

IMPACT OF AQUIFER HETEROGENEITY ON GEOMICROBIAL KINETICS

by

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THESIS ABSTRACT

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Title: Impact of Aquifer Heterogeneity on Geomicrobial Kinetics

Rates of microbial reactions are important in understanding groundwater chemistry and bioremediation. In aquifers, microbial rates depend on physicochemical and biological factors and also on how groundwater transport impacts microbial reactions at pore-scale. I numerically simulate microbial acetate consumption in a porous medium, focusing on how physical heterogeneity of the medium impacts the rates. My model is a 3-D cube, which represents a portion of a sandy aquifer. Acetate is supplied by groundwater flow through the cube, and microbes live on randomly distributed grain surfaces by oxidizing acetate. I simulated microbial acetate oxidation under a range of groundwater velocity, acetate concentrations, spatial heterogeneity, and other physicochemical conditions. The results demonstrate a significant gap in microbial kinetics between the pore-scale and continuum model. Specifically, microbial rates are larger in porous media of greater heterogeneity. For this reason, I propose that microbial parameters should not be applied directly to field-scale biogeochemical modeling.

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CHAPTER I

INTRODUCTION

Microorganisms are pervasive and active in the subsurface, from shallow aquifers to deep basins. They catalyze reduction-oxidation reactions, dissolve and precipitate minerals, introduce and remediate contaminants, and drive the global cycling of elements. The survival and growth of microorganisms depend on the physical and chemical conditions of the environment, and the environment in return influences the population sizes and activities of microorganisms.

An important question in studying natural microorganisms is how fast microbes catalyze geochemical reactions. Quantifying the rates of microbial metabolism will increase our ability to predict the environmental impact of microorganisms. It will also improve the predictive models of bioremediation, reactive transport, and climate change. For example, bioremediation has been used since the 1970's to clean contaminated water and soil in situ, and it continues to be a subject of active research (F. Chapelle 1999; Gharasoo et al. 2012). If we could accurately predict rates of microbial reactions in the environment, we could optimize bioremediation design and operation to be effective and efficient, and improve models of reactive transport to better represent complex systems of microbial and geochemical reactions in geological media.

If we are able to quantify the rate of microbial metabolism, we can also better describe the carbon cycle between aqueous and atmospheric reservoirs. Anaerobic microbial decomposition of organic material produces carbon dioxide and methane, both of which are significant greenhouse gases in the atmosphere. Understanding the potential

release of carbon dioxide and methane from subsurface reservoirs is important in our understanding of the consequences of those gases in the atmosphere. As climate variability increases, microbes will likely become more active in aquatic environments with an increase in nutrients from increased weathering and thawing of permafrost regions (Baron et al. 2012).

Microbial rates are often predicted from empirical models based on parameters derived from laboratory experiments (Monod 1949). However, the predicted rates are often orders of magnitude different than the field observations (Reeburgh 1983; Phelps et al. 1994). The discrepancies between the predicted and measured rates are likely the result of the idealized growth conditions in the laboratory in contrast to the diverse physicochemical conditions of the environment (Jin and Bethke 2005).

Spatial heterogeneity is an important feature of the groundwater environment, but is often left out in large scale studies. Field scale experiments and models often use the representative elemental volume technique to describe the average flow parameters of an entire aquifer or watershed. However, aquifer characteristics may differ between pores, a phenomenon that is called pore-scale heterogeneity. Aquifer heterogeneity influences microbial rates. For example, previous studies have shown that pore scale heterogeneity affects the local concentrations of chemicals and contaminants in aquifers (Brown et al. 2000), which results in heterogeneous microbial reaction rates (Jin et al. 2013).

This study focuses on how aquifer heterogeneity affects microbial kinetics. I investigate the impact of aquifer pore-scale heterogeneity on the metabolic rates of microbes, and the potential for upscaling microbial parameters for use in field scale modeling. Specifically, I develop a pore-sale model to describe aquifer heterogeneity.

While the pore-scale model captures the variation of parameters between pores, it treats each pore as a homogenous system. I simulated the progress of acetoclastic methanogenesis at pore-scale and at the continuum scale. I compare the results of the simulations to investigate how aquifer heterogeneity affects the rates of microbial metabolism.

CHAPTER II

LITERATURE REVIEW

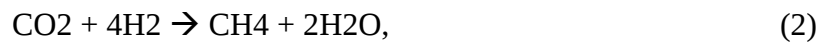
2.1. Methanogenesis

Methanogenesis is the production of methane by a group of archaea called methanogens (Conrad 2005). In aquifers, natural organic material is fermented to H₂ and acetate. Some methanogens partition acetate into methane and bicarbonate,



a pathway called acetoclastic methanogenesis (Madigan, Martinko, and Parker 2000).

Others oxidize H₂ by reducing bicarbonate to methane,



a pathway called hydrogenotrophic methanogenesis (Schlesinger 1997). They save the energy released from methane production by synthesizing ATP, which they can then break down for thermodynamically unfavorable biological processes. For the purpose of this study I focus on the reaction rate of acetoclastic methanogenesis.

Methanogenesis is an important microbial process for understanding groundwater chemistry and subsurface-atmospheric cycling of carbon. Methanogens are globally significant microorganisms that emit substantial quantities of methane and carbon dioxide into the atmosphere, but also have the potential for bioremediation in the subsurface. Methanogens living in deep subsurface aquifers were confirmed by the 1980's by various geologists (Belyaev and Ivanov, 1983; Godsy, 1980; Olson et al. 1981). They became an important microorganism to study with the realization that methane is a major component of atmospheric gasses (Levy 1973; Owens et al. 1982). While subsurface methanogens

are not the sole source of methane in the atmosphere, they are an important part of the biotic methane reservoir, along with wetlands, rice paddies, and farm animals (Cicerone and Oremland 1988).

In aquifers, methanogens compete with other respiring prokaryotes for H_2 and acetate, a process that results in microbial zonation – the segregation of microbial respiration along the groundwater flow path. For example, close to the recharge area, groundwater is often oxidic, and aerobic respiration occurs, but anaerobic respiration ceases. Once O_2 is depleted, iron respiration occurs if there are ferric minerals, but other anaerobic respiration, including sulfate respiration and methanogenesis, is not significant. After the ferric minerals are consumed, sulfate reduction occurs if there is sulfate in the groundwater. Finally, where all electron acceptors are exhausted, methanogenesis takes place (Figure 1).

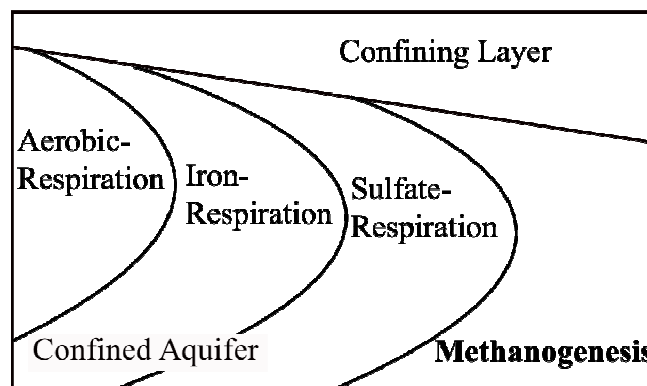


Figure 1. Zonation of microbial respiration along the flow path of groundwater (Modified from Lovley et al.1994).

Microbial respiration is zoned because respiration occurs where the energy in the environment is larger than the amount of energy saved by the microorganisms. Because the reduction of O_2 is favored significantly by thermodynamics, aerobic respiration can go forward even where the concentrations of electron donors are very small. On the other

hand, methane production proceeds close to thermodynamic equilibrium. Hence methanogenesis needs a large concentration of acetate and H_2 in the environment to remain thermodynamically favorable (D. Lovley et al. 1994; F. Chapelle and Lovley 1992; Bethke et al. 2011).

2.2. REV and Subsurface Heterogeneity

Aquifers are heterogeneous in their chemical and physical properties, which is a great challenge in studying the quality of groundwater. However, most groundwater models neglect the heterogeneity and complexity of aquifers and use the representative elementary volume (REV) to describe an entire aquifer or watershed (Bear 1972; Bachmat and Bear 1987). An REV is a relatively small volume of the natural system where physical parameters of the porous media are measured and then averaged to describe the whole system.

It is a common practice to use the REV in modeling groundwater flow and transport in porous media. This type of simulation intentionally disregards the natural pore scale variability over an entire aquifer. While this technique may capture the general flow of groundwater, it may not be detailed enough to accurately describe microbial rates. I suggest that the groundwater flow greatly affects microbial kinetics, and that using the REV in biogeochemical models will inaccurately predict microbial activities in groundwater.

The groundwater flow transports nutrients and substrates that microbes consume, thus the location and concentration of those chemical compounds will determine the possible reaction rates. In addition, the pore-scale flow regime could produce preferential flow patterns that concentrate or isolate nutrients and substrates from a microbial

population. The size of microbial cells is on the same order of magnitude as that of pores in many porous geological formations, which is why the pore-scale heterogeneity might be a significant factor in the reaction rates.

Pore-scale heterogeneity could affect local chemical concentrations in aquifers. For example, Brown et al. (2000) investigated the distribution of iron, sulfur, dissolved oxygen, hydrogen, and inorganic carbon within an aquifer that has high levels of dissolved iron. According to their results, the pore-water in the aquifer matrix had significantly higher concentrations of major ions than the samples collected from wells, and the chemical composition of groundwater samples was also inconsistent among wells. These results showed that pore-scale heterogeneity can lead to microenvironments with different chemical compositions.

2.3. Microbial Kinetics

Most groundwater models describe the rates of acetoclastic methanogenesis according to the Monod equation. The rate of methanogenesis is defined as the rate at which methane is produced or acetate is consumed,

$$r = \frac{\partial CH_4}{\partial t} = \frac{-\partial ac}{\partial t} \quad (3)$$

The rate is commonly described according to the Monod equation (Monod 1949),

$$r = k \times [X] \times \frac{[ac]}{[ac] + K_{ac}} \quad (4)$$

where k (mol/g/s) is the rate constant, $[X]$ (g/m³) is biomass concentration, $[ac]$ (mol/m³) is acetate concentration, and K_{ac} (mol/m³) is the half-saturation constant.

According to this equation, the rates of methanogenesis depend linearly on biomass concentrations, and hyperbolically on acetate concentrations (Figure 2).

Specifically, where acetate concentrations are small, the equation reduces to

$$r = k \times [X] \times \frac{[ac]}{K_{ac}} \quad (5)$$

and the rates increase linearly with acetate concentrations. Where acetate concentrations are very large, much larger than the half-saturation constant K_{ac} , the rate law reduces to

$$r = k \times [X] \quad (6)$$

Hence the product is also called the maximum rate, the rate where acetate concentration does not limit methanogenesis. Where acetate concentration takes the same value as K_{ac} , the rate law reduces to

$$r = \frac{k \times [X]}{2} \quad (7)$$

That is the rate that is half of the maximum rate. To accurately model the growth of microorganisms, cell maintenance and biomass synthesis must also be taken into account (Pirt 1965),

$$\frac{d[X]}{dt} = Y \times r - m \quad (8)$$

where Y is the growth yield, the amount of biomass produced per reaction, and m is the specific maintenance rate. In this study, I focus on the impact of aquifer heterogeneity on the rates of methanogenesis, and will not consider microbial growth and maintenance in aquifers.

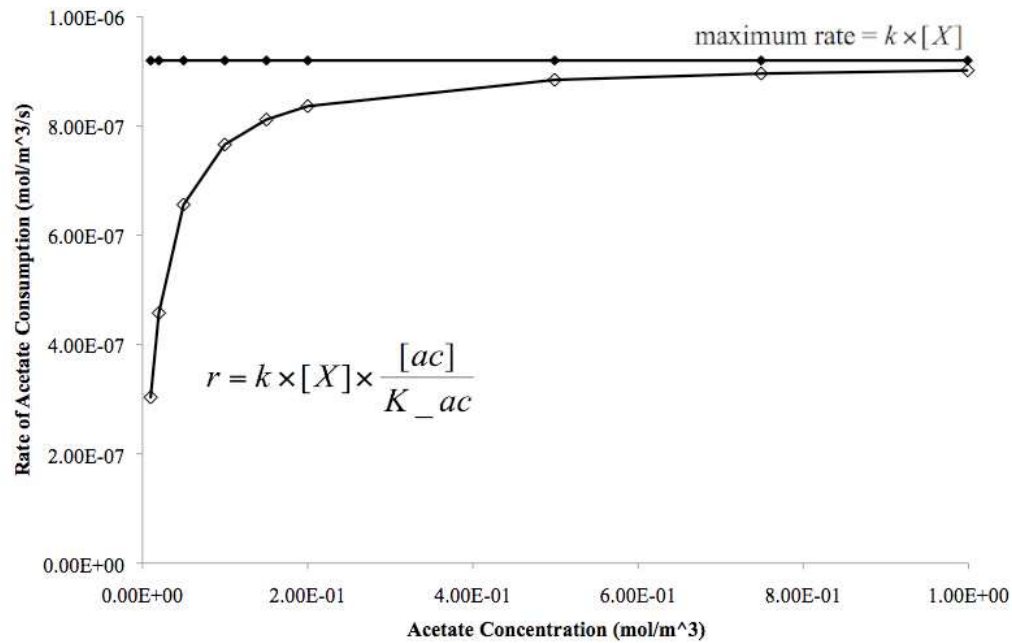


Figure 2. Variations in the rates of acetate consumption with acetate concentration. The line is calculated using the Monod equation and a half-saturation constant (K_{ac}) of 20 μM , a biomass concentration of $.92 \text{ mol/m}^3$, and a rate constant of $1\text{E-}06 \text{ mol/m}^3/\text{s}$. The maximum rate is calculated using Equation 6.

However, the Monod equation is developed to describe microbial metabolism in homogeneous laboratory reactors. Its application to natural system has not always been successful (F. H. Chapelle and Lovley 1990; Murphy and Schramke 1998). A comprehensive review of biogeochemical rates in anaerobic environments highlighted the discrepancy between microbial reaction rates measured in the laboratory and those observed in the field (Reeburgh 1983). Observing rates in the subsurface is difficult because microbial reaction rates are slow and the subsurface has limited accessibility. While some of the earlier geochemical models produced rates that were on the same order of magnitude as observed rates (R. Lovley and Klug 1986), more recent models have produced rates that are orders of magnitude smaller than the observed rates (Phelps et al. 1994).

The discrepancy between microbial rates in the lab and those in the field are not well understood. The parameters used in the biogeochemical models may not sufficiently represent the diverse natural conditions; some parameters should be determined for each specific site (Jin et al. 2013). Recent research into the thermodynamic aspect of geomicrobial kinetics has shown that thermodynamic factors influence respiration rates (Jin and Bethke 2005). The diverse physiochemical conditions of natural environments are likely responsible for the differences between the rates (Jin and Bethke 2005). Another factor to consider is the impact of geochemical conditions. For example, natural environments may contain inhibiting factors, such as sulfate, that impede the progress of methanogenesis (Bethke et al. 2011). Although the discrepancy in predicted and observed rates is well documented, how to upscale microbial rates at pore-scale to large biogeochemical systems remains as an open question.

CHAPTER III

HYPOTHESIS AND METHODS

3.1. Hypothesis

This study tests the hypothesis that the discrepancy between predicted and observed geomicrobial rates is partially a result of pore scale heterogeneity in natural environments. Because of aquifer heterogeneity, microbial parameters in nature differ from those in laboratory reactors. As a result, the parameters determined empirically in the laboratory should not be directly applied to field scale biogeochemical models, but should be upscaled to more accurately represent microbial metabolism in natural environments.

To test this hypothesis, I built one model that describes the aquifer as a heterogeneous medium with a heterogeneous flow field and another model of a homogenous medium. I will refer to the first model as the pore-scale model and the second model as the continuum model. I simulate acetoclastic methanogenesis in the two models with the same initial and boundary conditions, and then compare the reaction rates of the two models to test the hypothesis.

3.2. Aquifer Model

I built aquifer models using the software package COMSOL. The model is a 3-D porous cube of $5.0 \times 1.75 \times 5.0 \text{ mm}^3$, which represents a portion of an aquifer. The cube contains methanogens that consume acetate, which is supplied by the groundwater flow through the domain in the x direction.

The model has an interior mesh of 43750 cubes that are 0.1^3 mm^3 (50 x 17.5 x 50 in the x, y, z direction respectively) (Figure 3). I chose a cubic mesh so I could control the consistency of the heterogeneity. To investigate how the size of the meshing affects the accuracy of numerical simulation, I carried out the simulation using a range of mesh sizes from 0.05^3 to 0.25^3 mm^3 .

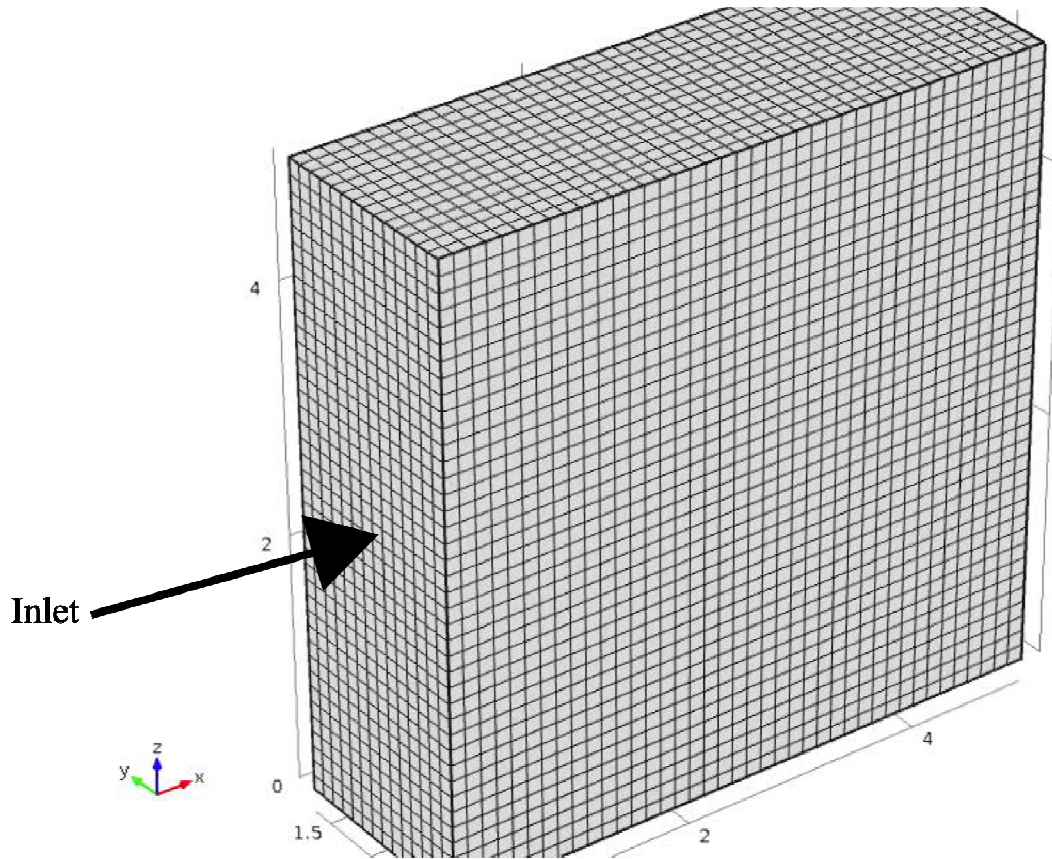


Figure 3. Meshing results of a model that represents a portion of a sandy aquifer. The dimensions of the model are 5 mm, 1.75 mm, and 5 mm. There are 43750 cubic cell; the length and volume of the cell are 0.1 mm and $1\text{E-}3 \text{ mm}^3$, respectively.

3.3. Pore-Scale Model

I use a pore-scale model to describe a hypothetical fine grained sandstone aquifer. I assume that the hypothetical aquifer has an average permeability k of $7.9\text{E-}14 \text{ m}^2$ (Li et

al. 2006), and a porosity n of 0.2. Permeability is a function of the medium, not the fluid; it is a measurement of how a specific medium allows a liquid to flow through it.

$$k = Cd^2 \quad (9)$$

where C is a constant of proportionality and d cm is the grain diameter (Freeze and Cherry 1979). Whereas hydraulic conductivity K m/s is a function of the fluid and the medium; it is a measurement of how quickly a fluid can flow through a medium.

$$K = \frac{k\rho g}{\mu} \quad (10)$$

The parameters that are specific to the fluid are the density ρ g/m³ and viscosity μ g/s/m, which is a fluids ability to resist deformation.

In the hypothetical aquifer, the mean diameter d_m of the sand grain can be calculated from the Kozeny-Carmen equation (Bear 1972),

$$k = \frac{d_m^2}{180} \times \frac{n^3}{(1-n)^2} \quad (11)$$

and the value is .032 mm.

The heterogeneity of the hypothetical aquifer is described explicitly based on the parameters of mesh cells. The mesh is labeled by the coordinates (x, y, z) at its center. I assume the pore volume ($v(x,y,z)$) of mesh cells is random, and follows a log normal distribution. The probability density function of pore volume (v) is

$$f(v) = \frac{1}{v\sigma\sqrt{2\pi}} e^{-\frac{(\ln v - \mu)^2}{2\sigma^2}} \quad (12)$$

where v is the pore volume at specific point, μ is the mean, and σ is the standard deviation (Figure 4). For the hypothetical aquifer, I set the logarithmic mean of the pore

volume, $\ln(v)$, at -3.42 mm^3 (Li et al. 2006). The magnitude of aquifer heterogeneity is defined by the standard deviation of the pore volume. In this study, I assume a logarithmic standard deviation of 0.25, 0.5, 0.75, and 1.0, describing an aquifer of relatively homogenous pore volumes to very heterogeneous pore volumes.

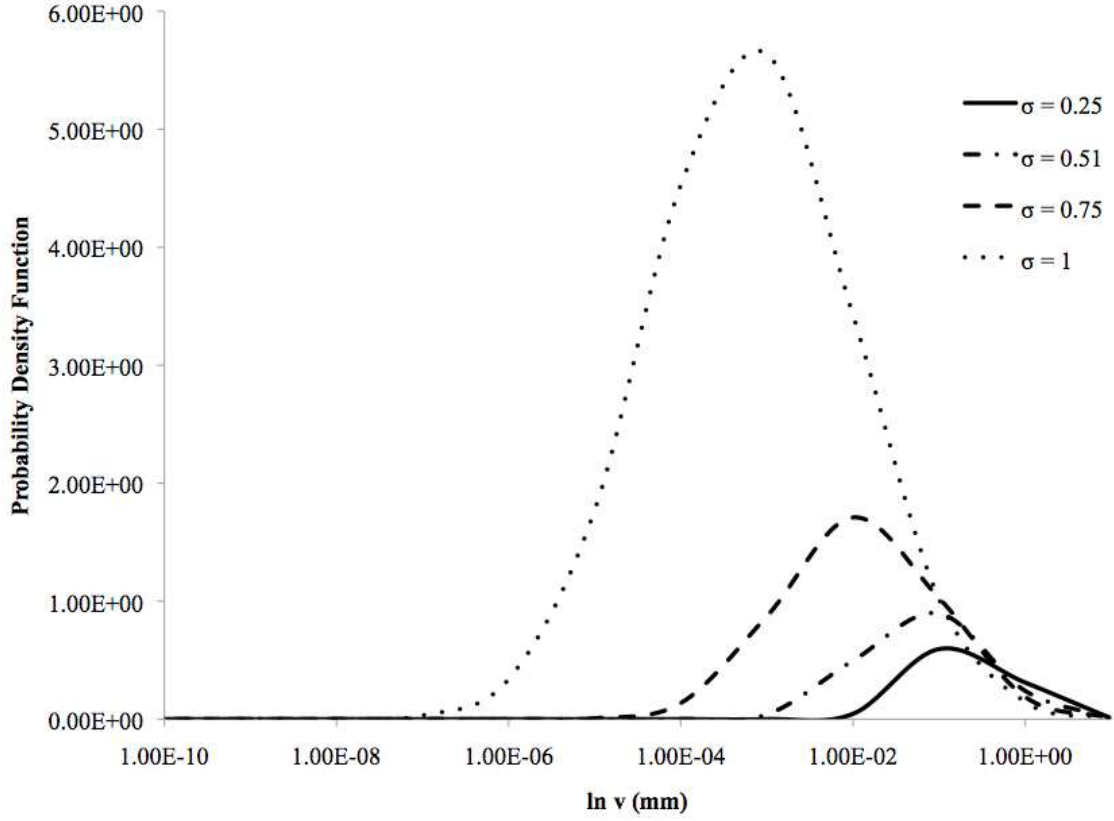


Figure 4. Probability density function with a log-normal mean of -3.42 and a range of standard deviations.

I then calculate the permeability, diffusion, and grain surface area of a mesh cell from a cell's pore volume. Taking permeability k as an example, the permeability $k(x, y, z)$ of a mesh cell at location (x, y, z) is calculated from the pore volume $v(x, y, z)$,

$$k(x, y, z) = \frac{\mu_k}{\mu_v} \times e^{\ln v(i, j, k)} \quad (13)$$

and

$$\ln v(i, j, k) = \ln v\left(c\left(\frac{x}{l}\right), c\left(\frac{y}{l}\right), c\left(\frac{z}{l}\right)\right) \quad (14)$$

where μ_k is the mean permeability, μ_v is mean pore volume, $v(i,j,k)$ is the spatially distributed pore volume, $c(\cdot)$ is a rounding function, and l is the resolution of spatial heterogeneity. The pore volume $v(x,y,z)$ is generated randomly based on the log normal distribution of pore volume. I assume that over the distance of l , aquifer permeability remains the same, but at distance greater than l , the permeability can differ significantly. For the hypothetical aquifer, I set the value of l as 0.2 mm.

The force of mixing from the groundwater flow dominates the dispersion of acetate, but diffusion is also an important dispersion mechanism. Diffusion is the movement of material, driven by a concentration gradient. The diffusive flux is a pore-scale process that is calculated with Ficks law,

$$\frac{\partial[ac]}{\partial t} = D \times \omega \times \left(\frac{\partial^2[ac]}{\partial x^2} + \frac{\partial^2[ac]}{\partial y^2} + \frac{\partial^2[ac]}{\partial z^2} \right) \quad (15)$$

where D is the diffusion coefficient m^2/s , ω is an empirical constant that accounts for the solid elements within a defined volume of space $xyz \text{ m}^3$. The ω is essentially an effective transport factor that will alter the rate of acetate dispersion depending on the pore volume at a specific coordinate. In a natural aquifer, the diffusion will be anisotropic and heterogeneous. I used a mean diffusion coefficient value of $1 \times 10^{-9} \text{ m}^2/s$.

I calculate biomass concentration in a mesh cell based on the surface area of the cell. This is because microorganisms are more likely to live on the surface of grains rather than just floating in the pore space. Specifically, I calculate the concentration of biomass $b(x,y,z)$ in a mesh cells at (x, y, z) according to

$$b(x, y, z) = \frac{\mu_{-b}}{\mu_{-SA}} \times SA(x, y, z) \quad (16)$$

where

$$SA(x, y, z) = svr \times e^{\ln v(i, j, k)} \quad (17)$$

where μ_{-b} is the mean biomass, μ_{-SA} is the mean surface area, and svr is the surface to volume ratio. For the hypothetical aquifer, I take the surface to volume ratio as $1 \times 10^4 \text{ cm}^{-1}$ (Li et al. 2006; Sen et al. 1990; Borgia et al. 1996).

3.4. Continuum Model

The continuum model has the same geometry, mesh, initial and boundary conditions as the pore-scale model. The values used for the permeability, diffusion, and biomass are the averages that were used in the functions in the pore-scale model. The parameters, and thus the aquifer and flow regime depict a homogenous medium. In this way, the continuum model represents an aquifer using the REV to calculate the flow field and geomicrobial kinetics.

3.5. Reactive Transport

The rate Q at which groundwater flows through mesh cells is calculated by Darcy's law,

$$Q = -K \frac{\Delta h}{\Delta L} A \quad (18)$$

where Q , which is the flow rate, will depend on the total area of the aquifer, K is $7.71 \times 10^{-7} \text{ m/s}$, and the hydraulic gradient $\frac{\Delta h}{\Delta L}$, which is the change in hydraulic head h over a distance ΔL , is 7.53×10^1 (Table 1).

Table 1.

Parameters used in calculating the flow field, mass balance, and reaction rate equations.

flow velocity (m/s)	5.80E-05
permeability (m ²)	7.90E-14
hydraulic conductivity (m/s)	7.71E-07
hydraulic gradient	7.53E+01
diffusion coefficient (m ² /s)	1.00E-09
biomass (mol/m ³)	9.20E-01
rate constant (mol/g/s)	1.00E-06
half saturation constant (mol/m ³)	2.00E-02

Acetate concentrations in the hypothetical aquifer are controlled by diffusion, advection, and methanogenesis. The concentration is calculated according to the mass balance equation:

$$\frac{\partial(ac)}{\partial t} + u \times \nabla(ac) = \nabla D(\nabla(ac)) + r_{ac} \quad (19)$$

where u is the velocity vector described by Darcy's law, D is the diffusion coefficient, r_{ac} is the microbial rate for acetate consumption, and is calculated using the Monod equation (Equation 3). I assume that acetate concentration at $x = 0$ remains constant at 20 μM and that acetate concentration at time 0, or initial acetate concentrations in the aquifer is 0.

3.6. Calculated Rates

I also calculated a rate using the Monod equation (Equation 3) and the average acetate and biomass concentrations from the model. It did not take groundwater flow or diffusion into account. The rate constant and half-saturation constant are empirically derived and taken from the literature.

CHAPTER IV

RESULTS

I solved numerically for acetate concentrations in both pore-scale and continuum models using the software COMSOL. Both models share the same initial and boundary conditions. I carried out the simulation on ACISS – the cluster computer of University of Oregon. I compare the simulation results, especially the rates of acetate consumption by methanogens, to test the hypothesis.

4.1. Size of Mesh Cells

The size of mesh cells determines the number of cells in the model and the numerical error in solving acetate concentrations in the model. In general, where the mesh cells are smaller, the error becomes less significant. However, the time for numerical simulation increases dramatically with the number of mesh cells. To search for an acceptable cell size, I ran a series of simulations for both pore-scale model and continuum model under the same conditions except for the mesh size.

Figure 5 shows the simulation results of the continuum model. At different cell sizes, there is little variations in the average rate of acetate consumption. In other words, the continuum model can be solved using cell sizes as large as 0.25 mm.

Figure 6 shows the simulation results of the pore-scale model. At mesh sizes of 0.05, 0.1, and 0.2 mm, the average rates fall into a narrow range of $8.92\text{e-}6$ to $8.94\text{e-}6$ mol/m³/s. At the mesh size of 0.15 mm, the rate is $9.0\text{e-}6$, slightly larger than the rates at other mesh sizes. At mesh size of 0.25 mm, the rate decreases to $8.91\text{e-}6$ mol/m³/s. The

variations in rates is likely due to the numerical error of the simulation. To test the hypothesis, I choose a mesh size of 0.1 mm

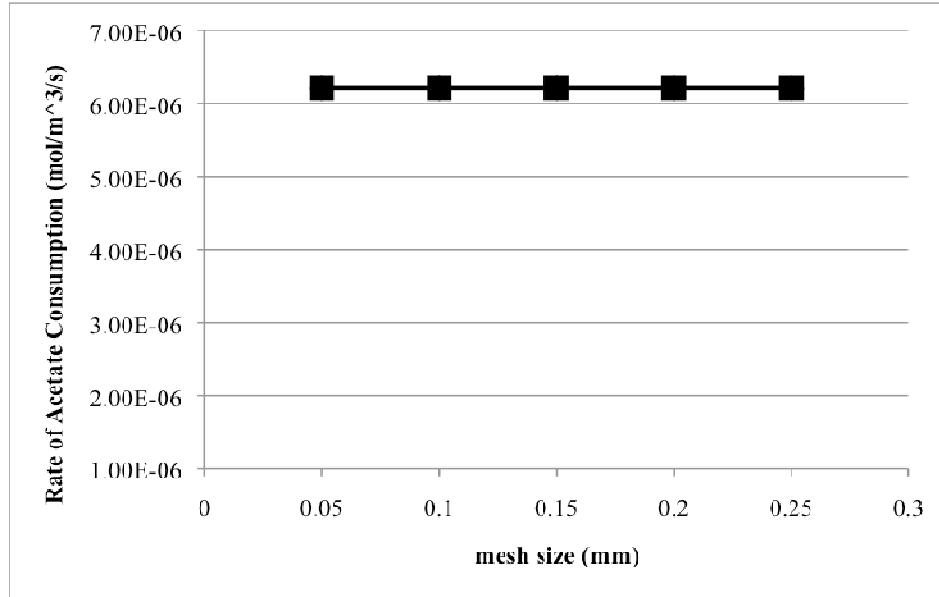


Figure 5. Average rates of acetate consumption calculated by continuum model at different mesh sizes. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , the inlet acetate concentration is $20 \text{ }\mu\text{M}$, and the standard deviation of the pore volume is $.051 \text{ mm}^3$.

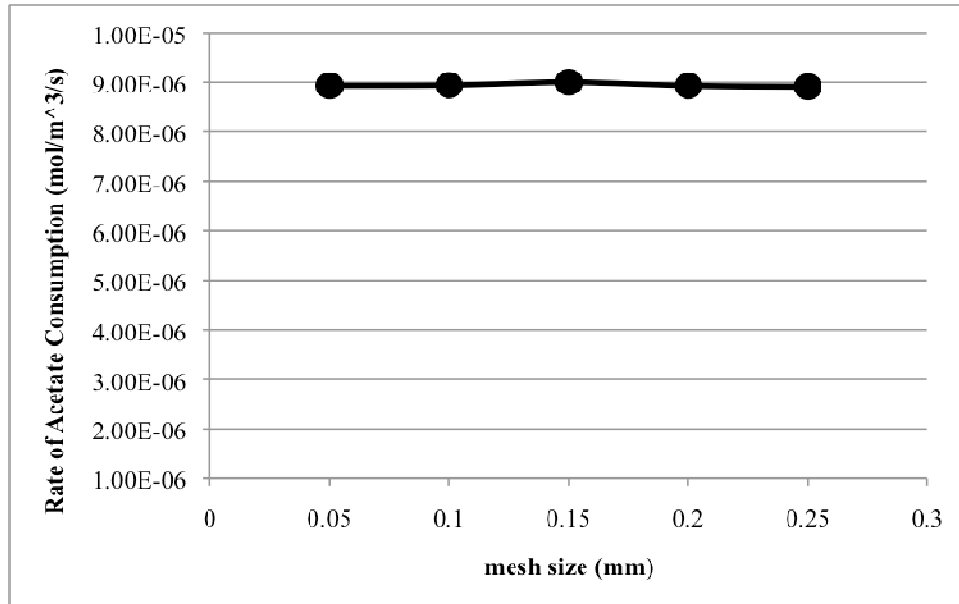


Figure 6. Average rates of acetate consumption calculated by pore-scale model at different mesh sizes. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , the inlet acetate concentration is $20 \text{ }\mu\text{M}$, and the standard deviation of the pore volume is $.051 \text{ mm}^3$.

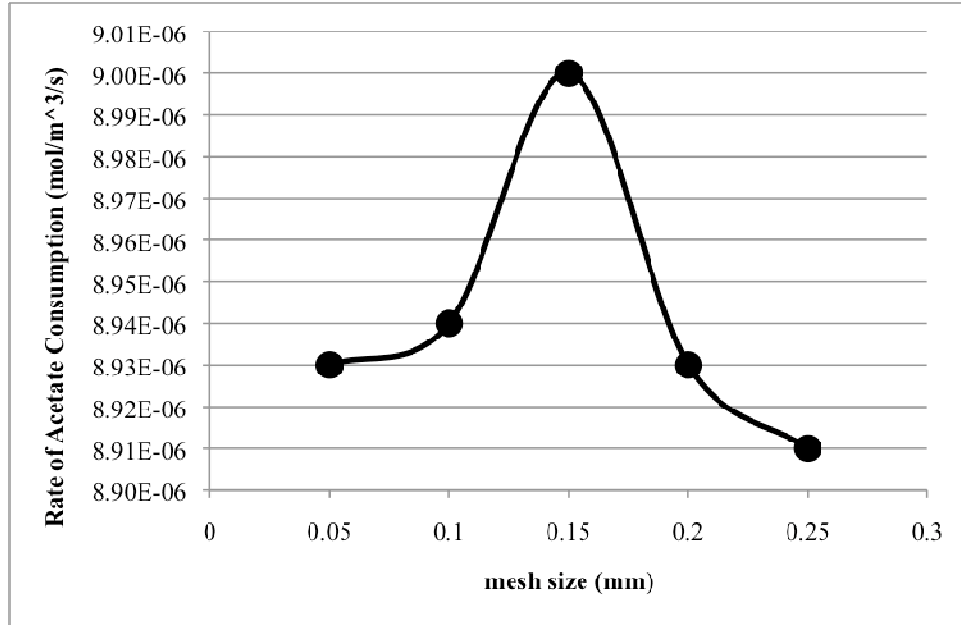


Figure 7. Close up of the average rates of acetate consumption calculated by pore-scale model at different mesh sizes.

4.2. Groundwater Flow

To test how groundwater flow influences the rates of methanogenesis, I changed the flow velocity in the hypothetical aquifer by changing the difference in water head between the inlet and outlet of the model, and kept all other parameters the same.

In the pore-scale model, the rate of acetate consumption increases with the velocity of groundwater flow. Specifically, where the flow velocity is small, the increase in the rate is very sharp. At flow velocity greater than $5\text{E-}7$ m/s, the rate increases gradually with increasing flow velocity. At flow rate greater than $5\text{E-}5$ m/s, the rate increases to a maximum value. Because the goal of the study is to evaluate the difference in reaction rate between the continuum and pore-scale model, in the following simulations, I use a flow velocity of $5.8\text{E-}5$ m/s which produces a maximum rate of acetate consumption. The maximum rate is the rate at which acetate concentration does not limit the reaction (Equation 6).

In comparison, the rates calculated by the continuum model are not greatly significantly by the flow velocity.

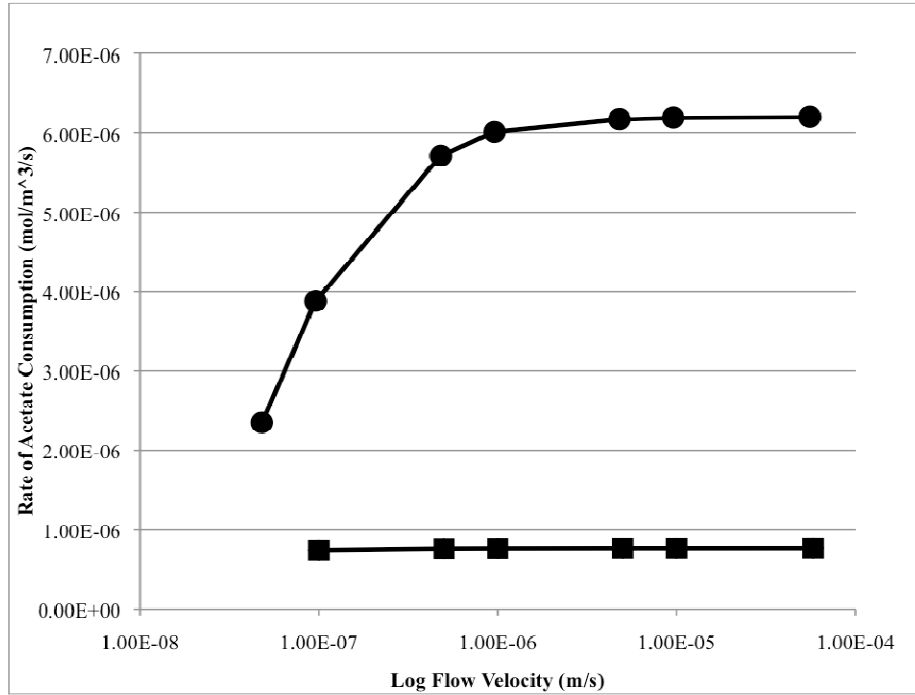


Figure 8. Impact of flow velocity on the rate of acetate oxidation. Pore-scale model results are depicted by the filled in circle, and continuum model results are the filled in squares.

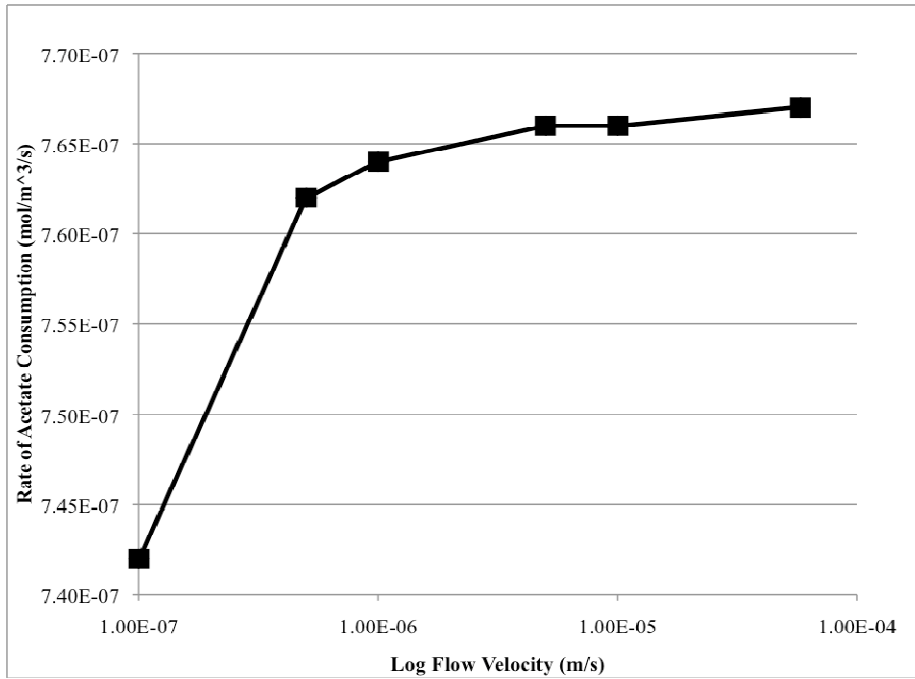


Figure 9. Close up of continuum model reaction rates change due to flow velocity.

4.3. Simulation Results of Pore-Scale Model

The simulation results from the pore-scale model show the spatial heterogeneity. As the figure shows, the heterogeneity in pore volume leads to large variations in the flow velocity (Figure 10) and biomass concentrations (Figure 11) in the model. Consequently, the rates of acetate consumption (Figure 13) and acetate concentrations (Figure 12) are also heterogeneous in the model.

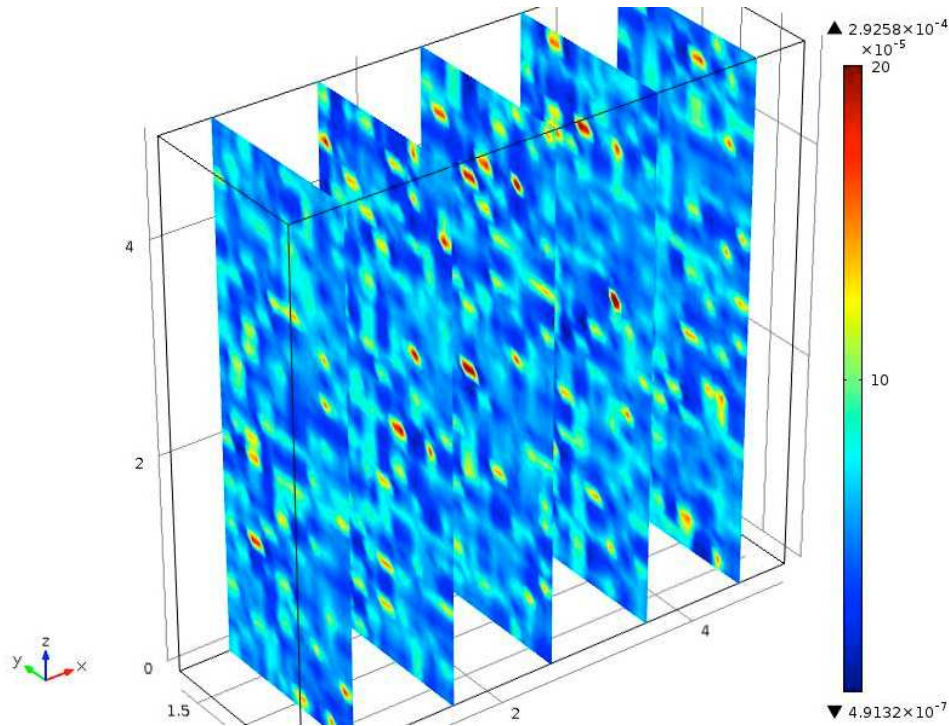


Figure 10. The groundwater flow velocity in the hypothetical aquifer. The flow velocity is calculated using the pore-scale model. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , and the standard deviation of the pore volume is $.051 \text{ mm}^3$.

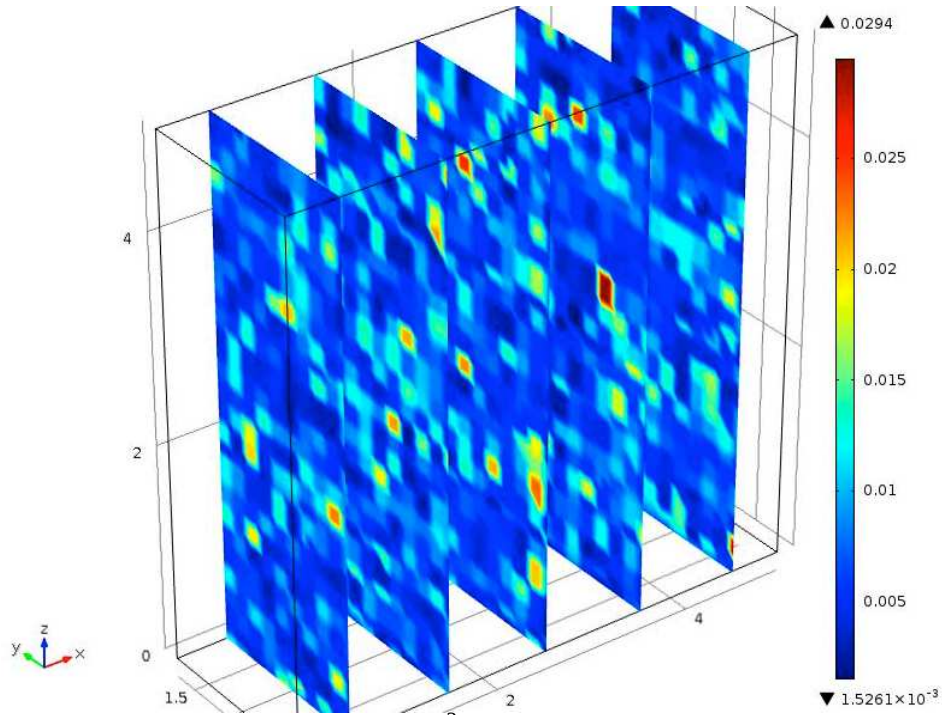


Figure 11. The biomass distribution mol/m^3 in the hypothetical aquifer. biomass distribution is calculated using the pore-scale model. The mean biomass is $.92 \text{ mol/m}^3$ and the distribution is calculated using Equation 16.

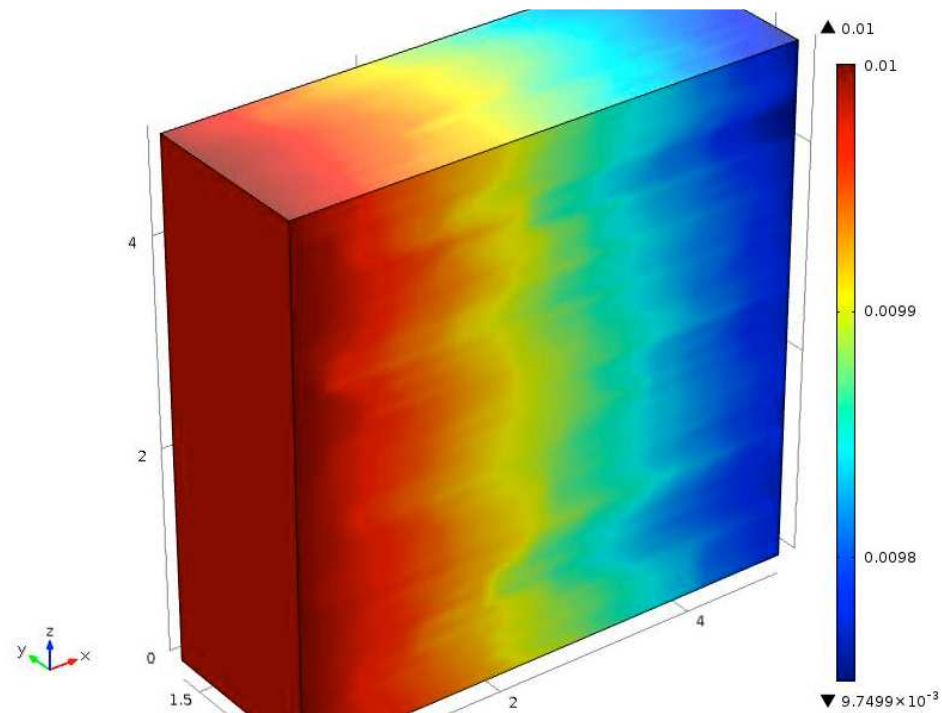


Figure 12. The acetate distribution (mol/m^3) in the hypothetical aquifer. The acetate concentration is calculated using the pore-scale model. The inlet concentration of acetate is $0.01 \text{ (mol/m}^3\text{)}$.

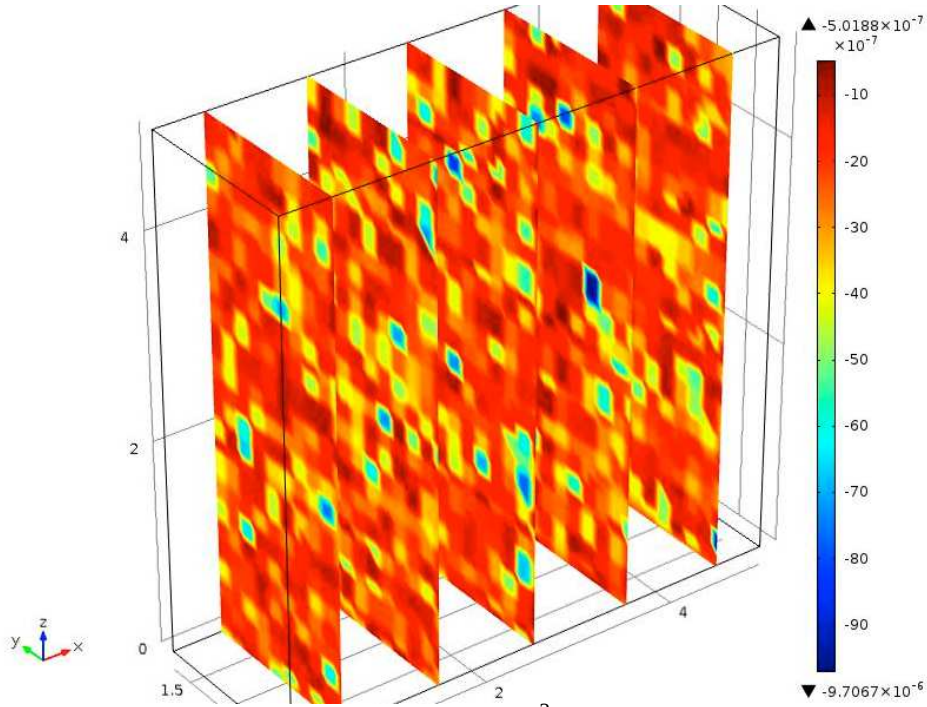


Figure 13. The rate of acetate consumption ($\text{mol/m}^3/\text{s}$) in the hypothetical aquifer. The rate is calculated using the Monod equation with the spatially distributed and heterogeneous acetate concentration and biomass.

The results of this investigation indicate that the reaction rates in the pore-scale model are faster than the reaction rate in the continuum or calculated model. This is a surprising result as we expected pore scale heterogeneity to decrease the reaction rate. Preferential flow patterns developed in the pore-scale model, and the groundwater flow rate did have an impact on the acetate oxidation rates.

4.3.1. Magnitude of Heterogeneity

To learn the impact of the degree of aquifer heterogeneity on the reaction rates, I changed the standard deviation of the logarithm of the pore volume. Specifically, I assumed a logarithmic standard deviation of 0.25, 0.51, 0.75, and 1.0 mm^3 . The standard deviation is the only parameter to change in these models, all other parameters are unaltered, specifically, the flow regime, acetate, and biomass concentrations. Increasing

the standard deviation of the pore volume increased the range of values for the Darcy velocity, and also created more preferential flow patterns in the hypothetical aquifer (Figure 14-17). Increases in the heterogeneity of the aquifer also increased the rate of acetate consumption (Figure 18). The difference between the reaction rates with standard deviation of 1.0 and those with standard deviation of 0.75 mm^3 is larger than the difference between the reaction rates with standard deviation of 0.75 and 0.51 mm^3 .

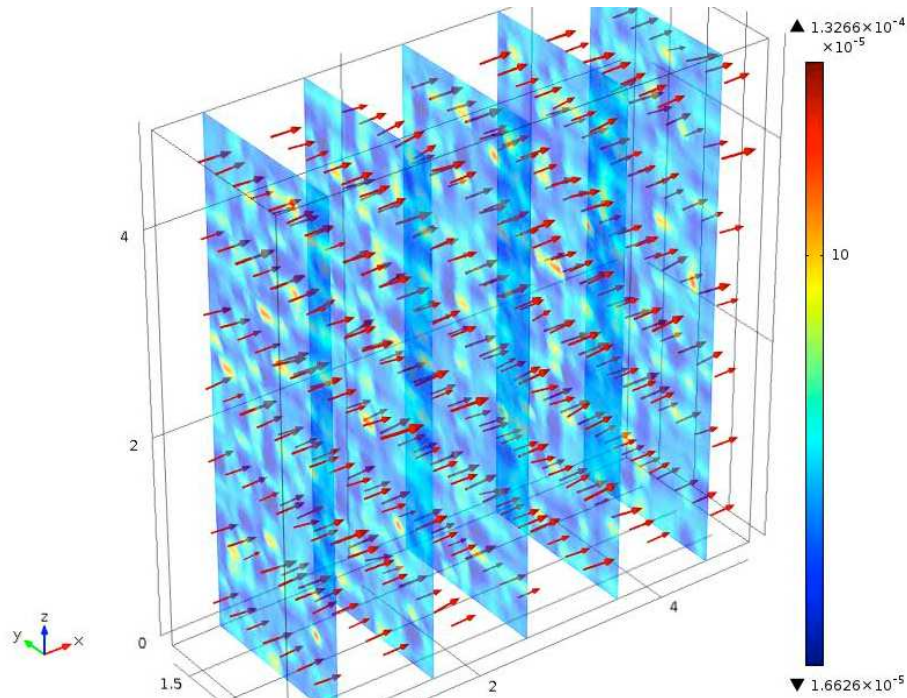


Figure 14. The groundwater flow velocity vector in the hypothetical aquifer. The flow velocity is calculated using the pore-scale model. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , and the standard deviation of the pore volume is 0.25 mm^3 .

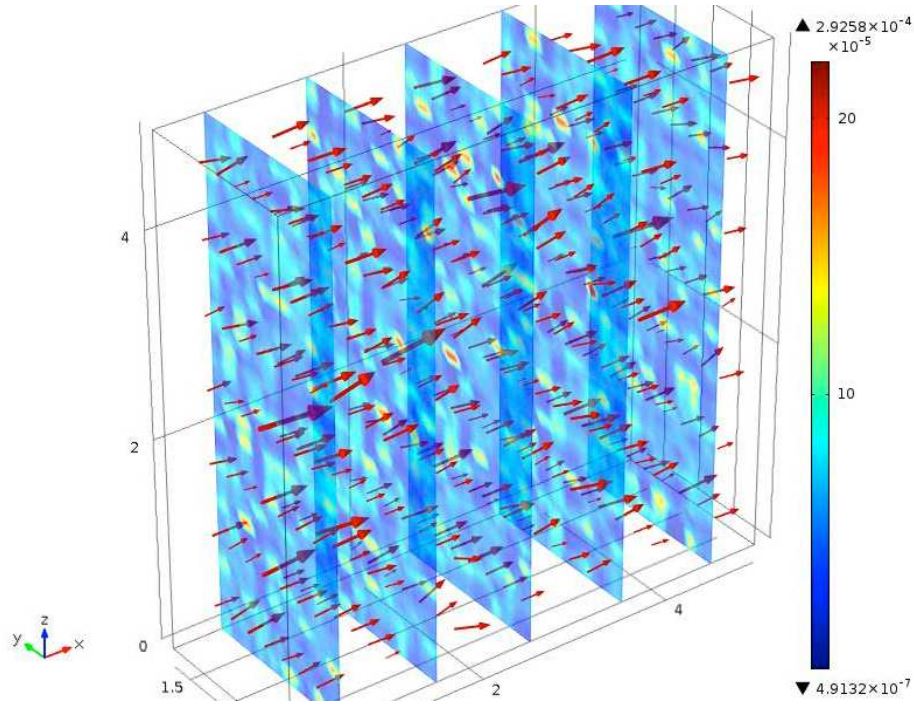


Figure 15. The groundwater flow velocity vector in the hypothetical aquifer. The flow velocity is calculated using the pore-scale model. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , and the standard deviation of the pore volume is 0.51 mm^3 .

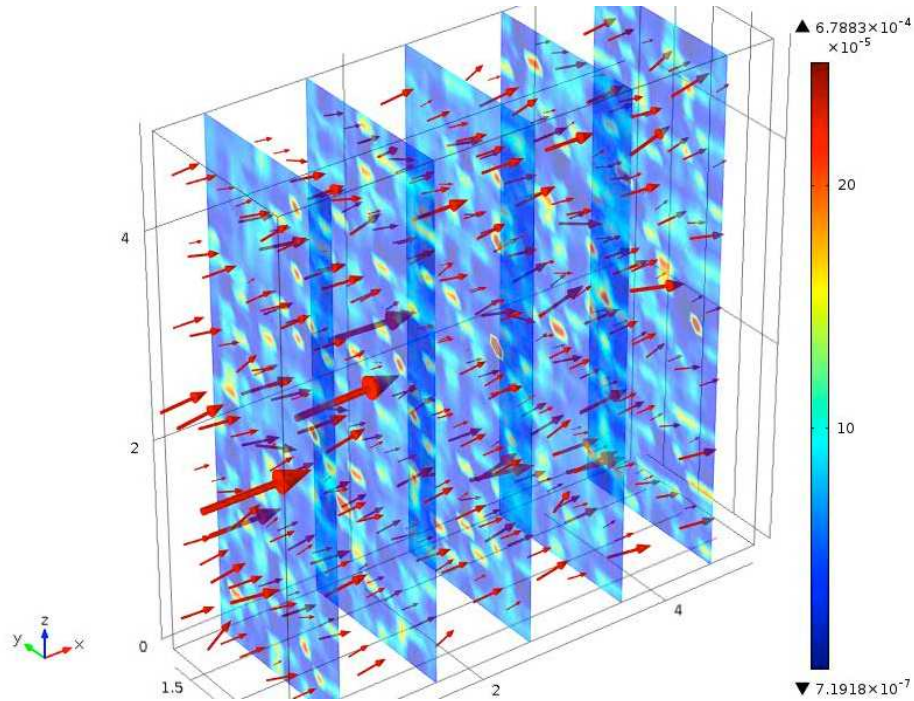


Figure 16. The groundwater flow velocity vector in the hypothetical aquifer. The flow velocity is calculated using the pore-scale model. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , and the standard deviation of the pore volume is 0.75 mm^3 .

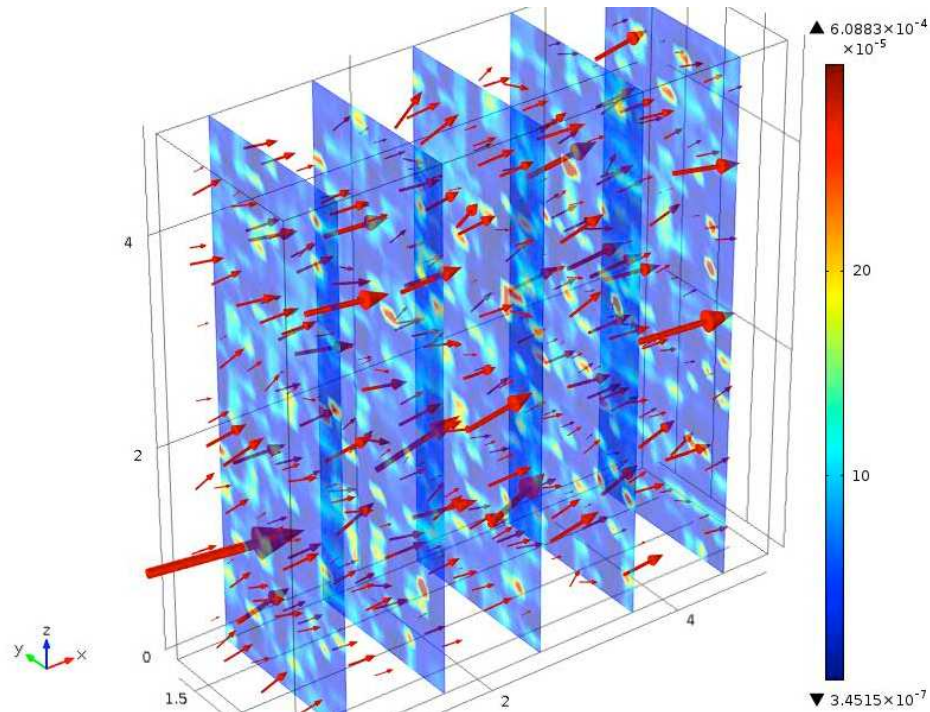


Figure 17. The groundwater flow velocity vector in the hypothetical aquifer. The flow velocity is calculated using the pore-scale model. The permeability of the aquifer is $7.9\text{E-}14 \text{ m}^2$, the inlet flow rate is $5.8\text{E-}05 \text{ m/s}$, the log pore volume is -3.42 mm^3 , and the standard deviation of the pore volume is 1.0 mm^3 .

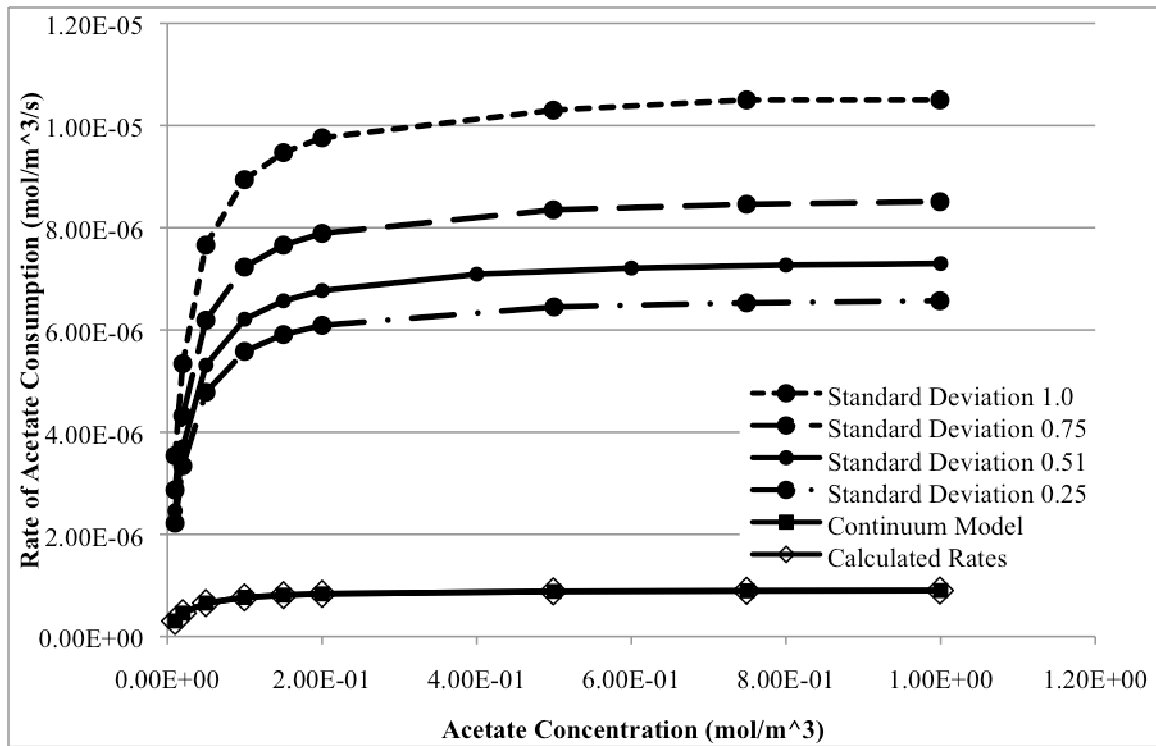


Figure 18. The impact of the degree of heterogeneity on the rates of microbial reaction.

4.4. Simulation Results of Continuum Model

The reaction rates from the continuum model were equivalent to the calculated reaction rates. The calculated reaction rates were based on the Monod equation (Equation 3) and average acetate concentration in the aquifer. This result suggest that the rates of acetate consumption in the continuum model are not affected significantly by the groundwater flow field.

CHAPTER V

DISCUSSION

The mesh convergence test validated the mesh element size that I used in the construction of this model. The continuum model results are not dependent on the mesh size in the range that I tested. The pore-scale model results do fluctuate using the mesh cell sizes from 0.25 to 0.01 mm, likely due to numerical error in the model. Nevertheless, the resulting reaction rates only fluctuate by 9×10^{-8} mol/m³/s. The models were run multiple times to ensure consistent results.

The simulation results of the pore-scale model indicate that increasing the groundwater velocity increases the rate of acetate consumption to a maximum rate. I tested the rates at flow velocities from 5×10^{-8} to 1×10^{-4} m/s. At small flow velocities, the rates are close to the simulation results of the continuum model. Where groundwater flow is very slow, acetate transport is controlled mainly by diffusion, and can diffuse into different pore space, creating a relatively homogeneous acetate distribution. On the other hand, where the flow is fast, the advection becomes the main process of acetate transport. Because of aquifer heterogeneity, advection distributes acetate through preferential flowpaths, creating heterogeneous distribution of acetate. As a result, the rates of acetate consumption differ significantly from the predictions of the continuum model.

The simulation results from the continuum model show that groundwater flow velocity also affects the rates of acetate consumption, but to a lesser degree than in the pore-scale model. In the continuum model, the rate increases to a maximum rate of

7.67×10^{-7} mol/m³/s, an order of magnitude smaller than the maximum rate of the pore-scale model (Table 2).

Table 2.

This table shows how the reaction rate of the pore-scale and the continuum model changed as a function of the flow velocity. It is clear that while the continuum model is affected by the flow velocity, it is a minor alteration when compared with the changes in the pore-scale reaction rate. See figures 7 and 8.

Flow Velocity (m/s)	Reaction Rates (mol/m³/s)	
	Pore Scale Model	Continuum Model
5.00E-08	2.35E-06	7.27E-07
1.00E-07	3.88E-06	7.42E-07
5.00E-07	5.71E-06	7.62E-07
1.00E-6	6.01E-06	7.64E-07
5.00E-6	6.17E-06	7.66E-07
1.00E-5	6.19E-06	7.66E-07
5.80E-05	6.20E-06	7.67E-07
1.00E-04	6.21E-06	7.67E-07

Based on the simulation results, the rates predicted by the pore-scale model are larger than those of the continuum model. Previous studies have demonstrated that microbial metabolism in lab experiments is typically faster than in natural environments. The simulation results are surprising because the pore-scale model represents more accurately natural aquifers than the continuum model, and hence should have smaller rates. A possible explanation could be the impact of preferential flow paths through the pore-scale model: in preferential flow channels, the combination of fast groundwater flow, abundant substrate, and absence of competition may lead to large reaction rates.

The results of the standard deviation test imply that the complexity of a system directly impacts geomicrobial kinetics. At a small degree of heterogeneity, the results of

the pore-scale model are close to those of the continuum model and calculated reaction rates. At a larger degree of heterogeneity, the rates are faster, orders of magnitude larger than the rates of the continuum model. Based on this result, I conclude that the REV underestimates the reaction rates in natural aquifers.

5.1. Model Limitations

The simulation is limited in that it only considers the concentrations of biomass and acetate. Microbial rates are also affected by the competition within diverse microbial communities, the limitation of growth nutrients, the thermodynamic factors, and the growth and decay of biomass.

The rate of microbial growth changes as a function of the rate of acetate oxidation, which creates a highly nonlinear function (Equation 8). While the growth and maintenance constants are empirically derived values, the rate of acetate oxidation and biomass concentration are values that get constantly updated at each time step. I tried to add this function to the pore-scale model but I could not get realistic results. I suspect this occurred because of the nonlinearity of the equations and the limitations of the model interface. The rate of acetate oxidation is calculated using the biomass concentration, which is calculated using the rate of acetate oxidation. In addition, the biomass is added to the model as a solute species with a diffusion coefficient of zero so it would be stationary, and even when the diffusion coefficient was changed to a very small number the error persisted. I was not able to successfully add the biomass growth (Equation 8) into my model, but that would make the models more accurate representations of natural aquifers.

CHAPTER VI

CONCLUSION

To investigate the discrepancy between microbial rates determined from laboratory experiments and field observations, I built two models to test and compare reaction rates under different conditions. I built a pore-scale model that described pore-scale heterogeneity of an aquifer, and a continuum scale model that used REV values for all of the parameters. The two models have identical geometry, mesh element shape and size, and initial and boundary conditions.

The model is a 3-D cube of $5.0 \times 1.75 \times 5.0 \text{ mm}^3$ in the x-, y-, and z- direction respectively, with groundwater flow in the x-direction. The mesh is an inner network of cubes with a volume of 0.1^3 mm^3 , which is half the size of the distribution of heterogeneity. Decreasing the mesh size to a smaller value than the level of heterogeneity ensures that all of the pore-scale variability will be captured by the mesh. I tested the mesh dependency of the models using a mesh convergence test and concluded that the mesh size, 0.1^3 mm^3 , was an appropriate mesh size to use.

The degree of heterogeneity was altered to test the impact on microbial rates, and I found that increasing the degree of heterogeneity of the aquifer increased the microbial rates. To test this, I set the pore volume of the aquifer as a random function with a logarithmic normal distribution, and changed the standard deviation of the pore volume function. The shape of the reaction rate curve as a function of substrate concentration remains the same regardless of the standard deviation, but the maximum rate increases

with a higher standard deviation. This implies that geomicrobial kinetics is directly related to the degree of natural variability in an aquifer.

I also concluded that the flow rate impacted the microbial rates in the aquifer. By only altering the flow parameters and keeping all other input and boundary conditions constant, I was able to show the effect of groundwater flow rate on the resulting microbial rates. While the flow rate did affect both the pore-scale and continuum model, the change in rates from the pore-scale model results were much more dramatic than the change in rates from the continuum model.

In general, I found that the microbial rates in the pore-scale model were faster than the rates in the continuum model. This was a surprise to me because I intended the pore-scale model to represent the natural environment and the continuum model to represent a batch laboratory experiment. Previous work has shown that reaction rates measured in the laboratory are often orders of magnitude larger than those rates measured or observed in the field.

These models put aside other variables that exist in natural environments to focus on the aquifer heterogeneity. For instance, I do not include the competition from a diverse microbial community, limitation of nutrients or substrates, or microbial growth or decay. I understand that these absences limit the results, but from these models, new functions could be added to account for other factors. I also do not have experimental data to compare these results with, but future work could include laboratory experiments and field observations to check the validity of the model.

There are many factors that impact the reaction rate. On a fundamental level, the physical characteristics of an aquifer are an important factor that is often overlooked. The

local geology of an aquifer will influence the chemical composition of the groundwater and the aquifer properties will influence the transport of the chemicals by groundwater flow. The microenvironments of local chemical concentrations will affect geomicrobial kinetics within an aquifer. Inherently slow rates in anaerobic environments make methanogenesis a difficult process to study in situ, but combining field observation, laboratory experiments, and numerical modeling should provide a comprehensive understanding of geomicrobial kinetics. With evolving methods and equipment, we must keep studying methanogenesis in order to fully develop our knowledge of the microorganisms that have such a large influence on our environment.

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