e+00;...
2.2822e-06     0    0.001 0.99915 1.457e-03;...
];

iguess = 0;

```

```

conversion % needed for new system
% Finally, call our fitting routines
Mainfit
%handfit

```

Monochloramine Challenge on a *Pseudomonas aeruginosa* Biofilm

```

% fit_mcl.m
%
% from (Sirinisavan, 1994)

% Data gleaned from the experiment
ASFd=[1.1e12 3.34e11 6.47e10 1.02e10 4.51e9 1.24e9 8.89e8 1.5e8 1.7e7]./1.1e12;
tdosed =(60*60).*[0.001 0.167 0.25 0.5 0.75 1.0 1.25 1.5 2.0];
Cb=4;

% what would we like our graph to look like?
Grph_num = 30;
Grph_title = 'Monochloramine Disinfection of P.aeruginosa Biofilm';
Grph_xlabel = 'tdose [sec]';
Grph_ylabel = 'Log10(X/Xo)';

% Parameters required for a minimixation:
% Ranges appropriate for our experiment to model
% (leave these alone unless you have some thoughts as to the values these should be
kr = [1e-1 1e-10]; % range of kdis
tpenr = [1 10000]; % range of tpen
esr = [1.0 0.9]; % range of es
pr = [1 1e-2]; % range of p
thieler = [0.01 10]; % range of thiele mod.

% (omitted)

InitGuess = [...
1.6757e-03    500      0    0.95287 1.780e-01;...
4.4075e-04    500      0      1 1.000e+00;...
9.8642e-04      1      0    0.90188 3.083e-01;...
2.4396e-03      0    0.864  0.90706 4.854e-01;...
4.8520e-03      0    0.969      1 1.000e+00;...
9.7024e-04      0    0.001  0.9074 3.112e-01;...
];

```

iguess = 0;

conversion % needed for new system

% Finally, call our fitting routines
Mainfit

Sodium Hypochlorite Challenge on a
artificial *Pseudomonas aeruginosa*
Biofilm

% fit_k10

%

% from unpublished data (Grobe, 1998)

% Data gleaned from the experiment
% From Kate Grobe's Data

% Experiment 5:

Cb5=9.33;

tdosed5=60.*[0.001 60 120 135 150 175 200 225]; % seconds

IXd5=[9.757053727 9.15880051 8.285342851 7.976808532 7.363970465
6.110665462 5.070869228 3.538510897];

% Experiment 6

Cb6=9.36;

tdosed6=60.*[0.001 60 120 135 150];

IXd6=[9.678222374 9.075837138 8.439577325 7.892246094 7.313863698];

% Experiment 7:

Cb7=9.65;

IXd7=[10.08348372 9.286025977 9.425382586 9.10933502 9.103994024

8.899885147 8.192735744 7.609774019 6.919123579 6.032193491

5.059574066 2.839540893];

tdosed7=60.*[0.001 10 15 25 35 60 100 115 130 145 160 180];

IASFd5=log10(10.^IXd5./max(10.^IXd5));

IASFd6=log10(10.^IXd6./max(10.^IXd6));

IASFd7=log10(10.^IXd7./max(10.^IXd7));

IASFd=[IASFd5 IASFd6 IASFd7];

ASFd=10.^IASFd;


```
tdosed=[tdosed5*Cb5 tdosed6*Cb6 tdosed7*Cb7]; % An out-and-out lie, but, hey...
```

```
Cb=max([Cb5 Cb6 Cb7]);
```

```
% what would we like our graph to look like?
```

```
Grph_num = 61;
```

```
Grph_title = 'Chlorine Disinfection of P.aeuruginosa Biofilm';
```

```
Grph_xlabel = 'tdose*Cb [mg/L-sec]';
```

```
Grph_ylabel = 'Log10(X/Xo)';
```

```
% Parameters required for a minimization:
```

```
% Ranges appropriate for our experiment to model
```

```
% (leave these alone unless you have some thoughts as to the values these should be
```

```
kr = [1000 1e-8]; % range of kdis
```

```
tpenr = [1 100000]; % range of tpen
```

```
esr = [.001 1]; % range of es
```

```
pr = [1 1e-8]; % range of p
```

```
thieler = [0.001 10]; % range of thiele mod.
```

```
% (omitted)
```

```
InitGuess = [...
```

```
2.2028e-01 5.8e+04 0 0.99 8.746e-05;...
```

```
1.2439e-05 5.8e+04 0 1 1.000e+00;...
```

```
9.4231e-02 1 0 0.0010941 9.424e-05;...
```

```
9.4231e-2 0 0.001 0.0010941 9.424e-5;...
```

```
9.3726e-06 0 0.275 1 1.000e+00;...
```

```
9.4231e-02 0 0.001 0.0010941 9.424e-05;...
```

```
];
```

```
iguess = 0;
```

```
conversion % needed for new system
```

```
% Finally, call our fitting routines
```

```
% handfit
```

```
Mainfit
```

APPENDIX D

MATLAB Code

The code used in MATLAB can be broken into several parts:

Main routines that constitute the core of the ASF model.

Code generating data for sensitivity analysis in Appendix A.

Prediction routines for predictions in Appendix B.

Data-fitting routines used to model data in Appendix C

The percent-symbol (%) is used by MATLAB to indicate a comment or information not to be used by the MATLAB compiler.

Main Routines

Roughly organized, these routines fall into five categories:

Stoichiometric model numerical solution

Catalytic model numerical solution

Physiology model analytical solution

Graph plotting

Support modules

Stoichiometric model numerical solution Three programs are used to solve the numerical solution to the stoichiometric model: ASFstoic.m, argapps.m, and trapz.m.

% ASFstoic.m

% solves the Stoichiometric model numerically

function ASF = ASFstoic (tau,psi,p,Es,res)

% returns a single ASF point for the above parameters

% now we get into cases:

if tau > 1 % Case 1: tdose > tpen

 %fprintf('Case 1.1, w/ tau:%3.3f psi:%3.3e p:%2.2e Es:%2.2f\n',tau,psi,p,Es);

 A = -psi/tau;

 B = 2*psi/tau;

 C = -psi;

 ASF=argapps(A,B,C,0,Es,res);

 ASF=ASF+argapps(p*A,p*B,p*C,Es,1,res);

else % Case 2: tdose < tpen

 A = -psi/tau;

 B = 2*psi/sqrt(tau);

 C = -psi;

 if sqrt(tau)<=Es % Case 2.1: penetration into sensitive zone only

 %fprintf('Case 2.1, w/ tau:%3.3f psi:%3.3e p:%2.2e Es:%2.2f\n',tau,psi,p,Es);

 ASF=1-sqrt(tau);

 ASF=ASF+argapps(A,B,C,0,sqrt(tau),res);

 else % Case 2.2: penetration throughout biofilm

 %fprintf('Case 2.1, w/ tau:%3.3f psi:%3.3e p:%2.2e Es:%2.2f\n',tau,psi,p,Es);

 ASF=1-sqrt(tau);

 ASF=ASF+argapps(p*A,p*B,p*C,Es,sqrt(tau),res);

 ASF=ASF+argapps(A,B,C,0,Es,res);

 %argapps(p*A,p*B,p*C,Es,sqrt(tau),res);

end

end

function area = argapps(A,B,C,l,h,res)

% designed to approximate the area of the function:

% $\exp(A \cdot x^2 + B \cdot x + C)$
 % over the boundaries: $l \rightarrow h$
 % with the resolution (x points of): res

$x = l:(h-l)/res:h;$

$y = \exp(A \cdot x.^2 + B \cdot x + C);$

area = trapz(y,x);

function a = trapz(y,x)

%

% a = trapz(y,x)

%

% find the area under the curve $y = f(x)$ using the trapezoidal rule

%

% x = vector containing the independent variable data points

% the elements of x need not be equally spaced

% y = f(x) = vector of the function values corresponding to the

% elements in x

%

% a = the area under f(x) in the interval defined by x

%

%

% Written by: Duane Hanselman, University of Maine, (207)-581-2246

%

$x = x(:)'; y = y(:)';$ % make sure x and y are row vectors

$ly = \text{length}(y);$

$lx = \text{length}(x);$

if ($lx \sim ly$), error('x and y must be the same length'),end

$a = 0.5 * ([0 \ y] + [y \ 0]) .* ([x \ 0] - [0 \ x]);$

$a = \text{sum}(a(1,2:ly));$

Catalytic model numerical solution Three programs are used to solve the numerical solution to the catalytic model: ASFcat.m, argappc.m, and trapz.m (already shown).

```
function ASF = ASFcat (psi,thiele,pen,p,Es,res)
```

```
% returns a single ASF point for the above parameters using the catalytic system.
```

```
if thiele >= 1 % Case 1: no C at substratum
```

```
    if pen<=Es % Case 1.1: penetration into sensitive zone only
```

```
        ASF=1-pen;
```

```
        ASF=ASF+argappc(-psi,thiele,thiele,0,pen,res);
```

```
    else % Case 1.2: penetration into sensitive and resistant zone
```

```
        ASF=1-pen;
```

```
        ASF=ASF+argappc(-p*psi,thiele,thiele,Es,pen,res);
```

```
        ASF=ASF+argappc(-psi,thiele,thiele,0,Es,res);
```

```
    end
```

```
else % Case 2: C at substratum
```

```
    if pen<=Es % Case 2.1: sensitive biofilm only (rare)
```

```
        ASF=argappc(-psi,thiele,thiele^2,0,1,res);
```

```
    else % Case 2.2: penetration throughout biofilm (common)
```

```
        ASF=argappc(-p*psi,thiele,thiele^2,Es,1,res);
```

```
        ASF=ASF+argappc(-psi,thiele,thiele^2,0,Es,res);
```

```
    end
```

```
end
```

```
function area = argappc(A,B,C,l,h,res)
```

```
% designed to approximate the area of the function:
```

```
%  $\exp[A*(B^2*x^2-2*C*x+1)]$ 
```

```
% over the boundaries: l -> h
```

```
% with the resolution (x points of): res
```

```
x=l:(h-l)/res:h;
```

```
y=exp(A*(B^2*(x.^2)-(2*C)*x+1));
```

```
area = trapz(y,x);
```

Physiology model analytical solution The physiology model, considered alone, has a analytical solution, so the computational effort spent on this model is greatly reduced. Only one file is used: ASFphys.m:

```
% ASFphys.m

% analytical solution possible with no transport phenomena

function ASF = ASFphys (psi,Es,p)
% returns a single ASF point for the above parameters

ASF = Es*exp(-psi)+(1-Es)*exp(-p*psi);
```

Graph plotting One program is used uniformly throughout the ASF MATLAB system: ASFgrph.m:

```
% ASFgrph.m

% set up standard graph format

function ASFgrph(f,xl,yl,tit)

figure(f);

clf reset;

set(gcf,'PaperOrientation','landscape','PaperPosition',[1 0.5 9 7]);

axes('position',[0.15 0.19 0.82 0.78]);
cla reset;
xlabel(xl,'FontName','Helvetica','FontSize',18,'FontWeight','bold');
ylabel(yl,'FontName','Helvetica','FontSize',18,'FontWeight','bold');
if ~strncmp('none',tit,4)
    set(gcf,'Name',tit);
end
set(gca,'FontName','Helvetica','FontSize',18,'FontWeight','bold','LineWidth',3);

hold on;
```

Support routines One custom program, ASFpath.m, is used to control the “path” variable from within MATLAB. Not shown here, as it is copyrighted, is MATLAB’s Optimization Toolkit, which is required for the minimization routines.

```
% ASFpath.m
%
% adds sub directories to path.
```

```
path(pwd,path);
path([pwd,'/fit'],path);
path([pwd,'/data'],path);
path([pwd,'/pred'],path);
path([pwd,'/support'],path);
path([pwd,'/figures'],path);
path([pwd,'/thesis'],path);
```

Sensitivity Analysis

Two routines are used to generate the data seen in Appendix A, sens_stoich.m and sens_cat.m:

```
% sens_stoich.m
%
% sensitivity analysis data generator

% Assume some values as our constants

svardefs
% select file to write to, in current directory, or absolute
filename = 'sens_stoich.txt';

% Ok, set the ranges for our four parameters, and our xaxis, psi
```


% Alternately, you can manually assign values, but the length of the
% array _better_ be equal to pvar.

```
kr = [1e-5 1];
tpenr = [1 2e4];
esr = [1.0 0.99 0.1]; % Note manual assignment
pr = [.1 1e-5];
tdoser = [10*60 5*60*60];
```

% How many variations of each parameter?

```
pvar = 3;
```

% How many variations of tdose?

```
tdosevar = 3;
```

```
kn = pvar;
```

```
tpenn = pvar;
```

```
esn = pvar;
```

```
pn = pvar;
```

```
todsens = tdosevar;
```

% Initialize storage array(s)

% Should have the form: [psi(1) psi(2) psi(3).. k tpen es p] X pvar^4

```
Resp = zeros(pvar^4+1,tdosevar+4);
```

```
if length(tdoser) == 2 tdose = min(tdoser):(max(tdoser)-min(tdoser))/(tdosevar-1):max(tdoser);
```

```
else tdose = tdoser; end
```

```
if length(kr) == 2 k = min(kr):(max(kr)-min(kr))/(pvar-1):max(kr);
```

```
else k = kr; end
```

```
if length(tpenr) == 2 tpen = min(tpenr):(max(tpenr)-min(tpenr))/(pvar-1):max(tpenr);
```

```
else tpen = tpenr; end
```

```
if length(esr) == 2 es = min(esr):(max(esr)-min(esr))/(pvar-1):max(esr);
```

```
else es = esr; end
```

```
if length(pr) == 2 p = min(pr):(max(pr)-min(pr))/(pvar-1):max(pr);
```

```
else p = pr; end
```

```
i = 1;
```

```
for ki = 1:pvar
```

```
for tpeni = 1:pvar
```

```
for esi = 1:pvar
```

```
for pi = 1:pvar
```

```
i = i + 1;
```

```
Resp(i,:) = [zeros(1,tdosevar) k(ki) tpen(tpeni) es(esi) p(pi)];
```

```
end
```

```
end
end
end
```

```
% Start our looping
for vi = 2:pvar^4+1
```

```
% k is located at Resp(vi,tdosevar+1), tpen is located at Resp(vi,tdosevar+2)
% es is located at Resp(vi,tdosevar+3), p is located at Resp(vi,tdosevar+4)
kl = Resp(vi,tdosevar+1);
tpenl = Resp(vi,tdosevar+2);
esl = Resp(vi,tdosevar+3);
pl = Resp(vi,tdosevar+4);
```

```
for hi = 1:tdosevar
```

```
% Parameters to ASFstoxic are tau,psi,p,Es,res
% ASFstoxic(tdose(l)/tpenl,k(kl)*tdose(l)*Cb,p(pl),Es(Esl),100);
```

```
Resp(vi,hi) = ASFstoxic(tdose(hi)/tpenl,kl*tdose(hi)*Cb,pl,esl,100);
if Resp(vi,hi)== 0 Resp(vi,hi)=eps; end
```

```
end
end
```

```
for hi = 1:size(Resp,2)
Resp(1,hi)=eps;
end
```

```
% write Resp matrix to file
dlmwrite(filename,Resp,sprintf('%t'));

```

```
% Write data file header
file=fopen(filename,'r+');
fseek(file,0,-1);
for l = 1:tdosevar
fprintf(file,'R%i:%0.3f\t',[l tdose(l)]);
end
fprintf(file,'%s\t','k');
fprintf(file,'%s\t','tpen');
fprintf(file,'%s\t','es');
fprintf(file,'%s\n','p');
fclose(file);
```

```

% sens_cat.m
%
% sensitivity analysis data generator

% Assume some values as our constants
cvardefs

% select file to write to, in current directory, or absolute
filename = 'sens_cat.txt';

% Ok, set the ranges for our four parameters, and our xaxis, psi
% Alternately, you can manually assign values, but the length of the
% array _better_ be equal to pvar.
kr = [1e-5 1e-2];
thieler=[1/2 1 1*2];
esr = [1.0 0.99 0.1]; % Note manual assignment
pr = [.1 1e-5];
tdoser = [10 500];

% How many variations of each parameter?
pvar = 3;
% How many variations of tdose?
tdosevar = 3;

kn = pvar;
thielen = pvar;
esn = pvar;
pn = pvar;
todsens = tdosevar;

% Initialize storage array(s)
% Should have the form: [psi(1) psi(2) psi(3).. k tpen es p] X pvar^4
Resp = zeros(pvar^4+1,tdosevar+4);

if length(tdosers) == 2 tdose = min(tdosers):(max(tdosers)-min(tdosers))/(tdosevar-
    1):max(tdosers);
else tdose = tdosers; end
if length(kr) == 2 k = min(kr):(max(kr)-min(kr))/(pvar-1):max(kr);
else k = kr; end
if length(thieler) == 2 thiele = min(thieler):(max(thieler)-min(thieler))/(pvar-
    1):max(thieler);
else thiele = thieler; end
if length(esr) == 2 es = min(esr):(max(esr)-min(esr))/(pvar-1):max(esr);
else es = esr; end
if length(pr) == 2 p = min(pr):(max(pr)-min(pr))/(pvar-1):max(pr);

```

```
else p = pr; end
```

```
i = 1;
for ki = 1:pvar
for thielei = 1:pvar
for esi = 1:pvar
for pi = 1:pvar
```

```
i = i + 1;
Resp(i,:) = [zeros(1,tdosevar) k(ki) thiele(thielei) es(es) p(pi)];
```

```
end
end
end
end
```

```
% Start our looping
for vi = 2:pvar^4+1
```

```
% k is located at Resp(vi,tdosevar+1), tpen is located at Resp(vi,tdosevar+2
% es is located at Resp(vi,tdosevar+3), p is locate at Resp(vi,tdosevar+4)
kl = Resp(vi,tdosevar+1);
thielel = Resp(vi,tdosevar+2);
esl = Resp(vi,tdosevar+3);
pl = Resp(vi,tdosevar+4);
```

```
for hi = 1:tdosevar
```

```
% Parameters to ASFstoc are tau,psi,p,Es,res
% ASFstoc(tdosec(l)/tpen(tpenl),k(kl)*tdosec(l)*Cb,p(pl),Es(Esl),100);
% function ASF = ASFcat (psi,thiele,pen,p,Es,res)
```

```
if thielel < 1
    pen = 1;
else
    pen = 1/thielel;
end
Resp(vi,hi) = ASFcat(kl*tdose(hi)*Cb,thielel,pen,pl,esl,100);
if Resp(vi,hi)== 0 Resp(vi,hi)=eps; end
```

```
end
end
```

```
for hi = 1:size(Resp,2)
Resp(1,hi)=eps;
```

end

```
% write Resp matrix to file
dlmwrite(filename,Resp,sprintf("\t"));
```

```
% Write data file header
file=fopen(filename,'r+');
fseek(file,0,-1);
for l = 1:tdosevar
fprintf(file,'R%i:%0.3f\t',[l tdose(l)]);
end
fprintf(file,'%s\t','k');
fprintf(file,'%s\t','thiele');
fprintf(file,'%s\t','es');
fprintf(file,'%s\n','p');
fclose(file);
```

Prediction Routines

Three routines are used to make the predictions found in Appendix B: svardefs.m, cvardefs.m, pred.m. The first two create a default set of parameters values for the stoichiometric and catalytic models, respectively. The third generates the data based on the default and user parameters.

```
% ASFsvar.m
% sets up defaults for Stoicometric system.
De = 1e-5;           % from 1 - "Improving...", P. Stewart, sub J. Env. Eng, 3/31/97
Yxb = 50;           % from 1
Xb = 1e4;           % from 1
p = 0.01;           % resonable, as corresponds to x500 resistance
Cb = 1;             % from 1
Lf = 1e-2;          % 400 um film
Kdis = 1;           % from 1
tdose = 10000;       % whatever
Es = 0.90;          % 50/50 mix of resistant/sensitive
```

```
res = 1000; % resolution of argapp.m
```

```
% ASFcvar.m
```

```
% sets up defaults for Catalytic system.
```

```
De = 1e-5;           % from 1 - "Improving...", P. Stewart, sub J. Env. Eng, 3/31/97
Xb = 1e4;            % from 1
p = 0.01;            % resonable, as corresponds to x500 resistance
Cb = 400;             % from 1
Lf = 1e-2;           % 400 um film
Kdis = 0.001;         % from 1
tdose = 10000;        % whatever
Es = 0.90;            % 50/50 mix of resistant/sensitive
Krxn = 100; % 10-4 - 10-3
```

```
res = 1000; % resolution of argapp.m
```

```
% pred.m
```

```
%
```

```
% predicts the effects of varying parametrs
```

```
Grph_xlabel = 'Kdis*Cb*tdose [ ]';
Grph_ylabel = 'Log10(X/Xo)';
```

```
setglobal % for gendata.m
```

```
figdir
```

```
ASFgrph(Grph_num,Grph_xlabel,Grph_ylabel,Grph_title);
```

```
fprintf('%s\n\n',Grph_title);
```

```
% Make sure we account for numerous values of these...
```

```
l = length(Krxn)+length(Cb)+length(Yxb)+length(De)+length(Xb)+length(Lf)-5;
```

```
tpen = zeros(1,l);
```

```
thiele = zeros(1,l);
```

```
tdosed = zeros(l,2);
```

```
l = 0;
```

```
for Krxn1 = 1:length(Krxn)
```

```
for Cbl = 1:length(Cb)
```

```
for Yxb1 = 1:length(Yxb)
```

```

for Del = 1:length(De)
for Xbl = 1:length(Xb)
for Lfl = 1:length(Lf)

l = l + 1;
tpen(l) = Lf(Lfl)^2*Xb(Xbl)/(2*De(Del)*Cb(Cbl)*Yxb(Yxbl));
thiele(l) = sqrt(Krxn(Krxnl)*Lf(Lfl)^2/(2*De(Del)*Cb(Cbl)));

if tdosemax == 0
    tdosed(l,:)= [0.0001 tpen(l)*1.5];
else
    tpen(l) = tdosemax;
    tdosed(l,:)= [0.001 tdosemax];
end

for Kdisl = 1:length(Kdis)
for pl = 1:length(p)
for Es1 = 1:length(Es)

if eval(printwhen)

    %kdis=Params(1); tpen=Params(2); thiele=Params(3); es=Params(4); p=Params(5);
    switch model
        case 1, Params = [Kdis(Kdisl) tpen(l) n_thiele Es(Es1) p(pl)];
        case 4, Params = [Kdis(Kdisl) tpen(l) n_thiele n_es n_p];
        case 3, Params = [Kdis(Kdisl) n_tpen n_thiele Es(Es1) p(pl)];
        case 2, Params = [Kdis(Kdisl) n_tpen thiele(l) Es(Es1) p(pl)];
        case 5, Params = [Kdis(Kdisl) n_tpen thiele(l) n_es n_p];
    end

    fprintf('Generating data for 0-%3.0f seconds,\n',max(tdosed(l,:)));
    fprintf(' with Kdis: %3.3g Tpen: %3.3g Thiele: %3.3g Es: %3.3g p: %3.3g\n',Params);

    [tdosec,ASFc] = gendata(Params,Cb(Cbl),tdosed(l,:),model);
    plot(tdosec.*(Cb(Cbl)*Kdis(Kdisl)),log10(ASFc),'-','LineWidth',2); hold on;

end

end
end
end
end
end
end
end

```

```

end
end
end

fprintf('Done.\n');

if ASFmin ~= 0
    ylim([ASFmin 0]);
    fprintf('Rescaled Y-Axis to %g.\n',ASFmin);
end
if PsiMax ~= 0
    xlim([0 PsiMax]);
    fprintf('Rescaled X-Axis to %g.\n',PsiMax);
else
    xlim([0 max(tdosed(:,2))]);
end

if length(fname)
    % kill xaxis formatting
    set(gca,'xtickmode','manual');
    set(gca,'xtick',[]);

    fname=[figuredir fname];
    eval(['print -dmfile ' fname]);
    fprintf('Graph written to %s.\n',fname);
end

zoom on;
fprintf('You can zoom with mouse (see "help zoom" for details).\n\n');

```

Data Fitting Routines

Numerous files are used in the data fitting system, and only three will be shown here, as they are notable in importance. The first, Mainfit.m, uses the parameter estimates and ranges, survival fraction data, and dose time data and concentration to set up the process of the constrained

minimization. There is an option to allow the computer to make a brute-force approach at the initial estimates for the parameters, though this process is not recommended. The second routine, minfun.m, is the true core of this system. Given a set of data and parameter, it calculates the sum of the squared residual errors and returns it to the constrained minimization routine, constr.m (not shown here; integral member of the Optimization Toolkit). The third, gendata.m, is used by minfun.m, and several other programs, to generate a set of ASF data given a dose time range and parameter values.

```
% Mainfit.m
%
% attempts a constrained minimization of error in predicted ASF values to data ASF

outdir
setglobal

sflops = flops;

% set up parameter matrix
% Store order: k, tpen/thiele, es, p
VLB=[min(kr) min(tpenr) min(thieler) min(esr) min(pr)];
VUB=[max(kr) max(tpenr) max(thieler) max(esr) max(pr)];

% guesstimate initial guesses based on VLB and VUB
if iguess > 0
    iguessfit % Makes iterative attempt to build 5-D contour plot of error
else
    if InitGuess == 0
        fprintf('Using means of ranges as estimate.\n');
        Params = (mean([VLB;VUB])'*[1 1 1 1 1])';
    else
        if size(InitGuess,1) ~= 5
```

```

        fprintf('Uh, Im sorry, but youre using an old version of the
system.\n');
        fprintf('Try including the line:\n');
        fprintf('\tconversion\n\n in your program, just before Mainfit is
called.\n');
        fprintf('\nMike, in his infinte wisdom, has taken this into account,
and has\n');
        fprintf('instructed me to do so...');
        conversion
        fprintf(' ...done.\nHit any key to continue, though you really
should look at conversion.m\n');
        pause
    end
    fprintf('Using user-specified initial guesses.\n');
    Params = InitGuess;
end
end

% Ok, now straighten out Params so that we null unused parameters
% Stoich_Full; Cat_Full; Phys; Stoich_Trans; Cat_Trans;]
Params(1,3)=0; % Don't use thiele
Params(4,3)=0; % Don't use thiele
Params(3,3)=0; % Don't use thiele
Params(2,2)=0; % Don't use tpen
Params(5,2)=0; % Don't use tpen
Params(3,2)=0; % Don't use tpen

% clear results
Results = zeros(5,7);
Rstring = {' ' ' ' ' ' ' '};

for PModel = 1:5

    if findstr(domodels,num2str(PModel)) % Should we run this model?

        % Some options for the constrained minimization routine
        foptions(10)=999; % Number of function and gradient evaluations
        foptions(14)=foptions(10); % number of
        foptions(2)=1e-4; % Tol on F
        foptions(3)=foptions(2); % tol on Params
        PrintErr = 1e99;

        switch PModel
            case 1,tParams=[Params(PModel,1) Params(PModel,2) Params(PModel,4)
Params(PModel,5)];

```

```

        tVUB=[VUB(1) VUB(2) VUB(4) VUB(5)];
        tVLB=[VLB(1) VLB(2) VLB(4) VLB(5)];
    case 4,tParams=[Params(PModel,1) Params(PModel,2)];
        tVUB=[VUB(1)*VUB(5) VUB(2)];
        tVLB=[VLB(1)*VLB(5) VLB(2)];
    case 3,tParams=[Params(PModel,1) Params(PModel,4) Params(PModel,5)];
        tVUB=[VUB(1) VUB(4) VUB(5)];
        tVLB=[VLB(1) VLB(4) VLB(5)];
    case 2,tParams=[Params(PModel,1) Params(PModel,3) Params(PModel,4)
Params(PModel,5)];
        tVUB=[VUB(1) VUB(3) VUB(4) VUB(5)];
        tVLB=[VLB(1) VLB(3) VLB(4) VLB(5)];
    case 5,tParams=[Params(PModel,1) Params(PModel,3)];
        tVUB=[VUB(1)*VUB(5) VUB(3)];
        tVLB=[VLB(1)*VLB(5) VLB(3)];
end

fprintf('Starting constrained minimization routine for model %i/5.\n',PModel);
[tParams,foptions]=feval('constr','minfun',tParams,foptions,tVLB,tVUB,[],Cb,tdose
d,ASFd,PModel,1);
fprintf('Minimization completed for model %i/6.\n',PModel);

% Send back some suitable information
% Parameters and other info stored in Results, which is defined [6 8]
% Each row contains [Error num_its kdis tpen thiele es p],

switch PModel
    case 1,Results(1,3:7)=[tParams(1) tParams(2) 0 tParams(3) tParams(4)];
    case 4,Results(2,3:7)=[tParams(1) tParams(2) 0 n_es n_p];
    case 3,Results(3,3:7)=[tParams(1) 0 0 tParams(2) tParams(3)];
    case 2,Results(4,3:7)=[tParams(1) 0 tParams(2) tParams(3) tParams(4)];
    case 5,Results(5,3:7)=[tParams(1) 0 tParams(2) n_es n_p];
end

Results(PModel,1)= foptions(8); % Our Error. Beware large numbers.
Results(PModel,2)= foptions(10); % Number of iterations. Beware low values.

% Build out nice formatted string
tstring = sprintf('Error of %3.3g\n Kdis=%3.3g ',foptions(8),Params(PModel,1));
if PModel == 1 | PModel == 4 | PModel == 3 % Stoich or Phys
    if PModel ~= 3
        tstring = [tstring sprintf('tpen= %3.3g\n',Params(PModel,2))];
    end
    if PModel == 1 % Full or Phys

```

```

        tstring = [tstring sprintf('Es= %3.3g
p=%3.3g\n',Params(PModel,4),Params(PModel,4))];
    end
else % Cat
    tstring = [tstring sprintf('thiele= %3.3g\n',Params(PModel,3))];
    if PModel ~= 5 % Full or Phys
        tstring = [tstring sprintf('Es= %3.3g
p=%3.3g\n',Params(PModel,4),Params(PModel,4))];
    end
end
Rstring(PModel)= cellstr(tstring);

fprintf('Fit Model %i with %s\n',PModel,tstring);
end

end

showres % show the results in a nice table.

% minfun.m
%
% attempts a constrained minimization, returning error of fit

function [Error,G] = minfun(Params,Cb,tdosed,ASFd,pmodel,Print)
global PrintErr
global n_tpen
global n_thiele
global n_es
global n_p

ASFc = zeros(1,length(tdosed));

switch pmodel
    case 1,kdis=Params(1); tpen=Params(2); thiele=0; es=Params(3); p=Params(4);
    case 4,kdis=Params(1); tpen=Params(2); thiele=0; es=n_es; p=n_p;
    case 3,kdis=Params(1); tpen=0; thiele=0; es=Params(2); p=Params(3);
    case 2,kdis=Params(1); tpen=0; thiele=Params(2); es=Params(3); p=Params(4);
    case 5,kdis=Params(1); tpen=0; thiele=Params(2); es=n_es; p=n_p;
end

% had to add sign check
if p < 0 | es < 0 | kdis < 0 | tpen < 0 | thiele < 0
    Error = PrintErr*1.1;

```

```

G=ones(length(Params));
return
end

if pmodel == 1 | pmodel == 4
    for l = 1:length(tdosed)
        % Parameters to ASFstoic are tau,psi,p,Es,res
        % Store order of Params is: k,tpen,thiele,es,p
        ASFc(l)= ASFstoic(tdosed(l)/tpen,kdis*tdosed(l)*Cb,p,es,100);
    end
elseif pmodel == 2 | pmodel == 5
    for l = 1:length(tdosed)
        % Parameters to ASFcat are psi,thiele,pen,p,Es,res
        % Store order of Params is: k,tpen,thiele,es,p
        ASFc(l)= ASFcat(kdis*Cb*tdosed(l),thiele,1/thiele,p,es,100);
    end
else
    for l = 1:length(tdosed)
        % Parameters to ASFphys are psi,Es,p
        % Store order of Params is: k,tpen,thiele,es,p
        ASFc(l)= ASFphys(kdis*Cb*tdosed(l),es,p);
    end
end

% Ok, so we've got the ASFcalc data, compare to ASFreal data:

% This is the RSS (Residual Sum of the Squares), in log form:
% We need this for the AIC calculation later, as it assumes we used the RSS to
% get our fits.
Error=sum((log(ASFd+eps)-log(ASFc+eps)).^2);

if Print & ( Error < PrintErr)
    PrintErr = Error;
    if pmodel == 1 | pmodel == 4
        fprintf('Error: %3.3g w/ k: %3.3g, tpen: %3.3g, es: %3.3g, p: %3.3g\n',Error,kdis,tpen,es,p);
    elseif pmodel == 2 | pmodel == 5
        fprintf('Error: %3.3g w/ k: %3.3g, thiele: %3.3g, es: %3.3g, p: %3.3g\n',Error,kdis,thiele,es,p);
    else
        fprintf('Error: %3.3g w/ k: %3.3g, es: %3.3g, p: %3.3g\n',Error,kdis,es,p);
    end
end
end

```

```
G = ones(length(Params)).*-1;
```

```
return
```

```
% gendata.m
```

```
% Given a list of parameters, model, Cb, dose data, returns a set of ASF values  
% predicted and the dose times predicted for.
```

```
function [tdosec,ASFc] = gendata(Params,Cb,tdosed,pmodel)
```

```
defglobal
```

```
divs = 50-1;
```

```
tdosec = min(tdosed):(max(tdosed)-min(tdosed))/divs:max(tdosed);
```

```
ASFc = zeros(1,length(tdosed));
```

```
kdis=Params(1); tpen=Params(2); thiele=Params(3); es=Params(4); p=Params(5);
```

```
if tpen == 0 tpen=n_tpen; end
```

```
if thiele == 0 thiele=n_thiele; end
```

```
if pmodel == 1 | pmodel == 4
```

```
    for l = 1:length(tdosec)
```

```
        % Parameters to ASFstoc are tau,psi,p,Es,res
```

```
        % Store order of Params is: k,tpen,thiele,es,p
```

```
        % ASFstoc(tdosec(l)/tpen(tpenl),k(kl)*tdosec(l)*Cb,p(pl),Es(Esl),100);
```

```
        ASFc(l)= ASFstoc(tdosec(l)/tpen,kdis*tdosec(l)*Cb,p,es,100);
```

```
    end
```

```
elseif pmodel == 2 | pmodel == 5
```

```
    for l = 1:length(tdosec)
```

```
        % Parameters to ASFcat are psi,thiele,pen,p,Es,res
```

```
        % Store order of Params is: k,tpen,thiele,es,p
```

```
        ASFc(l)= ASFcat(kdis*Cb*tdosec(l),thiele,1/thiele,p,es,100);
```

```
    end
```

```
else
```

```
    for l= 1:length(tdosec)
```

```
        ASFc(l)= ASFphys(kdis*Cb*tdosec(l),es,p);
```

```
    end
```

```
end
```

```
return
```

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