# EXPLORING MICROBIAL AND HYDRAULIC CONTROLS ON REACTIVE SOLUTE TRANSPORT AND REACTION MICROZONE FORMATION IN SURFACE-GROUND WATER INTERFACES

By

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#### ABSTRACT

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Several experimental studies have detected the presence of microzones in hyporheic sediments. These microzones are small scale anoxic pores, embedded within oxygen rich porous media and can act as anaerobic reaction sites producing reduction compounds such as nitrous oxide, which is a greenhouse gas. Microbes are one of the key controls on nutrient transformation in hyporheic sediment and microbial biomass growth is also capable of altering hydraulic flux in sediment, causing 'bioclogging'. In this thesis, I developed one of the first computational modeling approaches that combined hydraulics and microbial kinetics to explore the presence of microzones in stream sediments. The model was used to explore stream and sediment conditions with different hydraulic flux (0.1-1.0  $mday^{-1}$  Darcy flux), nutrient concentrations  $(O_2 = 8mgl^{-1}, Org \ C = 20 \ mgl^{-1}, NO_3^- = 1.5 - 3 \ mgl^{-1}, NH_3 = 1 - 0.5 \ mgl^{-1})$ , and bioclogging scenarios (with and without). The model domain was constructed from a pore network model with random sized pore throat radii resulting in a heterogeneous and anisotropic flow domain that resembled a streambed, comprising of medium sand with a hydraulic conductivity of  $0.8mday^{-1}$ . Results indicate that microzone formation is controlled by the hydraulic flux, the nutrient concentration and bioclogging strongly inhibiting microzone formation. Bioclogging scenarios typically produced unstable microzones, which perished a few days after formation. Overall, results from the modeling show that anoxic microzones are likely to form under many hyporheic zone conditions, but their distribution and biogeochemical function will be dynamic and difficult to measure in the field. Future investigations, will need to develop field investigations capable of observing microzones to aid in further model development and validation.

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# **KEY TO ABBREVIATIONS**

$O_2$	Oxygen	
<i>N</i> <sub>2</sub>	Nitrogen	
<i>N</i> <sub>2</sub> <i>O</i>	Nitrous Oxide	
<i>NO</i> <sub>3</sub>	Nitrate	
NH <sub>3</sub>	Ammonia	
d	Day	
С	Carbon	
$mgl^{-1}$	Milligrams per litre	
Da	Damkohler Number	
Pe	Peclet Number	
h,hr,hrs Hours		

#### **CHAPTER 1**

#### INTRODUCTION

The element nitrogen is a key part of all ecosystems and hence undergoes multiple inorganic and organic N forms and transformation pathways in the (Figure 1.1) [6]. However, contamination of groundwater due to nitrogen compounds such as excess  $NO_3^-$  has become a serious worldwide issue, and it is mostly due to anthropogenic loading caused by agricultural activities as well as human waste [28][31]. For example, this excess of nitrogen in the ecosystem is the leading cause for lake eutrophication and algal blooms which pose serious environmental hazards [9].



Figure 1.1: Schematic of the Nitrogen Cycle (Bothe et al 2007)

Recent research efforts, however, have turned their focus on another member of the nitrogen cycle,  $N_2O$ , and the various pathways that lead up to its formation(Figure 1.2). This gas is a greenhouse gas, that is 300 times more potent than  $CO_2$  [43][19][1][11]. The nitrogen cycling in nature is a complex process which not only is controlled by biogeochemistry, but is also profoundly impacted by the surrounding abiotic reactions mitigated by hydrology whether be it in lakes [32] or streams [26]. The hyporheic zone in watersheds is a known hotspot for the transformation of multiple nitrogen species, including the processes that create  $N_2O$  (Figure 1.2). The hyporheic



Figure 1.2: Nitrous Oxide production pathways(Zhu et al 2013)

zone is defined spatially as the subsurface domain along the wetted perimeter of a stream, surface water-groundwater exchange occurs[39](Figure 1.3).



Figure 1.3: Hydrologic exchange denoted by arrows in a section of stream and hyporheic zone(Stonedahl et al 2010)

Typically, this physically-driven surface water exchange brings oxic water and dissolved solutes into the surrounding subsurface, and they interact with anoxic groundwater conditions before reemerging downgradient back into the surface flow. This kind of hyporheic exchange is

distinct from typical groundwater due to its bi-directional nature and that it occurs over relatively small spatial (1mm-1km) and temporal (1s-1y) scales [5] compared to purely subsurface flow. This region is known as a biological hotspot with microbially mediated nutrient transformations, which can have a major impact on stream ecology and water quality[22]. The hyporheic exchange forces a flux of oxygen, carbon and nitrogen compounds into and out of the stream sediment where they are subjected to microbial transformations. The general understanding of this phenomenon is as follows:

- 1. Highly oxic water flows into the stream sediments as a result of down welling or pressure gradient created due to streambed morphology.
- 2. Energetically favorable aerobic respiration occurs within the sediment.
- 3. The oxygen content gradually diminishes along the flow pathway and the water turns anoxic.

This would generally be the case for homogeneous sediments[29]. However natural sediments are rarely homogenous. Studies looking at denitrification(Figure 1.4), an anaerobic respiration pathway, in natural heterogeneous hyporheic sediments have detected the presence of reduced nitrogen products in bulk oxic sediments[41]. When it comes to hyporheic modeling studies regarding this issue, simple flow and reactive transport models cannot seem to explain in this phenomenon. Several sophisticated modeling attempts have been made to capture the effects of nitrogen dynamics[13][21].

A popular method employed in understanding of reactive solute transport is the residence time approach[8]. In this method a parcel of water is visualized as moving through a porous domain and the time it spends moving is measured as a function of the length and flow velocity. And then the residence time is used to calculate nutrient transformation rates between any two locations in the domain by measuring the difference in residence time and nutrient concentration between those two locations[18].



Figure 1.4: Field data from  ${}^{15}NO_3^-$  tracer experiments clearly shows anomolous  ${}^{15}N_2$  enrichment in oxic zones. (shown as blue in the figure from Briggs et al., 2015)

When it comes to any nitrogen nutrient cycling in nature, microbes are often at the heart of the transformation process. They can be classified as aerobes, anaerobes or facultative. Strains such as *Nitrosomonas europaea* play a crucial role in  $N_2O$  production as it is capable of oxidizing ammonia both under aerobic and anaerobic conditions[19].

Microbes can also impact the sediment hydraulics itself through the phenomenon of bioclogging. Microbial biomass growth in soil systems is not a simple growth phenomenon as with microbes growing in colonies but is accompanied by generation of extracellular polymeric substances (EPS). This EPS is composed mainly of polysaccharides and proteins, and it allows microbes to better latch on to soil particle surfaces as well as acts as a buffer against dehydrating circumstances [38]. The actual microbial biomass in the biofilm consists of various species including bacteria fungi, yeast, protozoa etc. The EPS plays a major role in governing the hydrodynamics of the system by physical constricting the pore space and hence reducing the overall flow through the system with time[36]. Consequently, microbial activity is also directly impacting the hydraulics of the system and this aspect is rarely considered in hyporheic investigations and models.

The hyporheic zone is where the microbes, the hydraulics and the porous domain heterogeneity come together in streams and river corridors, to form an efficient bio-reactor system for nutrient processing[24]. A popular approach of modeling this transport phenomenon in the hyporheic zone is the dual-domain theory where you divide up your porous domain into mobile and less-mobile porosity to simulate a heterogeneous environment[14]. Recent work by Briggs et al 2013[7] outlines a method where by analyzing breakthrough curves, one can quantify the relative proportions of these two kinds of porosity. The interaction in terms of mass transfer between the less-mobile and mobile porosity leads to the formation of small scale anoxic regions or 'microzones.' So, these microzones are relatively isolated reactive sites, embedded in a porous domain which is primarily composed of mobile pore spaces.

In this thesis, I develop a new modeling appraoach that can quantify the impact of dynamic bioclogging on the formation of these reaction microzones as well as the generation of  $N_2O$  in the hyporheic zone. When appropriate, to help guide the research, specific objectives and testable hypotheses are presented in the subsequent chapters.

#### **CHAPTER 2**

#### MODEL DEVELOPMENT AND CODE IMPLEMENTATION

In this chapter, I define the geometry and formulation strategies used to construct the pore network grid, plus the physics and chemistry implemented to model the flow and transport of solute through the network as well as the bioclogging in the system. A conceptual overview of the entire model framework is presented in Figure 2.1. The framework shows the sequence of calculations in the modeling process. Calculations start with the hydraulic head distribution, which is followed by the biogeochemical kinetics consisting of calculating the nutrient uptake rate and the bioclogging effects due to biomass growth. Following that, the uptake rates and the calculated hydraulics are used to compute the nutrient transport, the results of which are used to calculate the biogeochemical parameters all the while adjusting the head distribution caused due to bioclogging. Then snippets from the actual source code are presented as part of model description to demostrate how different aspects of the model are computed using a combination of Fortran and MATLAB.



Figure 2.1: Model Flowchart

## 2.1 Model Geometry

The modeling framework uses a 2-dimensional pore network model, comprising of spherical pore bodies and cylindrical pore throats, with a coordination number of 4 to represent the porous medium[27]. Each element of the pore network model (i.e. pore throats and pore bodies) has a finite 3-dimensional volume, which when summed up, comprises the entire pore volume of the domain. Porosity is a volume property, so the porosity of the domain is calculated as :

$$\eta = \frac{V_{pore}}{LB \,\triangle \, z} \tag{2.1}$$

where  $\eta$  is the porosity,  $V_{pore}$  is the pore volume, *L*, *B* are the length and breadth of the domain respectively and  $\triangle z$  is a small length in the direction orthogonal to the plane of *L* and *B*, which is slightly larger than the pore body diameter.

Figure 2.2 presents a schematic of the structure of the pore network. Although the network is depicted as consisting of similar sized pore throats, the pore throats are in fact variable in size throughout the domain, simulating a heterogeneous porous media.

In order to achieve the heterogeneity, the pore radii were randomly drawn from two different Rayleigh distributions, differing by 2 orders of magnitude in their distribution values. Figure 2.3a represents the larger of the two distributions and forms the distribution for the bulk of the pore throats in the network. The remaining pore throats (20% for the simulation domain used) are drawn from the smaller distribution shown in Figure 2.3b. This produces a heterogeneous porous media with high spatial variability in flux movement.

The source code for generating the porous domain is provided in Figure 2.4. In lines 1 and 2 of Figure 2.4, I generate the total grid for the pore network and in lines 16 and 17, two sets of arrays are drawn from two Rayleigh distributions. Each arrays has two colums corresponding to one of the dimensions of the pore network. In line 20 a random permutation of the indices of the two arrays are made so that the substitutions made are completely random. The actual substitution happens in line 26 as 20% of the values in the arrays with the larger distribution values are substituted by



Figure 2.2: Schematic of the 2D pore network



Figure 2.3: Rayleigh Distributions used for generating the porous domain

values from the array with the smaller distribution values.

Then the next step in analysis of the model geometry is computation of the effective pore throat length and and the pore volume of the generated pore network. The effective length needs to be calculated as the pore throats are shortened by the pore bodies at both ends.

$$l_{j} = \frac{Length \ of \ the \ domain \ in \ direction \ j}{Number \ of \ pore \ bodies - 1} - 2 * R_{porebody}$$
(2.2)

where  $l_i$  is the effective length of the pore throat in *j* direction (either along the length or breadth

```
n=150; % Nodes in y direction
m=150; % Nodes in x direction
NM=n*m;
%% Generating the values
L=raylrnd(2.5e-5, NM, 2); % Rayleigh distribution
Lcon = ray lrnd(.5e-5, NM, 2);
%% Visulazing the values using histograms
figure
G=reshape(L, [2*NM, 1]);
hist(G, 40);
figure
H=reshape(Lcon, [2*NM, 1]);
hist(H, 40);
16% Generating a mixed vector for each pore throat
for j = 1:2
index = randperm (NM);
Pk=round(.20*NM);
% Choosing what percentage of the values are to be substituted.
%This can be
% considered as controlling the degree of heterogeneity.
%
L(index(1:Pk), j)=Lcon(index(1:Pk), j); \% substituting
end
```

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Figure 2.4: MATLAB Code for generating the Pore Throat Distribution

of the domain) and  $R_{porebody}$  is the pore body radius. This represents the actual throat length traversed by the hydraulic flux while traveling from one pore body to an adjacent one. The total pore volume of the domain is calculated as a summation of the total volume of the pore throats and pore bodies in the system.

$$V_{pore} = (N * M)(\frac{4}{3}\pi R^3) + (N - 1) * (M - 2) * (\pi r_{iy}^2 l_y) + (M - 1) * N * (\pi r_{ix}^2 l_x)$$
(2.3)

where  $V_{pore}$  = total pore volume, R = pore body radius,  $l_y/l_x$  = effective pore throat length in y or x direction,  $r_{iy}/r_{ix}$  = pore throat radius at the ith location in y or x direction

## 2.2 Flow physics

The flow in the pore network model is assumed to be a quasi-steady flow without any source or sink present anywhere within the network. The flow in each pore throat is computed using a modified Hagen-Poiseuille equation which can take into account the effects of bioclogging, microbial mass grows in the system [35].

## 2.2.1 Mass Balance

Equations were first derived to determine the hydraulic head distribution through out the netwrk. The mass continuity equation for a steady state, incompressible flow can be written as:

$$\vec{\nabla} \cdot \vec{v} = 0 \tag{2.4}$$

$$\iiint (\vec{\nabla} \cdot \vec{v}) dv = 0 \tag{2.5}$$

$$\iint (\vec{v} \cdot \vec{n}) da = 0 \tag{2.6}$$

$$\sum_{i} A_i V_i = 0 \tag{2.7}$$

where v= the instantaneous velocity at a point, A = area of the control surface, V = average velocity of flow Therefore, the hydraulic head distribution can be achieved simply by solving Equation 2.7 at every single pore node in the network.

#### 2.2.2 Momentum Balance

In this section, I am deriving the Hagen-Poiseuille equation for laminar flow through a cirular pipe of radius *R*. The momentum balance equation for a fluid is expressed by the Navier-Stokes equation (for a Newtonian fluid):

$$\frac{\partial(\rho\vec{v})}{\partial t} + \vec{v} \cdot \vec{\nabla}(\rho\vec{v}) = -\vec{\nabla}p + \mu \,\nabla^2 \,\vec{v} + \frac{1}{3}\mu\vec{\nabla}(\vec{\nabla} \cdot \vec{v}) + \rho\vec{g}$$
(2.8)

where  $\rho$  is the density of the fluid, v is the flow velocity, p is the pressure,  $\mu$  is the dynamic viscosity and g is the acceleration due to gravity.

In order to simplify Equation 2.8 certain assumptions are made:

1. The flow is incompressible

- 2. The pore throat is a cylinder, so equations are solved in the cylindrical coordinate system
- 3. The flow is in steady state
- 4. Gravity forces are neglected
- 5. Velocity only in longitudinal direction is considered

These assumptions allows Equation 2.8 to be simplified to

$$\frac{1}{r}\frac{\partial}{\partial r}(r\frac{\partial v_x}{\partial r}) = \frac{1}{\mu}\frac{\partial p}{\partial x}$$
(2.9)

Integrating equation 2.9 twice with respect to r:

$$v_x = \frac{1}{4\mu} \frac{\partial p}{\partial x} r^2 + C_1 \ln r + C_2 \tag{2.10}$$

 $v_x$  has a finite value at r = 0 and since ln r is undefined at r = 0,  $C_1 = 0$ .  $v_x$  is also 0 at r = R. From the second boundary condition  $C_2 = -\frac{1}{4\mu} \frac{\partial p}{\partial x} R^2$ Thus, the final Hagen-Poiseuille equation has the form:

$$v_x = -\frac{1}{4\mu} \frac{\partial p}{\partial x} (R^2 - r^2) \tag{2.11}$$

Based on Equation 2.12, the toal mass flux through the cross-section of a cylinder will be equal to:

$$q = -\int_0^R \frac{1}{4\mu} \frac{\partial p}{\partial x} (R^2 - r^2) \ 2\pi r dr$$
(2.12)

$$q = -\frac{\pi}{8\mu} \frac{\partial p}{\partial x} R^4 \tag{2.13}$$

In the pore throats of the network, the biofilm is assumed to grow symmetrically from the surface of the throat towards the center (Figure 2.6) and this way over time, it clogs the pore throat thereby reducing the hydraulic flux through the section (Figure 2.5) [35][12].

Applying Equation 2.13 separately to both the open section of the pore throat as well as the section covered by the biofilm, the total hydraulic mass flux through the pore throat comes out to



Figure 2.5: Constriction of flow through a pore throat. R is the unclogged radius while r represents the the shrinking radius due to bioclogging



Figure 2.6: Biofilm growing in a pore throat

be:

$$q_{total} = -\frac{\pi}{8\mu} \frac{\partial p}{\partial x} r^4 + \left(-\frac{\pi}{8\mu_{bio}} \frac{\partial p}{\partial x} (R^4 - r^4)\right)$$
(2.14)

Substituting  $-\frac{\partial p}{\partial x}$  by  $\frac{\rho g \triangle h}{l}$  the final form of the equation is obtained as:

$$q_{total} = \frac{\pi \rho g}{8\mu} \frac{\Delta h}{l} (r^4 + \frac{(R^4 - r^4)}{X})$$
(2.15)

 $q_{total}$  is the total flux through a bioclogged pore throat,  $\rho$  is the density of water,  $\mu$  is the viscosity of water, g is the acceleration due to gravity, l is the length of the pore the pore throat, X is the ratio of the viscosity of the biofilm to water, R is the total radius of the pore throat, r is the radius of the unclogged section of the pore throat.

Referring back to Equation 2.7, the mass balance equation required to be solved at every pore node in order to obtain the hydraulic head distribution is given by:

$$\sum_{i=1}^{i=4} \frac{\pi \rho g}{8\mu} \frac{\Delta h_i}{l_i} (r_i^4 + \frac{(R_i^4 - r_i^4)}{X}) = 0$$
(2.16)

The mapping between *r* and *R* is a function of the volume of biomass in a pore throat. Let the conentration of biofilm (biomass per unit pore volume) be *B*, the density of the biofilm be  $\rho_{bio}$  and the length of the pore throat be *l*. Then the relation between *r* and *R* can be written as:

$$B\pi R^2 l = \rho_{bio} \pi (R^2 - r^2) l$$
 (2.17)

$$r = R(1 - \frac{B}{\rho_{bio}})^{0.5}$$
(2.18)

## **2.2.3** Fortran coding for the flow physics

In this section, I present some snippets of the fortran code used for computing the hydraulic head distribution.



Figure 2.7: Convention for mapping variables in source code: Hydraulic conductivity

Some variables in the code have a specific convention with a number included either in the name or in the index. The numbers read as follows:

- 1 refers to the x-direction
- 2 refers to the y-direction
- 3 refers to the node itself

For example, Figure 2.7 shows the conductivity variable Ke, which holds the pore throat conductivities, referenced around a node (i, j). The way this type of referenced variables are read in

the the code is, in the x-direction, the value held by the variable is always to the left of the node and in the y-direction the value referenced is below the node. So in Figure 2.7, Ke1(i, j) refers to the conductivity of pore throat connecting *H* and *H*1 and Ke2(i, j) refers to the conductivity of pore throat connecting *H* and *H*4.

1

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3

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14

15 16

17

```
!$OMP PARALLEL DO reduction (+:Vx)
DO i=1,n
DO j=2,m
Vx=Vx+(Re1(i,j)**2)*lenx*3.14
END DO
END DO
!$OMP END PARALLEL do reduction (+:Vy)
DO i=1,n-1
DO j=2,m
Vy=Vy+(Re2(i,j)**2)*leny*3.14
END DO
END DO
!$omp end parallel do
por =(Vx+Vy+(n*m*4*3.14*(Ra**3)/3))*100/(Lx*Ly*2*Ra*1.01)
```

Figure 2.8: Code for calculating porosity

Variable	Definition
$V_x$	Pore volume of all pore throats aligned in x-direction
$V_y$	Pore volume of all pore throats aligned in y-direction
por	Porosity of the domain
Ra	Radius of pore bodies
$L_X$	Length of the domain in x-direction
$L_{y}$	Length of the domain in y-drection
Re	Radius of pore throats
n,m	Grid size parameters

Table 2.1: Variables in Figure 2.8

Figure 2.8 shows the code for calculating the porosity of the domain. The commented lines contain OpenMP directives in order to carry out the task using parallel computing.

```
DO i=1,n

DO j=2,m

Ke1(i,j)=((3.14*rho*g/(8.0*meu))*(re11(i,j)**4+ &

(Re1(i,j)**4 - re11(i,j)**4)/X))/lenx

Ke2(i,j)=((3.14*rho*g/(8.0*meu))*(re22(i,j)**4+ &

(Re2(i,j)**4 - re22(i,j)**4)/X))/leny

END DO

END DO
```

Figure 2.9:	Code for	calculating	the pore	throat	conductivities
-------------	----------	-------------	----------	--------	----------------

Variable	Definition
lenx	Effective length of pore throats aligned in x-direction
leny	Effective length of pore throats aligned in y-direction
rho	Density of water
g	Acceleration due to gravity
meu	Dynamic viscosity of water
Ke	Conductivity of pore throats
re11	Constricted radius of pore throats aligned in x-direction
re22	Constricted radius of pore throats aligned in y-drection

Table 2.2: Variables in Figure 2.9

The conductivity of a pore throat is caluclated as follows:

$$Ke = \frac{q_{total}}{\triangle h} \tag{2.19}$$

3

4

6

7

0

where  $\triangle h$  is the hydraulic head difference across a pore throat. Therefore from Equation 2.15, the analytical relation for *Ke* is

$$Ke = \frac{\pi\rho g}{8\mu l}(r^4 + \frac{(R^4 - r^4)}{X})$$
(2.20)

Figure 2.9 shows the formulation of Equation 2.20 in the code.

```
!$omp parallel do private(i,j) default(shared) schedule(runtime)
DO j = 2, m-1
   DO i = 2, n-1
      A1((j-1)*(n)+i) = ((Ke1(i,j)+Ke1(i,j+1)+Ke2(i-1,j)+Ke2(i,j)))
                                                                        4
      A2((j-1)*(n)+i) = ((-Ke2(i-1,j)))
      A3((j-1)*(n)+i) = ((-Ke2(i,j)))
                                                                        6
      A4((j-1)*(n)+i)=((-Ke1(i,j)))
                                                                        7
      A5((j-1)*(n)+i) = ((-Ke1(i, j+1)))
                                                                        8
                                                                        9
   END DO
                                                                        10
END DO
                                                                        11
!$omp end parallel do
                                                                        12
                                                                        13
!$omp parallel do private(i) default(shared) schedule(runtime)
                                                                        14
                                                                        15
DO i = 2, m-1
   A1((i-1)*(n)+1) = ((Ke1(1,i)+Ke1(1,i+1)+Ke2(1,i)))
                                                                        16
   A3(((i-1)*(n)+1)=(-Ke2(1,i)))
                                                                        17
   A4((i-1)*(n)+1)=(-Ke1(1,i))
                                                                        18
   A5((i-1)*(n)+1)=(-Ke1(1,i+1))
                                                                        19
                                                                        20
                                                                        21
   A1((i)*(n)) = ((Ke1(n,i)+Ke1(n,i+1)+Ke2(n-1,i)))
                                                                        22
   A2((i)*(n))=(-Ke2(n-1,i))
                                                                        23
   A4((i)*(n))=(-Ke1(n,i))
                                                                        24
   A5((i)*(n))=(-Ke1(n,i+1))
                                                                        25
                                                                        26
END DO
                                                                        27
!$omp end parallel do
                                                                        28
```

Figure 2.10: Code for formulating the coefficient matrix for the linear equations

The 2 dimensional flow field results in a sparse pentadiagonal coefficient matrix A for the equation Ax = b, where x represents the vector of unknown hydraulic head values and b is the vector containing the boundary conditions. Figure 2.10 shows the formulation of the coefficient matrix where the vectors A1, A2, A3, A4, A5 represents the diagonals of the matrix A. The solver used for determining the vector x is the PARDISO solver available as a part of the Intel Math Kernal Library.

# 2.3 Biogeochemical Kinetics

In this section, I discuss the methodologies implemented to calculated the biomass numbers as well as the nutrient values used in the model.

#### 2.3.1 Monod Kinetics

One of the most popular and robust methods of modeling microbial growth throughout the decades has been the Monod model [23][37][42]. In this study, I implemented the multiple Monod Kinetic model outlined in Widdowson et al 1988[37], which can track microbial biomass growth both through aerobic and anaerobic conditions ( $NO_3^-$  respiration) as summarized below.

$$\frac{1}{M}\frac{dM}{dt} = (\mu_o [\frac{s}{K_{so} + s}][\frac{o}{K_o + o}][\frac{a}{K_{ao} + a}] - k_o) + (\mu_n [\frac{s}{K_{sn} + s}][\frac{n}{K_n + n}][\frac{a}{K_{an} + a}] - k_n)I_n \quad (2.21)$$

M = microbial biomass

 $\mu_o$  = growth rate for oxic conditions

 $\mu_n$  growth rate for anoxic conditions

*s*, *o*, *n*, *a*= cocnetration for organic carbon substrate,  $O_2$ ,  $NO_3^-$  and  $NH_3$ 

 $K_n, K_{sn}, K_{an}$ = respective substrate saturation constants under  $NO_3$  respiration  $K_o, K_{so}, K_{ao}$ = respective substrate saturation constants under  $O_2$  respiration  $I_n$ = an inhibitor factor =  $(1 + \frac{O}{K_c})^{-1}$  where  $K_c$  is a paramter  $k_o, k_n$ = microbial decay coefficients under oxic and anoxic conditions

Equation 2.21 allows the tracking of microbial biomass under both oxic and anoxic conditions. The inhibition factor  $I_n$  modulates the  $NO_3^-$  respiration in Equation 2.21 by assuming small values when the  $O_2$  concentrations are high and gradually increases in value as the  $O_2$  gets depleted over time. Now when it comes to other available equations that can be used to track biomass growth, there are models which take into consideration only the nutrient which has the lowest limiting constraint instead of all the solutes, but Equation 2.21 has been employed here as it is one of the most frequently occuring and mechanistically accurate models in environmental engineering literatures.

Once the mass is calculated, the nutrients consumed in that process is then estimated using stoichiometric coefficients.

$$r_{s} = \frac{\mu_{o}}{Y_{o}} \left[\frac{o}{K_{o} + o}\right] \left[\frac{s}{K_{so} + s}\right] \left[\frac{a}{K_{ao} + a}\right] + \frac{\mu_{n}}{Y_{n}} \left[\frac{n}{K_{n} + n}\right] \left[\frac{s}{K_{sn} + s}\right] \left[\frac{a}{K_{an} + a}\right] I_{n}$$
(2.22)

$$r_n = \eta \mu_n \left[\frac{n}{K_n + n}\right] \left[\frac{s}{K_{sn} + s}\right] \left[\frac{a}{K_{an} + a}\right] I_n \tag{2.23}$$

$$r_o = \gamma \mu_o \left[\frac{o}{K_o + o}\right] \left[\frac{s}{K_{so} + s}\right] \left[\frac{a}{K_{ao} + a}\right]$$
(2.24)

$$r_{a} = \psi \mu_{o} \left[\frac{o}{K_{o} + o}\right] \left[\frac{s}{K_{so} + s}\right] \left[\frac{a}{K_{ao} + a}\right] + \epsilon \mu_{n} \left[\frac{n}{K_{n} + n}\right] \left[\frac{s}{K_{sn} + s}\right] \left[\frac{a}{K_{an} + a}\right] I_{n}$$
(2.25)

 $r_s, r_n, r_o, r_a$  = uprake rates for substrate,  $NO_3^-, O_2, NH_3$  respectively

 $Y_o, Y_n$  = yield coefficients under oxic and anoxic conditions respectively

- $\gamma = O_2$  use coefficients for heterotrophic biomass
- $\eta = NO_3^-$  use coefficient for heterotrophic biomass growth
- $\epsilon, \psi = NH_3$  use coefficients for  $NO_3^-$  and  $O_2$  respectively

When computing for the biomass using Equation 2.21 the following assumptions are made:

- 1. Planktonic microbial populations are neglected.
- 2. The microbial biomass growth includes the entire mass of the biofilm.
- 3. Biomass attachment and detachment mechanisms are not considered.
- 4. All biogeochemical reactions take place within only the biofilm.

Moreover, the biofilm in the system is considered to be permeable, allowing transport through the biofilm by means of both advection and diffusion.

## 2.3.2 Implemention of microbial kinetics in Fortran

The following figure shows the imlementation of the Monod Kinetics in Fortran.

```
DO i = 1, n
        DO j = 2, m
                                                                                    2
            DO k = 1, 2
                Ii(i, j, k) = (1 + Onold(i, j, k) / Kc) * (-1)
                BnNew(i, j, k) = ((mo*(Snold(i, j, k))/(Kso+Snold(i, j, k))) * \&
                      (Onold(i,j,k)/(KO+Onold(i,j,k))) &
                      *(Anold(i,j,k)/(KAo+Anold(i,j,k)))-Kdo)*Bnold(i,j,k
) &
                      +(mn*(Snold(i,j,k)/(Ksn+Snold(i,j,k)))* &
                      (Nnold(i,j,k)/(KN+Nnold(i,j,k)))*(Anold(i,j,k)/ &
                                                                                    9
                      (KAn+Anold(i,j,k)))-Kdn)*Bnold(i,j,k)*Ii(i,j,k))*
                                                                                    10
dtreac+Bnold(i,j,k)
                                                                                    11
                rso(i, j, k) = (mo*(Snold(i, j, k)/(Kso+Snold(i, j, k))) * \&
                                                                                    12
                      (Onold(i, j, k)/(KO+Onold(i, j, k))) \&
                                                                                    13
                      (Anold(i, j, k)/(KAo+Anold(i, j, k))))
                                                                                    14
dtreac * (1/Y_0)
                                                                                    15
                rsn(i, j, k) = (mn*(Snold(i, j, k)/(Ksn+Snold(i, j, k))) \&
                                                                                    16
                      *(Nnold(i,j,k)/(KN+Nnold(i,j,k))) &
                                                                                    17
                      *(Anold(i,j,k)/(KAn+Anold(i,j,k))))* &
                                                                                    18
                                                                                    19
                      Bnold(i, j, k) * dtreac * Ii(i, j, k) * (1/Yn)
                                                                                    20
                rs(i, j, k) = rso(i, j, k) + rsn(i, j, k)
                                                                                    21
                ro(i, j, k) = ga * Yo * rso(i, j, k)
                                                                                    22
                                                                                    23
                rn(i, j, k) = eta * Yn * rsn(i, j, k)
                ram(i, j, k) = psi * Yo * rso(i, j, k) + esp * Yn * rsn(i, j, k)
                                                                                    24
                rnos(i, j, k) = knos * rn(i, j, k)
                                                                                    25
                                                                                    26
                                                                                    27
                IF (BnNew(i, j, k) > rhob) THEN
                                                                                    28
                   BnNew(i, j, k) = rhob
                                                                                    29
                END IF
                                                                                    30
                                                                                    31
                Bnold(i, j, k) = BnNew(i, j, k)
                                                                                    32
            END DO
                                                                                    33
                                                                                    34
        END DO
     END DO
                                                                                    35
```

Figure 2.11: Code for calculating microbial growth and nutrient uptake rates, from Equation 2.21 to Equation 2.25

Variable	Definition
Ii	Inhibition factor
Bnold	Biomass concentration at the old time step
BnNew	Biomass concentration at the new time step
dtreac	Reaction time step
rnos	Nitrous oxide production rate
rhob	Density of biofilm
Anold	Ammonia concentration at old time step
Onold	Oxygen concentration at old time step
Snold	Substrate concentration at old time step
Nnold	Nitrate concentration at old time step
mo	Growth rate under toxic condition
mn	Growth rate under nitrate respiration
Kdo	Decay coefficient under oxic conditions
Kdn	Decay coefficient under anoxic conditions

Table 2.3: Variables in Figure 2.11

Table 2.3 lists out the variables for Figure 2.11 and any variable not listed in the table were defined prior to this. Figure 2.11 shows the computation for the microbial biomass and nutrient uptake rates, from Equation 2.21 to Equation 2.25.

# 2.4 Solute Transport

In the final section for this chapter, I discuss the numerical techniques implemented to bring together the biogeochemical parameters and the advection relations developed in previous sections in order to formulate a multispecies transport model.

#### 2.4.1 Transport equation for a solute

Solute transport is modeled using the standard advection-diffusion equation [4]. The formulation consists of a 1 dimensional transport equation at every pore throat and a 2 dimensional equation at every pore body.

$$\frac{\partial C}{\partial t} = D(\nabla \cdot (\nabla C)) - \nu \cdot \nabla C + S$$
(2.26)

where C is the solute concentration, v is the fluid velocity, D is the diffusion coefficient, S is a source/sink term for the solute. Integrating Equation 2.26 with respect to the control volume, the equation becomes

$$\iiint \frac{\partial C}{\partial t} dV = \iiint \left( D(\nabla \cdot (\nabla C)) - \nu \cdot \nabla C - (\nabla \cdot \nu)C + S \right) dV$$
(2.27)

$$\frac{\partial C}{\partial t}V = DA(\nabla C) + \sum Av_{average}C + SV$$
(2.28)

$$\frac{\partial C}{\partial t} = \frac{DA(\nabla C) + \sum Av_{average}C}{V} + S$$
(2.29)

where  $v_{average}$  is the average flux velocity across the control surface A.

Applying a finite difference explicit scheme to discretize the derivative terms and incorporating the modified Hagen-Poiseuille equation (Equation 2.15) to calculate the advective flux across the control surfaces:

$$C_{i}^{n+1} = C_{i}^{n} + \left(\sum_{i} \frac{\pi \rho g}{8\mu} \frac{\Delta h_{i}}{l_{i}} \left(r_{i}^{4} + \frac{(R_{i}^{4} - r_{i}^{4})}{X}\right)C_{i}^{n} \Delta t + \sum_{i} DA_{i} \frac{C_{i}^{n} - C_{i-1}^{n}}{l_{i}} \Delta t\right) \frac{1}{V} + S \Delta t \quad (2.30)$$

As mentioned earlier, the biofilm is treated as permeable, therefore it allows solute transport not only by advection, but also by diffusion. The diffuive flux is treated as two seperate quantities with one part flowing across the open section and other flowing across the bioclogged section. So the final form of the solute transport equation for a single specie is given by:

$$C_{i}^{n+1} = C_{i}^{n} + \left(\sum_{i} \frac{\pi \rho g}{8\mu} \frac{\Delta h_{i}}{l_{i}} \left(r_{i}^{4} + \frac{(R_{i}^{4} - r_{i}^{4})}{X}\right) C_{i}^{n} \Delta t + \sum_{i} (D_{w} \pi r_{i}^{2} + D_{b} \pi (R_{i}^{2} - r_{i}^{2})) \frac{C_{i}^{n} - C_{i-1}^{n}}{l_{i}} \Delta t\right) \frac{1}{V} + S \Delta t$$
(2.31)

n + 1 refers to the next time step while *n* refers to the current time step.  $D_w$  refers to the diffusion coefficient for solute flowing through the open section and  $D_b$  refers to the diffusion coefficient for solutes flowing through the bioclogged section.

Equation 2.31 is solved at every pore throat and pore body in the network individually for every single solute being tracked. Since the biogeochemical reactions are occuring only in the pore throats, S is zero for every solute at the pore bodies.

#### **2.4.2** Implementing solute transport in Fortran

This final code section and end of modeling chapter, shows the implementation of Equation 2.31 in fortran. The code is for solute transport of  $O_2$  through a pore throat and hence it is a one dimensional equation.

```
DO i=1,n

DO j=2,m

OnNew(i,j,1) = -ro(i,j,1) + (1-(ABS(H(i,j-1)-H(i,j))*Kel(i,j)*dt

+ ((Dw*3.14*(re11(i,j)**2)+&

Db*3.14*(Re1(i,j)**2-re11(i,j)**2))*dt/(lenx))*2)/(3.14*

lenx*Re1(i,j)**2))*Onold(i,j,1)&

+ ((ABS(H(i,j-1)-H(i,j))*Kel(i,j)*Dir(i,j,1)*dt+((Dw

*3.14*(re11(i,j)**2)+&

Db*3.14*(Re1(i,j)**2-re11(i,j)**2))*dt/(lenx)))/(3.14*

lenx*Re1(i,j)**2)*Onold(i,j-1,3)&

+ ((ABS(H(i,j-1)-H(i,j))*Kel(i,j)*aDir(i,j,1)*dt+((Dw

*3.14*(re11(i,j)**2)+&

Db*3.14*(Re1(i,j)**2-re11(i,j)*aDir(i,j,1)*dt+((Dw

*3.14*(re11(i,j)**2)+&

Db*3.14*(Re1(i,j)**2-re11(i,j)*aDir(i,j,1)*dt+((Dw

*3.14*(re11(i,j)**2)+&

Db*3.14*(Re1(i,j)**2-re11(i,j)**2))*dt/(lenx)))/(3.14*

lenx*Re1(i,j)**2))*Onold(i,j,3)
```

Figure 2.12: Code for calculating  $O_2$  concentration in a pore throat

Dir and aDir are multidimensional arrays of binary values 0 or 1 which corrects for the direction of the advective solute flux depending upon hydraulic head gradient.

#### **CHAPTER 3**

## COMPUTATIONAL MODELING AND ASSESSMENT OF THE FORMATION CRITERIA FOR HYPORHEIC ANOXIC MICROZONES

## 3.1 Introduction

In a valley bottom, along the river corridor, the hyporheic zone is a known control point for biogeochemical conditions in the watershed. Hyporheic zones, are regions of bi-directional flow in the river corridor, where surface flux enters the sediment and mixes with groundwater and then exits the sediment over relatively short spatial scales[5]. This flux of water also exchanges critical nutrients like  $NO_3^-$ , dissolved organic carbon (DOC) along with  $O_2$  in the sediment matrix, leading to enhanced microbial activity in the hyporheic zone relative to the surface water [13][40][41].

Oxygen is thermodynamically favored for microbial respiration in the hyporheic zone, leading to many hyporheic pore waters transitioning from bulk oxic conditions to bulk an anoxic conditions as the  $O_2$  is respired. However, recent field studies, including isotopic tracers of redox sensitive solutes, indicate the presence of anaerobic denitrification products, namely  $N_2O$  and  $N_2$  forming in bulk oxic porous domains of hyporheic zones[15][41][8]. The bulk oxic conditions should inhibit anaerobic processes, and yet anaerobic processes products exist. To explain this apparent paradox, the theory of anaerobic 'microzones' was created, or pore spaces zones depleted in oxygen embedded within oxygen-rich porous domain. These anaerobic microzones can arise from spatial heterogeneities of physical and biogeochemical conditions in the stream sediments (e.g., variable pore geometries, [8] variable carbon availability [29]). As indicated by the isotope tracer studies, these microzones, if present and stable, could facilitate multiple functions, not currently accounted for in hyporheic modeling studies, like enhanced formation of  $N_2O$ , a greenhouse gas over 300 times more potent than  $CO_2$  [11][19] [43]. So, accounting for the effect of microzones in any model trying to predict the production of  $N_2O$  may be crucial.

The microbial metabolic activity that likely plays a key role in the development of these

microzones, can also lead to the expansion of microbial biomass in the sediment matrix over time [23][37]. This gradual accumulation of biomass, which primarily appears as the formation of biofilms, attached to sediment particles, can alter the hydraulic flux through the sediment. This biomass accumulation can lead to a phenomenon known as 'bioclogging,' which causes a reduction in overall hydraulic conductivity and porosity [36] [34] [35]. Microbial biomass growth is comprised of microbial cells growth as well as the accumulation of extra cellular polymeric substances, which together comprise the structure of the biofilm[3]. A multitude of studies have observed the phenomenon of bioclogging and some have even showed an increase in hydraulic dispersivity values due to bioclogging [30][33]. Despite its potential importance, bioclogging has received little attention as a physical phenomenon in hyporheic hydraulic analysis and it should be a part of studies and models, especially when reactive transport due to microbial activity is under investigation [10]. However, several modeling efforts for porous sediments, unrelated to hyporheic zones, have tried to capture the mechanisms of bioclogging, either through colony formation [36] or by means of biofilm formations in pore network models [12][35].

As microzones in stream sediments is an emerging topic, modeling efforts on microzones formations in the hyporheic zone have been limited, with the most notable work being by Briggs et al. (2015) [8] and Sawyer (2015) [29], and most of the other research being experimental and inconclusive [16][41]. In the Briggs et al. (2015) simulation study, they did not account for the for the role of variable stream chemistry or bioclogging, as it was predicated solely on the assumption that the key controlling factors in determination of the microzone formation are residence time and Damkohler number for  $O_2$  (i.e.,  $DaO_2$ , a number indicating if anoxia is transport or reaction rate limited), as applied in previous hyporheic studies [42]. While the residence time was treated at the pore scale time of travel, the  $DaO_2$  was derived with a constant  $O_2$  uptake rate for each simulation throughout the model domain, thereby ignoring the heterogeneity in the distribution of biomass in a porous domain and the effect of availability of other nutrients on biomass growth. Consequently, the aim of the present study is to bring together the various process-based models available for hydraulics, nutrient transformations, and biomass development, to create more realistic mechanistic

simulations that can track dynamic hydraulic, microbial, and chemical conditions in a hyporheic zone that is experiencing different stream chemistry and microbial biomass growth conditions. Through the development and use of this new model, our objective was to assess the interacting controls on hyporheic pore water chemistry in space and time, with emphasis on revealing the understudied role of bioclogging. Our initial guiding hypothesis for the effect of bioclogging in the hyporheic zone was: the conditions that promote microbial biomass growth (e.g., nutrient and substrate availability) in the sediments would, in turn, create more heterogeneity in the flowpaths and residence times of the solutes and increase the abundance of anoxic microzones. To assess our objective and test this bioclogging hypothesis, we explored a range of realistic stream and hyporheic conditions that lead to or inhibited the formation and persistence of anoxic microzones occurring in bulk oxic pore water.

# **3.2 Model Formulation**

Please refer to Chapter 2 for details on the model formulation

# **3.3 Numerical Simulations**

The numerical experiments explored different hyporheic scenarios where realistic hydraulic flux, nutrient concentrations, and bioclogging conditions were varied. In total, 12 sets of simulations were performed comprising of 3 different hydraulic flux conditions run for two different states of nutrient conditions (i.e., "high" and "low" concentration) and finally with and without bioclogging occurring. The high and low concentrations were selected to represent typical more polluted "high" and pristine "low" conditions, while reflecting the general stoichiometry observed in freshwaters [20]. Each simulation utilized parallel processing with 40 Intel Xeon cores with a turbo clock speeds of 3.7 Ghz and 27 MB cache memory each and total time for 1 simulation set, depending on hydraulic parameters, ranged between 5 to 50 hours. The time step for numerical simulation is taken as the value satisfying both the Courant and diffusion number criteria. To reduce the computational load, the microbial kinetic step is computed every 30 seconds.

Parameters	Values	Source
$\mu_o$	$1.3e - 4s^{-1}$	Values assumed
$\mu_n$	$1.2e - 4s^{-1}$	Values assumed
$k_o$	$0.23e - 6s^{-1}$	Values assumed
$k_n$	$0.23e - 6s^{-1}$	Values assumed
Yo	0.34	Values assumed
$Y_n$	0.38	Values assumed
γ	1.4	Widdowson et al 1988
$\eta$	2.2	Widdowson et al 1988
$\epsilon$	0.122	Widdowson et al 1988
$ \psi $	0.122	Widdowson et al 1988
K <sub>n</sub>	$0.0026 \ mg cm^{-1}$	Widdowson et al 1988
K <sub>sn</sub>	$0.04 \ mg cm^{-1}$	Widdowson et al 1988
Kan	$0.04 \ mg cm^{-1}$	Widdowson et al 1988
Ko	$0.00077 \ mg cm^{-1}$	Widdowson et al 1988
K <sub>so</sub>	$0.04 \ mg cm^{-1}$	Widdowson et al 1988
Kao	$0.001 \ mg cm^{-1}$	Widdowson et al 1988
$D_{w}$	2e - 9	Briggs et al 2015
$D_b$	2e - 10	Ezeuko et al 2011

Table 3.1: Parameter values used for the microbial kinetics modeling

Variables	Values
Initial and influx $O_2$ cocentration	8mgl <sup>-</sup>
Initial and influx organic C concentration	$20mgl^{-}$
Initial biomass concentration(mass per unit pore volume)	$1e - 4Kgm^{-}$
Density of Biofilm	$20Kgm^{-}$
Viscosity ratio (X)	1 <i>e</i> 9
Total simulation time	30days
Initial and influx $NO_3^-$ concentration (HIGH)	$3mgl^{-}$
Initial and influx $NH_3$ concentration (HIGH)	$1mgl^{-}$
Initial and influx $NO_3^-$ concentration (LOW)	$1.5mgl^{-}$
Initial and influx $NH_3$ concentration (LOW)	$0.5mgl^{-}$

Table 3.2: Solute and biomass parameter values for simulations

# 3.4 Results and Discussions

#### **3.4.1** Bioclogging effects are significant control on microzones

#### 3.4.1.1 Temporal conditions with and without bioclogging

Numerical experiments where all hydraulic, biogeochemical, and microbial biomass were dynamic had similar outcomes. Namely, most scenarios start displaying significant bioclogging effects after 4 days with the simulations for higher nutrient flux displaying faster clogging than their low nutrient flux counter parts (Figure 3.1). Hence, bioclogging fundamentally shifts the



Figure 3.1: Hydraulic conductivity change over a 30d period. Each curve represents an initial Darcy flux value  $(0.1, 0.5, 1.0 \text{ md}^-)$ 

ability of water and solutes to move through the simulated hyporheic sediments. The pattern of reduction in hydraulic conductivity due to bioclogging shows similar behavior as seen in the 2 dimensional simulations for pore network models performed by Thullner and Baveye, 2008 [35] for rock type porous media. The general thresholding behavior for hydraulic conductivity (Figure 3.1)



Figure 3.2: a. Comparison of anoxic microzone population numbers under different nutrient and flux conditions, but with no biomass growth occurring. b. Comparison of microzone population numbers under different nutrient and flux conditions when biomass growth is occurring.

shows that that bioclogging initially proceeds slowly, but after reaching a threshold biomass volume level, results in a rapid decrease of the hydraulic conductivity. This decrease ceases at a very low, stable hydraulic conductivity that is determined and bounded by the hydraulic flux rate through the biofilm.

When we document and enumerate the overall anoxic microzone conditions through time, the microzone population that occurs under a given simulation set is provided in Figure 3.2. The percentage of anoxic pores which are formed in the bulk oxic region at any given time, where the oxic region is defined as the majority of pores (i.e > 50%) having  $O_2$  above  $2mgl^{-1}$ . We demarcated the border between the oxic and anoxic portions of the porous domain by selecting the column of the spatial grid where the number of oxic pores was less than or equal to 50% of the total pores along the grid column. The results clearly show that anoxic microzones are formed under most simulation conditions. However, stable microzones (Figure 3.3) (i.e., microzones that persist throughout the total simulation period) are only generated under circumstances where bioclogging is absent in the hyporheic zone. Figure 3.2 also shows that changing the hydraulic and nutrient flux conditions also determine the abundance and stability of microzones in the hyporheic zone.



Figure 3.3: Example spatial map of  $O_2$  concentrations for simulation of  $0.5mday^{-1}$  flux with high nutrient concentrations at 400 h, noting where the demarcation of bulk oxic and anoxic conditions is in this example, plus six examples of anoxic ( $< 2mgl^{-1}$ ) microzones are circled in red to highlight their location.

Under conditions without the presence of bioclogging, fluxes with higher nutrient concentrations start developing microzones much earlier than their low nutrient flux counterparts, but all the simulations tend to move towards an microzone equilibrium condition. While under the influence of bioclogging, the microzones do start forming but they are not sustained and disappear only hours after forming.

Previous simulation studies (Briggs et al., 2015) linked the formation of microzones to residence time with respect to a steady  $Da_{O_2}$ . But our results show that residence time alone cannot predict the total number of microzones as the heterogeneity in the distribution of biomass and its subsequent behaviors (i.e., growth, respiration) under different nutrient flux conditions, plays a key role in the emergence of the microzones.

#### 3.4.1.2 Spatial conditions with and without bioclogging

None of the bioclogging simulations were able to produce stable microzones across the hyporheic zone. This may be due to the fact that each simulation starts off as an advection dominated system, but due to increasing effects of bioclogging, slowly transitions into a diffusion dominated system. And, then, if given enough time, diffusion eliminates any concentration gradients in the domain leading to the loss of the anoxic microzones. For example, as the concentration profiles evolve through time (Figure 3.4), the bioclogged system approaches more or less uniform  $O_2$  concentration distribution.



Figure 3.4: a. Spatial oxygen profiles at 180 h, and b. for 240h for same hydraulic flux and nutrient concentrations (0.5md-1 flux and high nutrient concentrations).

To further illustrate the impact of bioclogging and the prevalence of advection dominating the outcome of microzone formation, we determined the evolution of the Peclet number (ratio of advective and diffusive flux) in the porous domain under the influence of bioclogging (Figure 3.5).

The resulting Peclet patterns show that every simulation starts with an advection dominated system (i.e., Peclet number > 1; shown as > 0 in Figure 3.5 due to log transformation in plot), but gradually over a period of days transitions into a diffusion dominated system. This transition occurred at roughly the same timeframe of 6-9 d when nutrients were high, but the transition was more gradual and took longer to achieve when nutrients were low.



Figure 3.5: Temporal evolution of system Peclet number for each of the bioclogging simulations. Each row is labeled with the corresponding hydraulic flux condition, while the first column corresponds to high nutrient flux and the second column refers to low nutrient flux conditions. The values are log10 transformed and the y=0 line corresponds to a Peclet number of 1.

In summary, the simulation results contradict our original hypothesis where we stated that bioclogging would enhance the microzone population.

This is because we presumed that the biomass growth would take place more or less throughout the domain, impacting the smallest pore throats preferentially. In fact, as Figure 3.6 demonstrates clearly, the major biomass growth takes place at the entrance of the hyporheic zone, creating a contiguous biofilm layer perpendicular to and effecting all flow through the domain. Recent results from an independent and more simplified modeling framework of hyporheic bioclogging [10] also observed this focused and effective bioclogging occuring directly at the sediment interface. These consistent findings suggest that bioclogging is likely to be a localized phenomena that is occurring near the influent (i.e., downwelling) region of hyporheic zones composed of sandy sediments as



Figure 3.6: Biomass profile for  $1.0md^{-1}$  flux with high nutrient concentrations at 160 h. with a. biomass concentration in pore throats aligned along the direction flow, and b. biomass concentration in pore throats aligned transverse to the direction of flow

in our study. We are also able to show that the biomass at the distal end of the flow paths does not attain the same growth levels because they are deprived of the essential nutrients required for their growth. Our findings are corroborated by field observations,[25][2] where microbial biomass was found to be accumulated at shallowest depths of streams, indication that bioclogging is indeed a localized phenomenon, controlled by biomass accruing at the infiltration sites of the sediment domain.

In the future, detailed field and laboratory investigations should be able to validate our simulated distribution of biofilm abundance. It should be acknowledged that this study is constrained by the fact the simulation conditions represents more of a controlled environment in a laboratory, rather than a natural setting in the field. In the field, access to studying these small scale issues can be challenging due to water depth, nondestructive sampling of sediments, and that stream beds are dynamic. This last point of bed dynamics is worth discussing because beds composed of medium sand, like that our simulations, are typically mobile (eroding and depositing) under most flow conditions [17]. This mobile bed results in a continuously changing streambed morphology and hence it is likely that the bioclogged layers forming at the influent of the sediment can be eroded and transported away resulting in the regular declogging of the system. However, given more stable

bed conditions, bioclogged sediments are likely to form in stream and river hyporheic zones [2].

# **3.4.1.3** Stable Microzones are modulated by transport(hydraulic flux) and reaction (nutrients) limitations

While bioclogging is critical to inhibiting microzones formation, the experiments without bioclogging reveal other criteria needed for stable microzone formation. First, stable microzone formation is not guaranteed for a hyporheic zone where bioclogging does not occur. For example, no microzones were stable at  $0.1md^{-1}$  Darcy flux and high nutrient concentrations without bioclogging (Figure 3.7a).The highest percentage of microzone obtained for this simulation was 16.67%, but the microzones were only ephemeral and disappeared by 300 h. This is mainly due to the fact that the high nutrient availability increased the oxygen respiration rates, while the hydraulic flux was not sufficient enough to replenish the oxygen consumed. Hence, the hyporheic domain rapidly



Figure 3.7: a Oxygen profile at 200 h for  $0.1md^{-1}$  flux and high nutrient concentrations and b. Oxygen profile at 400 h for  $0.1md^{-1}$  flux and low nutrient concentrations.

becomes bulk anoxic and no microzones can exist in such situations. However, at lower nutrient concentrations, the oxygen uptake rate is more limited (Figure 3.7b), allowing stable microzones (8.33% *of oxic domain*) to form even under the low hydraulic flux conditions.

The highest fraction of stable microzones formed under two types of conditions when neither transport or reaction limitations occurred. First, for intermediate hydraulic flux  $0.5md^{-1}$  and high nutrient conditions, (7.9% *of oxic domain*) and second for low hydraulic flux  $0.1md^{-1}$  and low nutrient conditions (8.33% *of oxic domain*). However, for the  $0.5md^{-1}$  flux with high nutrient concentrations (Figure 3.8a), the bulk oxic domain covered a substantially larger fraction of the domain when compared to the low flux scenario (Figure 3.7b). For low nutrient concentrations with  $0.5md^{-1}$  flux, (Figure 3.8b) the overall domain remains much more oxygenated and microzones formed are much lower (3.65% *of oxic domain*) due to less oxygen uptake, caused by the lack of nutrients. Figure 3.9a shows the spatial distribution of the microzones for a flux rate of  $1.0md^{-1}$ .



Figure 3.8: a Oxygen profile at 400 h for  $0.5md^{-1}$  flux and high nutrient concentrations and b. Oxygen profile at 400 h for  $0.5md^{-1}$  flux and low nutrient concnetrations.

These results are in line with expectations based on previous simulation studies [42][8], where the fraction of microzones in the bulk oxic domain (5.76% of oxic domain) decreased when compared to results obtained from lower flux conditions, primarily because more  $O_2$  is moved into the hyporheic pore waters. Figure 3.9b shows the dramatic impact of nutrients on reaction limitations, where, not only does the fraction of microzones change (1% of oxic domain), but the spatial distribution of microzones is also altered. This result suggest that while domain heterogeneity and hydraulic flux are key parameters in microzone formation, unlike previous modeling of hyporheic microzones [8], a dynamic  $Da_{O_2}$  ought to also be considered in order to completely analyze a hyporheic system.



Figure 3.9: a Oxygen profile at 400 h for  $1.0md^{-1}$  flux and high nutrient concentrations and b. Oxygen profile at 700 h for  $1.0md^{-1}$  flux and low nutrient concentrations.

#### **3.4.2** Transferable Findings and Implications for Future Research

In summarizing our observations based on the simulation results, it becomes clear that there are three main criteria that must be met in order for sandy hyporheic zones to develop and maintain stable microzones, and, with them, their ecological functions. These criteria are as follows:

- 1. It must be an advection dominated hyporheic flow systems.
- 2. There must be sufficient nutrient supplies to the hyporheic zone to stimulate oxygen uptake.
- 3. There cannot be stable bioclogging that forms in the hyporheic pores.

If these criteria are met, the actual quantum of stable microzones is likely to be a function of the transport and reaction limitations that control the nutrient flux into and distribution within the hyporheic zone. However, in order to maximize the amount of anoxic microzones formed in bulk oxic domain there must be a balance between the hydraulic flux and influx nutrient concentrations such that there is also a sizable bulk oxic domain to harbor these anoxic microzones. Under low flux conditions, the formation of microzones is more reaction limited, where only under a low nutrient concentration can a difference in oxygen concentration be obtained between a microzone and the surrounding oxic domain. On the other hand, in the case of very high flux conditions, the microzones are flux limited, where microzone numbers are diminished due to forcing of oxygen into the anoxic pore spaces.

## 3.5 Conclusion

The formation of microzones in hyporheic sediments is a complex and highly non-linear process, involving the interaction of hydraulic flux, nutrient availability, and biomass growth in the sediment matrix. This also results in variation in microzone abundance being dynamic through space and time, so this indicates that the potential ecological functions of microzones, such as greenhouse gas production, are also going to be dynamic through space and time. Although bioclogging turns out to be detrimental to stable microzone formation, the likelihood of microzones in nature, despite the presence of bioclogging, seems a real possibility. Sediment transport and mobile bed conditions might naturally act to de-clog biofilm that has accumulated at the influent of a hyporheic zone, thereby facilitating the persistence of anoxic microzones and bulk oxic pore waters. The simulation results for the microzones, suggest that while they may be small in extent relative to bulk oxic or anoxic pore volumes, they can develop and persist. Thus, they are still a viable hypothesis for the experimental and observation based studies that observe anaerobic products occurring in oxic pore waters of hyporheic zones. Additional modeling and field studies can use the microzone formation criteria revealed in this study before attempting to upscale the overall function of these microzones across different types of hyporheic zones and across spatiotemporal scales.

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