



**OXYGENATION OF THE ROOT ZONE AND TCE REMEDIATION:  
A PLANT MODEL OF RHIZOSPHERE DYNAMICS**

THESIS

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AFIT/GES/ENV/08-M07

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## **Abstract**

This study analyzes rhizosphere conditions that enhance the effective aerobic degradation of TCE in wetland bioremediation systems. A plant model was built using Stella 9.0 modeling software and uses numerical integration evaluation; it addresses movement of oxygen through plant vascular and aerenchymal systems, and into the rhizosphere where oxygen and other substrates influence bacteria. Methanotrophs and heterotrophs are assumed to be influential bacteria groups. Variations of humidity-induced-convection, methane, soil carbon, and copper concentrations are evaluated. Varying concentrations and hydraulic loadings of TCE are assessed with respect to TCE consumption rate and TCE treatment efficiency.

Soil conditions most directly affected TCE consumption, and hydraulic conditions most directly influenced treatment efficiencies. The research identified low carbon, low copper, high oxygen, and high methane concentrations as most conducive conditions for remediation. Variations in soil carbon had the highest impact on consumption rates; minimizing organic carbon concentrations of the influent may enhance remediation rates. It is recommended to first optimize soil conditions in a wetland treatment system, and then adjust hydraulic loading to achieve optimal treatment efficiencies. The model developed can be used to determine likely remediation rates and to then optimize efficiency by adjusting flow rates for a wetland bioremediation system.

AFIT/GES/ENV/08-M07

*To A, E, J, & E*

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Ian F. Thompson

## Table of Contents

	Page
Abstract.....	iv
Acknowledgments.....	vi
Table of Contents.....	vii
List of Figures.....	xii
List of Tables.....	xiv
I. Introduction.....	1
Model Assumptions.....	7
Problem Statement.....	8
Purpose Statement.....	8
Research Questions.....	8
II. Literature Review.....	10
Halogenated Aliphatic Compounds.....	10
Physiological Effects.....	13
Bioremediation.....	16
Anaerobic Bioremediation.....	17
Co-metabolism.....	17
Aerobic Bioremediation.....	18
Chlorinated Ethene Characterization.....	19
Treatment Methods.....	20
Wetland Characteristics.....	22
Water.....	24
Soil.....	24
Plants.....	26
Plant Physiology.....	27
Plant Cells.....	28
Plant Vascular System.....	30
Xylem.....	31
Phloem.....	32
Leaves.....	33
Photosynthesis.....	35
Stem.....	38
Roots.....	38
Root Components.....	39
Root Cap.....	40
Apical Meristem.....	40
Region of Cell Elongation.....	40

	Page
Hair Zone .....	41
Region of Cell-Differentiation .....	41
Hydrophyte Adaptations .....	42
Seasonal Variation and Photoperiodism .....	43
Circadian Rhythms and Diurnal Cycles.....	44
Plant Circulation .....	44
Phloem Loading .....	45
Plant Ventillation .....	46
Aerenchymal Tissue.....	46
Humidity Induced Convection.....	47
Oxygen Movement in the Roots .....	49
Oxygen Movement in the Rhizosphere.....	49
Oxygen Measurement .....	50
Exudation in the Rhizosphere .....	53
Carbon Sources .....	55
Exudation .....	56
Readsorption .....	56
Root Decay.....	57
Nitrogen .....	57
Microbial Communities .....	58
Microbial energy .....	58
Substrate Use .....	59
Cometabolism .....	61
Competitive Inhibition.....	62
Non-competitive Inhibition.....	62
Cell Toxicity .....	63
Methanotrophs .....	63
Methanotrophs and Nitrogen .....	64
Type I vs Type II.....	64
MMO and Copper Limitations.....	65
MMO and Energy .....	68
MMO and Reduced Iron .....	68
MMO and Oxygen Limitations.....	70
Cometabolism and Competitive Inhibition.....	71
Cometabolism and Non-Competitive Inhibition.....	72
Cometabolism and Bacterial Toxicity .....	72
MMO Inhibition vs Toxicity.....	75
MMO and Remediation .....	76
Heterotrophs.....	77
Methanogens .....	78
Quantification of Microbial Activity .....	79
Competition.....	80
Protozoa .....	81
Arbuscular Mycorrhizial Fungi.....	81
Soil and Microbial Influences .....	82

	Page
Models and Modeling .....	82
Plant Models .....	83
Air Movement .....	84
Rhizosphere Models.....	87
Root Quantification.....	87
Armstrong Rhizosphere Model.....	88
Bacteria Modeling.....	88
Trichloroethylene Treatment Modeling.....	89
Anderson Model.....	90
Tartakovsky Model. ....	91
Knowledge shortfalls .....	92
Soil Chemistry .....	93
Plant Dynamics .....	93
Microbial Characterization .....	94
III. Methodology.....	95
Modeling Development Process .....	96
Model Conceptualization .....	97
Expected Behavior .....	97
Leaf .....	98
Root.....	98
Soil .....	99
Microbial Mass .....	100
Model Assumptions .....	100
Fundamental Model Behavior.....	101
Environmental Factors .....	102
Plant factors .....	103
Leaf .....	103
Stem .....	105
Root.....	106
Soil .....	107
Microbes .....	108
Model Formulation .....	109
Leaf .....	110
Stem .....	114
Roots .....	114
Soil .....	115
Microbial Populations.....	116
Model Parameterization .....	119
Phragmites australis .....	120
Microbial Growth.....	120
Biodegradation of TCE.....	120
Model Testing and Validation .....	120
Behavior Reproduction Test .....	122
Extreme Conditions Tests.....	125

	Page
Behavior Sensitivity Testing.....	126
Model Application .....	127
Modular Testing.....	128
Dynamic Behavior Test .....	128
IV. Results and Analysis.....	131
Plant Model.....	131
Humidity Induced Convection.....	131
Plant Circulation .....	134
Bacteria Response.....	135
Oxygen.....	135
Copper.....	140
Methane and Carbon.....	141
TCE Response.....	142
Concentration.....	143
Toxic Threshold.....	148
Flow Rates .....	149
Rhizosphere Conditions.....	150
HIC Effects on Remediation.....	150
Carbon Effects on Remediation .....	151
Methane Effects on Remediation.....	151
Copper Effects on Remediation .....	152
Recommendations.....	153
V. Conclusions.....	156
1. What is the nature of the oxygen dynamic in the rhizosphere? .....	156
2. What are the most influential factors to microbial community populations in the root zone?.....	157
3. How can methanotroph populations be optimized to support aerobic remediation requirements for halogenated organics like TCE, TCA, DCE, and VC? .....	158
4. What are the influential factors of oxygen transport in a wetland plant? .....	161
5. How is oxygen level in the root zone affected seasonally? .....	162
Application.....	162
Model strengths.....	163
Model Limitations.....	163
Future research.....	164
Appendix A. Model Parameters.....	166
Appendix B. Venting Resistance Data.....	168
Appendix C. Rhizosphere Profile Comparison Data.....	169

	Page
Appendix D. Sensitivity Testing Data.....	170
Appendix E. HIC and Radiation Output Data.....	171
Appendix F. Soil Variable Testing Data.....	172
Appendix G. TCE Testing Data.....	177
Bibliography.....	186
Vita.....	191

## List of Figures

	Page
Figure 1. Metabolism of TCE in Human. ....	14
Figure 2. Upward Flow Constructed Wetland Remediation Sequence. ....	21
Figure 3. Wetland Oxygen Gradients. ....	27
Figure 4. Typical Leaf Mesophyll Cell.....	29
Figure 5. Symplastic/Apoplastic Pathways in a Root-Hair Region.....	30
Figure 6. Longitudinal Section of Plant Stem Vascular Bundle.....	31
Figure 7. Leaf Cross-Section. ....	34
Figure 8. Cross-Section Through a Monocot Stem.....	38
Figure 9. Longitudinal and Cross-Sectional View of Root.....	40
Figure 10. Oxygen Partial Pressures in Cortex and Root Surface. ....	43
Figure 11. Demonstration Model for Humidity Induced Convection.....	48
Figure 12. Root Cross-section Oxygen Measurements.....	53
Figure 13. Nutrient Adsorption and Exudation of Carbon/Nitrogen Sources. ....	54
Figure 14. Carbon Release of a Root System. ....	57
Figure 15. Andrews Model of Substrate-Inhibited Growth. (Vaccari, 2006:339).....	63
Figure 16. Thompson Conjecture of sMMO/pMMO Expression.....	69
Figure 17. Dissolved Oxygen Relationship to TCE Degradation.....	71
Figure 18. TCE Monooxygenase Cooxidation Pathways.....	73
Figure 19. Relationship of Contaminant Concentration and Degradation Rate. ....	74
Figure 20. <i>Phragmites australis</i> Parameters.....	84
Figure 21. Outflow Resistance Effect on HIC Flow.....	86
Figure 22. Dynamic Relationships Affecting Rhizosphere Oxygen.....	98
Figure 23. Oxygen Partial Pressures Along a <i>Phragmites</i> Root.....	99
Figure 24. Model Compartmentalization.....	102
Figure 25. Gas Exchange Process Inside the Leaf.....	104
Figure 26. Methanotroph Relationships in STELLA Format. ....	109
Figure 27. Thompson and Beckett Models Convective Flow vs Outflow Resistance...	123
Figure 28. Oxygen Profiles for Armstrong Electrode Study and Thompson Model Output. ....	124
Figure 29. Meth Initial Response Sc. A.....	129
Figure 30. Meth Initial Response Sc. B.....	129
Figure 31. Het Initial Response Sc. A.....	129
Figure 32. Het Initial Response Sc. B.....	129
Figure 33. Meth Final Steady-State A.....	130
Figure 34. Meth Final Steady-State B.....	130
Figure 35. RH Effect on Rhizome Oxygen Levels. ....	132
Figure 36. Relative Humidity Effect on Oxygen Levels in the Rhizosphere .....	133
Figure 37. Radiation Effect on HIC.....	134
Figure 38. Carbon Concentration and Heterotroph Growth.. ....	136
Figure 39. Bacteria Profiles in High HIC, Low C and CH <sub>4</sub> .....	137
Figure 40. Bacteria Profiles in Low HIC and C, High CH <sub>4</sub> .....	137
Figure 41. Heterotroph Profiles for Different Growth Conditions. ....	138

	Page
Figure 42. Heterotroph Effect on Oxygen Profile. ....	139
Figure 43. Oxygen Effect and Carbon Effect on Methanotroph Growth with 10, 35 ppm Carbon.....	140
Figure 44. Varying Conditions on MMO Expression.....	141
Figure 45. Effect of Carbon and Methane on Het:Meth Ratios.....	142
Figure 46. Effect of Raising TCE Concentration on MMO expression. ....	143
Figure 47. Effect of TCE Concentration on Methanotroph Profile in the Rhizosphere. ....	145
Figure 48. TCE Concentration Effects on Bioremediation.....	146
Figure 49. Bacterial Mass and TCE Concentration. ....	147
Figure 50. TCE Effect on Treatment Efficiency.....	148
Figure 51. TCE Tolerance varying MMO and Methane.....	149
Figure 52. TCE Treatment Efficiencies at Varying Flow Rates (TCE 50 ppb).....	150
Figure 53. HIC Effect on TCE Consumption .....	150
Figure 54. Carbon Effect on TCE Consumption .....	151
Figure 55. Methane Effect on TCE Consumption. ....	152
Figure 56. Copper Effect on TCE Consumption. ....	153
Figure 57. TCE Maximum Consumption Rates and Removal Efficiency at 500 ppb...	154

## List of Tables

	Page
Table 1. Porosity of Wetland and Non-wetland Species Grown in Drained and Saturated Medium. (Colmer, 2003: 19) .....	47
Table 2. Equilibrium Metal Speciation for Media with Different Copper Concentrations. (Morton, 2000: 1731).....	67
Table 3. Growth and Degradation Rates of OB3b Cells Expressing pMMO or sMMO at Various Contaminant Concentrations. (Lee, 2006: 7507) .....	75
Table 4. OB3b Chlorinated Ethylene Michelis-Menten Degradation Coefficients. (Lee, 2006:7505) .....	76
Table 5. Monod Half-Saturation Constants for Heterotrophs. (Van Bodegom, 2001:3591) .....	80
Table 6. Monod Half-Saturation Constants for Methanotrophs. (Van Bodegom, 2001:3592) .....	80
Table 7. Venting Resistance Profile vs Beckett, 2001: 278.....	123
Table 8. Extreme Conditions Testing for Microbial Growth.....	126
Table 9. Model Plant Sensitivity Results .....	127
Table 10. Treatment ranking of Testing Scenarios .....	155

# OXYGENATION OF THE ROOT ZONE AND TCE REMEDIATION:

## A PLANT MODEL OF RHIZOSPHERE DYNAMICS

### **I. Introduction**

Plants are amazing creations. Our lives depend upon them, yet there is still much we do not understand about them. The ability of plants to move oxygen and other exudates into the soil surrounding the root zone is a subject of special interest; these processes play important roles in supporting the symbiotic relationship between plants and microbial populations in the soil. Wetland environments, specifically, provide a significant obstacle to many plants; the reducing conditions associated with saturated soils limit oxygen availability in the soil. Wetland plants, termed *hydrophytes*, are specially suited to wetland soil conditions. They rely upon radial oxygen loss from their roots to aid in nutrient uptake for the plant, detoxify reduced elements in the soil, and to support beneficial microbial populations associated with the root zone. In these conditions, oxygen is used up quickly and plant rhizospheres are correspondingly thin; this complicates our ability to measure oxygen and other nutrient levels in the rhizosphere. Computer modeling of rhizosphere characteristics can help give important insight into oxygen concentrations, nutrient levels, and likely microbial interactions in the soil.

Rhizosphere dynamics are important to bioremediation. Chlorinated compounds like trichloroethylene (TCE) are a significant source of pollution in many groundwater systems. Bioremediation offers a cost-effective method of treating them. Since

hydrophytes are able to move oxygen and other exudates into the soil by diffusion from their roots, the rhizosphere helps to sustain communities of aerobic bacteria. These bacterial populations play important roles in the breakdown of halogenated organic compounds. Aerobic methanotrophs consume oxygen and methane available in the rhizosphere and produce harmless CO<sub>2</sub> and water. It is likely that the same mono-oxygenases used by the methanotrophs to digest methane also cometabolize TCE and other halogenated organic compounds. Consequently, constructed wetlands offer a viable way to remediate TCE and its associated byproducts in contaminated groundwater; a healthy population of methanotrophs is essential to the remediation process. Heterotroph populations also compete for oxygen in the rhizosphere; however, they do not have a known role in the remediation process. In order to create an optimum balance for bioremediation, better insight is needed into the movement of oxygen into the rhizosphere and its effect on associated microbial populations.

This study will use a modeling approach in order to calculate oxygen and other nutrient levels in the soils surrounding wetland plants. There are currently many unknowns in addressing rhizosphere dynamics; some assumptions are made in order to model the system. From a system dynamics perspective, the behavior of a system is a result of its causal structure. Precise variables are far less important in the modeling process than the structure that arises from the relationship between the model's elements. By accurately depicting the relationships of real-world components in the model with current knowledge, intuition can be gained on the behavior of the system. The model's system boundary includes the plant, its root structure, the plant's rhizosphere, and the microbial populations that exist near the oxygenated zones of the soil.

Oxygen levels in the soil quickly approach zero in the rhizosphere; this makes oxygen a limiting nutrient in wetland soils. This model will address the ability of plants to move oxygen from the leaves into the root zones. There are two likely ways that plants are achieving this end. The first process takes place in the bulk flow of solute inside the plant vascular structures, the phloem and xylem. Mature plant leaves use the process of photosynthesis to fix CO<sub>2</sub> and H<sub>2</sub>O into carbohydrates for plant energy needs. These sugars are mostly converted to sucrose for transport inside the phloem. The high concentration of sugar creates an osmotic potential inside the phloem that drives the flow of the solute towards the plant roots. High osmotic potentials (two to three mega-pascals) are common inside the sieve tubes, and transport velocities of .5 to 1.5 meters per hour are common in most plant species. (Salisbury, 1992:171-176) High levels of oxygen from photosynthesis diffuse into this solution and are transported by high pressure *bulk solute flow* down the stem to the roots.

Wetland plants have an additional source of oxygen that must also be modeled. While all plants have the ability to increase air channels in their tissue (known as aerenchyma) under conditions of oxygen stress, wetland plants have much higher concentrations of these air channels in their shoots, up to 45 percent by volume, than other plants. (Crawford, 1982; Salisbury, 1992) The volume lost to this tissue represents a significant sacrifice by the plant, but it plays a very critical role in plant survival. The opening of interior spaces provides a pathway for air inside the plant, a ventilation system that can be used to move oxygen and nitrogen down to the roots and to expel waste gases like methane and carbon dioxide from roots to the atmosphere. Though diffusion can play a role in the movement of air through these air passages, *bulk air flow* is the primary

method of transport in these channels. Two processes, bulk solute flow and bulk air flow, are important in delivering oxygen to the rhizome and both need to be modeled to accurately assess oxygen levels in the root zone.

Once in the plant rhizome, the roots permit delivery of oxygen into the rhizosphere primarily through diffusion processes. Concentration gradients provide the motive force for most exchanges between the roots and the soil, and diffusion allows a two way exchange between the plant roots and the soil around it. There are other factors, though, that affect the permeability of the roots and their exchange of water, oxygen, and other nutrients with the rhizosphere. Plant structures, tissue differences, and low permeability membranes give the plant a degree of control on the amount of oxygen that is exuded to the rhizosphere.

Nutrients in the soil provide a source of nourishment for microbial populations in the soil, and the consumption of these primary substrates is limited by oxygen. The heterotroph populations compete for carbon sources, consuming oxygen that is also required by the methanotroph populations who are valuable to remediation processes. Correspondingly, the movement of carbon sources into the root zone plays an important factor in determining microbial populations in the root zone. Modeling may provide a source of insight to better characterize the growth of bacteria resident in the rhizosphere.

Aerobic bioremediation is dependent upon methanotrophic bacteria, and those bacteria are primarily dependent upon the metabolism of methane. One additional area of concern in this study, then, is the concentration and influence of methane in the rhizosphere. Anaerobic processes in nearby wetland soil provide methane that is used by the methanotroph populations in the rhizosphere for energy. Roots create a matrix in the

soil through which the methane diffuses. The extent of oxygen's radial diffusion limits the ability of methanotrophs to utilize the methane existing in the nearby soil. Methane flux into the rhizosphere where oxygen levels support methanotroph growth could be a limiting factor to methanotroph populations. Root architecture could also play a role in determining the availability of methane to the methanotrophs that rely upon it; dense roots may preclude the flux of methane into the central portions of the root and limit methanotrophs to outer portions of the root zone. It also remains to be seen if a large population of methanotrophs is critically important for remediation purposes; a small population of these bacteria may be sufficient to metabolize the small amounts of compound that are available in the soil for remediation. It is also unknown to what extent the compounds that we are trying to remediate have a toxic effect on the microbes that are digesting them.

The model is an important tool. It will allow the manipulation of numerous variables that may not be available for manipulation in another setting such as a laboratory or field test. This gives the model a great amount of flexibility. Data can be generated quickly in response to changes in variables.

Modeling also has a great number of limitations. A number of assumptions will need to be made in the modeling process that will account for unknowns. It is important that all components of the model complement their function and behavior in the living system; this will allow comparisons of the model to empirical knowledge about plant systems. The model's output will also need to be scaled against measurements obtained in real systems; required data sets may not be available to scale the model and some assumptions need to be made in order to achieve characteristic results.

In this model, limited knowledge on plant oxygen movement necessitates the modeling of oxygen flow by bulk flow in both the solute and the air in the air spaces (aerenchymal tissue). The capacity of various parts of the root to transport oxygen is uncertain, and the root processes are divided to better account for the oxygen flow from the roots to the rhizosphere. Root permeability changes along the length of the root, and transfer coefficients are used for root segments since diffusion constants for the entire root are unknown. Plant species characteristics to include size, root structure, and respiration rates are also accounted so that they can be varied. Diurnal and seasonal variations are not inherent in the model, but constants can be varied to account for plant responses to changing external conditions.

With respect to modeling of microbial populations, Monod growth is assumed. Carbon sources are assumed to be a limiting nutrient for the heterotroph populations, and methane a limiting nutrient for methanotrophs. Oxygen limits the consumption of both these substrates, and has an effect on the enzymes produced by the bacteria. Space consideration for microbial cohabitation is not modeled; it is assumed that oxygen and nutrient levels are the limiting factors of growth. Basic inputs concerning soil conditions (saturation, porosity, hydraulic flow rates, and TCE concentration) and other potentially limiting nutrients (methane, carbon, and copper) are entered as constants. Inhibition of the bacteria by toxic compounds is accounted by the Andrews model, a modification of the Monod expression, and by degrading effects caused by toxic intermediates during TCE transformation

## Model Assumptions

The following assumptions apply to this model and its results:

1. Humidity induced convection (HIC) and plant photosynthesis are the main contributors to rhizosphere oxygen.
2. Nearly all rhizosphere oxygen is contributed through the plant's root hair zones.
3. The plant efficiently minimizes overlap of rhizosphere zones.
4. Mature and homogenous plant stand of *Phragmites australis* that ignores diurnal cycles (constant phloem/xylem flow and humidity/temperature/light levels).
5. Heterotrophs and methanotrophs are the only bacteria of treatment significance in the rhizosphere.
6. Primary carbon flow is from BOD in treatment water, and organic carbon is the primary substrate for heterotrophic bacteria
7. Methane is generated in anaerobic zones of the wetland treatment area and is the primary substrate for methanotrophic bacteria.
8. TCE is the only contaminant in the treatment water, and is consumed aerobically only.
9. Bacterial activity is the most significant sink of oxygen in the rhizosphere (ignores chemical oxidation, fungi, predation).
10. Copper availability, determined by total copper concentration and redox conditions, determines MMO expression.
11. sMMO and pMMO have greatly different transformation rates for TCE ( $k_{TCE}$ ) but have roughly equivalent affinities for methane and TCE ( $K_s$ ,  $K_{s, TCE}$ ) and TCE inhibition rates ( $k_{i, TCE}$ ).

12. A subsurface flow wetland treatment system with uniform flow and continuously-stirred-reactor assumption outside the rhizosphere.

### **Problem Statement**

The impact of radial oxygen loss and exudates in the rhizosphere of wetland plants is poorly understood. This knowledge gap limits the optimization of wetland bioremediation processes for halogenated organic compounds and other environmental contaminants.

### **Purpose Statement**

The purpose of this study is to mechanistically examine oxygen transfer into the soil in order to optimize aerobic remediation conditions for aliphatic compounds such as trichloroethylene (TCE). Increased intuition regarding the flow of oxygen and other key root exudates into the rhizosphere of wetland plants will aid in determining effects on microbial populations in the soil. This knowledge can be used to guide further research into radial oxygen loss by plant roots and to optimize conditions of constructed wetlands to support microbial populations that are critical in bioremediation processes.

### **Research Questions**

1. What is the nature of the oxygen dynamic in the rhizosphere?
2. What are the most influential factors to microbial community populations in the root zone?

3. How can methanotroph populations be optimized to support aerobic remediation requirements for halogenated organics like TCE, TCA, DCE, and VC?
4. What are the influential factors of oxygen transport in a wetland plant?
5. How is oxygen level in the root zone affected seasonally?

## **II. Literature Review**

Trichloroethylene and other organic halogenated organics are significant sources of pollution in the environment. Bioremediation can be a cost effective and efficient way to mitigate the hazards posed by many of these chemicals. In order to optimize wetland bioremediation techniques, an understanding of the microbial populations responsible for the remediation processes is required. Different bacteria exist in aerobic and anaerobic environments, both of which are present in a wetland. In a wetland, aerobic zones exist in the rhizosphere, the area around plant roots where oxygen diffuses into the soil. Scientific understanding of rhizosphere dynamics is limited. Three key components of understanding this dynamic are: 1) the plant processes that move oxygen and other exudates in and out of the soil; 2) the movement of oxygen in the soil surrounding the roots; and 3) the behavior of microbial populations living in the soil surrounding the rhizosphere. This literature review was conducted in order to develop a large scale view of rhizosphere activity and to focus that vision towards an accurate model of the rhizosphere dynamic.

### **Halogenated Aliphatic Compounds**

Trichloroethylene (TCE) is in a class of liquid organics known as chlorinated hydrocarbons. This class includes other compounds such as perchloroethylene (PCE), vinyl chloride (VC), carbon tetrachloride, and trichloroethane (TCA). The physical and chemical properties of TCE, TCA, and PCE, in particular, allow small amounts of these compounds to contaminate large supplies of groundwater. (Cheremisinoff, 2001:22)

Chlorinated hydrocarbons are also in a larger class of chemicals collectively grouped as solvents. Solvents have variable lipophilicity and volatility, a small molecular size, and lack charge. These characteristics allow them to be readily absorbed by the skin, gastrointestinal tract, and, most significantly, the lungs. Most of these solvents produce some degree of central nervous system depression. (Klaasen, 2003:361)

Chlorinated ethenes and ethanes are also produced by natural processes in the environment. PCE and TCE, for example, are produced by marine algae. (Field, 2004: 5; Abrahamsson, 1995) Vinyl chloride and other halogenated organics are generated during the production of humus in soil. (Field, 2004: 5; Hoekstra, 2003, 1998; Keppler, 2002, 2000; Laturus, 2002) Concentrations of these chemicals in nature, however, are relatively low. Production of chlorinated ethenes and ethanes industrially is approximately 8000 kt per year in the United States alone. Though a substantial decrease in inadvertent release has been made in recent years, releases of chlorinated solvents worldwide remains high. (Field, 2004: 5; Fetzner, 1998; Lecloux, 2003)

When these solvents are not disposed properly, they are able to volatilize into the atmosphere or to leak into the ground. Atmospheric vapors in an unconfined area typically are diluted and dispersed rapidly. Solvents that move into the ground, however, are extremely persistent and represent a significant threat to water sources. Spilled solvents percolate through the soil and enter the groundwater. Many volatile organic compounds, like TCE, are heavier than water. This causes them to sink to the bottom of aquifers and makes them very difficult to remove from the environment. (Klaasen, 2003:362) Since all solvents are somewhat soluble in water, they diffuse into the groundwater. This often causes unhealthy concentrations in water that is pumped to the

surface and impacts indoor air quality in areas with high water table fluctuations.

(Cheremisinoff, 2001:22)

Ingestion, absorption through the skin, and inhalation are normal routes of contaminant exposure. Gases in the air enter the lungs; blood levels equilibrate almost instantaneously and result in rapid uptake of chemicals into systemic circulation.

Solvents also easily permeate the digestive tract; one hundred percent of an oral dose is assumed to be absorbed by the body. Dermal exposure can have varying degrees of penetration by passive diffusion that depend on concentration, surface area exposed, duration, nature of the skin, and nature of the solvent. Once in the body, the lipophilic compounds partition into hydrophobic sites such as the phospholipids in cell walls, lipoproteins, and cholesterol that is present in blood. Though many compounds partition back to the air during normal respiration after the individual is removed from the source of exposure, concentrations in fatty tissues remain in the body for prolonged periods. In order to minimize the threats posed by solvents, the Occupational Safety and Health Administration has established a set of legally enforceable Permissible Exposure Limits (PELs). These limits are designed around an 8 hour workday/ 40-hour workweek in order to ensure the safety of exposed individuals through any likely metabolic pathway.

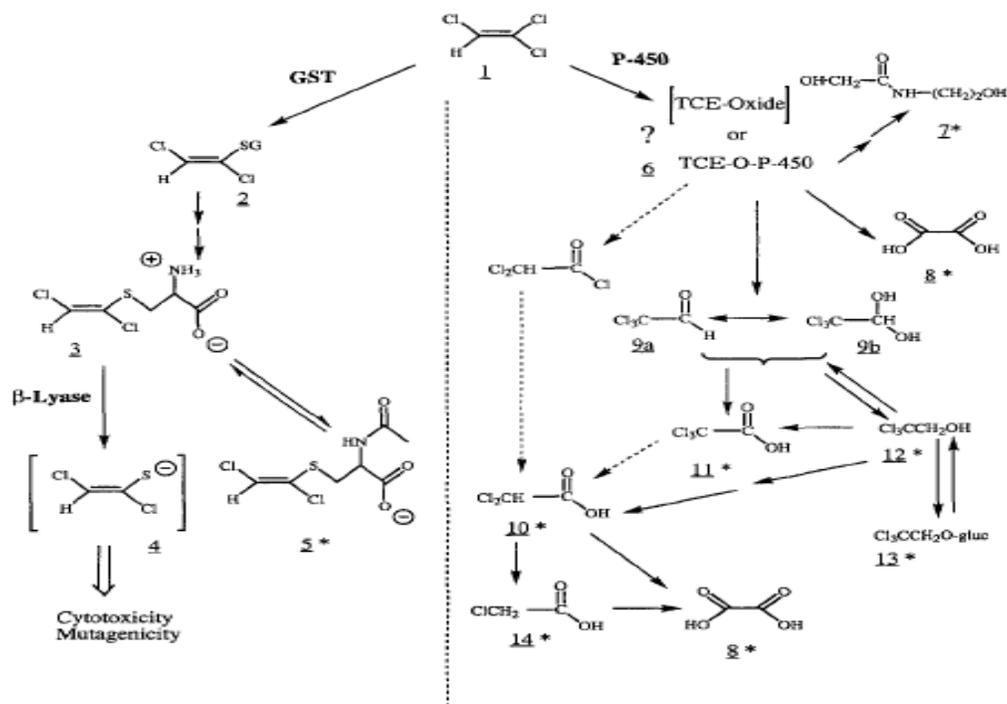
(Klaasen, 2003:362)

Trichloroethylene is a solvent of significant concern in the environment. It is a colorless, volatile liquid that is nonflammable under standard conditions. It is typically used for industrial and commercial degreasing; it is also used in the manufacture of PCE and plastic cement, and is used to process commodities such as coffee beans, cotton, and wool. Though current laws and safeguards limit the amount of contamination placed into

the environment today, large amounts of TCE have leaked into the soil in years past. This has led to high TCE concentrations in groundwater at many locations. The U. S. Environmental Protection Agency (US EPA) has classified TCE as a priority pollutant due to its widespread contamination, possible carcinogenicity, and its anaerobic conversion to the more potent VC. (Cheremisinoff, 2001:25) TCE and other volatile organic compounds are regulated as air pollutants subject to the Clean Air Act Amendments Title III.

#### Physiological Effects.

Though dermal absorption is not considered to be a major factor in risk assessments, TCE is considered to be an eye and skin irritant. It is readily absorbed across biological membranes, however, so inhalation and gastrointestinal absorption of TCE are very significant. (Lash, 2000:178) TCE is associated with Hodgkin's disease, multiple myeloma, and numerous cancers. Most of the toxicities due to TCE result from metabolites produced during reactions inside the body. (Chiu, 2006: 1450) The metabolites of TCE follow two major metabolic pathways, each of which has acute and chronic toxic effects in the body. The products of both pathways are depicted in Figure 1 below. The metabolic flux resulting from glutathione (GSH) and oxidative pathways differs for each tissue, and effects on each target organ differ correspondingly. (Lash, 2000:177) The metabolism of TCE and its effects on organs is fairly complex.



**Figure 1.** Scheme of metabolism of TCE. Metabolites marked with an asterisk are known urinary metabolites. Metabolites: 1 = TCE, 2 = DCVG, 3 = DCVC, 4 = 1,2-dichlorovinylthiol, 5 = NAcDCVC, 6 = TCE-P450 or TCE-oxide intermediate, 7 = *N*-(hydroxyacetyl)-aminoethanol, 8 = oxalic acid, 9a = chloral, 9b, chloral hydrate; 10 = dichloroacetic acid, 11 = trichloroacetic acid, 12 = trichloroethanol, 13 = trichloroethanol glucuronide, 14, monochloroacetic acid

Figure 1. Metabolism of TCE in Human. (Lash, 2001:178)

The major pathway for breakdown of PCE is an oxidation process facilitated by four different P450 cytochromes (right side of Figure 1). CYP2E1 appears to be the most active of the P450 isoforms. The majority of TCE likely undergoes chlorine migration to oxygenated TCE-P450 prior to transitioning to chloral hydrate. Chloral hydrate is rapidly transformed in the liver, but Clara cell injury in the lungs has been associated with the accumulation of chloral hydrate. (Lash, 2000:180) Specifically, TCE has been shown to cause lung cancer in the mouse, but not in the rat. (Klaasen, 2003:365) The liver has the highest P450 activity levels of any tissue, and P-450 metabolites have been directly linked to liver damage. Chloral hydrate subsequently breaks down to trichloroacetate or trichloroethanol. Trichloroacetate is the primary candidate of liver injury and cell

proliferation, and it is also the major metabolite of TCE in the circulatory system due to a high affinity for binding to blood plasma. (Lash, 2000:182) Trichloroacetate is broken down to dichloroacetate, also linked to liver damage. Dichloroacetate further breaks down in the body, but trichloroacetate and trichloroethanol are the major TCE metabolites that are recovered from urine. P-450 activity is also present in the kidneys, but nephrotoxicity and nephro-carcinogenic effects have only been linked to the GSH metabolic pathway. (Lash, 2000:177)

The second metabolic pathway (left side of Figure 1) breaks TCE down by glutathione (GSH). Far less is known about this pathway than the oxidative pathway. It is known that reactive metabolites from the GSH pathway are potent renal toxicants both in vitro and in vivo. (Chiu, 2006:1452) Kidney tumors are likely caused by reactions of GSH metabolites that alkylate cellular nucleophiles such as DNA. (Klaasen, 2003:36) TCE is broken down to S-dichlorovinyl glutathione (DCVG) which is subsequently broken down to S-dichlorovinyl-L-cysteine (DCVC). Bioactivation of DCVC may occur through the renal  $\beta$ -lyase metabolism, producing the reactive metabolites that are toxic to the kidneys. (Chiu, 2006:1453) This evidence as it applies to humans, however, is debatable since male rats are especially prone to the effects, while female rats and mice display lesser associations. It is hypothesized that the reactive metabolites of the GSH pathway may have a genotoxic effect on the proximal tubule of the human kidney. (Klaasen, 2003:365) The problem, then, is not a simple one and is a high priority issue for the field of toxicology. Modeling focus is placed on TCE and its major oxidative metabolites trichloroacetic acid and trichloroethanol. (Chiu, 2006:1450)

## **Bioremediation**

Removal of chlorinated contaminants in the environment can be a lengthy, difficult, and costly undertaking. Since PCE, TCE, and TCA all sink to the bottom of aquifers, they have been termed as “Dense Nonaqueous-Phase Liquids” or “DNAPLs”. Depending on subsurface conditions, the contamination can persist for years and most DNAPL sites are not fully remediable without extracting the entire contamination source by pumping. It is especially important in the case of DNAPLs, then, to determine the position, size, and hydrogeological situation of underground sources prior to any remediation effort. (Cheremisinoff, 2001:26) Once the site is characterized, bioremediation may offer an effective and cost efficient means to remove these contaminants from the environment. Bakst (1991) showed that bioremediation is one of the least expensive remediation techniques when its application is feasible. Monitored natural attenuation of TCE is also possible when the correct conditions are present. (Brigmon, 2001: 5-8)

Bioremediation is the use of naturally occurring organisms to effect remediation of a contaminant by reducing its concentration in the environment. Bioremediation is usually accomplished by microbial consortia that live in the soil and water. Microbes can often benefit from the contaminant directly by using it as a food source. The contaminant can act as an electron donor or carbon source that supports the growth of the microbe, and is chemically altered during the process. The successful application of bioremediation depends upon the ability to stimulate and enhance the desired microbial activity and bring the contaminant into contact with the microbial community performing the remediation function. (Brigmon, 2001: 3)

Actual change of the contaminant can be effected by a variety of biochemical reactions. During redox reactions, electrons flow from the contaminant to an electron donor. In aerobic conditions, oxygen is the normal electron acceptor. As the microbial population grows, the rate of biodegradation can also increase until the supply of contaminant is depleted.

#### Anaerobic Bioremediation.

Remediation of chlorinated compounds can also be performed anaerobically, without oxygen. In the absence of oxygen, other electron acceptors can be used for respiration. In nature, nitrates, iron, sulfates, and CO<sub>2</sub> are common alternate acceptors. Chlorinated compounds can also serve as electron acceptors in a process known as halorespiration. During halorespiration, microbes use the chlorine in the compounds as an electron acceptor to process another substrate; this is an energetically favorable reaction that results in the reductive dehalogenation of the contaminant (Field, 2004: 6; McCarty, 1997), normally resulting in a less toxic product.

#### Co-metabolism.

Microbes can also be used to remediate contaminants indirectly. Enzymes that are typically used to digest a primary substrate may also chemically alter the contaminant of concern. This is referred to as co-metabolism. Co-metabolism can occur both aerobically and anaerobically. The consumption of the contaminant, however, is not linked to the growth of the micro-organism, and the microbes in these systems rely upon other primary substrates and electron acceptors for growth. The degradation of the

contaminant is a fortuitous circumstance that can be amplified by supplying the microbes with the primary substrates and electron acceptors they require. In the case of chlorinated compounds, methane monooxygenases expressed by methanotrophic/methylotrophic bacteria to oxidize methane are also used to cooxidize the chlorinated compound of interest. (Field 2004:6; Wackett, 1995) Methanotrophs are physiologically versatile, living in a diverse range of hostile environments, and while other microbes are also capable of degrading chlorinated aliphatic compounds, methanotrophs are optimal in bioremediation when TCE is the primary concern. (Brigmon, 2001: 4) In this case of aerobic co-metabolism, the methanotrophs are dependent upon methane and oxygen.

#### Aerobic Bioremediation.

Aerobic bioremediation of TCE is dependent upon a co-metabolic process by methanotrophic bacteria, so a healthy population of methanotrophs is essential to the remediation process. The methanotrophs are a group of aerobic, gram-negative bacteria that use methane as their sole source of carbon and energy. It is likely that the same mono-oxygenases used by the methanotrophs to digest methane also metabolize TCE and other halogenated organic compounds. An advantage of methanotrophs in aerobic conditions limits the accumulation of undesirable metabolites. (Brigmon, 2001: 4)

Soil oxygen is also a limiting factor in bioremediation; the extent of oxygen's radial diffusion limits the ability of methanotrophs to utilize the methane existing in the nearby soil. Heterotrophic bacteria populations also compete for the oxygen in the rhizosphere; however, they do not have a known role in the remediation process and their presence may limit the oxygen available to methanotrophs. Oxygen is a limiting nutrient

in wetland soils and oxygen release by wetland plants accounts for as much as 90 percent of the oxygen entering the substrate. (Reddy and others, 1989; Allen and others, 2002:1010) The fine roots of wetland plants have a large surface to volume ratio and are especially conducive to growing the bacterial populations needed for aerobic degradation of TCE (Amon *et al.*, 2007: 64; Brigmon, 2001: 8), creating a biofilm matrix through which oxygen and methane diffuse.

#### Chlorinated Ethene Characterization.

For discussion purposes, chlorinated ethenes are often divided into two categories, lower and higher chlorinated compounds, due to differences in their behavior with respect to remediation. The lower chlorinated ethenes include vinyl chloride (VC), and the dichloroethenes (1-1-DCE, tDCE, cDCE). The higher category includes TCE and PCE. As a general rule, the higher chlorinated ethenes are more prone to anaerobic biodegradation and the lower ethenes are more prone to aerobic degradation. There is overlap in both categories with exception of PCE that typically only degrades anaerobically. A large number of reports show that TCE and PCE are naturally attenuated in the environment. The degradation of PCE and TCE was observed to proceed anaerobically to cDCE or to ethane and/or VC. (Field, 2004:27; Löffler, 2000; Pavlostathis, 1993; Fennel, 2001)

One unfortunate complication of remediation is the transition of TCE to dichloroethylene (DCE), and then to vinyl chloride (VC). VC is one of the most dangerous compounds in the group of halogenated organics and is a class A carcinogen. High CNS depression and death have been associated with acute exposure to VC.

(Vaccari, 2006:820) Any remediation process must also address the resulting formation of VC and further reduce it to a less harmful compound. In microbial remediation, VC is remediated by the same bacteria that also aerobically metabolize TCE, and DCE.

### Treatment Methods.

The limitations of in-situ remediation are recognized; pump-and-treat methods are the default choice for extracting chlorinated organics from soil and groundwater, but those methods are normally expensive, involving energy-intensive thermal or controlled biological processes, often costing millions of dollars for treatment alone. Additionally, adsorption and desorption of the chlorinated organics leads to extended treatment times and years or decades of pumping. Large volumes of water must be pumped with very low concentrations of contaminant to accomplish remediation. (Shelley *et al.*, 2002:6) A few alternative treatment methods have been recommended by Shelley *et al.* in order to maximize water treatment volume and to drastically reduce costs. One method involves introducing hydrogen and zero-valent metals into groundwater circulation wells (GCWs) in order to facilitate a reductive dechlorination of the ethenes. A second method is the use of a constructed wetland with an upward flow of water that will treat the contaminants by anaerobic and aerobic microbial processes. Sequential treatment in anaerobic and aerobic zones leads to the complete destruction of chlorinated ethenes. Plant roots are the primary contributor of oxygen in the root zone. The upward flow wetland is briefly described here and illustrated in Figure 2.

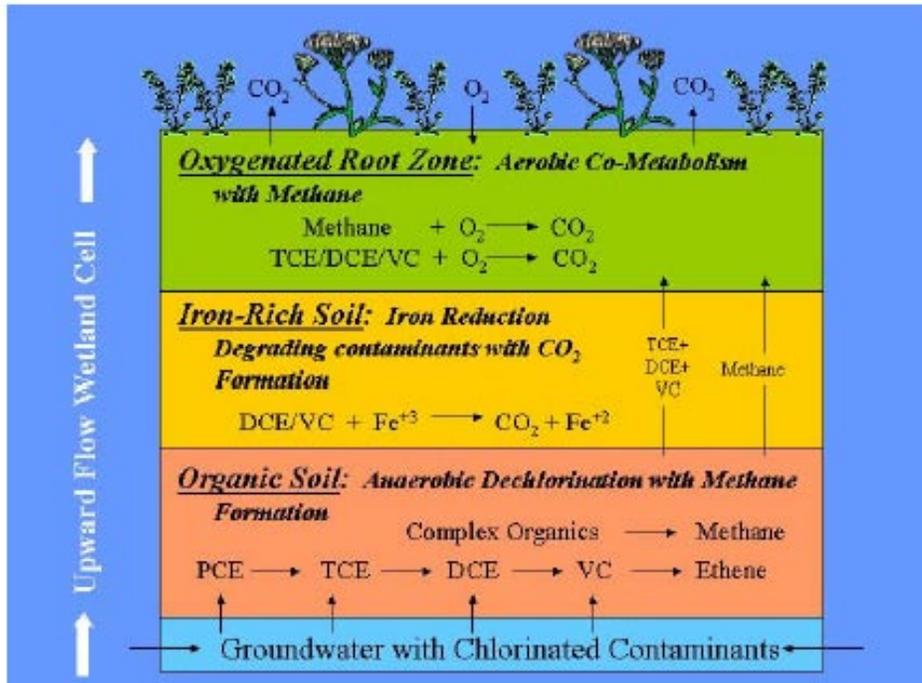


Figure 2. Upward Flow Constructed Wetland Remediation Sequence. (Shelley, 2002:14)

Water is pumped from the contaminated source and fed through pipes to the lower level of the constructed wetland. This region is dominated by anaerobic conditions due to the saturation of the soil. Anaerobic bacteria are able to convert chlorinated ethenes to progressively less chlorinated forms by reductive chlorination, using the chlorine from PCE, TCE, DCE, and VC as electron receptors. (Amon *et al.*, 2007: 52) PCE must be degraded anaerobically. Additionally, aerobic zones that exist around the roots (rhizosphere) of the wetland plants enable aerobic bacteria to co-metabolically consume the TCE, DCE, VC using the same mono-oxygenases used to digest methane in order to produce  $\text{CO}_2$  and water.

The maximum contaminant level for TCE allowed by the EPA is 5 parts per billion. In lack of definitive knowledge on the effects of TCE, this is a health protective measure. (Cheremisinoff, 2001:26) PCE is regulated by a reference dose of .01

mg/kg/day, the equivalent of 350 parts per billion in drinking water. The constructed wetlands at Wright Patterson Air Force Base effectively reduced PCE and TCE well below EPA limits while eliminating all VC generated during the remediation process. It is likely that this system can successfully remediate contaminant levels 100 times the EPA standards. (Amon *et al.*, 2007: 63) Plant mixes that optimize the flow of oxygen and other nutrients to the microbial populations in the soil that are responsible for the remediation effects could help to improve the degradation process (Amon *et al.*, 2007: 61), and are an important consideration in the engineering design of any constructed treatment wetland system. (Gersberg *et al.*, 1991; Bezbaruah and Zhang, 2004: 69) An additional possibility for remediation described by Amon *et al.* is to divert contaminants to the subsurface of natural wetlands, but this technique may depend upon EPA concessions to be permitted.

### **Wetland Characteristics**

Wetlands play important roles in nature. Numerous aspects associated with wetlands give them the ability to mitigate and remediate problems caused by pollutants in the environment. This section will describe wetlands, and the characteristics of the soil, water, and plants that contribute to wetland bioremediation capabilities.

Wetlands are ecosystems where land transitions to water; they can be found in every region of the United States and throughout most of the world. It is estimated that wetlands cover 4-6 percent of the Earth's land surface. (McGraw-Hill, 2007) As transitional zones between land and water; mixing environmental conditions contribute to their diversity and high productivity. Wetlands have one or more of the following

attributes: 1) at least periodically, the land supports predominantly hydrophyte vegetation; 2) the substrate is predominantly undrained hydric soil, and; 3) the substrate is nonsoil and is saturated with water or covered by shallow water for at least some portion of the growing season annually. (Hammer, 1992:5)

Wetlands play important environmental roles by stabilizing shorelines, controlling flooding, improving water quality, and acting as groundwater recharge areas. Like kidneys for the landscape, they provide natural filtration, sedimentation, control of organic matter, carbon sequestration, and decomposition of pollutants. (Hammer, 1992:5) “Assimilative capacity” is the ability to retain, process, or transform nutrients, organic matter, and contaminants; wetland soils and vegetation strongly influence this capacity. (AccessScience, 2007) These characteristics have been harnessed in many industrial remediation processes.

Wetlands are recognized as important natural resources. The Clean Water Act Section 404 is the principal tool for wetland protection in the United States. For regulatory purposes under the Clean Water Act, the term wetlands means "those areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs and similar areas." (EPA, 2007) Water saturation (hydrology) largely determines how the soil develops and the types of plant and animal communities living in and on the soil. (EPA, 2007) To understand the wetland ecosystem, the interaction between the water, soil, and vegetation must be understood.

### Water.

The defining characteristic of a wetland is the presence of water. Wetlands are areas where water covers the soil, or is present either at or near the surface of the soil all year or for varying periods of time during the year, including during the growing season. The prolonged presence of water significantly impairs the growth of any plants not suited for saturated conditions and creates conditions that favor the growth of specially adapted plants (hydrophytes).

Numerous variables impact the nature of the water chemistry. Clarity, pH, dissolved nutrients, ion concentration, salt concentration, flow velocity, dissolved oxygen, and temperature are a few factors influential in wetland conditions (Hammer, 1992:195). The influence of organic compounds can be a significant factor in the hydrogeochemistry. (Hite and Cheng, 1996:423) The anaerobic and reducing conditions created by water are also important in promoting the development of characteristic wetland (hydric) soils. (EPA, 2007)

### Soil.

The primary difference between most terrestrial and wetland soils occurs due to the anaerobic conditions that are present in a saturated environment. The saturation of the soil makes wetlands one of the major reducing ecosystems in nature, and is the dominant factor determining the nature of soil development. (Hammer, 1992:30; Dahl, 2006: 101)

Initial soil pH and buffering capacity are the most important factors that regulate the direction and magnitude of pH shifts in the rhizosphere. (Jones and others, 2004:467)

Buffering capacity is highest in calcareous soils where calcium carbonate acts as a storage sink for the bicarbonate system.

Depending on the saturation and clay content, wetland soils are classified as: 1) mineral soils < 12-20 percent organic matter, and 2) organic soils >12-20 percent organic matter. Mineral soils have approximately fifty percent pore space.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Na}^+$  are the dominant cations. Organic soils have lower density, >80 percent pore space, have greater cation exchange capacity, are  $\text{H}^+$  dominant, and more significantly limit water movement. Redox potential in the soils is typically -300 to 300 mV. (Hammer, 1992:30) The fine particle sizes of organic soils provide a larger surface area for the formation of biofilms, an important factor in any bioremediation process. (Amon *et al.*, 2007: 64)

Microbial populations living in the soils also exert a significant influence on soil characteristics (nutrient availability, metal speciation, pH). Populations include bacteria, fungi, and the protozoa that graze upon them. Most bacterial colonization of plant roots occurs in areas with the highest exudation levels, the root tips and root nodes. Beneficial bacteria in the rhizosphere have a complex symbiotic relationship with the plant and the other bacteria in the rhizosphere, accepting nourishment from plant exudates and sloughed off cells, and in return limiting the growth of harmful bacteria and providing nutrients in a useable form for the plant. (Kapulnik: 1996: 773) Even though microorganisms in wetlands have been classified using a variety of approaches, studies of wetland soil microbiology are limited and focused on bacterial groups engaged in key processes of interest. (Gutknecht, 2006: 24) A more specific discussion of bacteria and arbuscular mychorhizal fungi (AMF) is included below in the section on microbial

communities. The relative impact of bacteria and AMF as they relate to wetland plants and their root zones, specifically, is unclear.

### Plants.

Plants have a dynamic relationship with the water and soil around them. Water depth, frequency and duration of flooding, and water chemistry are the three most significant factors affecting wetland plants. (Hammer, 1992:195) The water influences the nature of the soil and creates anaerobic/reducing conditions. One important exception to the anaerobic characteristic of wetlands, however, is the presence of aerobic zones around the root zones of wetland plants. This originates from oxygen diffusion into the soil from rhizomes, roots, and rootlets. (Hammer, 1992:30) Since the soils of wetlands are often saturated, it is necessary for plants in aquatic, wetland, or flood-prone environments to supply oxygen into their root systems that lie below the water. (Colmer, 2003:17) It is widely accepted that wetland plants can transport oxygen into their roots, supporting aerobic respiration of bacteria and oxidizing phytotoxic compounds in the rhizosphere. Wetland observation has shown that roots typically extend to at least 100 cm below ground. (Amon et al, 2007: 54) The plant systems responsible for air movement will be covered in the subsequent section on plant physiology.

Air movement inside plant aerenchymal tissue, a combination of advection and diffusion, is one source of oxygen to the root zone. Additionally, water moving inside plant vascular tissue not only carries important sugars, amino acids, and organic acids, but also provides high concentrations of oxygen to the roots. Oxygen is delivered to the roots in two ways: bulk flow of air through the aerenchymal tissue, and bulk flow of

dissolved oxygen in the phloem sap. The effects of plant radial oxygen loss into the soil and the microbial activity associated with it have significant impacts on alkalinity, eH, and dissolved inorganic solutes in the soil. (Hite and Cheng, 1996:423) Figure 3 below demonstrates flow pathways in a generic wetland plant and shows how aerobic areas around the root can greatly increase the amount of oxygen moving into the saturated wetland soil.

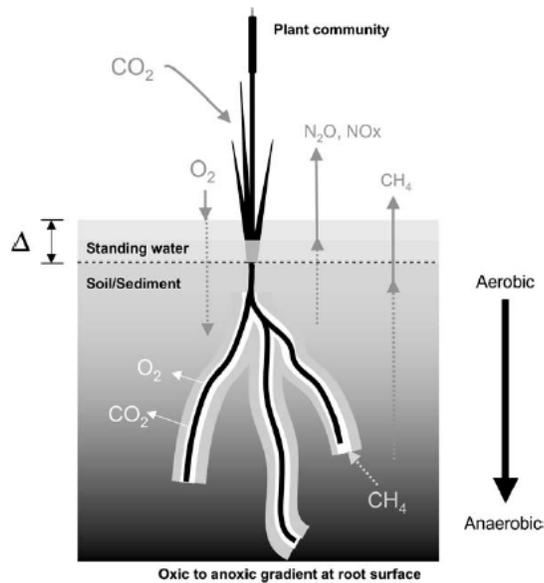


Figure 3. Wetland Oxygen Gradients. Numerous factors contribute to oxic and anoxic conditions in wetland soil, permitting a wide range of chemical and biological processes to exist. (Gutknecht, 2006: 18)

### Plant Physiology

In order to generate a working plant model, a discussion of plant physiology is essential. Though the topic indeed spans volumes in literature, processes that are reflected in the plant model will be discussed in abbreviated form. Plants are multicellular, photosynthetic, and eukaryotic organisms. There are four main plant groups: bryophytes, seedless vascular plants, gymnosperms, and angiosperms. The

angiosperms, or flowering plants, are the dominant group of plants on land, and are divided into two classes: the monocotyledons (monocots), and dicotyledones (dicots). The two differ substantially in the way that their vascular systems are arranged. (Vaccari, 2006: 146) Most plants adapted for wetland conditions are monocots that display lysigenous (vice schizogenous) aerenchyma, and monocot physiology is the primary focus of this review. (Visser *et al.*, 2000: 1237)

Being autotrophs, plants can generate all the amino acids and vitamins they require. The only nutritional requirements they have are inorganic nutrients. Carbon is mostly absorbed as CO<sub>2</sub>. Oxygen is absorbed as water or O<sub>2</sub>. Hydrogen is absorbed through water. Nitrogen is absorbed as either nitrate or ammonia. When nitrate is absorbed, it is converted to ammonium by the plant in a process known as amination. Most other nutrients are used as enzyme cofactors, intermediates in electron transfer reactions, and regulation of plant processes. (Vaccari, 2006: 153) In a wetland environment where reducing conditions exist, many critical nutrients may not be present in the form which they are absorbed by the plant; the influence of plant exudates and radial oxygen loss is a survival mechanism that allows hydrophytes to obtain plant nutritional requirements.

### Plant Cells.

The cells of a plant vary significantly by location and function. All cells play a role in the oxygen and nutrient cycle by consumption, respiration, excretion, and transport of molecules. Movement of solute through individual cells is primarily via diffusion. Since diffusion time increases by the square of the distance, diffusion also

plays a role in limiting the size of the cell. Membranes, specifically, provide the most restrictive barriers to diffusion in the cells. The outer covering of a typical cell, the cell wall, is composed of polysaccharides like cellulose that provide rigid structure for the cell as well as the entire plant. All solutes and water moving in and out of the protoplast must cross the cell wall. The cell wall has a large negative charge and acts differently with cations and anions. (Nobel, 1991: 33) Cell walls, however, are relatively porous and do not serve as the main barrier to the passage of water and small solutes like oxygen moving into the cells. Figure 4 shows a typical leaf cell.

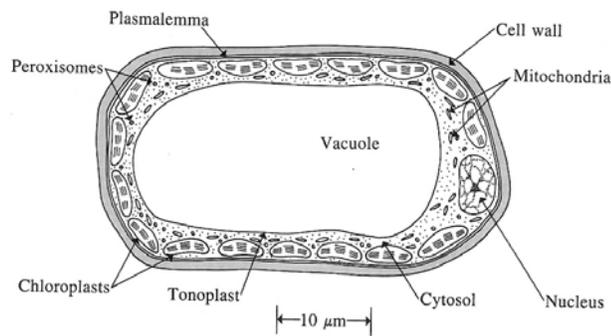


Figure 4. Typical Leaf Mesophyll Cell (Nobel, 1991: 2)

The plasma membrane, or *plasmalemma*, just inside the cell wall, is the primary cell barrier for the diffusion of solutes. The permeability of the plasmalemma varies with the particular solute, giving the plant cell a degree of regulation over flux into and out of the cell. (Nobel, 1991: 1) Permeability coefficients for small solutes moving across the plasmalemma typically range from  $10^{-10}$  to  $10^{-6} \text{ m}^2\text{s}^{-1}$ , a greater resistance to diffusion than the cell wall. (Nobel, 1991: 37) In addition to the outer membranes, plant cells also have numerous membranes within the cell that separate components within the cytoplasm and further restrict the movement of solutes to plant organelles.

Many plant cells are linked to each other through a series of openings in the cell walls termed *plasmodesmata*. Plasmodesmata typically occupy .1 to .5% of a cell's surface area. The passages themselves range from 20 to 200 nm and contain some constrictions that may control flow between the cells. These connections create a continuous cytoplasm, or *symplasm*, that speeds solute movement between cells. The symplasm is an effective transport pathway and can increase flux between cells more than a hundred times that of diffusion across the cell walls, a significant consideration in any plant transport calculation. (Nobel, 1991: 39) Solutes flowing outside cell walls follow an *apoplastic* pathway. Flow of solutes between adjacent cells is called a *symplastic* pathway. Both flow pathways are shown in Figure 5 below.

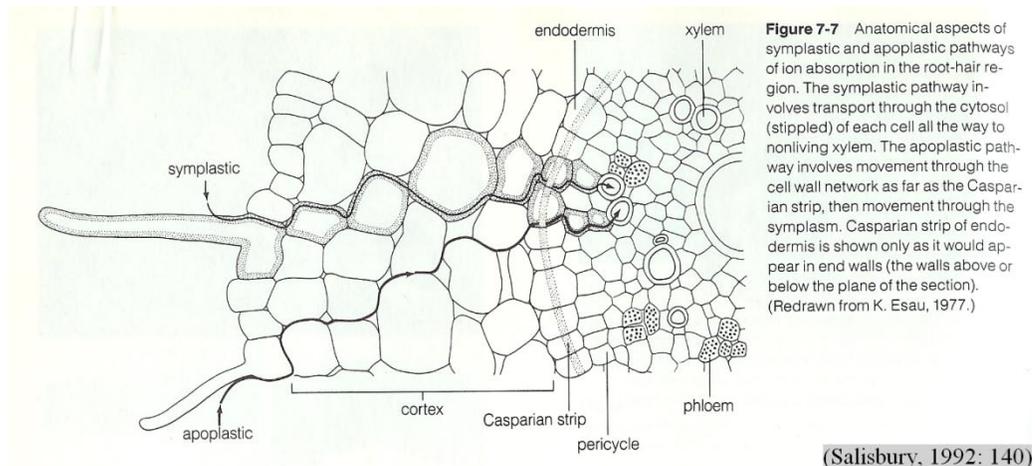


Figure 5. Symplastic/Apoplastic Pathways in a Root-Hair Region. (Salisbury, 1992: 140)

### Plant Vascular System.

Like human arteries, capillaries, and veins, plants also have a circulation system. In the plant, the xylem and phloem constitute the means to circulate water and solutes. “Thus, the xylem and phloem serve as the plumbing that connects the two types of plant

organs functionally interacting with the environment”, the leaves and the roots. (Nobel, 1991: 9) Both xylem and phloem originate from a vascular cambium and remain in close proximity to each other throughout the plant. The vascular bundle forms numerous branches throughout the plant in order to optimize the movement of water and nutrients.

Figure 6 shows a longitudinal section of vascular tissues in a plant stem.

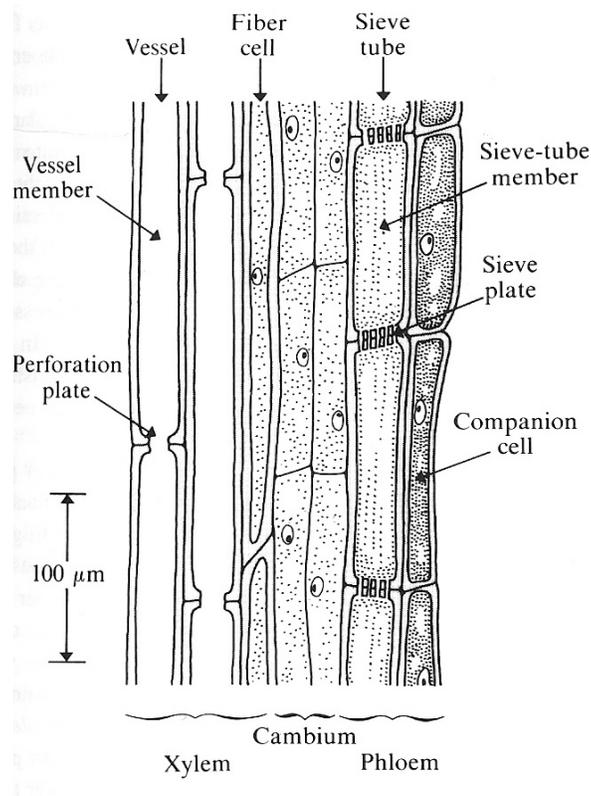


Figure 6. Longitudinal Section of Plant Stem Vascular Bundle. (Nobel, 1991: 5)

*Xylem.* Movement of water and nutrients from the soil up to the plant occurs primarily in the xylem. Xylem tissue is comprised of vessel members, parenchyma cells, and fibers. The vessel members are the conducting elements of the xylem and they typically have thick, lignified cell walls and no protoplast. The vessel cells are hollow, dead cells that form the low resistance pathway for solute movement. They are arranged

end to end with other vessel members in order to form a xylem vessel. The cells are separated on their ends by a perforation plate, a cell wall with small openings, which permit movement of solute. Xylem elements can vary in width from 8-500  $\mu\text{m}$ . (Nobel, 1991:507)

Fiber cells are long and thin, have lignified cell walls, and contribute to the structural support of the plant. Parenchymal cells serve an important role in storing carbohydrates and permit lateral movement of the solutes in and out of the conducting cells. The xylem flow is powered by hydrostatic pressure and will be examined in greater detail below in “Plant Circulation”. Typically, xylem sap contains 10 millimolar of inorganic nutrients and smaller amounts of organic molecules such as sugars and amino acids. (Nobel, 1991:6) It is likely that organic molecules are reabsorbed from the soil or diffuse from the phloem.

*Phloem.* The movement of photosynthetic products, mostly in the form of sucrose, is predominantly moved throughout the plant in the phloem. The phloem consists of sieve elements and companion cells. Unlike xylem vessel members, the phloem sieve cells are living cells that are filled with cytoplasm. The sieve cells are typically one to three mm long and are attached end to end in order to form a continuous sieve tube. The ends of sequential sieve elements are linked by sieve plates, a section of cell wall with numerous pores typically one to five  $\mu\text{m}$  in diameter. The sieve plates permit flow between the sieve elements and likely serve a clotting function during plant injury.

The companion cells have an important function in supporting the sieve elements. The companion cells typically have many mitochondria that produce ATP, an important

energy source for the cells. They also accumulate sugars and other solutes that could play a role in phloem loading (see “Plant Circulation” below). They are connected to the sieve elements by numerous plasmodesmata, permitting low resistance diffusion of cell contents, and may also actively transport contents between cells. (Nobel, 1991: 513)

Phloem solute typically contains 90% carbohydrates, mostly in the form of sucrose. Sucrose concentration ranges from 0.2- 0.7 M. Additionally, amino acids typically measure 0.05 M. Solutes typically move by bulk flow in the phloem at speeds of 0.2-2 meters/hour. Flow is towards the region of lowest osmotic gradient. (Nobel, 1991: 515)

### Leaves.

Leaves are the solar cells and industrial work centers of a plant. The large surface area of leaves is used to capture solar energy. Photosynthesis in the leaves provides a source of oxygen required for aerobic respiration and valuable sugars that are used by the plant for energy. A typical leaf cross section is shown in Figure 7. Individual leaves are typically only four to ten cells thick. The outer layer of the leaf, the epidermis, is typically a single cell thick and is covered by a cuticle comprised of cutin, a waxy material that helps to minimize water loss from the plant. Mesophyll cells make up most of the leaf. The layer of mesophyll below the upper epidermis, the palisade parenchyma, comprises approximately 70 percent of the mesophyll and is the main site for photosynthesis. The other mesophyll cells are termed spongy parenchyma, and have significant void volumes (15-40%) that facilitate the exchange of carbon dioxide, oxygen, and water vapor. (Vaccari, 2006: 148) Most of the individual cells are exposed to air in

the intercellular spaces, optimizing transfer of gases into leaf spaces. Additionally, individual mesophyll cells in the leaf are seldom more than a few cells from vascular tissue. (Nobel, 1991: 506) This optimizes the transfer of photosynthates from the leaf cells to the plant phloem, an important component of phloem loading that drives the circulation of solutes towards the roots. This process will be described in Plant Circulation.

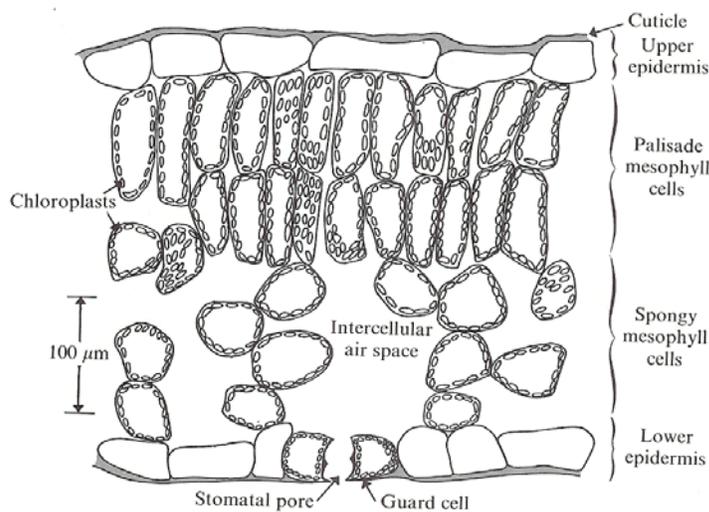


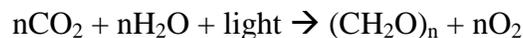
Figure 7. Leaf Cross-Section. Approximately thirty percent of a leaf is comprised of air space. (Nobel, 1991:3)

The leaves also act as an air valve for the rest of the plant, helping to control the flow of gases in and out of the plant. The entry and exit point for gases in and out of the leaf is through numerous pores in the leaf termed *stomata*. The size of the stomata can be varied by water pressure in a set of guard cells that surround the opening of each stoma. When the guard cells are filled with water they bow outward and cause the pore to open. The stomata, then, act as a vent control for airflow in and out of the leaves and to the rest of the plant. They can be used to regulate the amount of CO<sub>2</sub> entering the cells

for photosynthesis, and limit the amount of water vapor lost by the plant through transpiration, the evaporation of water from the plant into the atmosphere. (Nobel, 1991:

4) Their small size and variability is an important factor in the process of *humidity induced convection*, a process that forces convective currents of air to flow inside the plant. Humidity induced convection is covered in the section on plant ventilation.

*Photosynthesis.* Almost all plants are autotrophs, able to grow on inorganic carbon sources, and phototrophs, able to use light as an energy source through photosynthesis. Photosynthesis is a process of light-driven electron transport that converts CO<sub>2</sub> and water to a useable source of energy in the form of organic sugars. It is the primary source of energy fixation in the world, using solar energy to power the oxidation of water and the reduction of C O<sub>2</sub> to yield carbohydrates. Net production of glucose is summarized in the following expression:



The chloroplasts in leaves, filled with various chlorophyll molecules and beta carotene, are the sites of photosynthesis. They are especially sensitive to light in the blue/violet spectrum (400-500 nm) and red spectrum (620-690 nm). Light plays two essential functions in this process. It drives electrons from water to reduce NADP<sup>+</sup> to NADPH (Nicotinamide adenine dinucleotide phosphate), and it provides energy to form ATP from ADP and Phosphorous. As photons hit the chlorophyll molecules and beta carotene they are excited, and this excitation energy is transferred by inductive resonance to reaction centers in the leaf for the conversion of NADP<sup>+</sup>. NADPH is subsequently used in the reduction of C O<sub>2</sub> and acts as an energy conduit for the reaction. There is also an important balance between the absorption spectrum and the response of various

chlorophyll molecules known as the Emerson enhancement effect. Two separate groups of pigments termed Photosystem I and II cooperatively make use of multiple bands of light in order to synergistically enhance rates of energy adsorption and reaction rates. (Salisbury, 1992: 207-224)

Ultimately, the carbohydrates formed by photosynthesis are mostly transformed to sucrose in the cytosol of the leaves. (Salisbury, 1992: 244) The sucrose is a critical molecule both for its energy content, for its conversion to starch for storage, and because sucrose loading into the plant's phloem creates an osmotic gradient that results in sap movement. Starch also accumulates in leaves where it is formed directly from photosynthesis. It forms during the daylight hours when photosynthesis is occurring, and is consumed by respiration or translocation at night. (Salisbury, 1992: 245)

Rates of photosynthesis are governed by a variety of factors. These include water availability, CO<sub>2</sub> concentrations, light intensity, nutrient, temperature, plant age, and genetics. In most wetlands, water is normally not a limiting factor. Leaf photosynthetic capacity, the photosynthesis rate under optimal conditions, varies widely between different plant species. Species using the C-4 photosynthetic pathway, like some wetland plants, typically have the highest photosynthetic rates. (Salisbury, 1992: 253-254)

Two important quantifications of light intensity are the light compensation point, at which photosynthesis balances the rate of respiration, and the light saturation point at which increasing light intensity no longer increases photosynthesis. These points vary with species, temperature, and CO<sub>2</sub> concentration. Most leaves hit their light compensation point around two percent of full sunlight. (Salisbury, 1992: 255) The total amount of sunlight absorbed by a plant is also dependent upon the area of its leaves

exposed to light in relation to its footprint on the ground, a quantity termed the leaf area index (LAI). The grazing angle of the light can also reduce light adsorption, as is the case with leaves that are nearly vertical such as grasses and sedges commonly found in wetlands. (Salisbury, 1992: 260)

CO<sub>2</sub> saturation can be a significant effect in photosynthesis, and photosynthesis is usually limited by the amount of CO<sub>2</sub> diffusing into the chloroplasts in the leaf cells. (Nobel, 1991: 20) For most plants, this increase is noticeable during drought conditions when stomates are partly closed to minimize water vapor loss. CO<sub>2</sub> concentration also influences the light saturation point for many plants, only to a much lesser extent in C-4 plants. Even slight breezes can increase the effects of photosynthesis by reducing the depth of the air boundary layer around the leaf and making it easier for CO<sub>2</sub> to diffuse across the layer. (Salisbury, 1992: 260-261) Ventilation, then, can be an important factor in photosynthesis. CO<sub>2</sub> levels in plants can be significantly influenced by plant respiration and the by-products of the Krebs cycle. Respiration, in turn, is influenced by oxygen levels, substrate (sugar) availability, temperature, age, species of plant, and life cycle. (Salisbury, 1992: 275-288)

Plants can continue to photosynthesize over a broad temperature range which is largely species dependent. C-4 plants generally have higher temperature optima than C-3 plants. Increases in temperature usually result in an increase in photosynthetic ability until a point where plant molecules begin to denature. C-4 plants normally have temperature optima between 30 and 40 degrees Celsius. (Salisbury, 1992: 262)

## Stem.

The plant stem (Figure 8) functions as a support structure for the plant. It houses the main features of the plant vascular system, the xylem and phloem, which permit solute movement of food, nutrients, water, and oxygen throughout the plant. The xylem contains fiber cells that help to provide structural support to the plant. (Nobel, 1991:6)

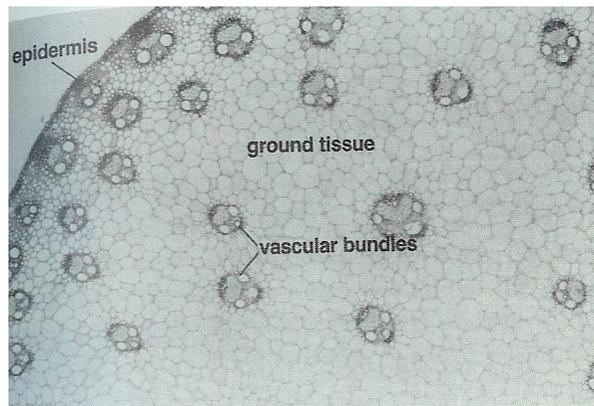


Figure 8. Cross-Section Through a Monocot Stem. (Salisbury, 1992: 97)

## Roots.

*Rhiz-* Greek - root

Roots provide anchorage for the plant, and provide for the uptake of nutrients and water from the soil. Secondary functions include storage of energy, chemical synthesis, propagation, and dispersal. Roots act as an osmotic sink by turning sugars into starch, transforming other compounds like amino acids and organic acids, and exuding them through the roots into the rhizosphere. Roots represent a capital investment for the plants, with both construction and maintenance costs that are usually constrained by carbon availability. (Fitter, 1996: 1) Accordingly, plants attempt to achieve a balance between root growth and their requirements for nutrients and water. Many root processes

vary along roots as a function of age, tissue structure, and anatomical differences.

(Doussan, 2003: 427) This section will describe the various components of the root, and root processes used in oxygen transport.

*Root Components.* Generally, roots can be classified into three main categories: primary, nodal, and lateral roots. Primary roots leads to a single-axis root (taproot) system with dominant vertical growth. Nodal roots, or adventitious roots, grow at specific locations and are usually a response to a environmental condition. The ability to produce adventitious roots is species specific. Lateral roots are the result of branching from a parent root axis. The formation of lateral roots results in acropetal branching, a pattern that generally follows the parent root axis outward. Lateral roots decrease in size as they go outward, but are limited by a minimum effective root diameter, and genetically by maximum branching orders. (Doussan, 2003: 421) At lower nutrient levels, fine roots approach a minimum diameter <100um, and coarse terminals (0.5-1 mm) develop in higher nutrient conditions. Roots are constantly growing and decaying, with half lives as short as 10 days. The terminal section of a root is shown in Figure 9.

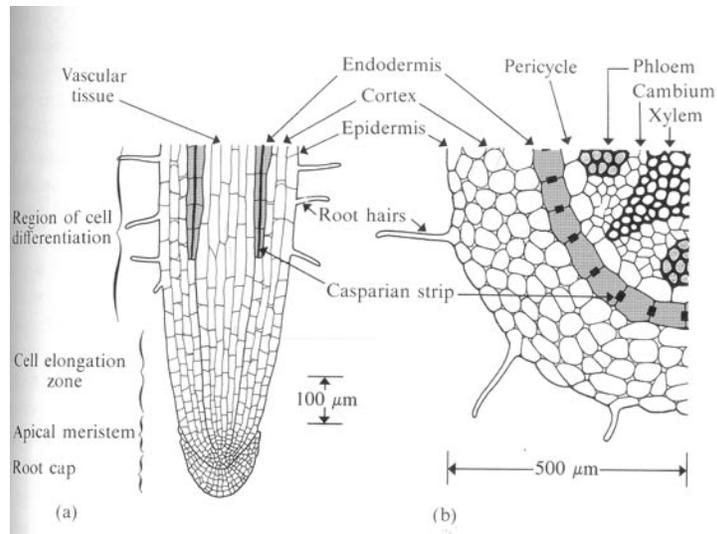


Figure 9. Longitudinal and Cross-Sectional View of Root. Roots have various zones that perform different functions. Cell arrangement plays an important role in movement of oxygen and other nutrients throughout the root structure. (Nobel, 1991: 7)

*Root Cap.* At the tip of every root, the root cap is an important part of a growing root. The main function of the root cap is to protect the apical meristem as the root grows through the soil and open a passage for the growing roots by *sloughing off* to produce root cap mucilage. (Sievers: 1996: 31)

*Apical Meristem.* Inside the root cap, the apical meristem is an area where cells rapidly divide. It exists in the terminal 1-2mm of roots. (Webster: 1996: 51)

*Region of Cell Elongation.* In this region, the cells elongate in the direction of the root axis. This pushes the root cap through the soil and causes cells to slough off.

Interestingly, the cylindrical shape of roots is a plant optimization. (Nobel, 1991: 8)

Since a cylinder has greater strength per unit cross-sectional area than other shapes, the shape of roots, along with the protective root cap, helps roots to grow and explore the soil most efficiently.

*Hair Zone.* Situated just behind the zone of active root elongation, the hair zone of most plants is one to four cm long. (Hofer, 1996: 116) A root hair is a modified epidermal cell with a filamentous extension that projects radially from the root up to 1.5 mm. (Salisbury, 1992: 137) Formation of numerous root hairs greatly increases the root's surface area for adsorption of water and nutrients. Root hairs form closest to the root tip, and new hairs do not develop among pre-existing ones. Older hairs tend to be longer, but epidermal cells cease to create root hairs as they become older. (Hofer, 1996: 116; Cormack, 1962) Consequently, the older roots towards the base root tend to have few or no root hairs. The presence of root hairs depends on the species of plant, but it is often minimized by soil conditions and microbial activity. (Salisbury, 1992: 138) In aqueous medium, root hair production can be increased by increasing oxygen content. (Hofer, 1996: 118)

*Region of Cell-Differentiation.* Cells here assume more functional roles. Cell walls thicken and cells cease to elongate. The epidermis becomes less permeable to water and other molecules closer to the main root. Root hairs can grow from the epidermis, further increasing root permeability. Inside the epidermis, the cortex is an area of tissue with numerous air spaces, facilitating diffusion of CO<sub>2</sub> and O<sub>2</sub>. Inside the cortex, the endodermis acts as a membrane that restricts movement of solute and water into plant vascular tissue. The cells of the endodermis are lined with a waxy material consisting of suberin, and form a barrier known as the casparian strip. In order for water or solutes to pass into the root, they must enter the cytoplasm of the endodermal cells. (Nobel, 1991: 8) The casparian strip acts as a low permeability hydraulic control between the cortex and the vascular tissues.

Inside the epidermis, the pericycle is a meristematic region that can produce cells for additional lateral roots. These form at nodes proximal to the root hair zone (towards tap root). Inside the pericycle, the vascular tissue, the xylem and phloem, are geometrically arranged to allow direct flow to either set of vascular tissue. The vascular cambium, that produces the xylem and phloem cells, is between the vascular tissues. (Nobel, 1991: 9)

### Hydrophyte Adaptations.

The aerenchymal developments of hydrophytes constitute one plant defense against anoxia. Additionally, the roots grow more impermeable to diffusive forces towards the basal side of the root, especially in stagnant and highly reducing soils. (Visser, 2000: 1243) As a result, the flux of oxygen, water, and other nutrients increases closer to the apex of the root. Oxygen losses from root tips helps to detoxify the soil around growing plant root tips, and nutrient exudation encourages beneficial microbial growth. Together, barriers to radial O<sub>2</sub> loss (ROL) in the basal zones and presence of aerenchyma in the roots enable the development of an aerobic rhizosphere around the root tip and enhance penetration of the root into anaerobic substrates. (Colmer, 2003:17)

Monocotyledonous species like *Phragmites australis*, specifically, tend to develop a strong barrier to radial oxygen loss in basal root zones while dicotyledonous species have a much weaker resistance to ROL. (Visser, 2000: 1237) Plants grown in highly reducing soils demonstrate a much greater ROL than those grown in oxic soils, and plants display a ROL saturation that is likely limited by root surface area; larger roots have a greater surface area and can potentially exude greater amounts of oxygen than small

roots. (Sorrell, 1999: 1591) Figure 10 shows a side profile of oxygen flux along a *Phragmites australis* root. The final 3 centimeters of the root, coincident with the root hair zone, is clearly responsible for the majority of oxygen flux from the root. Root aerenchyma facilitate diffusion of oxygen into the roots and low root permeability towards the base of the root assists in maintaining high cortex oxygen levels.

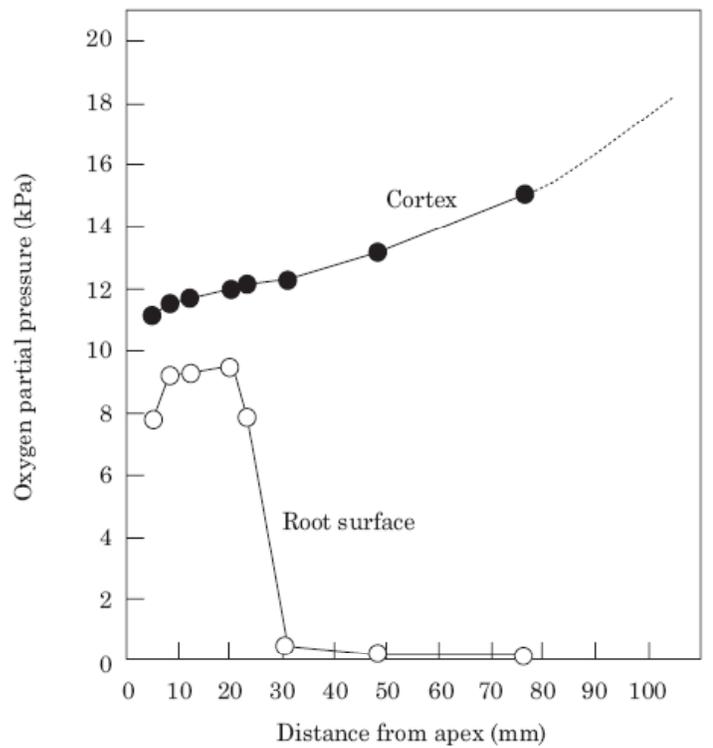


Figure 10. Oxygen Partial Pressures in Cortex and Root Surface. The root is a 110-mm long *Phragmites australis* root with a 1 mm diameter. (Armstrong *et al.*, 2000: 692)

Seasonal Variation and Photoperiodism.

Plants are closely tuned to light and change their growth patterns in response to it. For example, plants will turn towards a light source, a characteristic called phototropism. Another light-driven behavior is called photo-periodism. Photoperiodism directs the flowering cycle of the plant, and is controlled by a protein complex known as

phytochrome. Phytochrome acts as a detector for light and can induce phototropism or stem growth in order to seek the sunlight if the plant is shaded. Plants generally follow a 24 hour cycle in response to light conditions. In the absence of light, plants continue to show a cycle that is less precise, known as circadian rhythm. (Vaccari, 2006: 153)

Oxygen consumption by root respiration, which varies seasonally with temperature and plant growth, appears to be the major variable influencing root zone oxygen supply.

(Allen, 2002:1014) See also Salisbury (1992: 504-530).

### Circadian Rhythms and Diurnal Cycles.

Just as humans have a rhythmic response to the environment, plants also have a periodic cycle governed by light, temperature, and time clocks. These factors influence growth, respiration, and other chemical processes in the plants. (Salisbury, 192:471-484)

### **Plant Circulation**

Many wetland plants can have two circulation systems, a pressurized vascular system comprised of the phloem and xylem that moves solutes in water, and an air/gas circulatory system comprised of aerenchymal tissue. The latter will be covered in the following section. This section will focus on bulk flow and diffusion of solutes in the vascular tissues.

The contents of plant vascular systems are under substantial pressure, often near 0.4 to 0.5 megapascals (MPa). Flow in response to pressure differences is termed bulk flow, while movement due to the random movement of molecules down a concentration gradient is termed diffusion. Advection is the predominant long distance transport

process in plants, while diffusion plays a significant role over short distances only.

Diffusion flux rates are calculated from Fick's First Law:

$$J_j = D_j \frac{(C_{j1} - C_{j2})}{x} \quad (\text{Salisbury, 1992: 42})$$

where  $J_j$  is flux ( $M/L^2/T$ ),  $D_j$  is the diffusion coefficient,  $C$  is the concentration, and  $x$  is the distance

Advection rates are influenced by gravitational forces and potentials resultant from water or chemical potential. Water potential ( $\Psi$ ) is the chemical potential of water in a system and is expressed as units of pressure. Water diffuses in response to chemical potential in order to minimize the Gibbs free energy in the system. As water diffuses from areas of high potential to low potential, energy is released and has the potential to perform work such as moving water in the stem. In plant vascular tissue, this is known as root pressure.

### Phloem Loading.

Rates of phloem transport are 500-1500 mm/hr for most plant species. The transported solute consists of approximately 90 percent carbohydrates, mostly sucrose. Sugars are raised to high levels in phloem cells by a process called phloem loading that utilizes selective recognition of sugar carriers in the plasmalemma transporting sugars into the cytoplasm. The high concentration of sugar creates an osmotic potential that draws water into the phloem cells, increasing the hydraulic pressure and causing advection of the solution. Many other substances, such as  $O_2$  and  $CO_2$ , enter the phloem by diffusing in along their concentration gradients and are cotransported in the sap of the plant. (Salisbury, 1992)

## **Plant Ventilation**

A number of factors are influential in oxygen movement within plants. In wetland plants, aerenchymal tissue is a high volume conduit for gaseous oxygen. Humidity induced convection provides a motive force and helps to raise oxygen levels in the rhizomes, permitting greater diffusion of oxygen through plant roots. The roots themselves develop barriers to radial oxygen loss that increase the flow of oxygen through permeable areas of the root near the root tip.

### Aerenchymal Tissue.

Aerenchymal tissue plays a central role in the survival of wetland plants by assisting in the delivery of oxygen to the roots. Aerenchymal tissue forms when mature cells collapse and lyse, creating *lysigenous aerenchyma*. This creates large air corridors for gas exchange that begin in the leaf stomata, flow throughout the entire plant, and allow faster air movement (advection and diffusion) from the shoots to roots. The collapse is often signaled by ethylene formation, a product that frequently accompanies plant stress. (Salisbury and Ross, 1992:285) This permits the distribution of air entering through leaves and other portions above the water into the plant roots. Other gases from the plant roots, some of which may originate in the substrate, are also vented to the atmosphere in this manner. (Hammer, 1992:40) While terrestrial plants may create aerenchymal tissue in times of stress, wetland plants can routinely have large stem volumes occupied by aerenchymal tissue. Most hydrophytes have a developed system of air passages, or lacunae, which can occupy up to sixty percent of plant volume. This represents a large plant investment in a ventilation system. Table 1 demonstrates the

difference in root porosity of non-wetland and wetland species and shows the increase in aerenchyma that occurs in oxygen-deficient conditions.

Table 1. Porosity of Wetland and Non-wetland Species Grown in Drained and Saturated Medium. (Colmer, 2003: 19)

Species		Control	O <sub>2</sub> -deficient	Reference no.
Selected monocotyledonous non-wetland species				
<i>Triticum aestivum</i>	adventitious roots	3-6	13-22	1, 2, 3
<i>Hordeum vulgare</i>	adventitious roots	7	16	1
<i>Zea mays</i>	adventitious roots	4	13	4
<i>Festuca rubra</i>	entire root system	1	2	5
Selected dicotyledonous non-wetland species				
<i>Vicia faba</i>	entire root system	2	4	5
<i>Pisum sativum</i>	entire root system	1	4	5
<i>Brasica napus</i>	entire root system	3	3	6
<i>Trifolium tomentosum</i>	entire root system	7	11	7
Selected monocotyledonous wetland species				
<i>Oryza sativa</i>	adventitious roots	15-30	32-45	5, 8, 9, 10
<i>Typha domingensis</i>	adventitious roots	10-13	28-34	11, 12
<i>Phragmites australis</i>	adventitious roots	43	52	5
<i>Juncus effusus</i>	adventitious roots	31-40	36-45	5, 13
<i>Carex acuta</i>	adventitious roots	10	22	13
Selected dicotyledonous wetland species				
<i>Rumex palustris</i>	adventitious roots	15-30	32-45	5, 13
<i>Plantago maritima</i>	entire root system	8	22	5
<i>Ranunculus flammula</i>	entire root system	9-11	30-37	5, 14
Selected aquatic species (collected from natural habitats)				
<i>Zostera marina</i>	adventitious roots & rhizome		22-32	15
<i>Halophila ovalis</i>	adventitious roots		15	16

### Humidity Induced Convection.

While aerenchyma can permit diffusion of gases in the plant, a more significant movement of gases occurs by advection. The small aperture of leaf sheath stomata creates a partition that resists advective outflow more than it resists diffusive inflow. Constant humidification inside the leaves reduces the partial pressures of nitrogen, oxygen, argon, and CO<sub>2</sub>, creating a concentration gradient for diffusion. The inward diffusion of outside gases and constant humidification of the leaves creates leaf pressurization. The pressure drives the flow of gases along the conduit of least resistance, the plant aerenchyma. This creates significant movement of air inside the plant and helps to ventilate plant gases from the rhizome. This phenomena is termed

humidity-induced-convection (HIC). (Beckett, 2001: 270; Armstrong *et al.*, 1996; Dacey, 1981)

As demonstrated by Dacey, sunlight that warms plant leaves (or any other source of heat) is a significant factor in leaf humidification and explains the loss of HIC during darkness. He further showed that leaf pore sizes, transitional between Knudsen and Poiseuille flow, help to facilitate HIC. (Dacey, 1987) The pressure differentials in waterlilies facilitated airflow at 50 cm/min and flow of 22 liters of air per day entering a single leaf, a demonstration of the substantial thoroughflow possible by HIC. (Dacey, 1982) For most wetland species, mathematical models indicate that pressurization from humidity is the dominant factor in HIC. (Colmer, 2003: 35) Armstrong demonstrated HIC with a laboratory model, using micro-partition membranes to help quantify the effect. Figure 11 depicts the model used to physically demonstrate the phenomena.

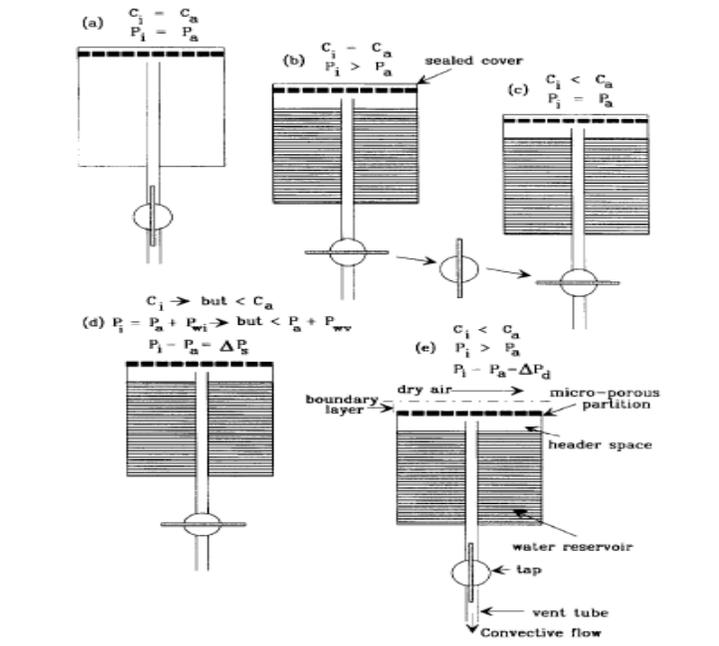


Figure 11. Demonstration Model for Humidity Induced Convection. Armstrong’s model demonstrated how humidity inside a micro-partition membrane contributes to elevated gas pressures and advective gas movement. (Armstrong *et al.*, 1996: 123)

### Oxygen Movement in the Roots.

Roots of many wetland species contain large volumes of aerenchyma (root porosity can reach 55%), while barriers to oxygen loss often develop in basal zones. These barriers combine to raise cortex oxygen levels in the root and restrict radial oxygen loss to apical root sections. Diffusion is the mechanism that moves gases inside the roots of all plant species, but HIC through-flows in the stem and rhizomes can raise O<sub>2</sub> concentrations in the rhizomes close to ambient oxygen levels. (Colmer, 2003: 17)

Oxygen levels in the soils are much greater in the day due to the influence of this advection. At night, there is little HIC in the plant stems, however plant rhizospheres still remain aerobic; this may result from a combination of gaseous diffusion in the aerenchyma, venturi-induced air currents, and oxygen saturation of plant tissue during the daylight hours that continues to supply oxygen in darkness hours (similar pattern to sucrose saturation in the leaves that maintains phloem loading during darkness).

### **Oxygen Movement in the Rhizosphere**

The top layer of soil/water in wetlands is oxidized by simple diffusion from the atmosphere. Air currents and thermal circulation affect the mixing conditions at the surface. Oxygen release by wetland plants, though, may account for as much as 90 percent of the oxygen entering a wetland substrate. (Reddy and others, 1989; Allen and others, 2002:1010) Flux of oxygen into the soil from root systems is termed *radial oxygen loss* (ROL). (Armstrong, 1979; Beckett, 1988; Colmer, 2003:21) Knowledge on the anatomical basis of radial oxygen loss in various species is scant (Colmer, 2003:17), though some studies have characterized roots of certain wetland species.

The plant's release of oxygen into the rhizosphere is not without justification; the efflux of oxygen across root membranes into the saturated soil provides oxygen to the rhizosphere and has numerous benefits to the plant:

- Reduces high redox potential around the roots
- Enhances nutrient availability
- Limits the amount of toxic ions around the roots
- Supplies oxygen for symbiotic microbial populations
- Allows venting of gases from the soil
- Enhances root penetration into anaerobic sediments

Under oxic conditions, consumption of O<sub>2</sub> in root and microbial respiration decreases redox potential and increases pH. (Jones and others, 2004:467) In anoxic waterlogged soils, minerals like Fe<sup>2+</sup> and Mn<sup>2+</sup> can cause rhizotoxicity (Armstrong and others, 1992); roots have been shown either directly or indirectly to induce the oxidation of Fe and Mn leading to their precipitation as plaques around the root. Recent studies have demonstrated that the presence of other phytotoxins in the rhizosphere can induce substantial cell wall lignification in the epidermal-hypodermal cylinder and reduce the permeability of the root. (Armstrong *et al.*, 2000: 697)

### Oxygen Measurement.

Measuring the oxygen released from root zones, however, is difficult. The quantification of oxygen flux from the root systems is also complicated by species and seasonal differences, spatial heterogeneity, and measurement issues. (Bedford and others, 1991; Sorrell and Armstrong, 1994; Allen and others, 2002:1010) Plant capacity for O<sub>2</sub> diffusion is determined by anatomical, morphological, and physiological characteristics, as well as environmental conditions like temperature and demand for oxygen in the rhizosphere from biological or chemical processes. (Colmer, 2003: 21; Gersberg and

others, 1986; Steinberg and Coorod, 1994, Jackson and Armstrong, 1999; Allen and others, 2002) Rhizospheres are characteristically thin; the oxic shell surrounding the roots varies from about .5 to 5 mm in thickness. Measurement of oxidation around the root tips is also affected by the reducing capacity of the soil; an increase in eH cannot be measured if there is an oxygen sink such as a reduced mineral (like  $\text{Fe}^{2+}$ ) or the organic compounds that typically surround most root systems. (Allen, 2002:1014) Oxygen flux is a saturating function that depends upon the incident intensity of light on the leaves (Christensen, 1994:847) The plant's capacity for diffusion also increases in time; as roots grow, their higher densities and oxygen releasing capacity increases the oxygen available in the soil (Van Bodegom, 2001: 3591), while senescence can reduce the plant's capacity for oxygen efflux. (Christensen, 1994:847)

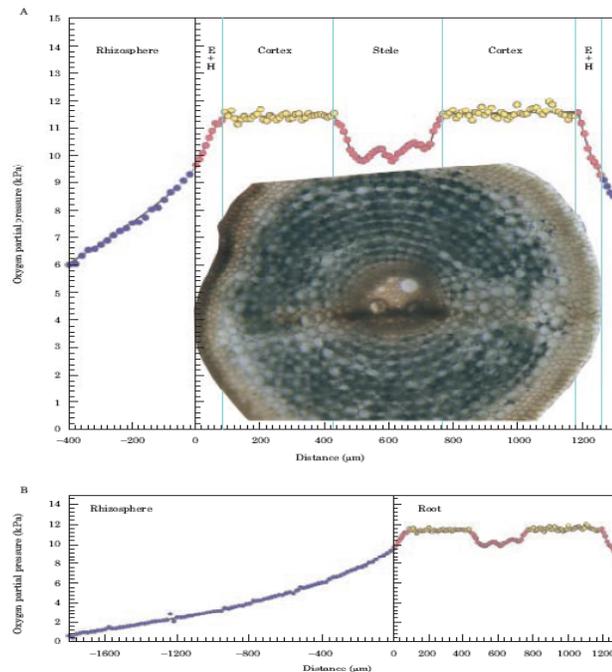
Since plants consume less oxygen during cold weather than warm weather, it is possible that the release of oxygen into the rhizosphere actually increases during cold weather. Allen and others demonstrated that temperature played a significant role in chemical oxygen demand (COD), dissolved organic carbon (DOC) removal, and root zone oxidation status of some wetland plant species. (Allen and others, 2002:1010)

The most successful rhizosphere studies have been conducted with microelectrodes. While test conditions for microelectrode measurements mimicked oxygen demand of a wetland to an extent, they may not be an accurate representation of conditions that would exist in a constructed treatment wetland. (Bezbaruah and Zhang, 2004: 68) Nonetheless, they offer the most accurate picture available regarding radial oxygen loss profiles. In contrast to most terrestrial plants, wetland plants usually exude oxygen from the root zone vice consuming it. Christensen *et al.* used microsensors to

analyze the dynamic between radial oxygen loss of the freshwater plant *Littorella uniflora* and the incident light intensity upon its leaves, mathematically modeling the profile obtained with a computer implementation of Fick's second law of diffusion. Results showed: 1.) oxygen continues to be released in the dark, though at rates tenfold less than during the light; 2.) light saturation occurred at 60-70  $\mu\text{mol}/\text{m}^2/\text{s}$ ; 3.) young roots had rates of exudation double those of older roots; and 4.) the major oxygen consumption in the agar medium occurred at the oxic/anoxic interface. (Christensen *et al.*, 1994: 847-851)

Armstrong *et al.* measured the oxygen profiles of *Phragmites australis* adventitious roots and interpreted the results using mathematical modeling. (Armstrong *et al.*, 2000: 687) This was also the earliest use of microelectrodes used to measure profiles in a wetland grass. Root oxygen profiles (Figure 12) showed higher concentrations in the cortex where aerenchyma was present and a slight deficit in the stele, offering evidence that HIC is responsible for increasing root oxygen levels. Relatively flat cortex profiles demonstrate a low oxygen demand in that area. (Armstrong *et al.*, 2000: 695) They concluded that the lateral roots, specifically the root hair zones, were likely the most important contributors to sediment oxygenation through radial oxygen loss. (Armstrong *et al.*, 2000: 698)

Figure 12. Root Cross-section Oxygen Measurements. Oxygen profile from microelectrode measurements taken in the root hair zone 7 mm from the root tip. Note the lower oxygen pressure (concentration) in the stele, elevated cortex level, and steep gradient across the permeable Epidermal-Hypodermal layer. (Armstrong et al., 2000: 694)



### Exudation in the Rhizosphere

A primary function of the plant roots is nutrient acquisition. Plants can enhance uptake of nutrients from soil by chemical (abiotic) and biotic means. Abiotic means directly affect soil chemistry and include water and ion uptake, release of  $H^+$  and organic compounds, and oxygen/ $CO_2$  flux. These processes modify the pH, eH, nutrient concentrations, water, and ionic potential of the soil, resulting in unique conditions in the rhizosphere. Abiotic release may also help to detoxify metals in the rhizosphere; anion channels in the root facilitate the release of malate and citrate in the presence of aluminum. (Jones and others, 2004:469)

Plant influences can also affect biota around the root and further enhance nutrient uptake for the plant through biotic processes. In addition to oxygen, other root exudates provide a source of nourishment, particularly carbon and nitrogen, which support microbial populations in the soil. Root-derived organic materials include exudates,

mucilages, and dead epidermal cells. Specifically, organic acids, amino acids, and sugars are the most abundant root exudates with organic acids being five times more abundant than the sugars. (Kuiper, 2004:11) These organic compounds are also the primary constituents in phloem flow. Jones gives evidence to show that plants can regulate this flow into the rhizosphere by regulating the exudation process or reabsorbing exudates from the soil. (Jones, 2004:460)

Microbial turnover of root exudates in the soil is a rapid process. Most sugars, amino acids, and organic acids have half lives in the soil of .5-2 hours. (Nguyen and others, 1999; Ryan and others, 2001; Jones and others, 2004:464) The heterotroph populations consume these root exudates as well as oxygen, often competing with the methanotroph populations valuable to remediation processes. Correspondingly, the movement of these exudates into the root zone plays an important factor in determining microbial populations in the root zone. Figure 13 demonstrates how plant absorption and exudation of nutrients result dynamic rhizosphere conditions.

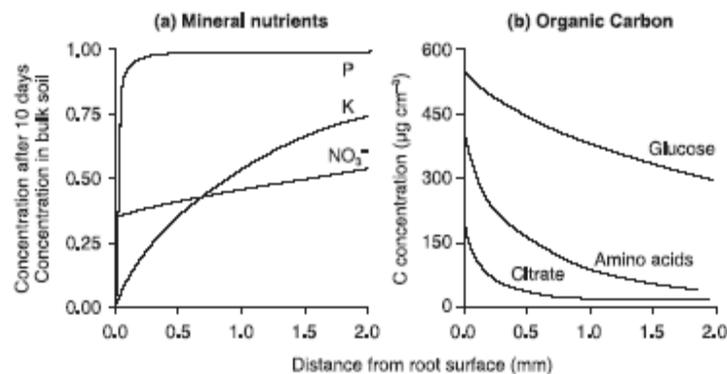


Figure 13. Nutrient Adsorption and Exudation of Carbon/Nitrogen Sources. (a) shows the depletion of N, P, and K from root uptake. (b) demonstrates the gradients of three organic solutes from root exudation. (Jones and others, 2004:464)

There are two classes of exudates: diffusive exudates that the plant does not control, and closely controlled exudation controlled by the opening of membrane pores.

Uncharged solutes follow the modified net flux density equation:

$$J = P (C_o - C_i)$$

where J = flux (M/T/L<sup>2</sup>)

P = membrane permeability coefficient of the solute (L/T)

C<sub>o</sub> = concentration in cytoplasm (M/L<sup>3</sup>)

C<sub>i</sub> = concentration in soil

There are difficulties parameterizing this equation. It is difficult to measure cytoplasmic solute concentrations. There is limited data on root membrane permeability coefficients and on the surface area available for exudation. (Jones, 2004:460) It is also likely that plant membranes are selectively permeable. Concentration gradients across the membrane are large, and Jones suggests this is maintained by the constant removal of exudates from the soil by microbial uptake, soil sorption, or readsorption of nutrients by the roots. (Jones, 2004:461-2)

### Carbon Sources.

Understanding the carbon cycling dynamic in terrestrial ecosystems is a prerequisite to understanding the fate of contaminants in the soil. The rate of carbon entry into the soil is relatively easy to measure, but the below ground exchange between the plant and soil pool is not well understood. (Jones, 2004: 460) Laboratory measurements of carbon flux are inaccurate; they often negate or ignore the effects of readsorption by the roots, and fail to account for the carbon added to the soil by dead or

dying roots. (Jones and others, 2004:463) Significant plant factors in carbon flux are exudation, readsorption, and root decay.

*Exudation.* It is clear that plants exudates a significant amount of carbon sources. Studies from vegetative and cereal crops show that carbon is transported from leaves to the external environment around the roots in less than an hour from photosynthesis. (Jones and others, 2004:463) Dilkes and others found exudation is a function of carbon flux into the root, and not necessarily coupled to rates of photosynthesis. (Dilkes and others, 2004; Jones and others, 2004:464) In barley and wheat plants, carbon exuded into the rhizosphere may account for 14-40 percent of all carbon fixed by the plant. (Hojberg, 1993: 431) Some of the exuded C is absorbed in microbial biomass with slower turnover (30-90 days). It is likely that a slight change in soil chemistry could result in significant changes in flux. (Jones and others, 2004:464)

The plant may be able to regulate microbial activity through the exudation of organic acids. Efflux of organic acids can be enhanced by an order of magnitude by opening organic acid-specific anion channels. (Ryan and others, 2001) Organic acids are not needed by the plant, and it does not actively readsorb them. Microbial communities use amino acids and sugars primarily for growth; organic acids are primarily used in respiration. Organic acids would not, then induce microbial proliferation in the rhizosphere, but could support resident populations. (Jones and others, 2003)

*Readsorption.* Plants can recapture amino acids and sugars, however there is no system to return organic acids back to the roots; it is speculated that organic acids play an important role in nutrient capture. This is consistent with findings that alkalinity values

in a fen reflect the presence of both bicarbonate and organic acid anions. (Hite and Cheng, 1996:423)

*Root Decay.* Root decay may be a more significant source of carbon than exudation. Fine roots grown by the plant are in a constant state of growth and decay, excreting root cap mucilage, losing cells, and dying back. This sequence is shown in Figure 14. Consequently, a large percentage of carbon in the soil is likely derived from fine plant roots. However, the residence time of the carbon from fine-roots is not well understood or quantified. (Strand *et al.*, 2008: 456)

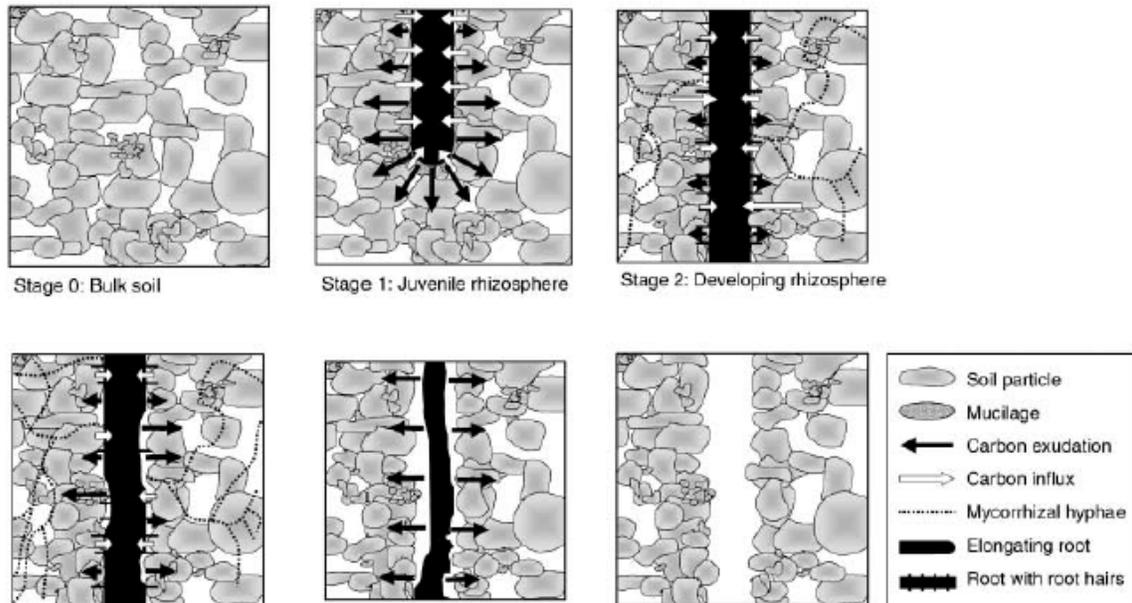


Figure 14. Carbon Release of a Root System. Sequence of root growth, mucilage exudation, and carbon release as the root dies back. Bacterial growth is highest during stage 3. (Jones and others, 2004:466)

### Nitrogen.

It is likely that plants can only access low molecular weight dissolved organic nitrogen such as amino acids, peptides, and urea. Low concentrations of dissolved organic nitrogen in the rhizosphere show that strong competition exists. (Jones and

others, 2004:470) It has also been shown that methane oxidation by methanotrophs is increased by nitrogen availability. Competition between methanotrophs, heterotrophs, and the plant itself may further restrict the growth of methanotrophs. (van Bodegom *et al.*, 2001: 3596)

### **Microbial Communities**

In nature, there is very rarely a pure culture; the rhizosphere is no exception and plays host to numerous bacteria, fungi, and other microbial organisms. The organisms interact with components of the plant, soil, water, and each other. Given constant inputs/outputs to the system for a long period, these components can establish a dynamic steady-state. Understanding the dynamics of wetlands is further complicated by the close proximity of greatly differing aerobic and anaerobic zones due to the presence of root structures. Currently, exact knowledge about microbial populations responsible for degradation processes is limited. (Kuiper, 2004:10) Additionally, the collective effect that microbes play on each other in the remediation process is uncertain. This study seeks to understand the behavior of the aerobic organisms used in remediation; it examines the energy and substrates available in the rhizosphere and the microbial interactions that affect oxygen levels.

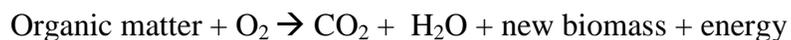
#### Microbial energy.

Most cells obtain energy by the oxidation of organic carbon compounds, reducing the available carbon to a more negative valence. Carbohydrates provide both the building materials for cells and energy that the cells need for metabolism. Energy can be obtained

by respiration where an inorganic molecule acts as an electron acceptor, or fermentation, where an organic molecule plays the role as an electron acceptor. Respiration reactions provide the most energy to the cells; the use of oxygen as an electron receptor provides the greatest amount of energy and is termed *aerobic respiration*. Organisms that use oxygen, then, are likely to dominate in areas of high oxygen, and oxygen will be used preferentially to other electron receptors. Eukaryotes are characterized by the ability to only use oxygen as a final electron receptor, however many prokaryotes, like the bacteria, can use alternative electron receptors for anaerobic respiration. With decreasing energy return, nitrate, nitrite, sulfate, some metals, carbon dioxide, and even carbon monoxide can be used by many bacteria for respiration. Additionally, alternative electron receptors, such as the halogens available in many environmental contaminants, permit a greater energy return than other available electron acceptors and are often removed from their parent compounds during anaerobic respiration processes. This results, conveniently, in the reductive dehalogenation of these contaminants, usually with the beneficial effect of reducing the toxicity of the contaminant.

#### Substrate Use.

Respiration must also be accompanied by an electron donor. This role is normally filled by a carbon source and the carbon is oxidized to a higher valence state by an electron acceptor like oxygen.



Monod growth: As long as all needed substrates are available in sufficient quantity, bacterial growth is not inhibited. However, when substrates are depleted below

a certain level, they begin to decrease the growth rate of bacterial communities that rely on that substrate. For modeling purposes, this is often represented by *Monod kinetics*, where the maximum growth rate of the bacteria is multiplied by a factor that decreases the growth rate at low concentrations of substrate.

$$\mu = \mu^{\wedge} * [ S / (S + K_S) ]$$

where  $\mu$  is the adjusted growth rate

$\mu^{\wedge}$  is the maximum growth rate for the population

S is the concentration of the required substrate or nutrient

$K_S$  is the half-saturation coefficient of the required substrate or nutrient

Organisms need many substrates: an energy source; electron acceptor; sources for carbon, nitrogen, and phosphorus, and other essential nutrients; and other organic growth factors.

*Liebig's law of the minimum* states that the nutrient in shortest supply will limit growth.

The same Monod approach can be applied to these requirements. (Vaccari, 2006: 323)

The addition of other limiting substrates, such as dissolved oxygen, can be accounted by adding additional expressions to the Monod model where A and B denote each particular substrate:

$$\mu = \mu^{\wedge} [ S_A / (S_A + K_A) ] [ S_B / (S_B + K_B) ]$$

As described above, however, there may be other electron acceptors that the bacteria are able to use in sequence according to either energy return or preference of the bacterial species. Likewise, they may not depend on only one substrate, and may be able to utilize several organic substances that are all available at different concentrations in the soil. This is sometimes addressed by using a general measurement of organic matter, such as biochemical oxygen demand (BOD) or chemical oxygen demand (COD) as a

representation of carbon source availability. Mixtures of micro-organisms are similarly lumped together in order to define characteristic growth behavior. The applicability of the Monod model, then, may depend upon the specific organism involved; knowledge of the substrates used by the organism will limit the compromise of this model. (Vaccari, 2006: 329) Microbial growth in the rhizosphere is thought to be primarily nitrogen limited. (Jones and others, 2004: 470)

The utilization of the substrates addressed by the Monod equation can also be calculated. The rate of substrate utilization is proportional to the growth rate and the rate of yield, expressed by a yield coefficient. Substrate removal can be calculated by:

$$dS/dt = [\mu^{\wedge}/Y] [ S / (S + K_S) ] X$$

where Y is the Yield [biomass produced / mass of substrate utilized]  
and X is the biomass of the consuming organisms

A value of .5 to .6 is a typical yield value for heterotrophic bacteria, but can be greater than 1 for many hydrocarbons as well as oxygen when being used as an electron receptor. (Vaccari, 2006: 328)

### Cometabolism.

Bacteria produce enzymes to digest the substrates used for growth and energy. Other compounds in the environment, however, can also be acted upon by the enzymes produced. This results in the breakdown of the secondary substrate, but has no beneficial return to the bacteria that produced the enzyme. In the case of chlorinated solvents, the methane mono-oxygenase (MMO) used to digest methane also breaks bonds in TCE, DCE, and vinyl chloride. In addition to the enzyme, oxygen and a source of reducing

potential (usually in the form of NADP) are also required to facilitate the reaction.

(Alvarez-Cohen and Speitel, 2001: 106)

*Competitive Inhibition.* The secondary substrates compete with the primary substrate for active enzyme sites. Enzymes facilitating cometabolic reactions often have several sites that can react with a number of various substrates, and when multiple substrates are available, additional competition can result in decreased transformation rates for each substrate. This results in *competitive inhibition* of bacterial growth by limiting the amount of primary substrate available for digestion.

*Non-competitive Inhibition.* Toxic agents can lower the overall growth coefficient of a bacterial population and decrease its reaction rate with a substrate. This is known as substrate inhibition or *non-competitive inhibition*. Inhibition may result from: 1.) a substrate normally used for growth at unhealthy high concentrations, 2.) a byproduct of cell metabolism, or 3.) other various external factors and substances. (Vaccari, 2006: 338) Each of these effects can be modeled by a modification of the Monod equation and is known as the *Andrews model* (analogous to a Haldane expression as applied to biological processes):

$$\mu = \mu^{\wedge} \left[ \frac{S}{S + K_S} \right] \left[ \frac{K_I}{S + K_I} \right]$$

where  $K_I$  is the half-inhibitory concentration.

When  $K_I \gg S$ , the expression reverts to the original Monod equation. As the concentration of inhibitory agent increases, the growth rate is reduced and asymptotically approaches zero at high concentrations as shown in Figure 15.

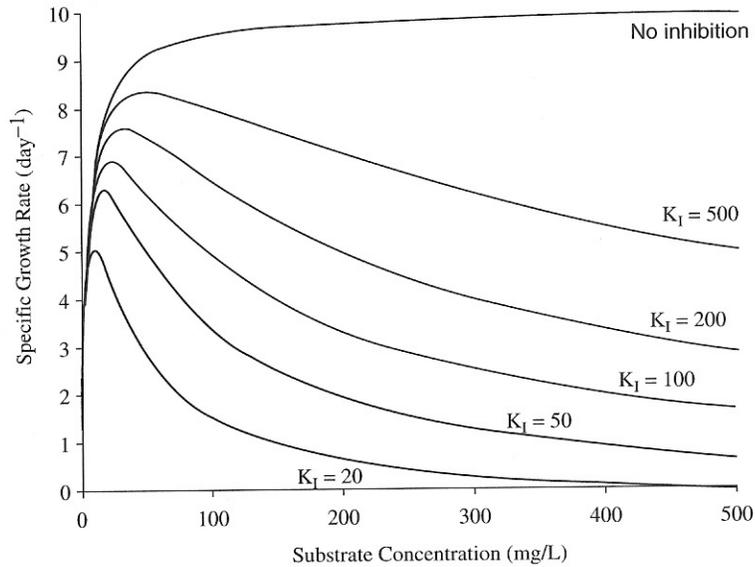


Figure 15. Andrews Model of Substrate-Inhibited Growth. (Vaccari, 2006:339)

*Cell Toxicity.* By-products of the secondary substrate can also be toxic to the bacteria producing the enzyme, degrading the enzyme or resulting in cell death. Toxic substances resulting from cometabolism broadly affect all cellular functions and result in cell inactivation that is proportional to the amount of compound degraded. (Chu and Alvarez-Cohen, 1999: 766; Alvarez-Cohen and Speitel, 2001: 108)

### Methanotrophs.

The methanotrophs/methylotrophs are a group of aerobic, gram-negative bacteria that use methane as their sole source of carbon and energy. They have a significant impact on the levels of methane in the soil. Oxygen availability limits the growth of the methanotrophs, and methane consumption rates are directly affected by the number of methanotrophs available. (Van Bodegom, 2001: 3591) The majority of methane in the soil is consumed by the methanotrophs; a smaller percent is vented through the stems and

leaves of wetland plants into the atmosphere. Calhoun attributed methane loss from methanotrophic activity associated with the wetland plants *P. cordata* and *S. eurycarpum* under oxic conditions to be 87.6 and 62.6% respectively; the remaining methane exited the soil by diffusive flux through the stems and leaves of the plants. (Calhoun, 1997: 3054) As a reference, methanotrophs comprised 1-2% of microbial biomass in rice paddy soils and tended to reach their highest numbers during flooded conditions. (Macalady *et al.*, 2002: 149) Growth factors significantly influencing methanotroph growth include oxygen, methane, and copper concentrations, nitrogen sources ( $\text{NO}_3$  and  $\text{NH}_4^+$ ), pH, and temperature. (Brigmon, 2001: 9)

*Methanotrophs and Nitrogen.* Like most bacteria, methanotrophs compete for nitrogen sources; while low additions of nitrogen increase methanotroph activity, high levels of nitrogen have resulted in decreased methane oxidation, likely due to competition from denitrifying bacteria. Few studies have focused on methanotroph response to nitrogen additions, (Bedard and Knowles, 1989; Conrad and Rothfuss, 1991; Van der Nat and Middelburg, 1998; Macalady *et al.*, 2002: 154) though Chu and Alvarez-Cohen (1999) did find that nitrogen-fixing methanotrophs may be responsible for enhanced TCE degradation activity (Chu and Alvarez-Cohen, 1999: 766), a result also likely from the low oxygen conditions associated with nitrifying conditions.

*Type I vs Type II.* Methanotrophs are divided into three groups, Type I, II, and X. Determining factors include intracytoplasmic membrane ultrastructure, enzymatic characteristics, fatty acid carbon lengths, G + C values, and 16S rRNA sequences. 16S RNA sequence analysis has identified eight genera of methanotrophs: *Methylococcus*, *Methylomonas*, *Methylomicrobium*, *Methylobacter*, *Methylocaldum*, *Methylosphaera*,

*Methylocystis*, and *Methylosinus*. These distinctions, however, are not all-encompassing. Type I methanotrophs utilize the *ribulose monophosphate* pathway to assimilate formaldehyde produced from the oxidation of methane; they usually have higher cell yields on methane than Type II strains. (Macalady *et al.*, 2002: 148) Type I possess bundles of intracytoplasmic membranes. Type II methanotrophs have their intracytoplasmic membranes arranged around the periphery of the cell and use the *serine* pathway for methane assimilation. This gives Type II strains greater oxygen affinity, allowing them to grow preferentially at low concentrations. (Macalady *et al.*, 2002: 148) Type X methanotrophs have characteristics of both Type I and II groups. (Brigmon, 2001: 2)

The methanotrophs and other bacteria are not limited to the rhizosphere alone. It is likely that both type I and II groups coexist in the rhizosphere, but occupy different niches; both groups are numerically important in wetland environments, specifically in rice paddies. (Macalady *et al.*, 2002: 153) In cases of extreme oxygen limitations, colonization of the root interior may be a possible methanotrophic behavior. (Calhoun, 1997: 3057)

*MMO and Copper Limitations.* Methanotrophs are able to express various forms of methane mono-oxygenase (MMO), the enzyme that is used to expedite the respiration of methane with oxygen. Two distinct forms of MMO have been reported: a soluble MMO (sMMO) that is found under copper-limiting conditions and is located in the cell's cytoplasm, and a particulate MMO (pMMO) that is seen in copper sufficient environments and is found in the intra-cytoplasmic membrane. (Field, 2004:31; Morton, 2000; Wackett, 1995) Most methanotrophs cannot express sMMO. (Murrell, 1992;

Hanson and Hanson, 1996; Alvarez-Cohen and McCarty, 2001: 113) Of those that can express sMMO, the polypeptides are only expressed at low concentrations of copper. sMMO acts over a much broader range of substrates than pMMO, and can also degrade a broader range of hydrocarbons. (Lee, 2006: 7503) Both forms of MMO are able to degrade pollutants like TCE, but at much different rates. (Morton 2000:1730) pMMO rates for TCE metabolism are often 0.1 to 1 percent those of sMMO, and pMMO cometabolism rates at low copper concentrations (50-300 ug/L) are even lower than those at high copper concentrations; at normal environmental concentrations < 150 ug/L, pMMO cometabolism rates are expected to be at their lowest values. (Alvarez-Cohen and McCarty, 2001: 113)

Copper can be a significant factor in the formation of methane monooxygenase, however it is unknown which forms of copper are bioavailable to methanotrophs. There is difficulty measuring the affects of changing copper concentrations due to testing that is artificially biased by culture growth medium. (Morton, 2000: 1730) A number of behaviors can be inferred from the Table 2 below which compares iron and copper concentrations in two different agar solutions. As copper levels are increased, iron precipitates, free Cu increases, and precipitated Cu increases. In both solutions, though, the precipitated Cu to free Cu ratio climbs as more copper is added, with free Cu<sup>+1</sup>, the reduced form, created by the oxidation of the iron. In the NMS solution, this ratio is reduced only after 100% iron precipitation is achieved and the iron can no longer force the reduction of copper back to Cu<sup>+1</sup>. There appears to be a copper saturation effect by 5 uM when cells cease to incorporate copper and precipitated copper accumulates more quickly than free copper. The iron rich NMS culture results in higher levels of sMMO

activity, further showing that sMMO is expressed when copper exists in its reduced form. Altogether, the study shows the important relation that iron plays on copper availability; the presence of abundant copper may also oxidize iron in low oxygen conditions, however, iron is normally exceedingly abundant in natural settings.

Table 2. Equilibrium Metal Speciation for Media with Different Copper Concentrations. (Morton, 2000: 1731)

Medium	Metal speciation	Result obtained with total copper ( $\mu\text{M}$ )				
		0.02	0.11	5	10	20
M2M	Chelated Cu (%)	100	100	99	96	94
	Precipitated Cu (%)	0	0	0.5	3.6	5.6
	Free Cu (%)	0.089	0.048	0.013	0.014	0.015
	pCu <sup>b</sup>	10.7	10.3	9.2	8.8	8.5
	Soluble Fe (%)	16	9	14	11	11
	Precipitated Fe (%)	84	91	86	89	89
NMS	Chelated Cu (%)	100	94	93	70	59
	Precipitated Cu (%)	0	5.9	7.2	30	38
	Free Cu (%)	0.16	0.14	0.08	0.21	2.8
	pCu	10.3	9.8	8.4	7.7	6.3
	Soluble Fe (%)	99	99	68	28	0
	Precipitated Fe (%)	1	1	32	62	100

<sup>a</sup> M2M and NMS have total iron concentrations of 0.9 and 9  $\mu\text{M}$ , respectively.

<sup>b</sup> Free copper concentrations at equilibrium, reported as the negative log of  $[\text{Cu}^{2+}]$ .

In a study of a eutrophic freshwater lake in Switzerland, Xue and Sigg (1993) found that free copper concentrations were 6-7 orders of magnitude lower than the total concentration of copper present. The free  $[\text{Cu}^{2+}]$  measured in the lake was low and could not be explained by the presence of EDTA alone. It is likely that the presence of organic ligands that strongly complex with Cu(II) resulted in low free  $[\text{Cu}^{2+}]$ . (Stumm, 1996:625)

In the rhizosphere, it is possible that organic ligands that are present could complex with Cu(II) and result in low levels of available Copper. In natural aqueous systems, free  $\text{Cu}^{2+}$  dominates copper species up to pH 6.  $\text{CuCO}_3$  dominates from pH 6-9.3,  $\text{Cu}(\text{CO}_3)_2^{-2}$  from 9.3-10.7,  $\text{Cu}(\text{OH})_3^-$  from 10.7-12.9, and  $\text{Cu}(\text{OH})_4^{-2}$  beyond pH 12.9. (Stumm, 1996: 399)

Most natural wetland soils are buffered around pH 7, although radial oxygen loss does lower the pH at root surfaces. (Bezbaruah and Zhang, 2004: 65)

While copper concentrations can be a limiting factor at low levels, high levels can also have adverse effects; high concentrations of copper are toxic to microbes. Kalabina *et al.* (1944) showed an appreciable decrease in bacteria beyond 0.1 mg/L of copper, and concentrations above 0.5 mg/L retard all microbiological processes. (Stumm, 1996)

*MMO and Energy.* Oxygenase enzymes consume molecular oxygen as well as reductants like NADP during the oxidation of cometabolites and substrates alike. Primary substrates provide energy that can be used to regenerate reductant, but they also interfere with the consumption of the cometabolite by competitive inhibition. Cometabolic reactions, however, do not replenish the energy they consume. Consequently, the rate of cometabolic reaction can be limited by the amount of reductant available. Consequently, high concentrations of cometabolite can lead to rapid exhaustion of reductant sources. The use of alternate electron receptors, such as formate, that assist the regeneration of reductant have been shown to sustain high TCE transformation rates. (Alvarez-Cohen and Speitel, 2001: 107)

*MMO and Reduced Iron.* There is evidence that iron also affects sMMO activity. (Morton 2000:1732) The presence of iron in reduced form may have an effect on copper availability by reducing the copper as the iron is oxidized. Cells may only be able to absorb the copper in its oxidized free form,  $\text{Cu}^{+2}$ . This may suggest that cells are unable to express pMMO in low eH environments due to the oxidized copper limitation, and would resort to sMMO activity in those environments, likely absorbing  $\text{Fe}^{+2}$  and using it

in place of  $\text{Cu}^{+2}$  in the enzyme. The higher eH around the root zone likely oxidizes both copper and iron, making copper bioavailable for use in pMMO enzymes.

Figure 16 represents a theoretical relationship between iron, copper, and MMO expression. In high redox conditions, copper is in oxidized form and the full concentration that exists is available for pMMO expression. When substrate/cometabolite oxidation occurs, the copper of pMMO is reduced and needs an outside electron acceptor, like NADH, to oxidize the copper and allow it to reactivate the pMMO. In low redox conditions, reduced iron reduces the available copper and makes it unavailable for pMMO expression unless the copper is oxidized by NADH. When no NADH is present to oxidize the copper, reduced iron can take the place of copper in the MMO, creating sMMO that is less selective than the pMMO formed in high eH conditions. Oxidation by sMMO may result in the sequential dehalogenation of TCE.

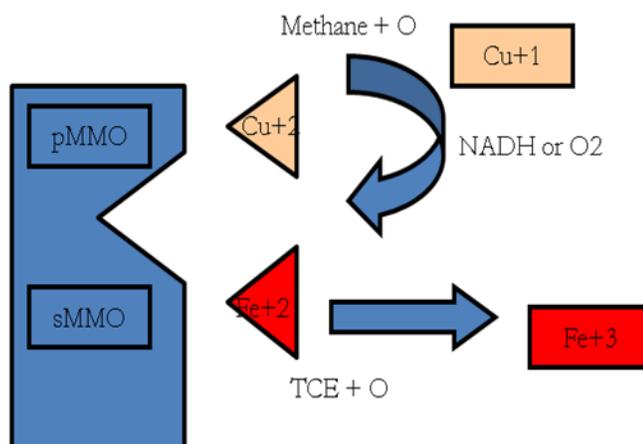


Figure 16. Thompson Conjecture of sMMO/pMMO Expression.

It was previously believed that Type II (*Methylocystis*, and *Methylosinus*) and X (*Methylococcus*) were the only groups that could produce sMMO (sMMO covered in greater depth below). A Type I strain 68-1 of *Methylomonas methanica*, however, was

shown to have the ability to produce sMMO and demonstrated significantly greater rates of TCE degradation than the most popularly studied bacterium capable of TCE cooxidation, *Methylosinus tichosporium* OB3b. (Field, 2004: 31) Napthalene oxidation assay, an indicator of sMMO activity, however, showed that 68-1 sMMO substrate affinity was substantially lower than that of OB3b. (Koh, 1993:960) While the strains had little genetic homology, the expression of a similar enzyme is a common characteristic across the groups. Another methanotroph, *Methylocella silvestris*, has been identified as being a possible facultative anaerobe. (Lee, 2006: 7508) This species possesses only the sMMO, further validating the possibility that sMMO is limited by copper availability and inability to use the Cu+1 that is present in the reducing environments favorable to the strain. It is possible that Type II and X species that typically produce sMMO are more adapted to surviving in low oxygen conditions; expression of sMMO is simply a result of the environmental conditions in which they live. (Thompson conjecture)

*MMO and Oxygen Limitations.* TCE degradation activity is unstable in the presence of oxygen. This is likely a result of the oxidation of copper that occurs during high redox conditions. While some oxygen is required in order to maintain methanotroph activity, oxygen concentrations greater than 2 mg/L result in decreasing TCE degradation rates. Aeration of cells with oxygen results in damage specifically to the sMMO enzyme and has little effect on the cells themselves. (Chu and Alvarez-Cohen, 1999: 766) Below 2 mg/L, oxygen becomes the rate limiting step in methanotroph growth, however TCE degradation is unstable in the presence of oxygen. Two mg/L represents an optimal point that balances TCE degradation and methanotroph growth. (Uchiyama *et al.*, 1995: 611)

Figure 17 shows the influence of oxygen levels on TCE degradation. A dynamic relationship also exists between Type I and Type II methanotrophs; Type I grow rapidly in higher oxygen levels by using the more selective pMMO, while Type II grow more slowly at low oxygen concentrations and exhibit the less-selective sMMO enzyme that results in higher degradation rates but also increased cell toxicity. The 2 mg/L maximum observed may be a result of that dynamic relationship, showing that sMMO degradation is balanced by pMMO expression.

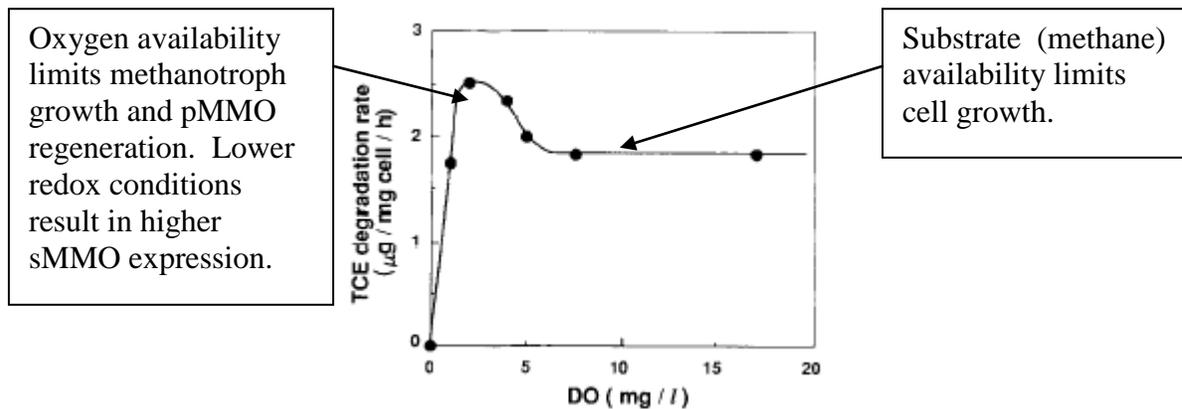


Figure 17. Dissolved Oxygen Relationship to TCE Degradation. Point of maximum TCE degradation by a methanotrophic culture shows the balance between sMMO expression that optimizes degradation and high oxygen that optimizes methanotroph growth. (Uchiyama *et al.*, 1995: 610)

*Cometabolism and Competitive Inhibition.* Primary substrate is required in order to sustain bacteria growth and regeneration during cometabolic reactions. High concentrations of the primary substrate, however, may be detrimental to remediation effects due to the competition with the cometabolic substrate for enzyme sites. For methanotrophs, TCE degradation rates have been shown to increase with the addition of low amounts of methane up to 0.1 mM. Beyond 0.1 mM, competitive inhibition results in a decrease of TCE degradation rate. (Alvarez-Cohen and Speitel, 2001: 107)

*Cometabolism and Non-Competitive Inhibition.* It is well-accepted that cometabolism of chlorinated solvents has a negative effect on the growth of methanotrophs, and it has been demonstrated that methanotrophs expressing sMMO have a lower growth rate than those that express pMMO. (Lee, 2006: 7504) Exposure to TCE often leads to the inactivation of the MMO enzyme and indirectly inhibits cell growth, decreasing cell activity in proportion to the accumulation of inhibitory products. (Arp and Hyman, 2001; Alvarez-Cohen and Speitel, 2001: 109; Chu and Alvarez-Cohen, 1999: 766) Growth inhibition is caused by the inability of sMMO to derive energy from the compounds being cometabolized. It is possible that TCE inactivation of sMMO occurs through loss of iron from the hydroxylase component of the enzyme or reaction with TCE epoxide hydrolysis products. (Koh, 1993: 965)

*Cometabolism and Bacterial Toxicity.* When cells process TCE, they suffer from adverse effects that lead to enzyme dysfunction and cell death. TCE itself does not cause direct toxicity to cells. When MMO inhibitors were applied to methane and ammonia oxidizing cultures, the cells no longer showed toxic effects from TCE. It is likely, then, that the oxidized intermediates of TCE like TCE epoxide, rather than the TCE itself, are toxic to bacteria. The degradation process, not the TCE itself, is responsible for cell inactivation. (Field, 2004: 32; Alvarez-Cohen and McCarty, 1991; Rasche *et al.*, 1991) The intermediate products are transient, do not accumulate appreciably, and the effects of the intermediates on the cells are likely immediate. (Alvarez-Cohen and Speitel, 2001: 109; Arp and Hyman, 2001) Knowledge of the specific nature of the toxicity to the cells and their ability to recover is not known; it is difficult to experimentally distinguish between active cells and cells that suffer from toxic effects. (Alvarez-Cohen and Speitel,

2001: 120) TCE toxicity does play a limiting role in bioremediation, especially at high concentrations of contaminant.

In the case of TCE, byproducts DCE, VC, and other chlorinated intermediates can also have toxic effects on the cells. Normal pathways of TCE metabolism by bacteria expressing the MMO enzyme are shown in Figure 18.

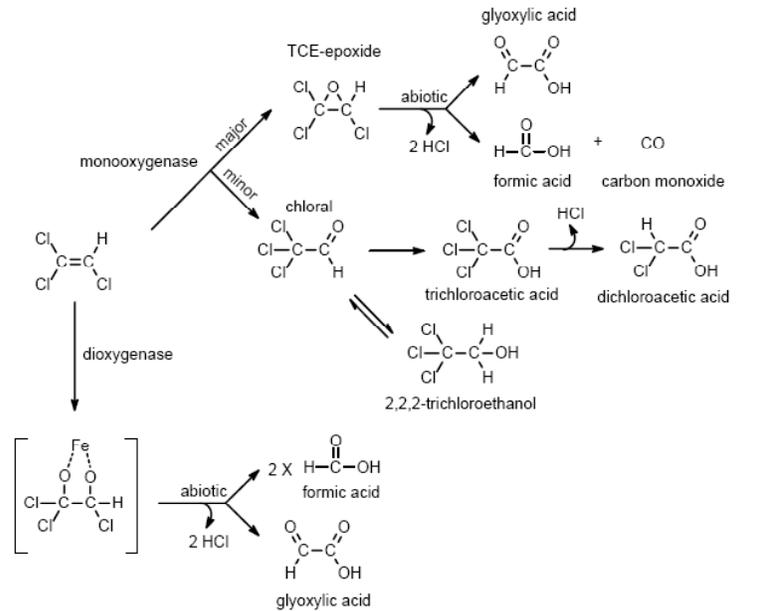


Figure 18. TCE Monooxygenase Cooxidation Pathways. (Field, 2004: 33; Wackett, 1995)

A 1996 study of four methane-oxidizing cultures by Chang and Alvarez-Cohen found that the transformation capacity for chlorinated aliphatic hydrocarbons was, generally, inversely proportional to its chlorine content. Product toxicity of chlorinated compound mixtures was found to be cumulative and was predictable using parameters measured for the compounds individually by the following equation (Chang and Alvarez-Cohen, 1996: 3372):

$$dX = \sum_i dX_i = \sum_i \left( \frac{dS_i}{T_{ci}} \right)$$

where X is the bacterial population

S is the cosubstrate consumed

And  $T_{ci}$  is the transformation capacity by the bacteria for the i'th cosubstrate

Degradation rates reflected affinity of the substrate for the oxygenase enzyme and different levels of inhibition from methane. Notably, 1,1 DCE exerted a much higher toxic effect than cDCE and tDCE (Chang, 1996: 3375), possibly owing to the asymmetric arrangement of chlorines. (Chang, 1996: 3371; Dolan, 1995) Figure 19 shows that TCE is initially degraded at a faster rate than cDCE and VC. This may be due to a higher selectivity of MMO for TCE. This is significant because, at low concentrations, degradation products of TCE (cDCE and VC) can accumulate, increasing competitive inhibition for MMO, and increasing cumulative toxic effects on the bacteria. At higher concentrations, cDCE and VC degrade more quickly than TCE.

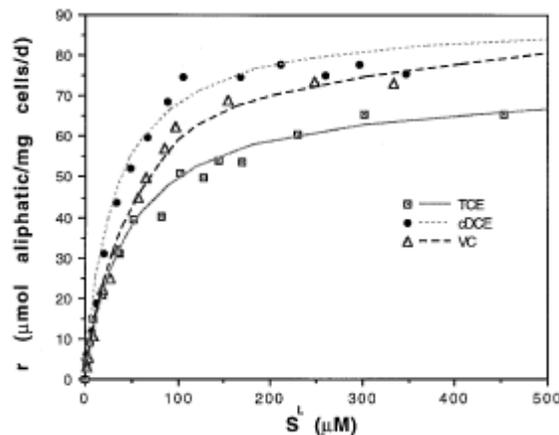


Figure 19. Relationship of Contaminant Concentration and Degradation Rate. Michaelis-Menten (Monod) curves for TCE, cDCE, and VC degradation by a mixed methanotroph culture. (Chang and Alvarez-Cohen, 1996: 3374)

*MMO Inhibition vs Toxicity.* The competing high transformation rates of sMMO and high yield rates of pMMO expressing methanotrophs result in optimum degradation of TCE at different concentrations. At pollutant concentrations less than 10 $\mu$ M, sMMO-expressing cells tend to degrade pollutants most quickly; the broader substrate range of the enzyme likely enables the sMMO cells to degrade a larger fraction of the pollutants than pMMO cells. At pollutant concentrations above 100 $\mu$ M, however, the pMMO methanotrophs grow fastest and ultimately digest more pollutant. (Lee: 2006:7503)

Table 3 shows that sMMO expressing methanotrophs have a higher growth rate when no contamination is present, but when contaminant is introduced, their growth rates quickly slow. pMMO types, however, maintain higher growth rates at higher contaminant concentrations since the pMMO enzyme is more specific (lower  $K_s$ ) to methane (Table 4).

Table 3. Growth and Degradation Rates of OB3b Cells Expressing pMMO or sMMO at Various Contaminant Concentrations. pMMO expression results in higher maintained growth at higher pollutant concentrations where sMMO cell densities are lower due to impaired growth. (Lee, 2006: 7507)

Enzyme	Substrate(s)	$\mu$ ( $h^{-1}$ ) (SD) <sup>a</sup>	$\mu/\mu_0$ (SD) <sup>a</sup>	Max OD <sub>600</sub>
pMMO	CH <sub>4</sub>	0.052 (0.005)	1.0	0.51
	CH <sub>4</sub> + 10 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.030 (0.002)	0.58 (0.07)	0.43
	CH <sub>4</sub> + 30 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.036 (0.001)	0.69 (0.07)	0.50
	CH <sub>4</sub> + 50 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.018 (0.0005)	0.35 (0.04)	0.42
	CH <sub>4</sub> + 100 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.014 (0.0005)	0.27 (0.03)	0.27
sMMO	CH <sub>4</sub>	0.064 (0.004)	1.0	0.49
	CH <sub>4</sub> + 10 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.025 (0.001)	0.39 (0.03)	0.40
	CH <sub>4</sub> + 30 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.031 (0.001)	0.48 (0.03)	0.42
	CH <sub>4</sub> + 50 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.016 (0.001)	0.25 (0.02)	0.20
	CH <sub>4</sub> + 100 $\mu$ M each VC, <i>t</i> -DCE, and TCE	0.007 (0.001)	0.11 (0.02)	0.14

<sup>a</sup> Numbers in parentheses represent the standard deviations of collected samples.

Table 4. OB3b Chlorinated Ethylene Michelis-Menten Degradation Coefficients. This shows the much higher rates of contaminant degradation ( $V_{max}$ ) achieved by sMMO expressing cells. The much higher half-saturation constants ( $K_s$ ) reflect lower enzyme specificity for a compound. Degradation rates are lowest for TCE, however the affinity of MMO for TCE is greater than that for t-DCE or VC. (Lee, 2006:7505)

Enzyme	Substrate	$V_{max}$ (nmol · min <sup>-1</sup> · mg protein <sup>-1</sup> )	$K_s$ (μM)	Reference or source
pMMO	CH <sub>4</sub>	82	8.3	32
	VC	42	26	This study
	t-DCE	61	42	This study
	TCE	4.1	7.9	32
sMMO	CH <sub>4</sub>	726 <sup>a</sup>	92	36
	VC	2,100	160	This study
	t-DCE	662 <sup>a</sup>	148	36
	TCE	580 <sup>a</sup>	145	36

<sup>a</sup> Converted from reported units of nmol · min<sup>-1</sup> · mg cells<sup>-1</sup> assuming that the cell dry weight is 50% protein.

*MMO and Remediation.* The differences between methanotrophs expressing sMMO and pMMO make them suitable for different remediation strategies. Sites with high pollutant concentrations (VC, DCE, TCE > 30 μM) should stimulate pMMO expression, possibly by the addition of copper or the raising of eH. Were sMMO strains to be used in high concentrations of contaminants, they would quickly exhaust growth by counterproductive oxidation of the contaminants and toxic accumulation. sMMO strains may degrade a wider variety of contaminants at low concentrations and can be stimulated at contaminant concentrations below 30μM, the point of negative net rate growth substrate turnover. (Lee, 2006: 7508) This could be stimulated by the addition of reduced forms of iron that would reduce available copper and make it unavailable to the methanotrophs, resulting in the expression of sMMO. Growth of pMMO strains was also limited by the presence of chlorinated ethylenes at high concentrations, but to a much lesser extent than the effect on sMMO strains. (Lee, 2006: 7507)

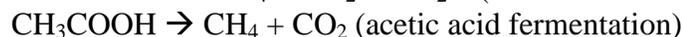
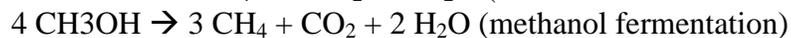
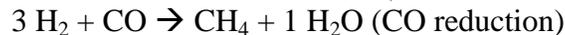
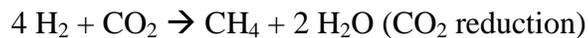
Cometabolism of a contaminant reduces the MMO used and requires an expenditure of energy. To restore the transformational capacity of the MMO enzyme, it must have a source of reducing power. NADH must be used to regenerate MMO after it transforms either methane or TCE. Depletion of NADH, then, can also limit the extent of TCE degradation and energy requirements must be considered in a wetland treatment system. When digesting methane, the energy resulting from transformation can satisfy this requirement. Optimal conditions for TCE degradation in methanotrophic bioreactors generally exist between 4% and 20% methane. (Brigmon, 2001: 9; Strandberg *et al.*, 1989) Addition of formate as an exogenous electron acceptor has also been shown to increase rates of TCE transformation. (Anderson, 1994, 383; Alvarez-Cohen, 1991; Brussea, 1991; Henry and Grbic-Galic, 1991; Oldenhuis, 1991)

### Heterotrophs.

For heterotrophic organisms, the availability of organic material is normally the limiting factor for growth. In soils and wetlands, survival may depend upon the ability to survive on low levels of organic substrates and the ability to grow quickly where higher concentrations are available. (Vaccari, 2006: 398) The root-zones of wetland plants are one such area of high carbon concentration, and the heterotrophs are able to capitalize on the availability of the organic substrates that are released by the plants. Organic acids are especially crucial to some bacteria. Lugtenberg *et al.* (1999) demonstrated the significance of organic acids on soil bacteria by studying auxotrophic mutants, showing that those with an impaired ability to use organic acids were significantly impaired in root colonization.

## Methanogens.

Methanogens are not actually bacteria. Although considered bacteria for many years, methanogens are now recognized as belonging to the Euryarchaeota kingdom in the domain of Archaea. Methanogens, however, are the Archaea of greatest scientific due to the critical role they play in the carbon cycle. They are strict anaerobes that live in a range of environments including freshwater wetlands and the digestive tracts of animals, including humans. Methanogens are characterized by their exclusive role in methanogenesis. Methanogenesis, the production of methane (CH<sub>4</sub>), is a reaction where carbon is reduced to methane, usually by oxidation with hydrogen. Most commonly CO<sub>2</sub> is used as the electron receptor, although CO is also reduced. Many methanogens can also obtain energy by fermentation of formic acid (CHOOH), methanol (CH<sub>3</sub>OH), and acetic acid (CH<sub>3</sub>COOH), oxidizing some molecules to CO<sub>2</sub> while reducing others to CH<sub>4</sub>. (Vaccari, 2006: 266, 395)



In the wetland environment, the methanogens play a crucial role for the methanotrophs: they provide substrate that the methanotrophs depend upon; the anoxic areas in the wetland result in high levels of methane as methanogens produce it by the reduction of carbon-dioxide. Additionally, they consume acetate and H<sub>2</sub> being produced by fermentative bacteria that could build to inhibitory concentrations.

### Quantification of Microbial Activity.

With all the species of bacteria available, difficulty with pure cultures in laboratories, and trouble quantifying bacterial colonies, determination of the number of bacteria in a wetland environment is limited. A 2001 study by Van Bodegom and all focused on incubation of microbes from a specific wetland community, a rice paddy. The study showed that heterotrophs and methanotrophs were the most abundant bacterial groups at all tested conditions. Based on rate constants, it is likely that heterotrophic and methanotrophic respiration are the most important microbial sinks of oxygen. (Van Bodegom, 2001: 3590) Other microbial groups played a minimal role in the consumption of oxygen in the rice rhizosphere. (Van Bodegom, 2001: 3589)

The most abundant species of methanotrophs and heterotrophs were isolated and tested under various growth conditions. Methanotrophs showed a lower  $K_{S,O_2}$  and  $u_{max}$  than the heterotrophic cultures. This means that the methanotrophs were at a disadvantage to the heterotrophs in terms of a lower maximum growth rate, but they had an advantage over heterotrophs since that growth was less inhibited at low oxygen levels. A measurement of heterotrophic and methanotrophic oxygen consumption,  $O_2, crit$  was compared. Heterotrophs likely consume most oxygen close to the root surface, while methanotrophs are more prolific further from the root surface at lower oxygen concentrations. (Van Bodegom, 2001:3590)

Methane consumption was correlated to oxygen consumption, and oxygen was found to limit methane oxidation rates under most conditions. The authors speculated that significant methane oxidation could occur in the rice rhizosphere at microaerophilic, low acetate, and high methane concentrations and will thus occur at very specific

microsites within the rice paddy. They additionally cited nitrate as playing a limiting role in methanotroph growth (Van Bodegom, 2001:3596) Distinctions are also made between Type I and II methanotrophs. Type II methanotrophs outcompete Type I in conditions of ample methane due to their ability to fix nitrogen and ability to use lower levels of oxygen (low  $K_{S,O_2}$ ). Monod substrate and growth relationships for heterotrophs and methanotrophs are included as Table 5 and Table 6.

Table 5. Monod Half-Saturation Constants for Heterotrophs. (Van Bodegom, 2001:3591)

Culture(s)	$K_{s,O_2}$ (M)	$K_{s,Ac}$ (M)	$\mu_{max}$ (h <sup>-1</sup> )
<i>Pseudomonas</i> sp. strain HET-1 C limited	n.d. <sup>d</sup>	$0.36 \times 10^{-3}$	0.054
<i>Pseudomonas</i> sp. strain HET-1 O <sub>2</sub> limited	$15 \times 10^{-6}$	n.d.	0.062
<i>Rhodococcus</i> sp. strain HET-2 BOM	n.d.	n.d.	0.12
<i>Rhodococcus</i> sp. strain HET-2 C limited	n.d.	$1.3 \times 10^{-3}$	0.12
<i>Rhodococcus</i> sp. strain HET-2 O <sub>2</sub> limited	$9.0 \times 10^{-6}$	n.d.	0.098
Published pure	$(9.4 \pm 12.7)^a \times 10^{-6}$	$(0.58 \pm 0.39)^b \times 10^{-3}$	$0.23 \pm 0.19^c$

<sup>a</sup> See references 6, 24, 41, and 54.

<sup>b</sup> See references 4, 23, and 58.

<sup>c</sup> See references 6, 24, 35, and 58.

<sup>d</sup> n.d., not determined.

Table 6. Monod Half-Saturation Constants for Methanotrophs. (Van Bodegom, 2001:3592)

Culture(s)	$K_{s,O_2}$ (M)	$K_{s,CH_4}$ (M)	$\mu_{max}$ (h <sup>-1</sup> )
<i>Methylocystis</i> sp. strain MOX 1 C limited	n.d. <sup>a</sup>	$28 \times 10^{-6}$	0.018
<i>Methylocystis</i> sp. strain MOX-1 O <sub>2</sub> limited	$1.9 \times 10^{-6}$	n.d.	0.017
Published pure	$(6.7 \pm 9.4)^b \times 10^{-6}$	$(29 \pm 22)^c \times 10^{-6}$	$0.12 \pm 0.04^d$

<sup>a</sup> n.d., not determined.

<sup>b</sup> See references 24, 30, and 47.

<sup>c</sup> See references 29, 30, 47, and 54.

<sup>d</sup> See references 24, 30, 47, and 54.

### Competition.

Bacteria and other microflora in wetlands are extremely diverse. (Amon *et al.*, 2007: 64) While some microbes may be mutually beneficial to each other, there is intense competition for all nutrients in a wetland, especially for oxygen. Microbial grazing by protists can also be a significant factor.

*Protozoa.* Protozoa are single celled organisms that contain both organelles and a formed nucleus. They are considerably larger than bacteria and can have a significant impact on bacteria in the rhizosphere by microbial grazing. They normally have flagella or cilia that help them to move in the soil.

*Arbuscular Mycorrhizal Fungi.* AMF are the most-common form of endomycorrhizae, a symbiotic and mutualistic fungus that lives in the vicinity of plant roots. In terrestrial plants, mycorrhizal fungi have been shown to have a significant impact by supplying plants with essential nutrients, especially phosphate,  $\text{NH}_4^+$ ,  $\text{K}^+$ , and  $\text{NO}_3^-$ . (Salisbury, 1992: 139) Root exudation patterns can be altered by AMF colonization; AM fungus is a large carbon sink (Douds and others, 2000; Graham, 2000, Jones and others, 2004: 472), alters carbohydrate metabolism, and increases root respiration. AMF can also alter microbial composition in the rhizosphere (Jones and others, 2004: 473) There has been relatively little study, however, of mycorrhizal associations in freshwater wetlands. It is often assumed that fungi are not as dominant as bacteria in wetlands, generally due to oxygen limitations in the saturated soils. (Mentzer, 2006; Gutknecht, 2006: 26) Their influence, however, can also be significant. In studies of arbuscular mycorrhizal fungi (AMF), Bohrer and all found significant colonization by AMF that was linked to plant growth patterns, specifically root production and vegetative growth; the highest levels of colonization occurred during high water tables. (Bohrer, 2004: 335) Despite substantial impacts of AMF and other fungi in the energy and carbon cycle, the overall impact of bacteria in wetland soils significantly outweighs that of fungi throughout the year. While the total mass of fungi in wetlands was greater than that of

bacteria, high growth rates and turnover times made bacteria the primary mediators of carbon flow. (Buesing, 2005: 596, 601)

### Soil and Microbial Influences.

Microbes around plant roots are largely dependent upon exudates and oxygen coming from the soil roots. They are also significantly influenced by factors in the soil itself. Some significant influences include metal concentrations, pH, eH, toxins, and hydrologic flow that moves water through the root zone.

### **Models and Modeling**

In wetland conditions, oxygen is used up quickly and plant rhizospheres are correspondingly thin; the oxic shell surrounding the roots varies from about .5 to 5 mm in thickness. (Christensen, 1994: 847) This complicates our ability to measure important characteristics of the rhizosphere. Computer modeling can help give important insight into oxygen concentrations, nutrient levels, and likely microbial interactions in the soil. Mathematical models can provide a theoretical basis for plant functions and identify gaps in knowledge.

Computer modeling is an important tool since it allows the manipulation of numerous variables that may not be changeable in another setting such as a laboratory or field test. This gives a model a great amount of flexibility. Data can be generated quickly in response to changes in variables. By accurately depicting the relationships of real-world components in the model with current knowledge, intuition can be gained on the behavior of the plant-soil system. The model does have limitations, however;

simplifications, lack of accurate data, and computing limitations all contribute to uncertainty. Despite uncertainties, a model may give qualitative insight that can be used to guide further research and offer explanations for system behavior. Mathematical models have been developed to describe plant processes, biological processes, and cometabolism.

### Plant Models.

*Phragmites australis* (common reed, carrizo), is a tall, rhizomatous reed with robust stems, tapering leaves, and a deep root structure. It is one of the more widespread, prolific, and useful remediation plants and is the most widely studied of the wetland plants. In 2000, Armstrong *et al.* conducted probe measurements of oxygen levels through the rhizosphere of adventitious roots in an agar solution, offering an accurate look at oxygen levels throughout the root and solution. A dynamic plant growth model of a *Phragmites* stands was developed by Asaeda and Karunaratne based on empirical data from numerous field studies. (Asaeda and Karunaratne, 2000) It includes calculations for photosynthesis and carbon fixation. The model was limited by lack of data on physical and biological growth factors, but it successfully reproduced all growth trends of the *Phragmites* stands studied. The study correlated higher growth rates of *Phragmites* with long growing seasons, higher solar radiation, and higher ambient temperatures. The model was later expanded and refined by Asaeda et al (Asaeda et al, 2002). Figure 20 shows some of the parameters measured for the stands.

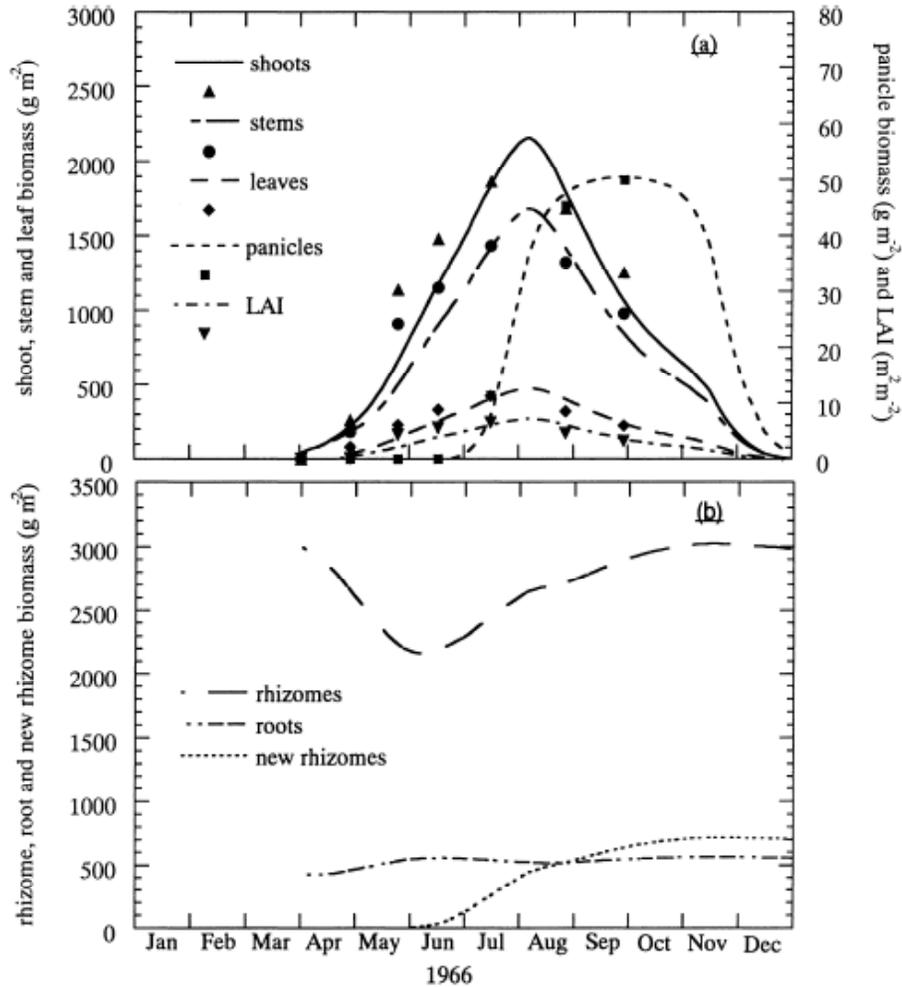


Fig. 1. Seasonal variation of (a) above-ground and (b) below-ground biomass of *P. australis* (Nesyt fishpond, Czech Republic). Observed data (represented by symbols) from Kvet et al. (1969). LAI: leaf area index.

Figure 20. *Phragmites australis* Parameters. (Asaeda et al, 2002)

### Air Movement.

In 1996, Armstrong and all used a mathematical model to demonstrate the humidity induced convection concept (HIC) based on the earlier model of Leuning (1983). It incorporated the effects of Knudsen diffusion for pores smaller than 0.1  $\mu\text{m}$  and Poiseuille flow resistance for pores greater than 0.1  $\mu\text{m}$ . The model was mathematically less rigorous than the Leuning model but gave nearly identical results.

The findings reiterated earlier findings that plant leaf pore diameters were optimized to support high dynamic pressures while still maintaining high airflow rates, and suggested that the flows in *Phragmites* result from optimal pore sizing and low venting resistance in the plant. Fastest flow rates were generated with 0.2  $\mu\text{m}$  pore sizes. The model can be used to calculate the water movement out of the leaves and the resulting convective flow into an air passage. The formulae, involving Knudsen resistances, can also be used to calculate flows of the various gases into the leaf pore spaces. The results of the model were compared against a physical model that used micro-porous partitions in place of a membrane. (Armstrong, 1996)

P. M. Beckett et al also used a modeling approach to analyze pressure flow in *Phragmites* stands. Static pressure ( $P_s$ ) was defined as the pressure generated inside the culm under zero flow conditions. Dynamic pressure ( $P_d$ ) is the pressure when flow is taking place. The delivery coefficient ( $1-P_d/P_s$ ) is a measure of the degree to which a culm is achieving its full convective flow potential. The model was developed in FORTRAN77 and the mathematical formulation solved simultaneous equations that represented leaf sheaths as large series of humidifying units. Input parameters assumed a porosity of the stomatal surface as 0.027 %, pore depth was 5 $\mu\text{m}$ , and pore slit width as 0.2 $\mu\text{m}$ . Culm and nodal resistances were  $0.4 \times 10^8 \text{ s m}^{-3}$ , and rhizome nodal resistance was  $0.5 \times 10^8 \text{ s m}^{-3}$ . The experiment assumed a maximum pressurization of 750 Pa (function of humidity and diffusive resistances), with a maximum  $P_s$  of 466.5 Pa in the blocked flow condition. The corresponding flow generated was 0.5457 L/hr. Flow outputs are summarized in Figure 21 below. (Beckett, 2001: 269-277)

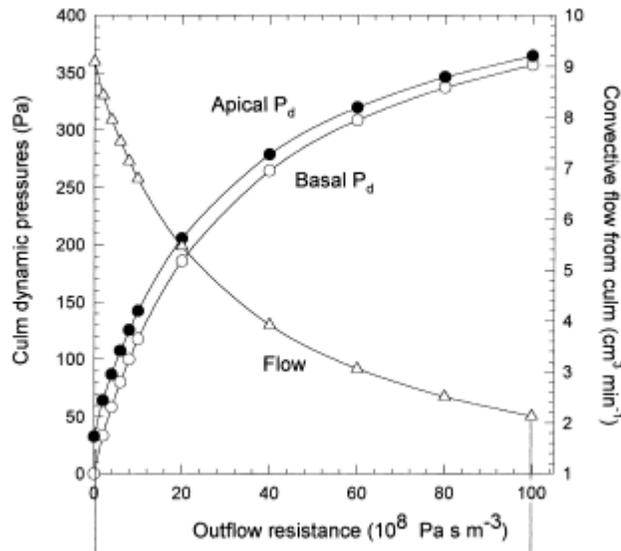


Figure 21. Outflow Resistance Effect on HIC Flow. (Beckett et al, 2001: 278)

The study showed that increasing the venting resistance from the culm of the plants reduces flows curvilinearly from a maximum flow rate at zero resistance. Increasing venting resistance increases the dynamic pressure, but reduces the pressure drop along the culm, resulting in lower flow rates. The study asserts that all leaf sheaths contribute to advective flow in the plant, but that the most apical (upper) leaves should contribute much less to overall flow than those in the middle region of the plant. In the field, however, many of the lower leaves senesce early in the growing season, possibly due to the higher humidity near the surface (Beckett, 2001: 289) or to lower incident radiation. The culms furthest from the rhizome should develop the highest dynamic pressures but will generate the least airflow, again due to the lower total pressure drop along the culm.

The study also examined dynamics of air flow and resistances in a Phragmites stand. Since the plants are connected to numerous other culms through underground rhizomes, the airflow of one plant can also create back pressure on another. (Beckett,

2001: 290) Stands with higher numbers of dead culms generated the lowest dynamic pressures and the highest convective flows; the dead culms behave as venting stacks for rhizome air flow and lower total venting resistance. (Afreen, 2007: 7)

### Rhizosphere Models.

*Root Quantification.* There is really no one root classification system; plant root systems vary greatly depending on species, soil characteristics, water availability, and other factors. (Fitter, 1996) In wetlands, root density varies by depth and species and may not be proportional to above ground biomass. (Amon *et al.*, 2007: 61) The most important goal, then, is to quantify branching patterns and relate them to root function. Most roots systems are trivalent branching structures. This means that each root *node* has three root links emerging from it, where a node is defined as the origin of a branch in a root system. Roots grow by branching, with lateral roots emerging from main roots at nodes prior to the root tip. The geometry can be expressed as a function of several components:

1. The number of links in the system- those that terminate in a meristem are referred to as exterior links and those that connect other links are called interior links. The magnitude of a link is the number of exterior links it serves, and is always one less than the number of interior links it serves.
2. Length of the links.
3. Distribution of branches.
4. Branching angles.
5. Relative diameter of the links as they increase in magnitude- This varies greatly by species, and has been studied little. (Fitter, 1996: 5-6)

Static modeling usually relies upon synthetic description. Fractal geometry assumes that the root system is homogenous across a large range of space scales and describes how a root fills geometric space. Topological Modeling describes the way root

systems branch according to globally established rules. It is useful for examining root system optimization under varying conditions such as the formation of nutrient depletion zones around roots. (Doussan, 2003: 423) In real situations, however, the assumption about regular distribution of roots does not hold. Global parameters such as root depth and density are not sufficient when investigating the development and functioning of root systems. (Doussan, 2003: 424) Due to the number of variables involved, it is often beneficial to focus the root model on a specific process and approximate trends of other inputs. (Doussan, 2003: 429)

*Armstrong Rhizosphere Model.* Armstrong *et al.* used a diffusion-based model of the root and rhizosphere using a series of concentric cylinders: inner stele, outer stele, cortex, epidermis/hypodermis, and rhizosphere. Oxygen is supplied to the outer cylinders from the cortex by radial liquid phase diffusion. Oxygen flux is calculated from the slope of the oxygen gradient using a log-linear relationship. The oxygen deficit across the epidermal/hypodermal cylinder is a function of diffusive resistance of the cylinder, oxygen consumption, and radial oxygen transfer. A convex profile across the epidermal/hypodermal cylinder may demonstrate that the root is most impermeable on the outer surface of the root. (Armstrong *et al.*, 2000: 691)

### Bacteria Modeling.

While there are few sources that accurately identify bacterial masses in the rhizosphere, there are numerous studies that provide growth constants for wetland bacteria that can be used in the modeling process. (Calhoun, 1998; Kaku, 2000; Erkel, 2006)

See above information in Bacterial Communities. Van Bodegom (2001:3591) found that ten weeks is not long enough to estimate steady-state populations of bacteria in low oxygen conditions. He also reaffirms the importance of methanotrophs and heterotrophs as the most important microbial sinks in a wetland.

### Trichloroethylene Treatment Modeling.

TCE is the most widely studied chlorinated solvent in aerobic cometabolism. Many mathematical models have been created to predict microbial responses to various TCE and substrate inputs. All equations begin with the basic expressions for competitive inhibition, non-competitive inhibition, and bacterial toxicity outlined above. Most use Monod expressions to represent saturation kinetics. A unique expression that is useful for quantifying cell response to a contaminant is the transformation capacity ( $T_c$ ). It indicates the amount of chlorinated solvent transformed per unit mass of bacteria cell prior to cell inactivation. Alvarez-Cohen and McCarty used this term to represent the amount of compound degraded per mass of cells inactivated in the process.

$$\frac{dS_c}{dX} = T_c$$

where  $T_c$  is the transformation capacity for a specific cometabolic substrate  
 $dS_c$  is the change in cometabolic substrate  
and  $dX$  is the change in active cell mass (Alvarez-Cohen and McCarty, 2001: 110)

TCE transformation capacity usually ranges from 25-150 ug TCE/ mg cells, although much larger values have been reported for some mixed cultures. (Alvarez-Cohen and McCarty, 2001: 113) This approach implies that the toxic effects decrease

overall cellular function. It can be combined to determine the net specific cell growth rate as a function of growth on substrate ( $r_g$ ) and cell inactivation by cell death and toxicity:

$$\mu = \frac{r_x}{X} = Y \frac{r_g}{X} - \frac{1}{T_c} \frac{r_c}{X} - b$$

where  $r_g$  is the rate of substrate consumption and  $r_c$  is the rate of cometabolite consumption (Alvarez-Cohen and McCarty, 2001: 110)

Modifications of this representation have been used frequently.

Ely *et al.* (1995) incorporated enzyme inactivation constants that were previously defined by Oldenhuis *et al.* (1991). This accounted for the potential recovery of enzyme that had been deactivated by toxicity at lower energy cost than cell regrowth, decoupling enzyme recovery from the cell growth. Cell inactivation models, though, have been questioned since many factors affect cell recovery. (Chu and Alvarez-Cohen, 1999: 770) Criddle (1993) and Change and Criddle (1997) included considerations for reductant energy by using a stoichiometric coefficient to account for the amount of growth substrate used to generate reductant. Their expression accounts for energy required during a cometabolic reaction, but does not account for cell growth. Chang and Alvarez-Cohen (1995) modeled reductant as a saturation kinetic expression. (Alvarez-Cohen and McCarty, 2001: 112)

*Anderson Model.* In 1994, Anderson and McCarty generated a time-dependent model for the treatment of trichloroethylene by methanotrophic biofilms. They modeled methane and TCE transport by diffusion, Monod growth kinetics, competitive inhibition between the methane and TCE, TCE product toxicity, and inactivation of the bacteria.

No prior published model had addressed TCE transformation product toxicity.

Methanotrophic biomass was subdivided into three categories: active biomass capable of utilizing methane and co-oxidizing TCE, secondary biomass that operate at a reduced state due to TCE toxicity, and inert cell material. Oxygen was assumed to not be rate limiting. Methanotrophs, however, appear to be most competitive at low oxygen conditions; sMMO expression is also optimized at low oxygen levels. The model assumed that a high copper concentration lead to pMMO expression and used a TCE rate coefficient ( $k_c$ ) 100 times smaller than that used for sMMO expression. The  $k_c$  used likely accounted for low TCE transformation rates by the simulated biofilm. Further, the model did not account for competition between bacterial populations. The model did suggest that TCE transformation was limited at high methane concentrations and that TCE transformation would be optimal near the  $K_s$  value for methane. It noted a balance that exists between the low concentrations of methane optimal for remediation and the higher concentrations sufficient for methanotroph growth and survival; TCE flux is limited by methane available for growth and is also limited by competitive inhibition by methane. It also indicates that highest rates of TCE flux is achieved at higher TCE concentrations. (Anderson, 1994: 389-390) Parameters for the model are included in Table 2 (Anderson, 1994: 388)

*Tartakovsky Model.* A 2005 model by Tartakovsky *et al.* modeled a single stage anaerobic-aerobic granular biofilm. The model established three bacteria groups: anaerobic methanogens, aerobic heterotrophs, and aerobic methanotrophs. The model assumed complete mineralization of TCE and its dechlorination intermediates to  $\text{CO}_2$ . Bacterial growth kinetics used multiplicative limitation and non-linear dependencies.

The growth of the bacteria was modeled based on methane limitations, inhibition by TCE and its dechlorinated intermediates, and oxygen limits caused by the competition by heterotrophs. Ethanol was used as a primary carbon source for heterotrophs. Oxygen penetration, however, was calculated linearly using Cartesian geometry, neglecting the influence of the bacterial biomass on the corresponding oxygen concentrations. The model did not include effects from different forms of MMO. The model also incorporated space limitations on bacteria growth by setting population caps. The literature review showed a wide range of values for specific growth and substrate transformation constants, and model parameters were varied to achieve qualitative agreement with experimental results. (Tartakovsky, 2005: 75) Parameters used are shown in the table below. The model predicted an optimal aeration rate for the reactor as 430 mg O<sub>2</sub> / liter/ day. At high aeration rates, heterotrophic bacteria prevailed over both methanogens and methanotrophs, limiting TCE transformation. The process performed best at high TCE loading rates until degradation of the chlorinated intermediates became rate-limiting. (Tartakovsky, 2005: 76)

### **Knowledge shortfalls**

Applied bioremediation science covers a broad area of study. Soil and hydrology characterization of a remediation site is essential to creating a treatment plan, and the treatment must optimize use of available resources to maximize the return of investment with respect to treatment goals. This literature review has shown that many unknowns currently limit the effective application of bioremediation, specifically as it relates to TCE remediation in wetland environments. Wetland soil chemistry, the behavior of

wetland plants, and the characterization and behavior of wetland microbial communities are critical areas of study. Wetland construction parameters including contaminant loading, soil and plant selection, and nutrient addition is limited by these knowledge gaps. (Amon *et al.*, 2007: 63) There is a need to better integrate plant physiology and molecular biology with soil chemistry, physics, and mesofaunal ecology. (Jones *et al.*, 2004:474)

#### Soil Chemistry.

Hydric soil is a resource that is associated with wetlands and takes time to develop, however its specific characteristics are not fully understood. The wide variety of conditions in various wetlands limits the direct study of single variables as they apply to all wetlands universally. Especially important to understanding soil chemistry is knowledge of plant exudates that act as electron receptors in the environment, lowering eH and potentially buffering pH.

#### Plant Dynamics.

In the past, plant studies have focused on agriculturally significant crops. Recent realization of the significance of wetlands, the plants that reside there, and their unique physiological characteristics has initiated deeper interest in the study and protection of these critical habitat areas.

The relationship between the plant and its surrounding soil is especially important. It is clear that the balance between carbon and nitrogen in the soil is crucial, but we have no knowledge of this ratio in soil. While there are many studies of amino

acids and sugars in root exudates, they are largely qualitative (Jones *et al.*, 2004:470)  
Understanding of organometallic complexes in the rhizosphere and the corresponding  
plant response is especially limited. (Jones *et al.*, 2004:468)

### Microbial Characterization.

Our current understanding of microbial community dynamics associated with  
rhizodeposition is limited. (Butler, 2003:6793) While traditional culture methods are  
being replaced by 16S RNA methods, characterization of bacteria and their roles in  
remediation continues to be an area of uncertainty. (Amon *et al.*, 2007: 64)

Methanotrophs are apparently key microbes in the natural attenuation of TCE.  
However, there have been no extensive studies of how mixtures of chlorinated  
compounds affect methanotrophs expressing sMMO or pMMO. (Lee, 2006:7504)  
Copper is a critical component of the MMO but, it is unknown which forms of copper are  
bioavailable to methanotrophs. (Morton, 2000:1730) Knowledge on the effect of TCE  
and its degradation by-products is also lacking. Fortunately, methanotrophs are one of  
the most-studied of wetland bacterial species. There are many other wetland species that  
need to be classified and studied in hope that other useful remediation characteristics may  
be discovered.

### **III. Methodology**

A mechanistic modeling approach is used to generate data in this study. Modeling is an effective tool since it allows the exploration of numerous variables quickly. STELLA version 9.0 (Isee Systems, 2007, formerly distributed by High Performance Systems) provides model output in a visually friendly format, and deterministically captures the dynamic characteristics of a system (the software being an ordinary differential equation solver in time and able to numerically solve partial differentials). It accounts for both deterministic and dynamic qualities of the elements that affect conditions in the rhizosphere. The model's system boundary includes the plant, its root structure, the plant's rhizosphere, and the microbial populations that exist in the oxygenated zones of the soil. It requires inputs for atmospheric, soil, plant, and microbial variables.

The model uses a discretized compartmental approach to account for oxygen gradients that exist inside the plant and the soil. Each compartment includes stocks of oxygen measured by mass. The model also calculates carbon dioxide, nitrogen, methane, soil carbon, microbial, and TCE masses in required areas. Flows between the stocks are generated by mathematical expressions, and the Stella software calculates flows along an incremented timeline, resulting in a numerical integration. An extended simulation of the software usually results in a steady-state expression of a model variable. This result can be compared to empirical data already collected for correctness, thereby validating the model. Once the model is validated, a sequence of simulations will be used to explore

the research questions posed in Chapter 1. The actual model is included on the accompanying disk with this thesis.

### **Modeling Development Process**

The first priority of modeling is to maintain the integrity of the system. In short, the model must address the research questions and not try to accommodate the answers. (Shelley, 2007: 43) This model attempts to simulate systems that exist in a generic wetland plant. From a system dynamics perspective, the behavior of a system is a result of its causal structure. It is important, then, that all components of the model realistically represent function and behavior in the living system; this will help maintain the integrity of model behavior and allow better comparisons of the model to empirical knowledge about plant systems.

Modeling has a number of limitations. There are many uncertainties associated with a plant model; not all processes inside plants are fully understood. The model requires quantification of “soft” concepts represented by variables that may not have a measured value. A number of assumptions are made in the modeling process that account for unknowns. Numerical precision is sacrificed when there is a lack of measured data, and consequently it is important to capture the dynamic relationship of the system. (Shelley, 2007: 55) The model’s output is scaled against measurements obtained in real systems; when comparable data sets are not available to scale the model, performance assumptions must be made. The method of approach must use available quantitative data for the mechanistic processes we do understand in order to qualitatively assess the behavior of processes we do not fully understand.

The model is developed and then tested in stages. (Shelley, 2007: 56; Forrester and Senge, 1980) Four stages of model construction are conceptualization, formulation, testing, and implementation. (Randers, 1980:285) These stages are followed and are discussed in this chapter.

## **Model Conceptualization**

### Expected Behavior.

Many system relationships affect this model; some relationships are well understood, while others to a lesser extent. Whether by measurement or calculation from a prior model, this model seeks to follow the available historical data for plant systems. Where information is unavailable, reference modes must be asserted based on known behavioral relationships. Closed feedback loops within the model are a key component to the model behavior; it is the closed-loop relationships that are neither intuitive nor able to be calculated directly by normal formulation. (Shelley, 2007: 48)

Numerous relationships in a system can create dynamic behavior; the influence of one factor can significantly affect oxygen levels in the plant and its rhizosphere. Figure 22 demonstrates the major relationships at work in the model. Photosynthesis and humidity-induced convection are the main contributors to oxygen in the rhizosphere. Oxygen outflow, plant respiration, microbial mass, and soil chemicals are negative influences on rhizosphere oxygen (as denoted by arrows and positive/negative signs).

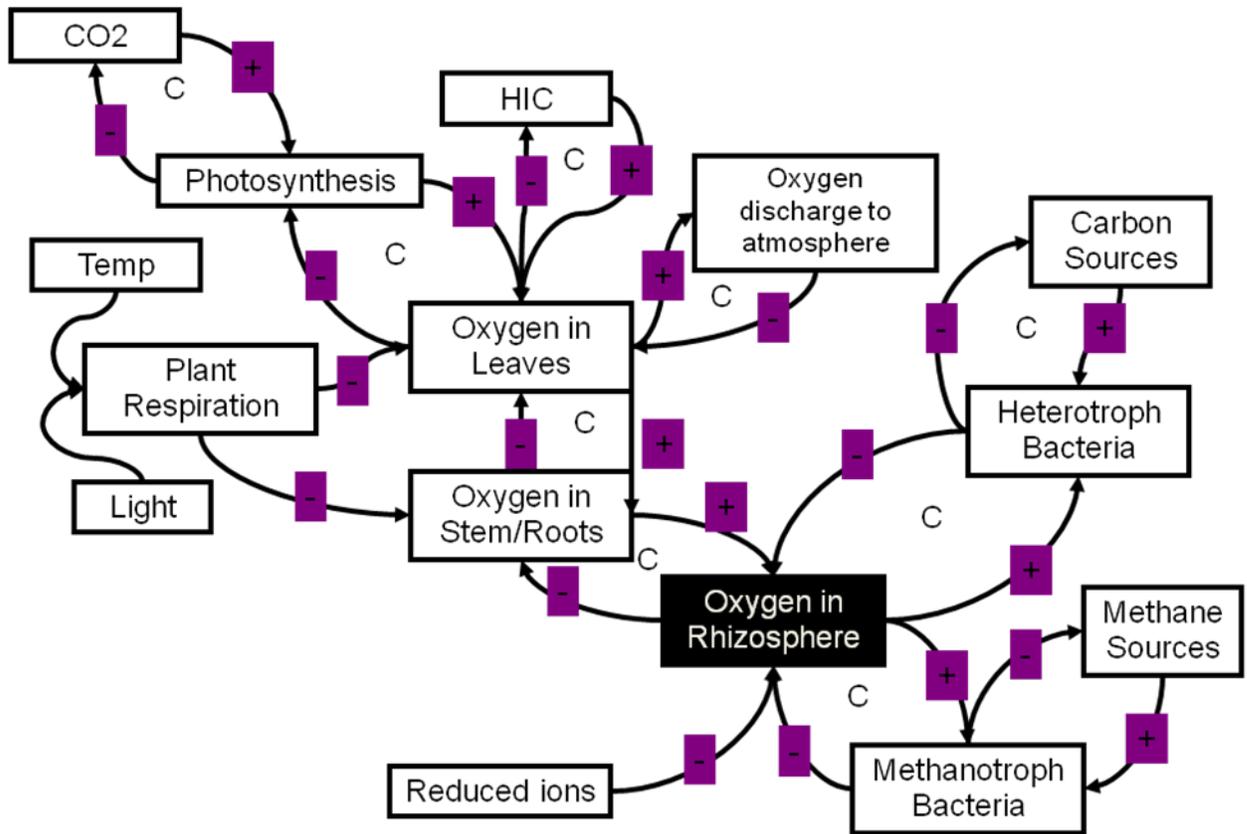


Figure 22. Dynamic Relationships Affecting Rhizosphere Oxygen.

*Leaf.* CO<sub>2</sub> concentrations in the leaf should fall below ambient concentrations, and oxygen concentrations should rise above ambient. Nitrogen and other partial pressures should fall below ambient concentrations due to pressure created in the leaf by water vapor. As venting path resistance in the plant decreases, humidity induced convection must reach a maximum due to the constraint of water flow to the leaf.

I expect to see most plant tissue concentrations stabilize slightly below the oxygen concentration in air due to plant respiration. Photosynthesis by the plant should compensate for plant respiration and result in a net oxygen increase around the plant.

*Root.* Root concentrations should decline along lateral roots and cortex concentrations should be higher than stele concentrations due to aerenchymal air flow

and oxygen transfer between the xylem and phloem in the stele. The root hair zone is the primary oxygen pathway into the surrounding soil. Most oxygen loss through the roots occurs in the root hair zone where root hairs are abundant, approximately the last 3 cm of the root as shown in the measurements of Figure 23.

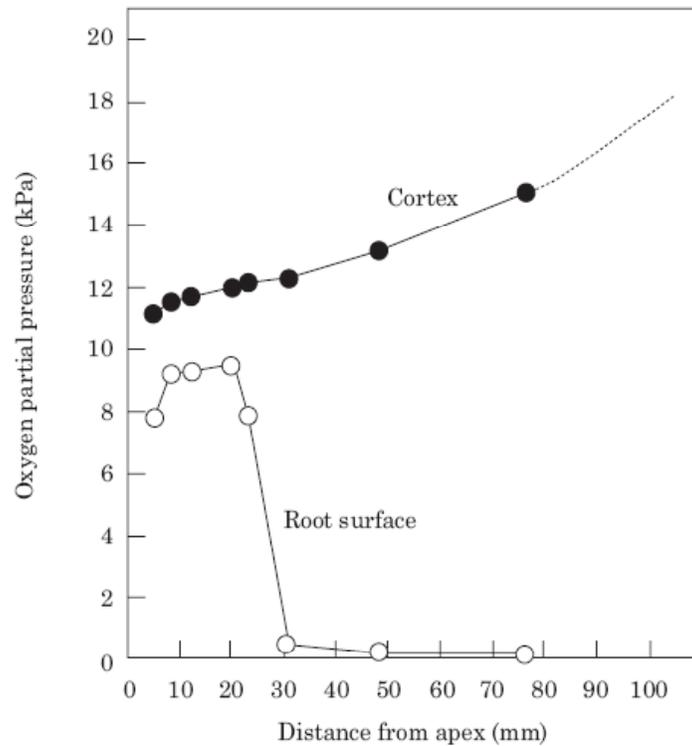


Figure 23. Oxygen Partial Pressures Along a *Phragmites* Root. The root was 110 mm long and 1 mm in diameter. (Armstrong *et al.*, 2000: 692)

*Soil.* When inputs are constant, oxygen concentrations in the plant and soil should approach a steady state. The root-zone should show a radial decline in soil oxygen concentrations that goes to zero at infinity. When the microbes are introduced into the system, the effect will be amplified by microbial consumption of oxygen. Oxygen levels should then approach zero within approximately 5 mm of the root surface.

*Microbial Mass.* Microbes consume oxygen rapidly and should create a steep concentration gradient resulting in more oxygen flow to the rhizosphere. Masses will vary greatly in response to changes in oxygen and substrate levels, and heterotroph consumption of oxygen will indirectly affect methanotroph growth. A heterotroph : methanotroph population of 100:1 is typical in wetland environments. Heterotrophs should grow in higher oxygen areas near the root, and methanotrophs should occupy the outer rhizosphere. TCE should cause toxicity to sMMO producers around 4ppm and pMMO producers around 13 ppm as identified by Lee *et al.*, 2006.

*Model Assumptions.* Given the reference modes above, the following assumptions apply to this model.

1. Humidity induced convection (HIC) and plant photosynthesis are the main contributors to rhizosphere oxygen.
2. Nearly all rhizosphere oxygen is contributed through the plant's root hair zones.
3. The plant efficiently minimizes overlap of rhizosphere zones.
4. Mature and homogenous plant stand of *Phragmites australis* that ignores diurnal cycles (constant phloem/xylem flow and humidity/temperature/light levels).
5. Heterotrophs and methanotrophs are the only bacteria of treatment significance in the rhizosphere.
6. Primary carbon flow is from BOD in treatment water, and organic carbon is the primary substrate for heterotrophic bacteria. Although plants may also exude significant amounts of carbon into the rhizosphere (organic acids, sugars), flow is assumed to originate from outside the rhizosphere.

7. Methane is generated in anaerobic zones of the wetland treatment area and is the primary substrate for methanotrophic bacteria.
8. TCE is the only contaminant in the treatment water, and is consumed aerobically only.
9. Bacterial activity is the most significant sink of oxygen in the rhizosphere (ignores chemical oxidation, fungi, predation).
10. Copper availability, determined by total copper concentration and redox conditions, determines MMO expression.
11. sMMO and pMMO have greatly different transformation rates for TCE ( $k_{TCE}$ ) but have roughly equivalent affinities for methane and TCE ( $K_s$ ,  $K_{s, TCE}$ ) and TCE inhibition rates ( $k_{i, TCE}$ ).
12. A subsurface flow wetland treatment system with uniform flow and continuously-stirred-reactor assumption outside the rhizosphere.

#### Fundamental Model Behavior.

Model behavior is a result of formulation that reflects significant influences in the system. The significant motive forces for oxygen movement in this model are: 1.) advection resulting from bulk flow of oxygen in the aerenchymal tissue (gas) and vascular tissue (dissolved); and 2.) diffusion down oxygen concentration gradients. Limited knowledge on plant oxygen movement necessitates the modeling of oxygen flow by bulk flow in both the solute (phloem/xylem) and in the air spaces (aerenchymal tissue). This model calculates oxygen flow in the leaves, stem, and roots of a monocot wetland plant, and then combines that flow with other factors in the soil that affect

microbial growth. Consequently, the model is subdivided into components to account for plant processes in its real-world counterparts: leaf, stem, root, soil, and microbes. Figure 24 shows the relationship of the major model components. The concentric cylinder around the root hair zone represent the rhizosphere levels with oxygen flowing outward and methane, soil carbon, and TCE flowing inward by diffusion and advective movement of water. Copper exists as a steady concentration in the soil.

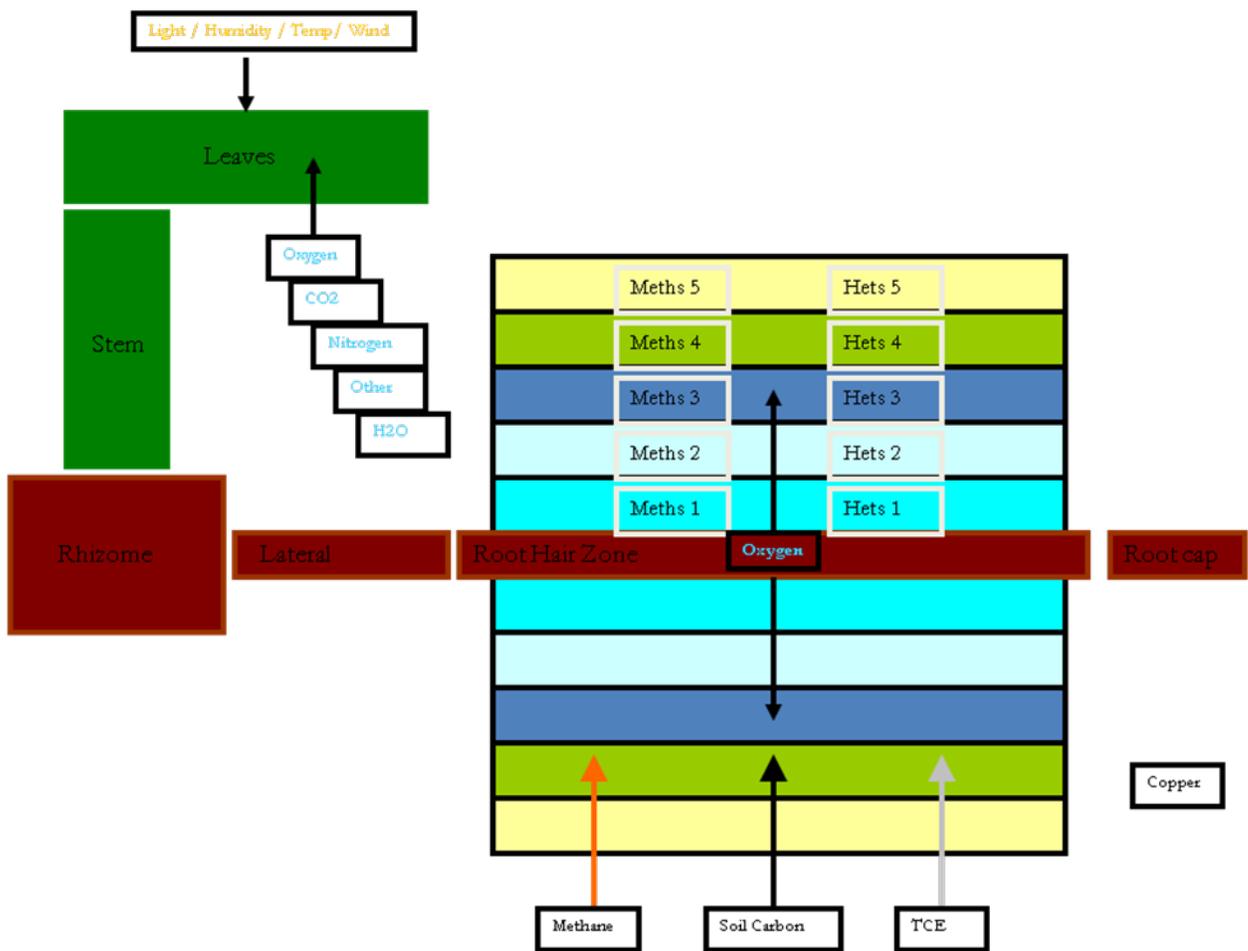


Figure 24. Model Compartmentalization.

*Environmental Factors.* Numerous operator inputs are required to establish environmental constraints that remain constant throughout the simulation: atmosphere

(temperature, humidity, wind speed, light level, atmospheric pressure, ambient gas concentrations); soil (saturation, porosity, methane, carbon, and copper concentrations); and hydraulic parameters (flow rates, wetland surface area, TCE input concentration). Diurnal and seasonal variations due to circadian rhythms, photoperiodism, and other biological clocks are not inherent in the model, but plant parameters (like photosynthetic and heat responses, mass, leaf area index, xylem/phloem flow rates, venting path resistance) can be varied to account for the effects of seasonal changes.

*Plant factors.* Plant species characteristics including leaf area, photosynthetic rates, size, root structure, and respiration rates are incorporated. As examples: 1.) Leaf area index is used to calculate leaf area for an individual plant; 2.) leaf pore size, root tip diameter, stem height, aboveground and belowground masses, and numbers of roots are entered as constants; 3.) a single plant respiration rate is used to calculate oxygen use in each plant zone on a mass basis.

*Leaf.* The leaf component incorporates the processes of photosynthesis and humidity induced convection (HIC); HIC generates the pressure used to drive airflow in plant aerenchymal tissue (also see discussion in Chapter 2). HIC is a function of humidity, light level, external temperature, external air concentrations, leaf area, and pore (stomata) size. Water vapor supplied by the plant raises the internal leaf pressure, reducing leaf gas concentrations in the leaf and causing external gas to diffuse down the new concentration gradient through the stomata. The small pore openings of leaf stomata permit diffusion but limit pressurized flow. Partially closed stomata resist the outward flow of air (Poiseuille flow resistance increases as pore size gets smaller), and the increased pressure of water vapor and other gases generates internal convection that

carries leaf gases into the plant. Resistance coefficients are calculated for three areas, a boundary layer, pore space, and header space. The model tracks partial pressures of all gases (nitrogen, oxygen, CO<sub>2</sub>, and “other”) inside the leaves, translates these pressures for heat generated by the leaf, and uses resistance from the stem aerenchyma and culms to translate these partial pressures into volumetric airflow. This generates a mass flow of oxygen out of the leaf down stem aerenchyma. Water vapor diffuses outward and it is assumed that the plant maintains 100% relative humidity inside the leaf. The model also incorporates a limit to water flux that limits the maximum HIC possible. Figure 25 demonstrates how oxygen in the leaf can flow in the plant by two pathways, aerenchymal movement of air, and vascular movement of solute.

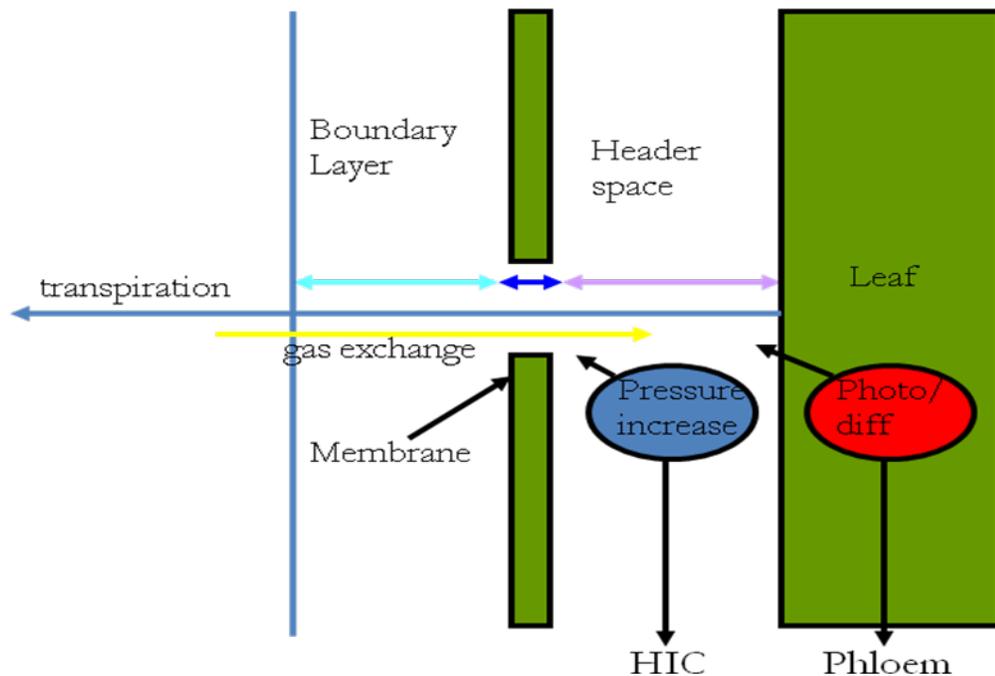


Figure 25. Gas Exchange Process Inside the Leaf.

The surface of leaf mesophyll cells is a transition zone, and gases partition from gas to liquid phase according to Henry’s Law; liquid concentration at the cell surface

equilibrates with gaseous concentration accordingly. Diffusion accounts for oxygen flow between cells and is represented by transfer coefficients. Phloem tissue moves oxygen out of leaf tissue as a function of the leaf concentration and the phloem volumetric flow rate. Xylem flow moves oxygen and CO<sub>2</sub> into the leaf tissue by bulk flow. In this model, a constant phloem/xylem flow is assumed. Since neither sugar loading of the phloem nor hydraulic conduction in the xylem are calculated, volumetric flows inside vascular tissue are operator inputs representative of flows according to plant type, size, and season in accordance with available literature.

Photosynthesis is a function of temperature, light, plant characteristics, and CO<sub>2</sub> available. Plant respiration can be a significant source of CO<sub>2</sub> for photosynthesis. When CO<sub>2</sub> was found to be limiting photosynthesis in the model, additional feedback pathways from plant respiration were added to more accurately represent CO<sub>2</sub> availability. Leaf, stem, and rhizome CO<sub>2</sub> was recirculated to generate this flow raising CO<sub>2</sub> levels in the leaf, and permitting greater oxygen production. CO<sub>2</sub> is allowed to partition into the aerenchyma and flow in the phloem/xylem.

*Stem.* Oxygen flow is moved from the leaf component to the stem component by three primary flows: aerenchymal movement using bulk airflow, and xylem and phloem flows using bulk water/solute flow. Inside the stem tissue, transfer between cortex, vascular, and aerenchymal tissue is accounted by diffusion. Like leaf spaces, transfer to/from gas phase incorporates Henry's calculations. Oxygen transfers between cortex, aerenchyma, xylem, and phloem according to transfer coefficients down diffusion gradients. Bulk flow moves oxygen from the spaces as a function of O<sub>2</sub> concentration and volumetric flow rate.

*Root.* The bulk flow continues into the root zone. The root component is subdivided into three main components to reflect changes in plant anatomy along the length of the root: the rhizome, the lateral roots, and the root tips. Root permeability changes along the length of the root and limits epidermal flux; transfer coefficients are used to facilitate gas flows through root segments (lateral zones, root hair zone, root cap); diffusion constants for the root segments are unknown. The rhizome is assumed to be an impermeable barrier for oxygen diffusion to the soil; in the model it represents the area of the root that is heavily lignified or suberized. The rhizome also vents bulk oxygen flow from aerenchymal tissue to the atmosphere, as it does in real plant systems, through other stems or dead culms in the plant stand. The elevated oxygen concentration in the rhizome then provides a motive force for diffusion into the lateral root aerenchymal tissue.

In the lateral roots, a separate stele and cortex component are created; this represents the influence of the casparian strip that surrounds the vascular tissue in the roots and limits diffusion between cortex and stele tissue to the symplastic pathway. Cortex tissue is in contact with aerenchymal tissue since the aerenchymal passages flow through them. Xylem and phloem transfer into stele tissue before diffusing to each other or into the cortex. The lateral roots are further segmented into lateral root sections in order to accurately portray the oxygen flux into the soil at different lengths along the root; this allows accurate representation of increasing permeability towards the root tip.

Vascular tissue ends in the last segment of the lateral roots prior to the root tip. The root tip is further divided into the root hair zone and the root cap. The root hair zone

represents the root area of highest permeability. The root cap represents the area of root growth, and all oxygen diffuses to it through the stele tissue of the root hair zone.

*Soil.* The rhizosphere is modeled as five concentric cylinders that run parallel to the axis of the root, creating a series of rhizosphere layers (or “levels”). Each level contains methanotroph, heterotrophy, methane, carbon, TCE, and oxygen stocks that account for mass existing in the rhizosphere level. The cylinder widths can be varied to examine oxygen concentrations at variable distances from the root. The volume of each sequential cylindrical section increases proportionally to the square of the distance from the root, creating a radially decreasing oxygen concentration profile. One limitation on the modeling program drives a component of this model; STELLA software is not equipped to perform partial differentials for radial oxygen loss from the roots. In order to compensate for multiple variables, transfer coefficients are used to establish radial oxygen diffusion into each rhizosphere cylinder in a step-like manner; oxygen levels can then be graphed in contour.

While typical diffusion would result in a trail of oxygen that tapers to infinity, this model simulates a biofilm incorporating diffusion with reaction. In the rhizosphere, oxygen is consumed by either organic or inorganic processes. It is assumed that reaction rate of the organic processes, such as microbial consumption, is catalyzed by enzymes and greatly exceeds the reaction rate of inorganics in the soil. For this reason, microbes are the only component considered for reaction in this model.

The model does not account for overlapping rhizospheres, and an assumption is made that the plant, attempting to conserve energy and carbon, minimizes the overlapping effect as it explores the soil for nutrients. Oxygen flows outward by

diffusion and TCE, methane, and carbon flow inward by diffusion. Advection from soil water also makes inputs and outputs to rhizosphere concentrations.

*Microbes.* Bacterial populations exist as mass stocks within each of the soil zones around the root. Heterotrophs and methanotrophs are identified as the dominant bacteria in wetland soils; these populations consume the oxygen as a function of their growth and metabolism. While this creates an oxygen drain, it also creates a higher diffusion gradient that draws more oxygen into the soil from the roots, increasing the total oxygen flux from the plant.

Relationships between the bacterial populations are largely determined by the respective abilities of each type to utilize limiting substrates and oxygen. Carbon sources (organic acids) are assumed to be a limiting substrate for the heterotroph populations, and methane a limiting substrate for methanotrophs. Both bacteria are limited by their ability to procure oxygen, and oxygen stoichiometrically limits the amount of primary substrate that can be consumed. With respect to modeling of microbial populations, Monod growth is assumed. Monod growth makes the assumption that there is a maximum rate at which organisms can grow. The half-rate constant,  $K_s$ , denotes the concentration of substrate at which microbe growth is one half its maximum rate. Values for  $K_s$  are found empirically. Space consideration for microbial cohabitation is not modeled; it is assumed that the oxygen and other substrate levels are the limiting factors of growth.

Adverse effects from non-competitive inhibition of TCE is accounted by the Andrews model (see Chapter 2), a modification of the Monod expression; effects are applied to both methanotroph and heterotrophy populations equally. Cumulative effects for multiple contaminants and daughter products (DCE, VC) are not included in this

model. Competitive inhibition of methane and TCE applies for methanotrophs, both as they consume methane and process TCE. Additionally, methanotrophs are proportionally affected by TCE toxicity according to their transformation capacity. Cell inactivation was not considered in this model since it has had questionable results in past models. (Chu and Alvarez-Cohen, 1999: 770) Cell inactivation is accounted by cell growth, toxicity, and decay constants. An example methanotroph stock formulation is shown in Figure 26. Heterotroph consumption of carbon and non-competitive inhibition by TCE have similar relationships.

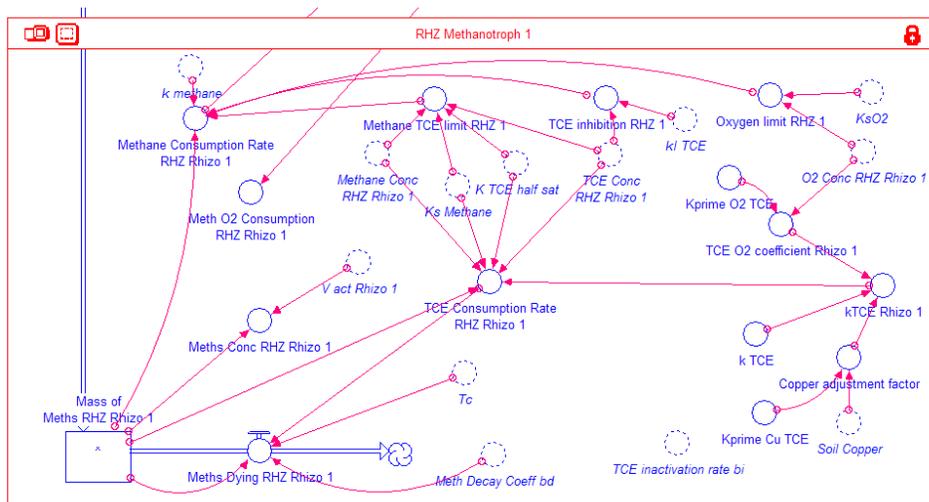


Figure 26. Methanotroph Relationships in STELLA Format. Methanotrophs grow by consuming methane. Methane and oxygen availability can limit methane consumption. Non-competitive inhibition can be caused by TCE and decrease the methane consumption rate. Competitive inhibition by TCE also limits available MMO for methane consumption. Oxygen and copper levels affect MMO expression and determine TCE consumption rate; TCE consumption results in cell death. Death is also caused by natural aging processes.

### Model Formulation.

The equations in this section mathematically express various relationships in the model. They are described in sequence as oxygen flows through the model. Specific STELLA formulation is included in the accompanying DVD.

*Leaf.* The mathematical formulation used for Humidity Induced Convection (HIC) accounts for Knudsen diffusive and Poiseuille flow resistance of gasses. It is described by W. Armstrong *et al.* (1996: 121-135) and is summarized below:

$$J_w = (2.337/P_a) / (R_h + R_{md} + R_b),$$

$\Delta P_{w(m)} = P_a (J_w \times R_{md}) \text{ kPa.}$

$$J_{o,n} = (\Delta P_{w(m)} / P_a) \times 1 / R'_{md} \text{ m}^3 \text{ s}^{-1}. \quad (\text{Max potential HIC})$$

$$(\Delta P_{ps} - \Delta P_d) / P_a \times 1 / R'_{md} = \Delta P_d / R_{VP}.$$

The convective flow, HIC, is then given by:

$$\text{HIC} = \Delta P_d / R_{VP}.$$

The leaf boundary layer calculation was used to determine the resistance of diffusion through the boundary layer (Nobel, 1991: 365):

$$L_b = 4.0 * \left( \frac{\text{Leaf\_width}}{\text{Wind\_Speed}} \right)^{.5}$$

where  $L_b$  is the leaf boundary layer ( $\mu\text{M}$ )  
 Leaf\_width = leaf length in the wind direction (m)  
 Wind\_Speed (m/s)

Additionally, to more accurately calculate HIC, a water flux limit coefficient was incorporated that adjusts the amount of water vapor available for HIC as conductive flow approaches its maximum value.

$$\text{HIC} / J_{on, \max} = \text{J fraction}$$

$$\text{WaterFluxLimitCoeff} = 1 - \text{J fraction} * (100 - \text{Relative Humidity\%}) / 100$$

The WaterFluxLimitCoeff is used to calculate water flux  $J_{w, \text{realized}}$  that is lower than  $J_w$ :

$$J_{w, \text{realized}} = \frac{\text{WaterFluxLimitCoeff} \cdot \left( \frac{p_{\text{Vap}}^{\text{leaf}} - p_{\text{Vap}}^{\text{air}}}{R_b + R_h + R_{\text{md}}} \right)}{R_b + R_h + R_{\text{md}}}$$

where  $J_{w, \text{realized}}$  (g/hr)  
and  $R_b$ ,  $R_h$ , and  $R_{\text{md}}$  are resistance coefficients for the leaf boundary layer, header space inside the leaf, and the membrane pores (stomata) respectively ( $\text{s/m}^3$ )

$J_{w, \text{realized}}$  is used to calculate  $J_{\text{on, realized}}$ , the actual flow rate into the leaf (g/hr). Knudsen coefficients were calculated for all atmospheric gases independently (not just for “air”). Gaseous oxygen and  $\text{CO}_2$  flow into the leaf were calculated using bulk flow based on

$J_{\text{on, realized}}$ :

$$\frac{dM_{O_2}}{dt} = \left[ \frac{J_{\text{on, realized}} \cdot \text{PoiseuilleBackflow}}{R_{\text{on, realized}}} \right],$$

and diffusion:

$$\frac{dM_{O_2}}{dt} = \frac{\left( \frac{D_{O_2} \cdot (C_{O_2}^{\text{air}} - C_{O_2}^{\text{leaf}})}{R_{\text{diff}}} \right)}{R_{\text{diff}}}$$

Gaseous oxygen and CO<sub>2</sub> flow out of the leaf [g/hr] were calculated using bulk flow and diffusion in the aerenchyma:

$$\frac{dM_{O_2}}{dtL} = \left[ \frac{HIC}{V_{stemaerenchyma}} + \frac{D_{O_2,air} \cdot A_{stemaerenchyma}}{L_{stem}} \right] \cdot ([O_2]_{leaf} - [O_2]_{air})$$

where HIC [cm<sup>3</sup>/hr]  
 [O<sub>2</sub>] is the oxygen concentration (g/cm<sup>3</sup>)  
 D<sub>O<sub>2</sub>,air</sub> is the diffusion coefficient for oxygen in air (770.4 cm<sup>2</sup>/hour)  
 A<sub>stemaerenchyma</sub> is the cross sectional area of the stem aerenchymal tissues (cm<sup>2</sup>)  
 L<sub>stem</sub> is the length of the stem (cm)

and for advection in the vascular tissues:

$$\frac{dM_{O_2}}{dt} = [O_2]_{leaf} \cdot \text{BulkFlowRate} \quad (M/T)$$

In order to calculate nitrogen and “other” (argon, helium, NO<sub>2</sub>, etc) gas pressures in the leaf, an estimate was made that accounted for a fractional decrease from atmospheric pressure based on the realized gas flow into the plant:

$$\text{Fraction\_decrease} = J_{on\_realized} \cdot R_{md\_prime},$$

and the estimate of gas pressures in the leaf is expressed by:

$$PP_{N_2} = \frac{(1 - \text{Fraction decrease}/1.1) \cdot N_2 \text{ Partial Pressure in Air} \cdot \text{Leaf Temperature}}{\text{Temperature}}$$

The combined gas law is used to compute gaseous concentrations and pressures:

$$PV = nRT$$

Henry's law is used to compute equilibrium concentrations between gaseous and liquid phases at the cell surfaces:

$$H = \frac{P}{(nV)}$$

where H = Henry's constant (L atm mol<sup>-1</sup>)

P = Pressure (atm)

And n/V = molar concentration (moles/ Liter)

Oxygen from photosynthesis of CO<sub>2</sub> was calculated by modifying the formulation of Asaeda *et al.* (2000). Plant senescence was not considered. Photosynthesis was averaged across the entire plant:

$$Ph_{shoot} = P_m \cdot \theta^{(T-20)} \left( \frac{Plant_{PAR}}{K_{PAR} + Plant_{PAR}} \right)$$

$$Plant_{PAR} = Lightlevel \cdot (-kLAI)$$

$$Lightlevel = \frac{Aboveground\_area\_mass}{Plant\_area\_density}$$

where Ph<sub>shoot</sub> = CO<sub>2</sub> photosynthesis by shoot (g/hr)

P<sub>m</sub> = maximum specific net daily photosynthesis rate of the plant (g/g/hr)

b<sub>shoot</sub> = the total shoot biomass for the plant (g)

Aboveground\_area\_mass (g/ m<sup>2</sup>)

K<sub>PAR</sub> = half saturation constant of photosynthetically active radiation (mol/m<sup>2</sup>/hr)

I<sub>PAR</sub> = Incident photosynthetically active radiation (mol/m<sup>2</sup>/hr)

Lightlevel = global radiation level (mol/m<sup>2</sup>/hr)

LAI = Leaf area index (m<sup>2</sup> leaf area/ m<sup>2</sup> plant)

*Stem.* Gaseous flow in the stem accounts for advection and diffusion. Liquid phase movement occurs in the xylem and phloem. Both are calculated as above. Oxygen and CO<sub>2</sub> partitioning to the aerenchyma is calculated using a mass transfer coefficient:

$$\frac{dM_{O_2}}{dt} = \left( [O_2]_{(aq)} * \text{Phloem\_Transfer\_Coefficient} \right) \text{ent}$$

$$\frac{dM_{O_2}}{dt} = O_2\_Consumption\_Stem\_Tissue = \text{Plant\_Stem\_Mass} * \text{Plant\_Tissue\_O}_2\_Usage\_Rate$$

where [O<sub>2(aq)</sub>] (g/L)  
and Phloem\_Transfer\_Coefficient (L/hr)

Oxygen consumption in the tissues is calculated by using a Temp\_Consumption\_factor to account for changing plant responses at temperatures from freezing to 350K. This is used to calculate an oxygen consumption rate for the specific tissue group:

$$\frac{dM_{O_2}}{dt} = O_2\_Consumption\_Stem\_Tissue = \text{Plant\_Stem\_Mass} * \text{Plant\_Tissue\_O}_2\_Usage\_Rate$$

$$\text{Plant\_Tissue\_O}_2\_Usage\_Rate = \beta_{\text{plant}} * \text{Temp\_Consumption\_factor}$$

where Plant\_Tissue\_O2\_Usage\_Rate (g/g/hr)  
Plant\_Stem\_Mass (g)  
And  $\beta_{\text{plant}}$  = the averaged plant respiration rate (taken as .004 g/g/day from Asaeda *et al.*, 2002)

*Roots.* Oxygen is transferred into the soil across a theoretical root epidermis of designated thickness, and uses an oxygen transfer coefficient that is estimated by Fick's Law of Diffusion:

$$\frac{dM_{O_2}}{dt} = SA_{root} O_2 \text{fluxcoefficient}$$

$$O_2 \text{fluxcoefficient} D = \frac{D_{O_2, EH} \left( \frac{O_2}{L} \right)_{\text{epidermal-hypodermal membrane}}}{EH\_dermal\_thickness\_RHZ}$$

where  $O_2 \text{fluxcoefficient}$  ( $M/L^2/T$ )

$SA_{root}$  = the surface area of the root hair zone for the entire plant

and  $D_{O_2, EH}$  is the permeability of the epidermal-hypodermal membrane (.45  $cm^2/day$  as validated)

For calculation purposes, the volume and surface areas of root zones are calculated as a combined total for the plant; each zone is treated as a cylinder with actual root radius, but the length is factored by the number of roots of that size that exist in the plant. This permits a straightforward calculation of the entire plant and minimizes use of small numbers that could detract from the accuracy of calculations.

*Soil.* A surface loading rate and root hydraulic retention time is calculated to determine mass flow of water through each rhizosphere:

$$\text{LoadingRate} = \frac{Q_{water}}{\text{TreatmentArea} * \eta_{soil}}$$

$$Q_{plant} = \frac{\text{LoadingRate} * \text{OverlapPercent} * \text{RootArea}}{100}$$

$$\text{PlantHRT} = \frac{V_{rootssystem}}{Q_{plant}}$$

$$Q_{RHZ1} = \frac{V_{rhizo1}}{\text{PlantHRT}}$$

where  $\text{LoadingRate}$  ( $L/m^2/hr$ )

$\eta_{soil}$  is the soil porosity ( )

$\text{OverlapPercent}$  accounts for plants with shared root zones ( )

$Q_{RHZ1}$  is the volumetric flow rate through the rhizosphere level ( $L/hr$ )

Inputs and outputs for oxygen, methane, carbon, and TCE in the rhizosphere result from water movement moving mass proportional to its concentration.

$$\frac{dM_{CH_4}}{dt} = Q C_{RHZ}^{*} C_{soil}$$

$$\frac{dM_{CH_4}}{dt} = - Q C_{RHZ}^{*} C_{RHZ}$$

Oxygen, methane, carbon, and TCE also diffuse along a concentration gradient. Transfer coefficients are estimated (see below) for methane, carbon, and TCE diffusion in the soil.

$$\frac{dM_{CH_4}}{dt} = D_{CH_4} * \left( \frac{C_{CH_4} - C_{RHZ}}{\text{Rhizo\_Increment}} \right)$$

*Microbial Populations.* Methane consumption uses a double Monod formulation with stoichiometrically constrained substrate limitation, competitive inhibition, and non-competitive substrate inhibition (Andrew's model) terms:

$$\frac{dM_{CH_4}}{dt} = k_{methane} X_{meths} * \left( \frac{C_{CH_4}}{K_{CH_4} + C_{CH_4} + \frac{C_{TCE}^2}{K_{TCE}}} \right) * \left( \frac{K_{TCE,ORHZ}}{K_{TCE,ORHZ} + C_{TCE}} \right)$$

where  $k_{methane}$  = the maximum methane consumption rate taken as 2.2 g CH<sub>4</sub>/ g cells/day (Smith, 2000)

$X_{meths}$  = the microbial mass in the rhizosphere level (g)

$C$  = concentration of substrate in the rhizosphere level (g/L)

$K_s$  = Monod half-saturation constant for methane (g/L)

$K_{TCE}$  = Monod half-saturation constant for TCE in the rhizosphere level (g/L)

$K_{I,TCE}$  = TCE inhibition constant taken as 0.005 g/L (Tartakovsky, 2005: 80)

$K_{s,O_2}$  = Monod half-saturation constant for oxygen (g/L)

The model does not permit more methane to be consumed than is available, nor can methane be consumed if oxygen is not available. Oxygen consumption is stoichiometrically linked to methane consumption (Noguera, 2000: 241):

$$\frac{dMO_2}{dt} = 1.66 * \frac{dM_{CH_4}}{dt}$$

The model calculates a transformation rate in each rhizosphere level that represents a mix of sMMO/pMMO using available copper and oxygen concentrations. It scales the highest known reaction rate ( $k_{TCE}$ ) for mixed methanotrophic culture: 9.6 g/g/d (Alvarez-Cohen, 1996) by a factor of .01-1 for copper and a factor of .01-1 for oxygen:

$$\text{TCE O}_2 \text{ coefficient Rhizo 1} = .99 * \left( \frac{.01 \cdot K_{\text{prime\_O}_2\_TCE}}{K_{\text{prime\_O}_2\_TCE} + C_{\text{O}_2, \text{Rhizo1}}} \right)$$

$$\text{Copper adjustment factor} = .99 * \left( \frac{.01 \cdot K_{\text{prime\_Cu\_TCE}}}{K_{\text{prime\_Cu\_TCE}} + C_{\text{Cu, soil}}} \right)$$

$$k_{\text{TCE, Rhizo 1}} = k_{\text{TCE}} * \text{Copper\_adjustment\_factor} * \text{TCE\_O}_2 \text{\_coefficient\_Rhizo\_1}$$

where  $K_{\text{prime O}_2 \text{ TCE}}$  is the oxygen half-saturation for sMMO taken as 2 mg/L (Uchiyama, 1995: 611)

$K_{\text{prime Cu TCE}}$  is the copper half-saturation for sMMO taken as 16ug/L (Alvarez-Cohen, 2001: 113; Tsien *et al.*, 1989)

$k_{\text{TCE}}$  (g TCE/g methanotrophs/day)

TCE consumption results from the reaction rate in the rhizosphere level representing the affinity for TCE, competitive inhibition of the enzyme by methane, concentration levels that exist in the rhizosphere level, and the mass of methanotrophs:

$$\frac{dM_{TCE}}{dt} = k_{TCE,Rhizo}^{**} X_{meths} \left( \frac{C_{TCE,RHZ}}{K_{TCE,RHZ} + C_{TCE}} \right) * \left( \frac{1}{1 + \frac{C_{CH_4}}{K_s}} \right)$$

where  $k_{TCE,Rhizo}$  = the maximum TCE consumption rate in the rhizosphere level as calculated above (g/g/hr)

$X_{meths}$  = the microbial mass in the rhizosphere level (g)

$C$  = concentration of substrates (g/L)

$K_s$  = Monod half-saturation constant for methane (g/L)

$K_{TCE}$  = Monod half-saturation constant for TCE (g/L)

Methanotroph growth results from the consumption of methane and is expressed using the yield (Y) taken as 0.7 g methanotroph/g methane (Tartakovsky,2005):

$$\frac{dX_{meths}}{dt} = 0.7 * \frac{dM_{CH_4}}{dt}$$

Methanotroph death results from TCE toxicity and natural decay ( $b_d$ ).  $b_d$  is taken as 0.1/day (Anderson, 1994: 388), and TCE toxicity is expressed using the Transformation Capacity ( $T_c$ ) taken as .21 g TCE/ g cells (Smith, 2000):

$$\frac{dX_{meths}}{dt} = - \frac{dM_{TCE}}{dt} * \frac{1}{T_c} - b_d X_{meths}$$

Heterotroph growth has similar calculations, but is simplified to account for carbon, oxygen, and non-competitive inhibition effects only. Heterotroph growth has a 0.67 g/g yield (Y) and a 1:1 ratio with oxygen for carbon consumed:

$$\frac{dM_{CKC}}{dt} = K_{X_{het}} \frac{M_{CRHZ}}{M_{CRHZ} + M_{CKC}}$$



$$\frac{dX_{het}}{dt} = 0.67 * \frac{dM_{CRHZ}}{dt}$$

$$\frac{dM_{CRHZ}}{dt} = \dots$$

Model Parameterization.

Parameters for the model were taken from a variety of sources. Appendix A identifies parameters used in the model. A few parameters deserve specific attention. Plant masses were adapted from Asaeda (2000) and used Nesyt fishpond data from Kvet et al., (1969). *Phragmites australis* has wide growth ranges depending upon environmental conditions, and the ones chosen represent moderate climate. One respiration rate was averaged for the entire plant rather than assigning specific values to each tissue group. The leaf pore diameter chosen to generate data represents a plant that is optimizing HIC. The resistance of the venting path (Rvp) was obtained by matching measured HIC flow rates of Afreen *et al.* (2007) using like conditions.

Microbial parameters were picked to represent a mixed-methanotroph culture. Kprime O2 TCE was determined by assertions made by Uchiyama (1995: 611); in oxygen concentrations > 2mg/L, activity decreased with increasing concentration. Kprime Cu TCE was based on observation that sMMO is only produced in wild bacteria at very low copper concentrations (<16ug/L). (Alvarez-Cohen, 2001: 113; Tsien *et al.*, 1989) kTCE is the highest known value expressed by mixed-cultures and is assumed to be

the maximum rate for TCE consumption. A higher  $k_{TCE}$  value is expressed by mono-cultures and may be a better representation of enzyme capacity. Pure-culture sMMO max is 55 g/g/d or 2.292 g/g/h. (Anderson, 1994; Oldenhuis, 1991) Regarding  $K_{TCE}$ , the half saturation affinity of MMO for TCE, sMMO has a slightly lower  $K_m$  (higher affinity) for TCE of 35uM or .0046 g/L (Field, 2004: 31) and also has a slightly higher affinity for methane. These small differences are not distinguished in the model.

Additional summaries of modeling data are available.

*Phragmites australis*. Armstrong, J., Armstrong, W. and Beckett, P.M. (1990); Armstrong (1991); Armstrong (2005); Asaeda, T. and S. Karunaratne (2006).

*Humidity Induced Convection*. J. W. H. Dacey (1987); J. Armstrong *et al.* (1996); W. Armstrong *et al.* (2000); Beckett, P., W. Armstrong, and J. Armstrong (2001); Afreen *et al.* (2007).

*Microbial Growth*. Calhoun (1998); van Bodegom *et al.* (2001); Field (2004); Vaccari (2006).

*Biodegradation of TCE*. Anderson and McCarty (1996); Watson, Stephen, Nedwell, and Arah (1997); Alvarez-Cohen and Speitel (2000); Noguera, Pizarro and Clapp (2000); Field and Sierra-Alvarez (2004); Tartakovsky, Manuel, and Guiot (2005).

## **Model Testing and Validation**

In order for a model to provide reliable information, it must be validated through testing. Validation is the process of establishing confidence in the soundness and usefulness of the model. (Shelley, 2007, 59) Once the framework and mathematical structures of the model are in place, the model is scaled against known measurements in

order to create a precise fit of the empirical data available. Comparing the model system output to reality corroborates or refutes the model within constraints of the objectives. Deviations from empirical data need to be accounted and the model corrected to obtain correct parameterization. In this case, the model must behave in the same manner as the real plant it represents, produce an accurate soil oxygen concentration gradient, and produce microbial responses that mirror empirical findings. The following tests are used to validate this model:

- 1.) Structure verification test- this model has been built to reflect the actual structures that exist in a real plant system. Oxygen and substrates flow in a similar manner to a real plant/rhizosphere system. See Model Assumptions for limitations.
- 2.) Parameter verification test- Parameters must correspond to real life both conceptually and numerically. Parameters for this model have been obtained by surveys of the scientific literature available (in Model Parameterization). Any parameter that cannot be verified through current sources is justified by the Behavior Reproduction test.
- 3.) Behavior Reproduction test- The model must generate realistic modes of behavior without further adjustment to the model structure. While model parameterization will fit the data to a small set of empirical data, the model must respond correctly to changes in plant variables, air, and soil conditions. Realistic changes to variables should not cause the model to generate unpredicted behavior and should generally correspond to empirical data.

- 4.) Extreme Conditions Test- Acceptable behavior at the extreme ends of model parameters is a demonstration of the model's flexibility and generally improves model performance in the operating range. (Shelley, 2007: 60) Extreme conditions testing was applied to the microbe simulations to ensure their response was adequate. In a series of 16 simulations, TCE, carbon, methane, and copper were sequentially set at maximum and minimum conditions in order to verify the model behaved realistically at extreme limits. This was not done for the plant components; plant response was captured by sensitivity testing.
- 5.) Behavior Sensitivity Testing- In order to determine specific characteristics and limitations of this model, many variables are tested to determine their relative impact on model performance. Parameters are varied from original settings +/- 10, +100% and -50%. Sensitivity is reported as high, medium, or low impact based upon the effect on steady state oxygen level in the soil.

#### Behavior Reproduction Test.

Humidity induced Convection (HIC) is the major input for oxygen in this model. In order to validate the model, comparisons were made to an analytical model by P. Beckett, W. Armstrong, and J. Armstrong (2001). Model parameters were programmed similarly and results for HIC relative to the resistance of the venting path are shown in Table 7. Testing used a max pressure of 759 at 32% RH vice 750 Pa. Beckett static pressure for no-flow condition was 462 Pa and Thompson model was 466 Pa. This

indicates that the Thompson representation for nitrogen and other gas pressures is a good approximation for the HIC calculation. Results of the Thompson model closely approximate those of Beckett *et al.* The Thompson model includes a limit on water flux from the plant, so low resistance flow is lower than Beckett model as shown in Figure 27.

Table 7. Venting Resistance Profile vs Beckett, 2001: 278.

Rvp		Flow (L/hr)	Beckett	static pressure (Pa)
0	0		0.5457	
1.00E+08	1	0.49	0.53	14
2.00E+08	2	0.47	0.5064	26
4.00E+08	4	0.45	0.48	50
6.00E+08	6	0.43	0.4518	71
1.00E+09	10	0.38	0.41	105
2.00E+09	20	0.31	0.324	173
4.00E+09	40	0.23	0.24	254
6.00E+09	60	0.18	0.18	298
8.00E+09	80	0.15	0.15	325
1.00E+10	100	0.13	0.126	348
1.00E+12	10000	0.002	(0)	466

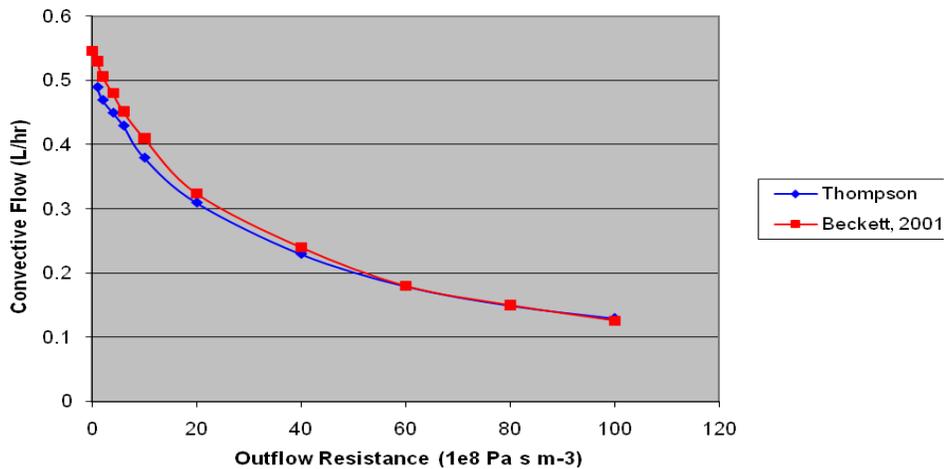


Figure 27. Thompson and Beckett Models Convective Flow vs Outflow Resistance. Thompson model predictions for HIC closely approximate the analytical solution of Becket *et al.* (2001)

A microelectrode and modeling study with *Phragmites australis* by W.

Armstrong *et al.* in 2000 provides an accurate portrayal of oxygen concentrations generated in the root zone by *Phragmites australis*. (Armstrong, 2003) A cross-section profile 7 mm from the root tip is shown below. After determining the resistance of the venting path by matching to data from Afreen *et al.* (2007), the model outputs were compared to those of Armstrong; model parameters were adjusted to obtain a similar profile. The Thompson and Armstrong rhizosphere profiles are shown below in Figure 28. It is possible that the agar solution in the Armstrong study resulted in a steeper profile due to advection currents in the solution; this is not inherent in the computer model. Despite the steepness of the gradients, the fundamental concentration levels of the root and rhizosphere are similar and reflect the same behavior.

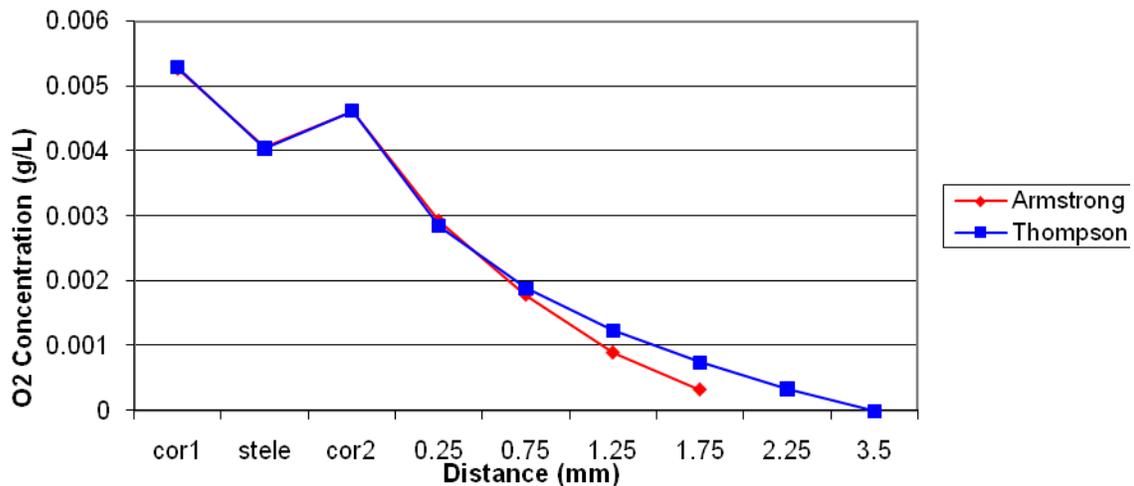


Figure 28. Oxygen Profiles for Armstrong Electrode Study and Thompson Model Output. Cor 1 is the cortex concentration of the lateral root while stele and cor2 are concentrations in the root hair zone.

Since accurate bacterial concentrations are not available at the scale of this model, it is not possible to assess microbial growth directly. In the initial runs of the model,

neither heterotroph nor methanotroph growth was being limited by substrate shortages. This was corrected by limiting substrate consumption to the oxygen that was stoichiometrically available. The primary substrate stocks also had to be directly routed to bacteria growth using yield as a unit converter. This limited the growth of the bacteria to only the substrate immediately available. Though different soil conditions were explored with the model, microbial behavior generally coincides with empirical observation. Het:meth ratios ranged from 4:1 to 137:1 under different soil conditions. Methanotrophs tended to colonize the outer rhizosphere, and heterotrophs occupied areas closer to the root where oxygen was readily available. After changing conditions, simulations frequently had to be run for 3-4 months simulation time before a steady-state was reached. These findings match behaviors observed empirically by Van Bodegom (2001:3591). Further comparisons related to TCE remediation are examined in Chapter IV Results and Analysis.

#### Extreme Conditions Tests.

Microbial performance was suitable at all soil conditions. Table 8 summarizes the testing.

Table 8. Extreme Conditions Testing for Microbial Growth

O2	Expectation	Result
MAX	hets/meths grow	+
0	no growth	+

Test	TCE (mg/L)	C (mg/L)	CH4 (ug/L)	Cu (ug/L)	Expectation	Result
1	0	0	0	0	bugs die	+
2				300	bugs die	+
3			1000	0	meths grow, sMMO	+
4				300	meths grow, pMMO	+
5		200	0	0	hets grow, meths die	+
6				300	hets grow, meths die	+
7			1000	0	het competition, sMMO	+
8				300	het competition, pMMO	+
9	500	0	0	0	bugs die	+
10				300	bugs die	+
11			1000	0	meths grow, sMMO toxicity	+
12				300	meths grow, pMMO degradation	+
13		200	0	0	hets grow, meths die	+
14				300	hets grow, meths die	+
15			1000	0	het competition, sMMO toxicity	+
16				300	het competition, pMMO degradation	+

Behavior Sensitivity Testing.

Plant parameter sensitivity testing measured output in Rhizo 1. Humidity was set at 80% for the tests. No microbes or advection were active during plant sensitivity testing. The model was most sensitive to changes in humidity. Overall, the model was most sensitive to the effects of humidity, but seemed less sensitive to light levels. Though light levels did not appear to have a direct effect on oxygen flow to the roots, increased photosynthesis resulted in elevated oxygen levels throughout the plant. EH dermal thickness was also a significant factor, but was not varied during subsequent variable testing. Table 9 summarizes the results.

Table 9. Model Plant Sensitivity Results. Sensitivity tests were run at 80% RH.

Adjustments	Value	Sensitivity					Comments	Sensitivity		
		-0.5	-0.1	ORIG	0.1	2X		High	Med	Low
Humidity *	50	0.003864	0.003864	0.003864	0.003846	0.002218	(10, 40, 50, 60, 98)	X		
Global radiation level	1500	0.003671		0.00366		0.003674	(200, 1000, 1500, 2000, 3000)			X
Temperature	298	0.00366		0.00366	0.003519	0.00265	(278, 295, 298, 301, 318)		X	
Atmospheric Pressure	101325	0.00304	0.00337	0.00366		0.003791			X	
Pm (Photosynthetic capacity)	0.21	0.00366		0.00366		0.00366				X
K CO2	0.00044	0.00366		0.00366		0.00366				X
Leaf Area Index	7	0.0036		0.00366		0.0037				X
CS Diffusion Transfer Coefficient	0.025	0.00366	0.00366	0.00366	0.00366	0.00366				X
O2 RHZ Diffusion Coeff	0.45	0.00279	0.00352	0.00366	0.00379	0.00439			X	
Lat root air transfer coeff	0.41	0.00317	0.00361	0.00366		0.00394			X	
RZ1 phloem rate coefficient	0.005	0.00366		0.00366		0.00366				X
Rvp	6.90E+09	0.00374	0.00367	0.00366	0.00366	0.00352				X
Leaf Tissue Percent	70		0.00367	0.00366	0.00367					X
Leaf Pore Diameter	0.2	0.00368	0.00367	0.00366	0.00366	0.00364				X
Fractional Porosity	0.00027	0.0036	0.00366	0.00366		0.00371				X
Plant Tissue O2 UsageRate	0.004	0.00398		0.00366		0.00304			X	
Leaf CO2 Transfer Coeff	5	0.00367		0.00366		0.00367				X
Phloem bulk flow rate	0.004	0.00366		0.00366		0.00367				X
Xylem bulk flow rate	0.004	0.00369		0.00366		0.00363				X
Number of Roots	10	0.00408	0.00374	0.00366	0.0036	0.0031			X	
Root Magnitude	4	0.00408		0.00366		0.0031			X	
Root tip radius	0.5	0.00328		0.00366		0.00363	(.25, .5, .8)			X
Hair Zone Length	3	0.00408		0.00366		0.0031			X	
Plant area density	300	0.00295	0.00357	0.00366		0.00394			X	
Rhizome mass fraction	0.85		0.00348	0.00366	0.00388				X	
Above/Below Area Mass	1500/3000	0.00396		0.00366	0.00361	0.00308			X	
Wind Speed	0.056		0.00369	0.00366	0.00364	0.00364	(.01, .056, 5, 15)			X
CO2 fraction	0.001	0.0367		0.00366		0.00367	(.0005, .001, .01)			X
EH dermal thickness RHZ	0.09	0.0044		0.00366	0.00353	0.00275		X		

## Model Application

Once the model is validated, it is then used to gain intuition about behavioral dynamics in the rhizosphere. This study makes a number of comparisons that would otherwise be unavailable or cost-prohibitive for field-testing. It first examines the impact of humidity and light through 15 different scenarios. Three scenarios are then selected that result in high, med, and low oxygen levels in the rhizosphere. For each oxygen scenario, three variables (carbon, methane, and copper) were systematically adjusted to determine microbial response in a clean (toxin-free) environment. The results are contained in Appendix F Soil Variable Testing Data.

Once baseline behavior was determined, seven specific scenarios were explored by varying TCE concentrations and loading rates. Additional testing was also conducted to make comparisons supporting the findings. In both cases, heterotroph and

methanotroph populations arrived at steady-state or approached zero. Due to timestep limitations on the STELLA program, steady-state values were achieved by exporting stock values, reimporting them back into the program, and repeatedly running the simulation until values no longer changed significantly.

#### Modular Testing.

For testing, the program had to be run in two separate simulation modules; small plant volumes required a small timestep for HIC calculations (0.00005 hr) that limited the simulation to 3 hours, but bacterial growth developed over scales of hours, weeks, and months and required a larger time step (0.01 hour) which allowed a 1320 hour simulation (multiplied by export/import iterations). All scenarios were initiated with the microbial growth module by setting a rhizosphere mass flow rate, establishing steady-state, and verifying the matching rhizosphere flow with the plant module output.

#### Dynamic Behavior Test.

An additional scenario was explored to check model behavior: two simulations were performed that drastically varied initial conditions of methanotroph/heterotroph populations. Although steady-state took over four months to develop in one scenario, both runs arrived at the same steady state values, a testament that the dynamic response of a system is independent of its initial conditions. Tests A and B were conducted and are recorded as Soil Variable Tests 25 and 25B in Appendix F Soil Variable Testing Data.

Figure 29 and Figure 30 show the initial response of methanotroph populations. Figure 31 and Figure 32 show the initial response of heterotroph populations. Figure 33 and Figure 34 show the steady-state population of methanotrophs at the end of the simulation. Heterotrophs also reach the same steady-state masses. Were the simulations to run longer, Figure 33 and Figure 34 would be identical. X-axis is time (hours). Y-axis is microbial mass (grams). The numbers on the lines represent the rhizosphere level.

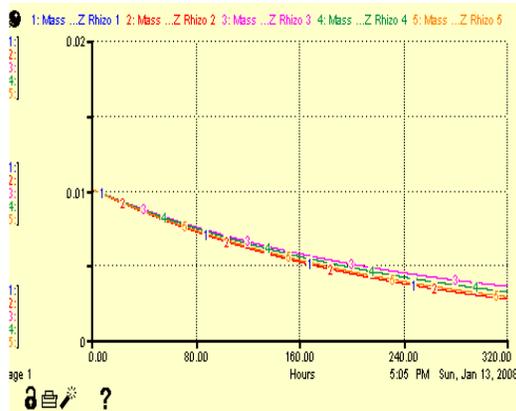


Figure 29. Meth Initial Response Sc. A

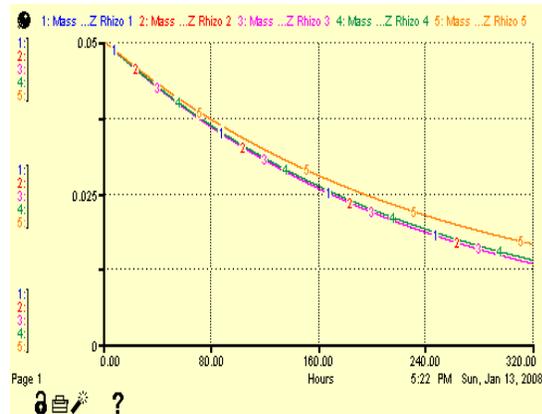


Figure 30. Meth Initial Response Sc. B

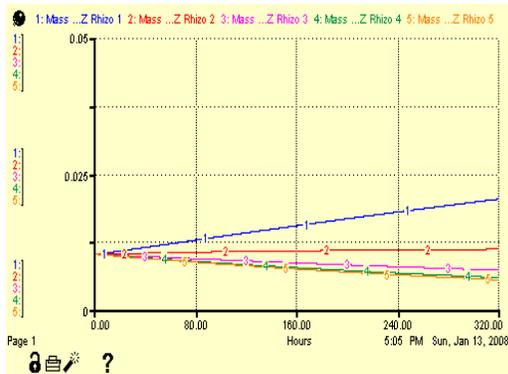


Figure 31. Het Initial Response Sc. A

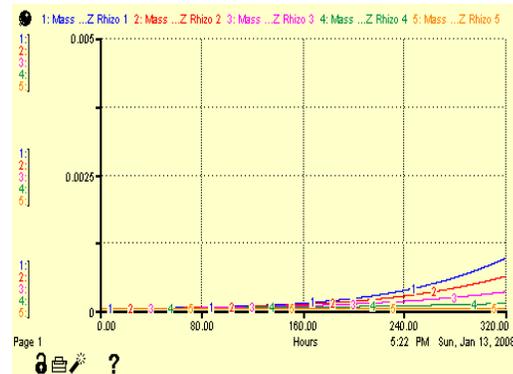


Figure 32. Het Initial Response Sc. B

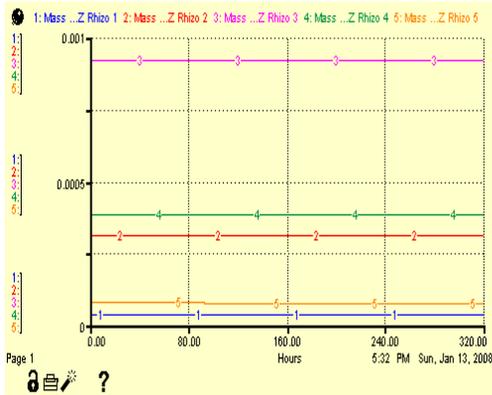


Figure 33. Meth Final Steady-State A

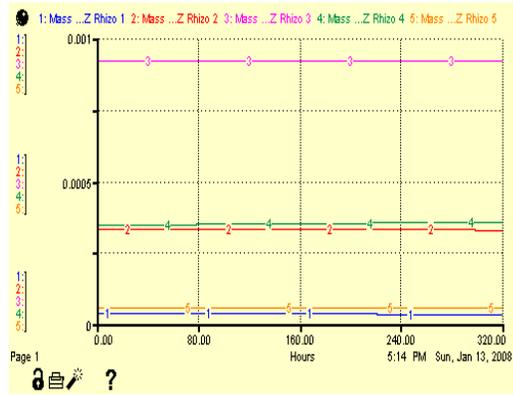


Figure 34. Meth Final Steady-State B

## IV. Results and Analysis

The results from this model may offer insight into the effects of oxygen, copper, methane, and carbon sources on TCE bioremediation. The results can be analyzed by looking at three outputs: the plant model and rhizosphere profile, bacterial growth, and TCE response. Each analysis shows specific behavioral patterns that can be important considerations in any bioremediation engineering effort. HIC and Radiation Output Data, Soil Variable Testing Data, and TCE Testing Data are included as Appendix E, F, and G respectively. Excel spreadsheet data is also included with the accompanying disk.

### **Plant Model**

HIC and photosynthesis are two significant inputs to oxygen levels inside the plant. HIC was shown to have the greatest influence on rhizome oxygen levels.

#### Humidity Induced Convection.

Model calibration and parameter sensitivity was covered in Chapter III. It shows that numerous variables can significantly influence oxygen concentrations in the root zone. Due to its role in humidity induced convection, relative humidity (RH) has a significant influence on rhizome oxygen levels. The plant, however, shows that it is very efficient at inducing air flow through its aerenchyma, even at high humidity. As shown in Figure 35, HIC remains substantial even in humid conditions, contributing to oxygen levels over ninety percent atmospheric even at relative humidity above 80%. A significant tapering effect does not begin until approximately 90% RH.

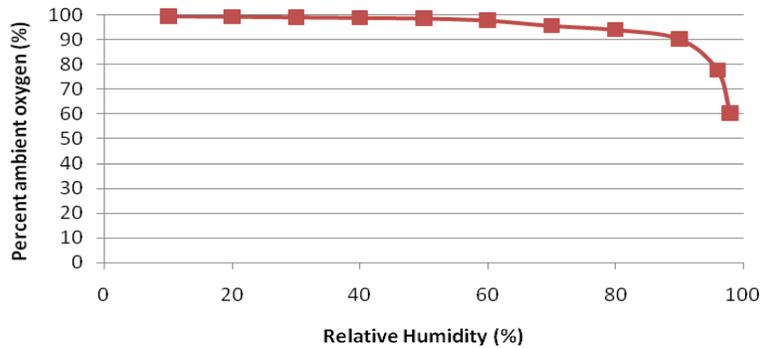


Figure 35. RH Effect on Rhizome Oxygen Levels.

The effect of HIC on rhizosphere oxygen concentrations is significant, but is less pronounced. Even at 98% RH, this model predicts that rhizosphere oxygen will still be  $\approx 60\%$  that obtained in low humidity conditions. Normal changes in relative humidity (10-80%) will have minimal effect on rhizosphere oxygen as shown by the close and overlapping lines shown by Figure 36. Oxygen delivery to the rhizosphere will be affected only at high humidities. This may imply that wetland treatment systems could be significantly degraded during extended rainy conditions; higher oxygen levels permit high consumption rates of methane and aid in methanotroph recovery from toxic effects of TCE (covered in greater detail later in chapter). Without considerations for bacterial consumption, the root profile is concave. This results from the diffusion of oxygen into a volume that increases by the square of the distance from the root surface.

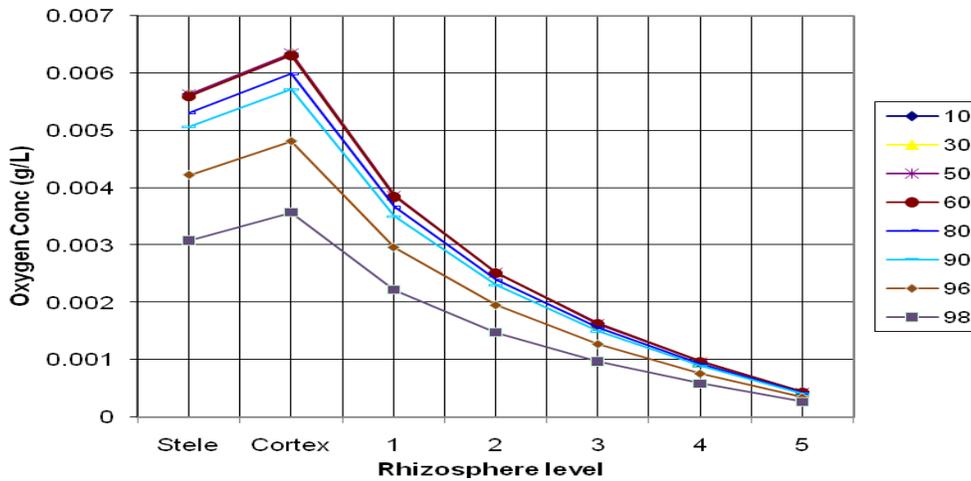


Figure 36. Relative Humidity Effect on Oxygen Levels in the Rhizosphere. 10, 30, and 50% RH lines coincide with the 60% line, showing that normal relative humidity variations will have minimal effect on rhizosphere oxygen concentrations.

Though the venting path resistance is listed as low sensitivity for the model, this parameter can fluctuate greatly. It is difficult to calculate this resistance in plants due to the tortuosity of venting paths, the dynamic effects of pressurization (increased pressurization also increases venting resistance), and dieback cycles of the plants; the presence of numerous dead culms can significantly lower the venting resistance of plants in a stand and significantly increase HIC through flow. Other parameters like pore width can vary constantly throughout the day in order to minimize plant water loss and maximize the CO<sub>2</sub> available for photosynthesis. Either a fully closed or fully open stomata would decrease HIC. Consequently, wetland treatment systems may exhibit different treatment efficiencies throughout the day as plant responses to atmospheric conditions change.

## Plant Circulation.

As summarized in model sensitivity testing, photosynthesis seemed to have little direct effect on root oxygen levels directly. The resulting HICs were the same for low and high radiation levels, and the oxygen concentrations in the rhizomes were also the same as shown by Figure 37. However, the effect of radiation on leaf-heating is not directly modeled, and high light levels cause heating of leaf gases which would increase the effective HIC. Were radiation level and internal leaf temperature correlated, the model is set up to calculate its effect on HIC. Photosynthesis did result, though, in higher oxygen levels throughout the plant tissues. The higher oxygen saturation obtained during the day may have a role in maintaining aerobic plant conditions during the night; oxygen stored during the day can supplement plant respiration requirements at night when HIC is reduced or non-existent. At night, diffusion and Bernoulli effects in the aerenchyma along with oxygen in the continuously flowing phloem are more significant inputs to rhizosphere oxygen.

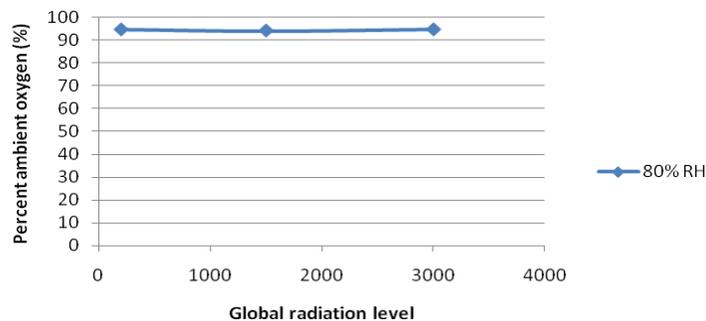


Figure 37. Radiation Effect on HIC.

Photosynthesis does reach saturation at low light levels. However, given the plant's relatively high half-saturation value for  $\text{CO}_2$ ,  $\text{CO}_2$  is the most limiting factor on plant photosynthesis. Recirculation of  $\text{CO}_2$  generated by plant respiration was needed to

increase CO<sub>2</sub> available in the leaves and maintain net positive oxygen production, suggesting that plant “rebreathing” of CO<sub>2</sub> could significantly increase carbon fixation and oxygen production.

### **Bacteria Response**

Bacteria in adjacent rhizosphere levels have a dynamic effect on the growth and decay of each other; substrate consumed in one level is unavailable to diffuse into adjacent levels, resulting in growth limitations for neighboring bacteria. Conversely, when bacteria are starving and dying in one level due to a deficiency of one substrate, it frees up supplies of the non-limiting substrates for their neighbors as well. Oxygen, methane, and carbon supplies, then, are all interconnected. Copper has no direct growth effects, but MMO expression changes significantly based on the oxygen concentration available in the rhizosphere level. The MMO expression of the bacterial mass contributes to dynamic behavior when TCE is introduced.

### Oxygen.

Oxygen profiles in the root zone vary greatly depending on concentration of soil substrates; specifically, the presence of high levels of carbon in the soil promotes heterotrophic growth and results in the most significant oxygen sink. At low and medium HIC, the model showed that oxygen was the limiting factor on growth as shown by the asymptotic behavior of the Low and Med HIC curves in Figure 38. Doubling HIC resulted in a doubling of microbial mass. Greatly multiplying the carbon concentration (6X) resulted in doubling microbial mass at low HIC, but had a slightly greater effect

(2.5X mass) at high HIC, showing that oxygen has less growth inhibition at high HIC conditions. Clearly, high oxygen and high carbon conditions in the soil are favorable to heterotroph growth.

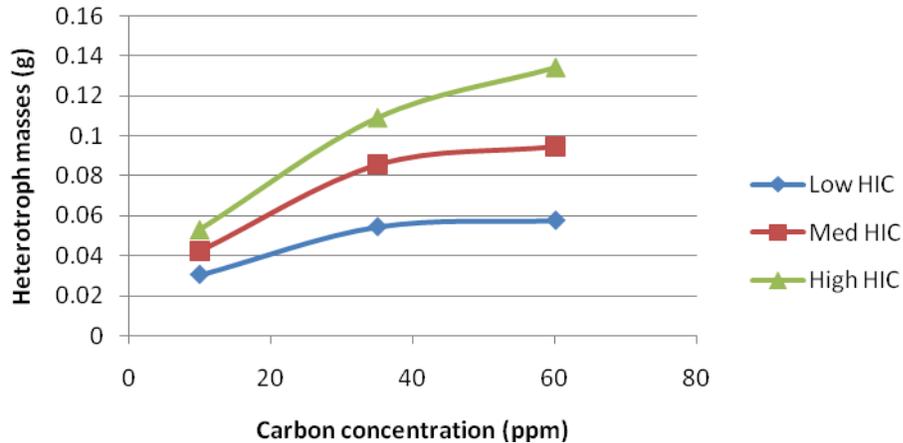


Figure 38. Carbon Concentration and Heterotroph Growth. Carbon consumption is limited by oxygen levels at low HIC. Carbon concentration remains a limiting growth factor at higher HIC.

In the rhizosphere, the bacteria grow inside a biofilm with growth being limited by the nutrient available in the smallest quantity. The profiles shown in Figure 39 and Figure 40 demonstrate how oxygen limits consumption of the primary substrate and limits bacterial growth. In Figure 39, heterotrophs are constrained by oxygen in the outer rhizosphere and by carbon on the inner rhizosphere (since it had been consumed in the outer levels). Methanotroph growth is constrained by methane. The limitation of methane diffusion is likely the reason that methanotrophs are normally found in the outer rhizosphere; though it is possible for methanotrophs to grow in high oxygen conditions, growth towards the interior is curtailed by low methane concentrations. In Figure 40, methanotrophs are constrained by oxygen in the outer rhizosphere and methane towards the inner rhizosphere. The convex profile demonstrates that methanotrophs will grow

near the root surface if high methane levels facilitate inward diffusion. Heterotrophs are constrained by oxygen. Any profile constrained by two substrates results in a convex profile.

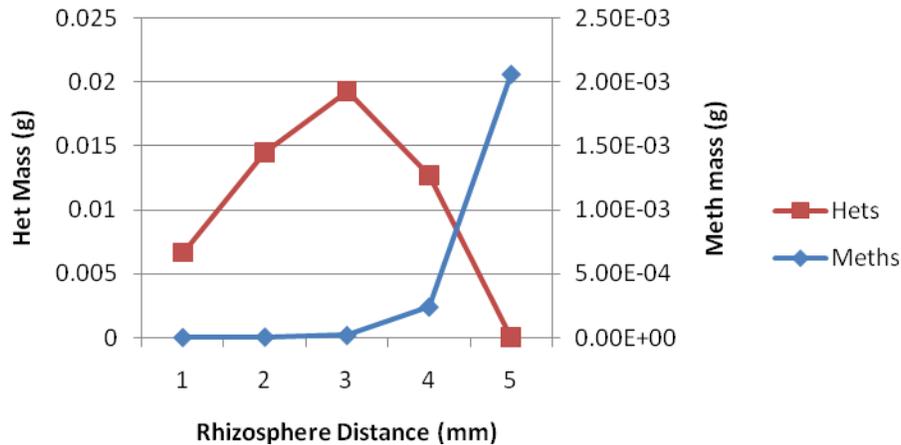


Figure 39. Bacteria Profiles in High HIC, Low C and CH<sub>4</sub>. Heterotrophs, showing a convex profile, are constrained by carbon consumption in the inner rhizosphere and oxygen in the outer rhizosphere. Methanotrophs are only limited by methane availability.

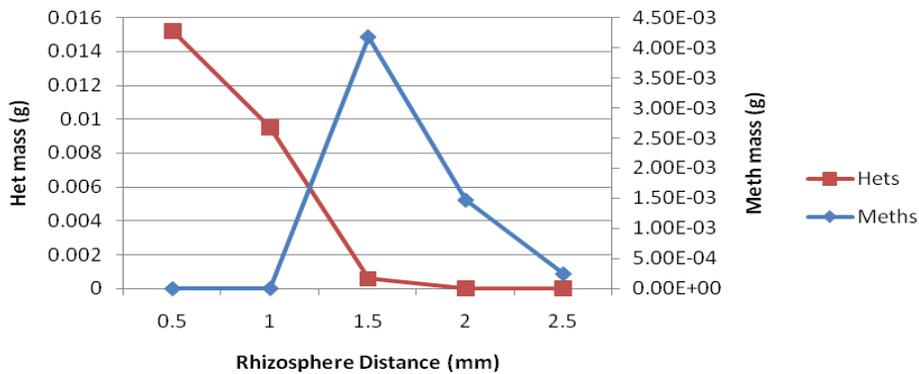


Figure 40. Bacteria Profiles in Low HIC and C, High CH<sub>4</sub>. Methanotrophs, showing a convex profile are constrained by methane in the inner rhizosphere and oxygen in the outer rhizosphere. Heterotrophs are constrained by oxygen availability only.

High carbon levels permit diffusion of carbon to the inner rhizosphere where adequate oxygen results in high heterotroph growth. Low oxygen levels likewise limit the growth of the heterotrophs. When carbon concentrations are low, carbon becomes the limiting nutrient and heterotroph populations shift towards the nutrient, creating a

different rhizosphere profile. Profiles for these conditions are shown in Figure 41. In High HIC/ High C and Low HIC/Low C, oxygen is the limiting nutrient. In medium HIC/Low C, oxygen is a limiting nutrient to the outer rhizosphere, but carbon is a limiting nutrient in the inner rhizosphere and results in a convex profile with a greater heterotroph population in the mid-rhizosphere.

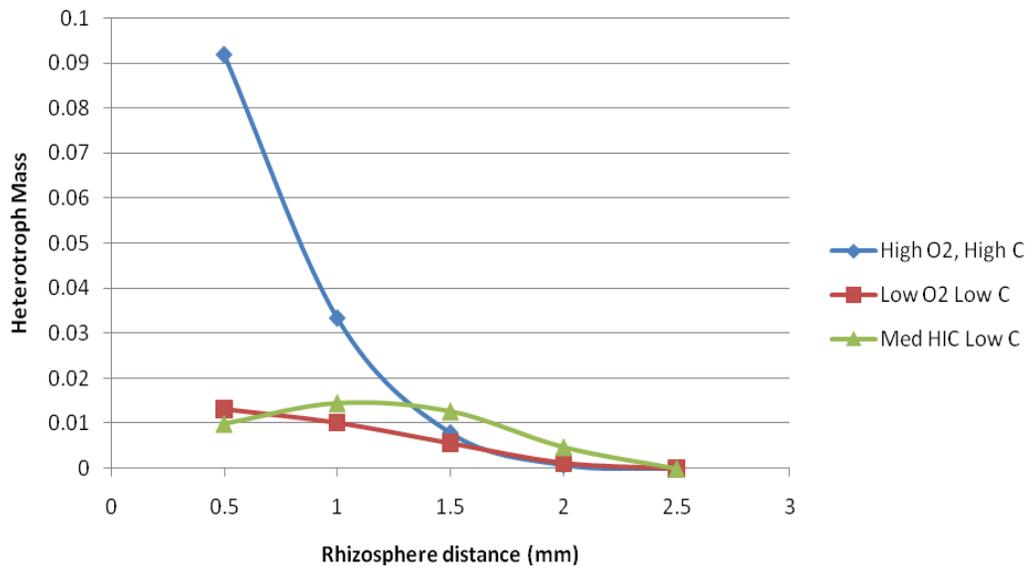


Figure 41. Heterotroph Profiles for Different Growth Conditions.

High heterotroph growth leads to a much steeper decline of oxygen levels in the rhizosphere and significantly more oxygen flux from the root system as shown in Figure 42. Since oxygen levels decline rapidly, high heterotroph populations are confined to the inner rhizosphere. Low growth conditions result in more oxygen in the rhizosphere that is available for use by other bacteria like the methanotrophs. High HIC and low carbon concentrations results in the highest rhizosphere oxygenation.

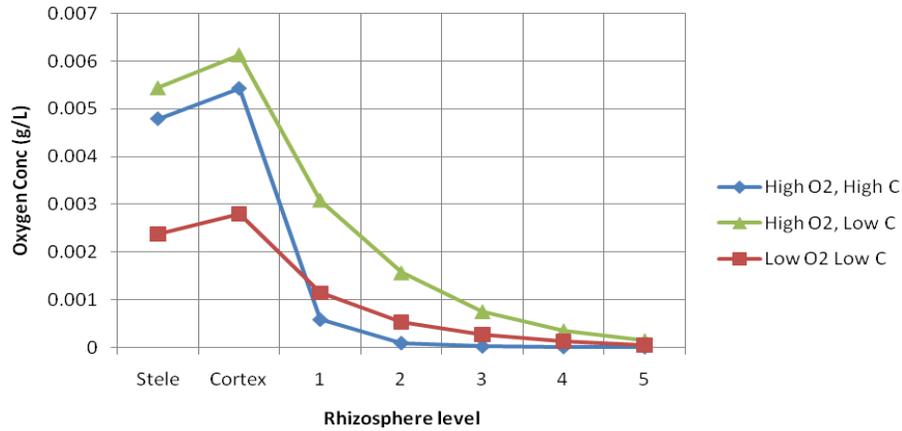


Figure 42. Heterotroph Effect on Oxygen Profile. Each level in this profile represents 0.5 mm from the root surface.

The oxygen demand by heterotrophs exerts significant influence on the methanotroph populations. Figure 43 shows how carbon concentration has a much greater effect than oxygen availability on methanotroph growth. All low carbon concentration conditions create much greater methanotroph mass than high carbon concentrations; since heterotroph growth is curbed, more oxygen is available to support methanotroph consumption of methane. It is also clear in this figure that oxygen is the limiting growth factor for methanotrophs in low HIC, and methane is the limiting factor in high HIC conditions. The highest methanotroph populations were found at high methane and low soil carbon concentrations.

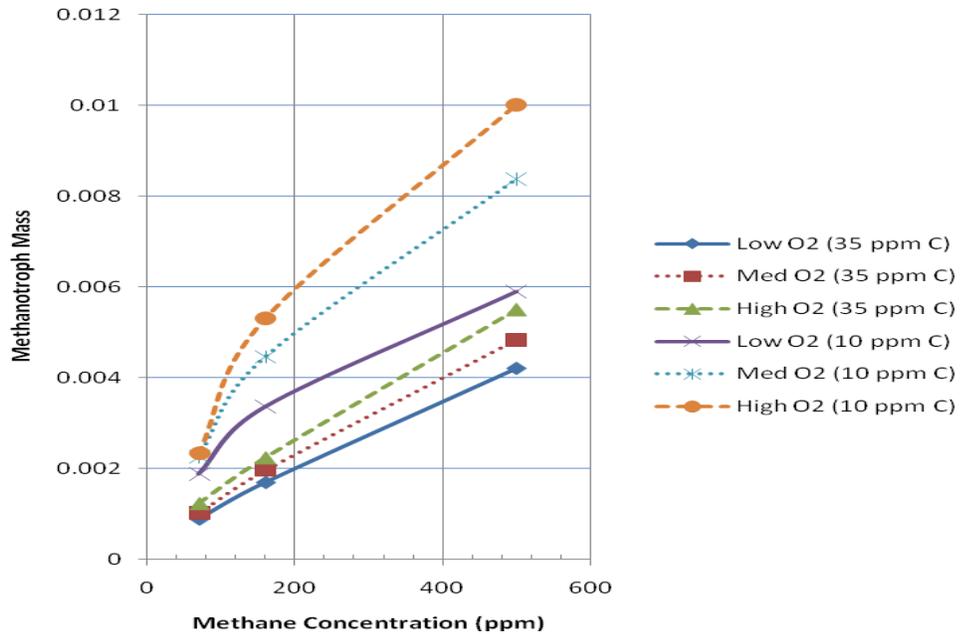


Figure 43. Oxygen Effect and Carbon Effect on Methanotroph Growth with 10, 35 ppm Carbon. Due to its role in heterotroph growth, carbon concentrations are the most significant factor in determining methanotroph populations.

### Copper.

In order to achieve full sMMO expression, both low oxygen conditions and low copper concentrations must exist in order to limit the copper available for expression of pMMO. Figure 44 demonstrates that copper concentrations effect MMO expression and that at normal copper levels of 100-200 ppb, pMMO will be the dominant enzyme present. sMMO will be present towards the outer rhizosphere where lower oxygen concentrations exist, and pMMO expression will be amplified near the root where oxygen concentration is higher. The highest percent of pMMO expression occurred at high HIC, low soil carbon, and low methane. Since methane and carbon limited growth, more oxygen was available for pMMO expression. The maximum sMMO expression occurred at high C, high methane, and medium HIC where oxygen, as the limiting substrate, was more quickly consumed. sMMO expression was next highest with low HIC, but lower

het/meth ratios at the low HIC (13:1 vice 20:1), allowed higher oxygen levels in the outer rhizosphere where the methanotrophs were present in greatest mass and enabled slightly more pMMO expression. The main influence of MMO expression in this model is correlated to copper concentration since methanotrophs tended to live in the outer rhizosphere where oxygen levels were already low. When TCE is introduced to the system, however, expression percentages of sMMO change significantly.

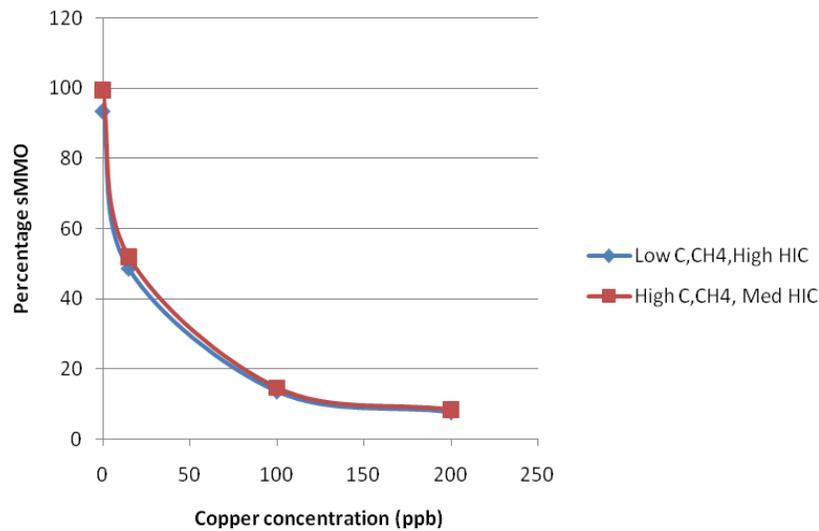


Figure 44. Varying Conditions on MMO Expression. When no TCE is present, copper level is the most significant influence on MMO expression; the effect of oxygen is negligible since most methanotrophs live in outer shells of the rhizosphere where oxygen is low.

### Methane and Carbon.

Heterotroph and methanotroph consumption of their primary substrates is limited by the amount of oxygen available for consumption. The effect of carbon and methane on the two bacteria can be seen by looking at ratios of the two populations. High carbon and low methane resulted in a ratio over 137:1 in high HIC conditions, and reverse conditions (low carbon, high methane) resulted in a minimum ratio of 4.3:1 at low HIC conditions. These het:meth ratios were calculated by summing mass throughout the

entire rhizosphere. Figure 45 depicts lower het:meth ratios at higher methane concentrations. Ratios in a wetland (rice paddy), where methane concentrations can range from 160 ppb to 16 ppm would favor low het:meth ratios likely below 20:1 (5% by mass), higher than prior calculations of 1-2%. (Macalady, 2002: 151-2) Differences between observed and simulated ratios could be accounted by nitrogen limitations since nitrite and/or ammonium in the soil may have an inhibitory effect on methanotrophs and decrease methane oxidation. (Macalady, 2002: 154; Hanson and Hanson, 1996) It should be noted that declining het:meth ratios are not due to heterotroph decline, but from methanotroph increase due to methane availability; het populations largely remain constant in stable carbon levels.

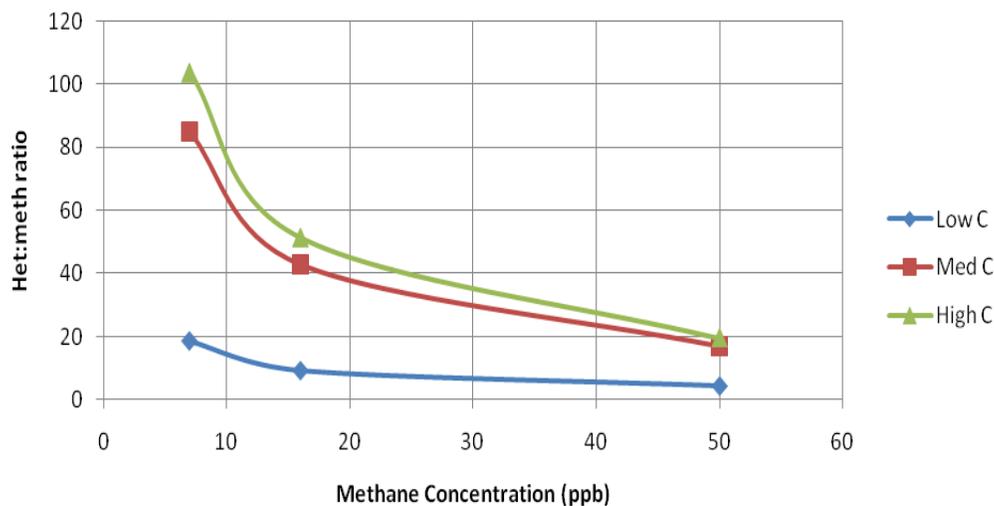


Figure 45. Effect of Carbon and Methane on Het:Meth Ratios. These graphs were based on medium HIC. The high carbon values were limited by oxygen availability in this scenario.

### TCE Response

TCE concentration and wetland hydraulic loading rate were varied on seven different treatment conditions to explore the dynamic effects of remediation. TCE

concentration was found to be a critical factor in bacterial growth, resulting in dramatic shifts in methanotroph profiles. TCE concentrations were modeled without regard to normal TCE saturation in water; this occurs at 1100 mg/L, so conditions above this level may not be present in real systems. Hydraulic loading rates were the most significant factor in controlling treatment efficiency.

### Concentration.

TCE concentration inputs to the system resulted in dynamic shifts in bacteria masses. The methanotrophs in the outer rhizosphere and low oxygen conditions expressed more sMMO and were more susceptible to toxic effects from TCE transformation due to the higher transformation capacity of sMMO. Raising TCE concentration results in a gradual shift towards the safer pMMO expression by methanotrophs as shown in Figure 46.

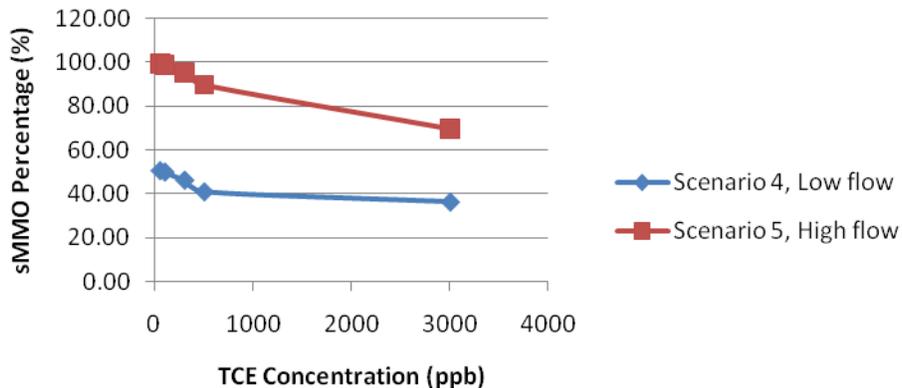


Figure 46. Effect of Raising TCE Concentration on MMO expression.

Since higher oxygen conditions lead to more pMMO expression, methanotroph near the root express more pMMO. When methanotrophs in the outer rhizosphere died, more methane was able to diffuse to the interior rhizosphere and allowed methanotrophs

there to grow. The growth of methanotrophs expressing pMMO remained high until much higher TCE concentrations. The variation of MMO expressed throughout the rhizosphere gives treatment wetlands an inherent capability to recover from inadvertent exposure to high TCE concentrations, however recovery of treatment efficiency from such releases could take months. This model did not take bacterial migration into account, however, and actual recovery times may be less.

Figure 47 depicts the time response of methanotrophs to an increase in TCE concentration (100 to 300 ppb), showing a significant redistribution of methanotroph masses in the rhizosphere. When TCE concentrations were increased, there was a significant decline in methanotroph mass in rhizosphere levels 3-5 where low oxygen levels allowed greater sMMO expression. Death of methanotrophs in the outer rhizosphere permitted greater methane to flow to the interior where pMMO expressing methanotrophs, more resistant to TCE toxicity, could use the methane for growth. This profile shows a response out to 320 hours. Simulation times often took in excess of 3000 hours (125 days) to arrive at a new steady state, supporting assertions by van Bodegom *et al.* (2001) that steady-state values for bacteria biomass may not be obtained until four months have elapsed.

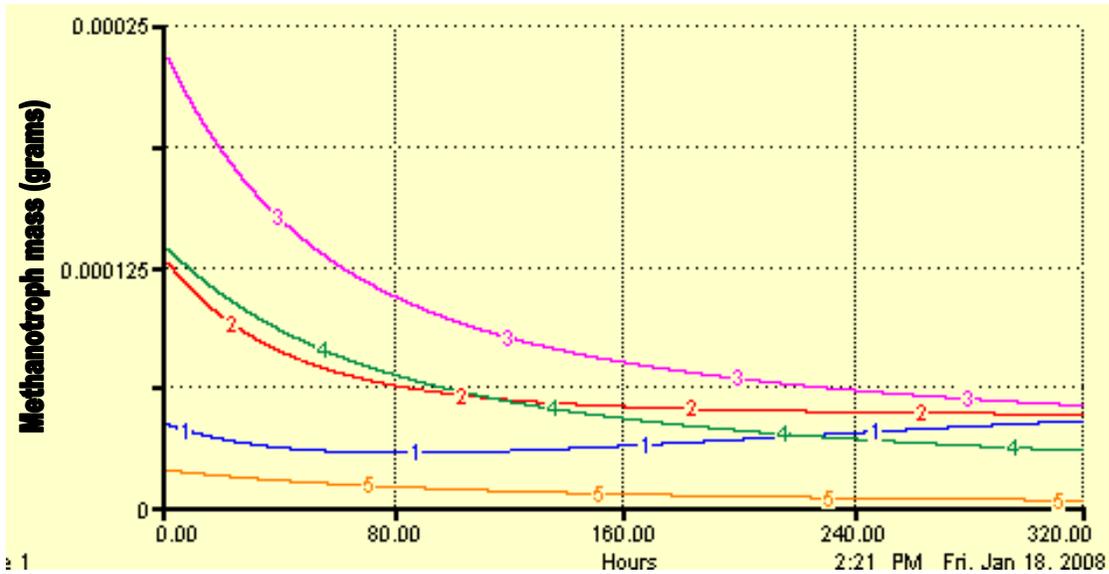


Figure 47. Effect of TCE Concentration on Methanotroph Profile in the Rhizosphere. This graph was generated at the medium loading rate of 2.4 L/m<sup>2</sup>/hr.

Figure 48 shows another representation of TCE impact on methanotrophs in the rhizosphere. Treatment efficiency and degradation rate quickly fall as sMMO expression gives way to pMMO expression. TCE degradation rate rises as TCE through flow approaches the bacteria's max consumption rate. As TCE concentration rises, methanotrophs expressing sMMO disproportionately suffer from toxic effects. This results in a reduction in treatment efficiency. MMO shifts towards pMMO expression as methanotrophs grow closer to the root where higher oxygen levels exist.

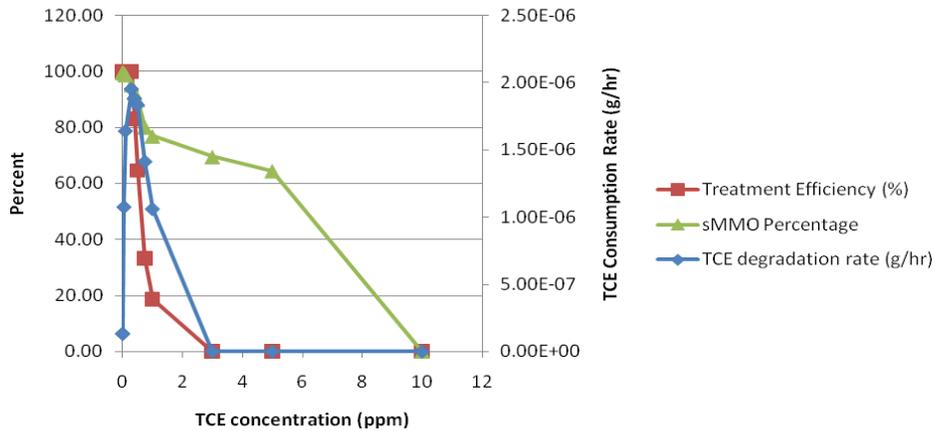


Figure 48. TCE Concentration Effects on Bioremediation. Output from Scenario 5 (sMMO, high methane, high HIC, and high carbon)

Figure 49 examines the difference between sMMO and pMMO toxic effects. Heterotrophs in both scenarios, affected only by non-competitive inhibition by TCE, showed a slight decline as TCE concentrations decreased, but did not suffer acute toxic effects like the methanotrophs. The slow loss of heterotrophs did free up some oxygen and permitted a slightly higher rate of recovery for the ailing methanotrophs, but by that time, competitive inhibition by TCE was substantial and also limited methane consumption. sMMO expression results in greater toxicity, but also results in high treatment efficiencies. pMMO expression results in resilience and higher overall mass, but poor removal rates at the low concentrations of TCE processed in a normal treatment system. A mixture of s/pMMO methanotroph masses lies between the two lines and would normally be weighted towards the pMMO line in a normal treatment system. Highlighted boxes indicate the concentration at which maximum TCE consumption was achieved, a balance between toxicity and maximum bacterial growth rates.

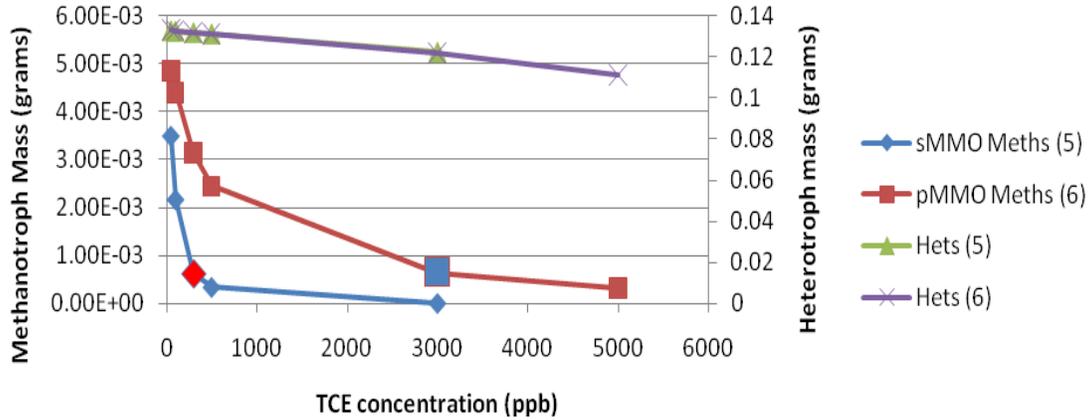


Figure 49. Bacterial Mass and TCE Concentration.

Raising the TCE concentration input to a wetland has the effect of raising TCE consumption to a saturation point, at which methanotrophs will begin to dieback at a greater rate than they recover and grow. The dashed lines in Figure 50 represent TCE consumption as a function of TCE concentration. pMMO permits methanotrophs to tolerate much higher levels of TCE, but the mass flux of TCE flowing through the system at those levels makes treatment at those levels inefficient. The solid lines represent efficiency curves; note how pMMO treatment quickly declines and crosses the sMMO line at the low treatment efficiency of 20%. Consequently, pMMO treatment is not beneficial at normal environmental concentrations <50 ppb. Also see *Copper Effects on Remediation* below.

## TCE Effect on Efficiency and Consumption

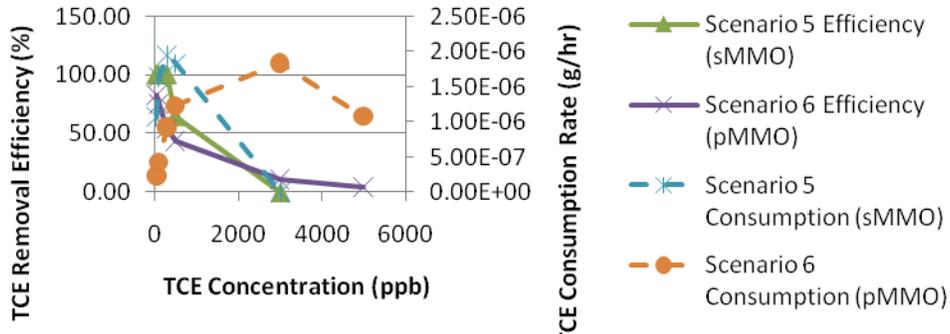


Figure 50. TCE Effect on Treatment Efficiency. At TCE concentration less than 1000 ppb, sMMO has both greater TCE removal capacity and efficiency.

*Toxic Threshold.* pMMO producing methanotrophs had a much higher resistance to the effects of high TCE concentration. For the low flow conditions tested in this simulation, high methane allowed pMMO meths to tolerate over 3000 ppb TCE prior to degradation. sMMO producing varieties with high methane tolerated much lower concentrations of 300 ppb before performance was degraded from TCE toxicity. Lower carbon levels helped slightly since they made more oxygen available to support methane consumption. Though copious oxygen helped to improve methanotroph recovery, methane was the driving factor in TCE resistance. In treatment systems dealing with TCE concentrations greater than 100 ppb, methane levels need to remain high to facilitate methanotroph growth and recovery. Figure 51 depicts the increased resistance to TCE toxicity afforded by abundant methane sources.

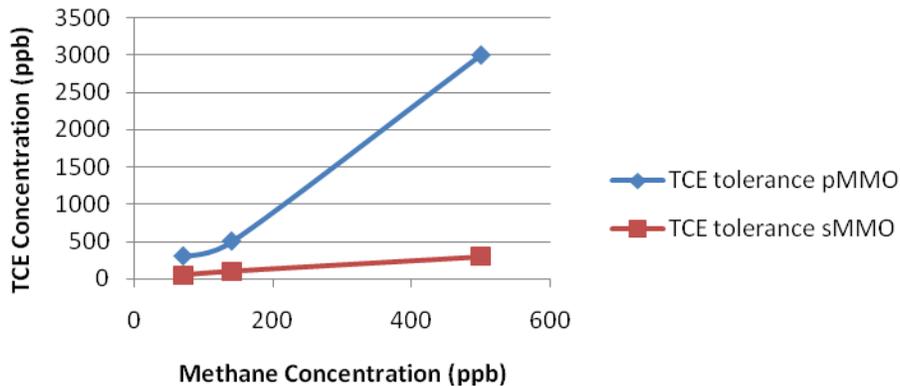


Figure 51. TCE Tolerance varying MMO and Methane. Methane concentration drives the resistance of both sMMO and pMMO producers to higher TCE concentrations. pMMO producers are naturally more resistant due to the decreased affinity of the enzyme for TCE.

### Flow Rates.

Flow rates had little effect on bacterial growth; similar masses of methanotrophs or heterotrophs were found at both high and low flow conditions. Diffusion of substrates into the rhizosphere proved to be a more significant factor than advective substrate flow. Flow rates do, however, affect treatment efficiency significantly; raising water flow (hydraulic loading rate) in the treatment system results in lower treatment efficiencies and allows a larger portion of the contaminant mass to pass the system untreated. High treatment efficiencies can be maintained at lower loading rates, but the best systems will maintain high efficiencies at higher loading rates as Series 5 in Figure 52. In order to optimize a treatment system, then, it is recommended that soil conditions first be analyzed in order to determine likely TCE steady-state consumption rates. Flow rates can then be adjusted in order to achieve the maximum efficiency (rate at which all TCE is consumed) or to achieve an efficiency that will result in meeting protective limit concentrations.

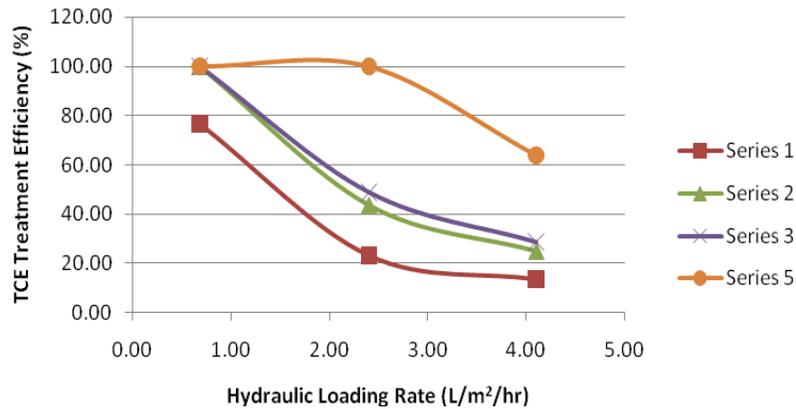


Figure 52. TCE Treatment Efficiencies at Varying Flow Rates (TCE 50 ppb).

Rhizosphere Conditions.

*HIC Effects on Remediation.* Higher oxygen encourages pMMO expression and should lower TCE consumption rate, but that loss is surpassed by the higher efficiency of consuming methane for growth/repair. In Figure 53, the medium HIC scenario shows a higher capacity for TCE remediation. High HIC conditions will amplify these effects still further.

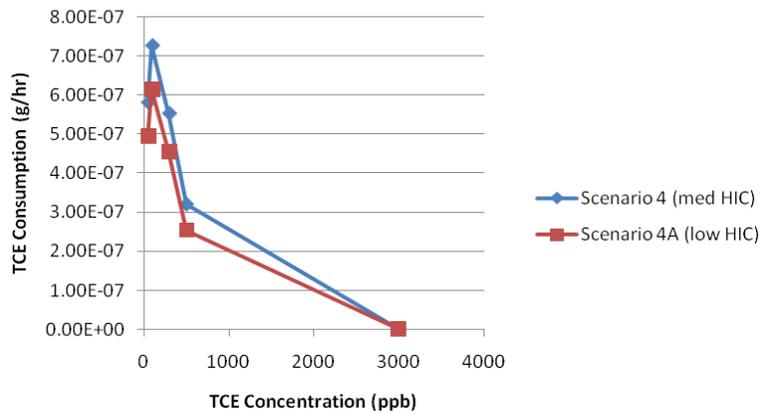


Figure 53. HIC Effect on TCE Consumption. Higher HIC permits methane consumption for recovery and results in higher rhizosphere capacity for TCE consumption.

*Carbon Effects on Remediation.* The presence of carbon in the rhizosphere increases heterotroph populations that consume oxygen as they consume carbon. This reduces oxygen available for methanotroph respiration and growth, resulting in reduced capacity to consume and recover from TCE toxicity. Figure 54 shows that high carbon concentrations can decrease methanotroph TCE consumption by a factor of three at moderate TCE concentrations (100-500 ppb).

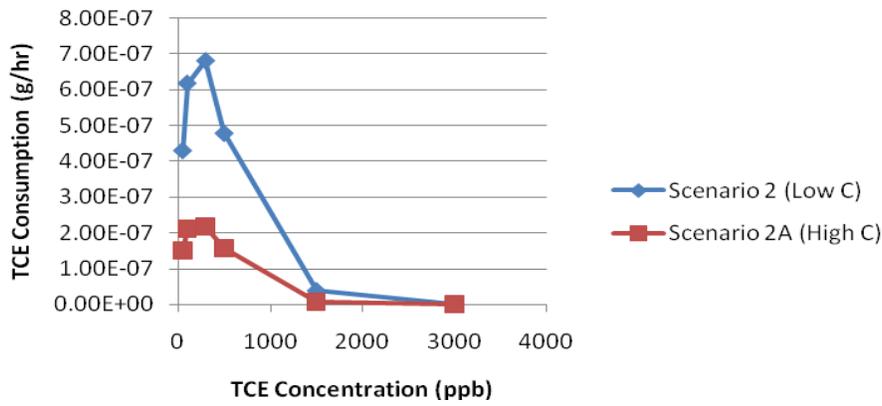


Figure 54. Carbon Effect on TCE Consumption. High levels of carbon in the soil can significantly lower methanotroph abilities to remediate TCE in the soil.

*Methane Effects on Remediation.* In addition to raising the tolerance to TCE concentration, higher methane concentrations also facilitate greater TCE consumption. The comparison of two sMMO scenarios in Figure 55 shows the significant increase in TCE consumption that accompanies high methane levels. Oxygen facilitates the consumption of methane and may play a factor in recovery where it is the limiting nutrient towards the outer rhizosphere. Consequently, methanotrophs towards the outer rhizosphere are the first to decline in mass when high TCE concentrations are introduced.

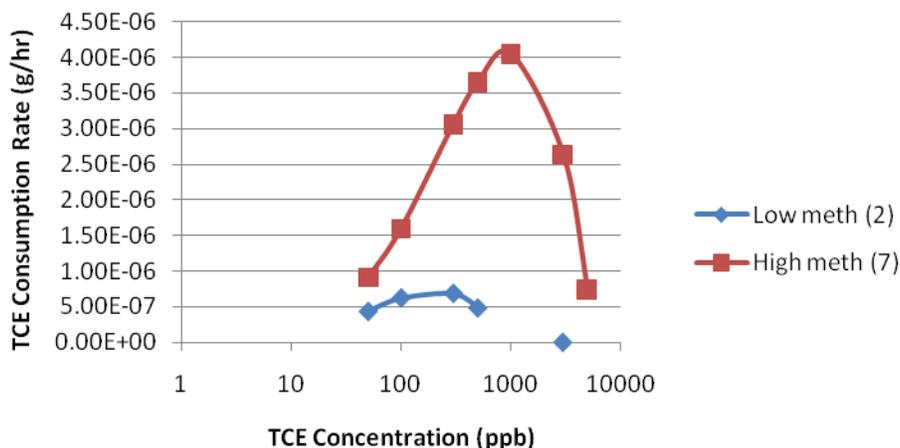


Figure 55. Methane Effect on TCE Consumption.

*Copper Effects on Remediation.* As shown above in “Bacteria Response”, copper is the driving factor in MMO expression in the rhizosphere. sMMO facilitates greater TCE consumption by methanotrophs resulting in high TCE consumption at low environmental concentrations. pMMO meths, more selective for methane, grow more efficiently and the higher mass of methanotrophs can have comparable consumption capacity at higher concentrations, however this will also result in much lower treatment efficiencies. Figure 56 shows the comparison of two scenarios with low and high copper. sMMO expression results in both high consumption and TCE treatment efficiency allowing small populations of sMMO producers to degrade a substantial mass of TCE at lower concentrations and at higher treatment efficiencies. Reducing copper to low levels in the treatment system may not be achievable, however limiting copper flowing into the wetland treatment system through chemical treatment and precipitation may help increase treatment efficiencies.

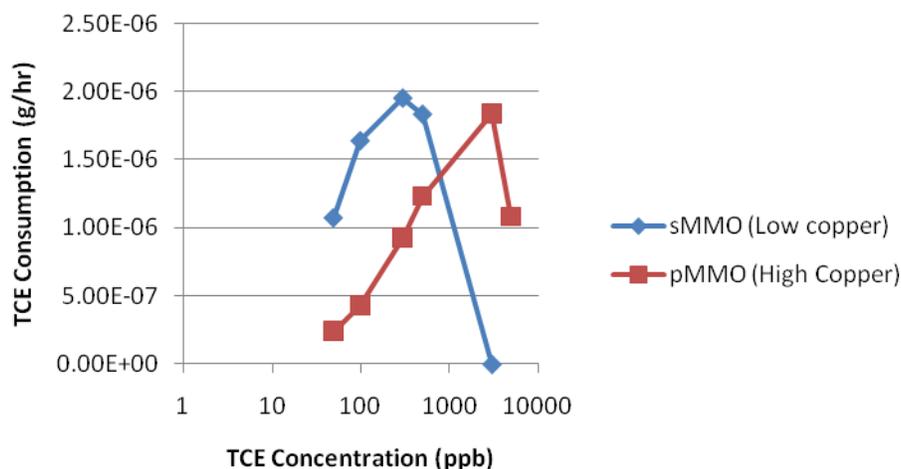


Figure 56. Copper Effect on TCE Consumption.

### Recommendations

The keys to successful remediation include managing the TCE consumption rate, and optimizing efficiency for one pass operation. The most favorable combination for remediation is a high consumption rate with a corresponding high efficiency. Figure 57 shows a snapshot of maximum TCE consumption rates and removal efficiency at for each scenario at 500 ppb. TCE maximum removal rates represented a balance between highest methanotroph growth rates and the effects of TCE toxicity. Scenario 7 has the highest potential for remediation of high through flows of TCE. Scenario 6 shows that high removal rates may be possible with pMMO expression, but only at high concentrations, resulting in very low removal efficiencies. Such high concentrations are not normally present in the environment.

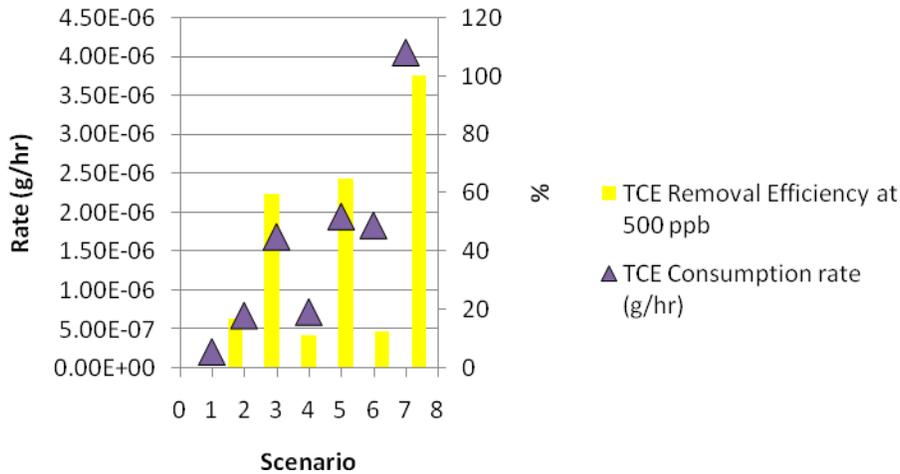


Figure 57. TCE Maximum Consumption Rates and Removal Efficiency at 500 ppb. Scenario 1 had nearly 0% efficiency at 500 ppb due to methanotroph toxicity.

Table 10 ranks the scenarios in order of overall TCE treatment by examining treatment efficiencies for each loading rate. Scenario 7, with high methane/HIC and low carbon, showed a substantially superior TCE consumption rate; high oxygen and methane permitted rapid methanotroph growth that compensated for toxic effects. This scenario may be representative of conditions that normally exist in a wetland (copper, methane, carbon, HIC), and helps explain the successful remediation of TCE even in natural wetlands.

Though the sMMO expression in Scenario 5 is ideal, it is likely not feasible since it may not be possible to reduce copper concentrations to the low levels needed to fully support sMMO expression. Scenario 3 is the more feasible pMMO scenario with good treatment rate and efficiencies resulting from low carbon conditions. The best remediation combination, then, is to minimize copper in the soil and treatment water, maximize methane, maximize HIC into the soil by using plants with high oxygen

Table 10. Treatment ranking of Testing Scenarios. Consumption rates for Scenario 6 were estimated based on the treatment profile.

TCE Profile	Conditions	2 GPM 100%		7 GPM 50%		12 GPM 20%		Avg rank
		rate	rank	rate	rank	rate	rank	
1	sMMO, low HIC, low methane, med C	1.57E-07	6	2.00E-07	6	5.80E-07	5	5.7
2	MMO mix, med HIC, low methane, low C	6.18E-07	5	3.00E-07	5	2.20E-07	6	5.3
3	pMMO, med HIC, med methane, low C	8.31E-07	3	4.83E-07	4	9.00E-07	3	3.3
4	MMO mix, med HIC, med methane, med C	7.27E-07	4	5.83E-07	3	<b>7.29E-07</b>	4	3.7
5	sMMO, high HIC, high methane, high C	<b>1.95E-06</b>	2	1.70E-06	2	1.90E-06	2	2.0
6	pMMO, high HIC, high methane, high C	8.00E-08	7	5.00E-08	7	8.00E-08	7	7.0
7	MMO mix, med HIC, high methane, low C	3.65E-06	1	3.04E-06	1	3.63E-06	1	1.0

TCE Profile	Comments
1	oxygen and methane recovery limited
2	methane availability limited recovery
3	methane availability limited recovery, pMMO limited degradation
4	moderate C limited O2 availability for meth recovery
5	high methane allowed recovery but was limited by O2 availability
6	pMMO limited TCE consumption, high carbon limited meth recovery
7	Low carbon is a more significant factor than sMMO expression

exudation rates, and avoid any additional inputs of carbon into the treatment system such as high BOD wastewater. The plant will exudate some carbon sources on its own, but this source will be rapidly consumed by the heterotrophs. Conditions favorable to HIC (high sunlight and 0-80% RH) will ensure that carbon is the limiting substrate and make excess oxygen available to methanotrophs, allowing methane to be consumed to recover from TCE toxic effects. Once soil conditions are known, the flow of the wetland can then be adjusted to achieve the maximum rate of removal at the efficiencies required to bring waste stream concentrations below required limits.

## **V. Conclusions**

The bioremediation of TCE can be enhanced by selection of plants that aid in the oxygenation of the rhizosphere, knowledge of soil conditions, and the control of flow rates to optimize treatment efficiency. This chapter will examine the stated research questions, address application of model findings, and discuss future research directions.

### **1. What is the nature of the oxygen dynamic in the rhizosphere?**

Oxygen has numerous effects in the rhizosphere, both direct and indirect. It constrains bacterial growth as a limiting nutrient which must be consumed with the primary substrate. Advantageous heterotrophic populations are favored in high oxygen conditions. Methanotrophs are likely to thrive in lower oxygen conditions where heterotrophs must look for other electron receptors, and where high methane concentrations which support growth are unhindered due to methanotroph affinity for oxygen.

Oxygen raises the eH in the soil and increases the availability of soluble copper that is present. This effects the expression of MMO by methanotrophs, greatly influencing both their remediation capability and their resistance to the effects of TCE. High oxygen levels increase methanotroph resistance to TCE by lowering sMMO expression and by assisting cell growth/ recovery by optimizing methane consumption. Low oxygen levels increase methanotroph transformation capacities by increased sMMO

expression, but also permit greater toxic effects by intermediate compounds during transformation. As TCE concentrations increase, the methanotroph population center shifts towards higher oxygen conditions closer to the root that favors pMMO expression. At low TCE concentrations, methanotrophs fill a niche in low oxygen conditions in the outer rhizosphere where methane is most abundant. Oxygen gradients, then, contribute to areas of high and low remediation capability within 5 mm of the root surface.

It must be remembered that oxygen is a tool used by the plant; if the plant did not benefit from the associated microbial activity and detoxification of metals, it would not exude the oxygen. Correspondingly, rhizospheres constantly change in response to plant growth and nutrient requirements. Assumptions about root characteristics must be based on average plant performance, but it should be recognized that areas of high and low productivity are widespread across a wetland. As an engineering consideration, steady state assumptions must be made. The selection of plants with high radial oxygen loss should result in greater remediation ability in the wetland setting; even at low TCE concentrations where sMMO expression is desired, low oxygen conditions will still exist at the outer edges of the rhizosphere where sMMO expressers will be exposed to the highest levels of methane in the rhizosphere.

## **2. What are the most influential factors to microbial community populations in the root zone?**

Carbon concentrations above 0.035 g/L (35 ppm) result in heterotroph growth that is limited by oxygen, and heterotroph growth is balanced by opposing gradients of carbon and oxygen. Carbon concentrations below .035 g/L restrict heterotroph growth and allow

oxygen to be used by other organisms. The methanotrophs are directly aided by low carbon concentrations since they live near the outer rhizosphere; higher oxygen availability allows them to consume methane more efficiently. Methanotroph growth is balanced by opposing gradients of methane and oxygen.

Methanotroph tolerance to TCE is determined by the concentration of TCE, the expression of MMO, and capacity for cell repair based upon oxygen and methane levels. Maximum TCE consumption is dependent upon methane and oxygen levels, and remains constant through all loading rates. Treatment efficiency varies according to flow rates, but consumption rates by the bacteria remain relatively constant. Toxic effects are normally fully expressed by 300 ppb, though toxic effects can begin as low as 10 ppb for sMMO expressing cultures in low methane conditions. High ROL helps to combat this by enabling maximum use of available methane. pMMO expression results in a significant increase of TCE tolerance, but greatly reduces treatment efficiency. Non-competitive inhibition effects on heterotrophs have a linear effect which gradually results in higher oxygen levels in the rhizosphere at high TCE concentration. By that time, toxic effects on methanotrophs have resulted in cell incapacitation, and the higher oxygen level has little helpful effect.

### **3. How can methanotroph populations be optimized to support aerobic remediation requirements for halogenated organics like TCE, TCA, DCE, and VC?**

If plant parameters and soil nutrient levels are known, a TCE concentration for maximum remediation can be determined. Loading rates (flow) should then be adjusted in order to permit treatment efficiencies that bring the output concentration below

permitted levels. This will allow the maximum overall through flow to be achieved.

Upper constraints will be limited by wetland hydrologic constraints (washout). In lack of specific guidance, do not raise input TCE concentrations beyond 100 ppb. This will ensure methanotroph viability even at low methane and oxygen levels. Dilution may be required for treatment of higher TCE concentrations, though cases with these treatment requirements are rare. Generally, high concentrations are more efficiently handled at low flow rates. Past the maximum TCE consumption rate, increases in flow result in lost treatment efficiency.

High methane levels, moderate oxygen flow, and relatively low copper levels maximize remediation effects for TCE by methanotrophs. Wetlands naturally optimize methane production in anaerobic zones that are rich with CO<sub>2</sub>. Wetland plants create an aerobic biofilm around their roots that is maximized during sunny days with low humidity, but is also substantial even at high humidity and low-light conditions.

If copper concentrations are high, high carbon levels are especially detrimental (the high copper limits TCE treatment due to pMMO expression, while the presence of carbon reduces oxygen available for growth leading to low methanotroph populations.) Addition of ferrous iron may help to reduce available copper on a short-term basis, but oxygen from the plants will ultimately oxidize both iron and available copper. The methanotrophs will be forced to expend additional NADH to re-oxidize the copper, resulting in an energy loss and reduced growth. Additionally, short-term application of iron may result in greater sMMO expression and disrupt the methanotroph steady-state. Therefore, application of iron is not encouraged. Minimizing the concentration of copper in influent is recommended.

If one must choose between low carbon and high methane, lower carbon levels result in better remediation. Provided methane levels are above 160 ppb, low C will limit heterotrophic growth, permit higher oxygen availability even at low HIC conditions, and thereby assist methanotroph recovery from TCE toxicity. For this reason, harvesting of remediation wetlands should be considered; harvesting not only reduces phosphates and nitrates leaching into wastewater during decomposition, but also reduces carbon concentrations that would otherwise reduce effectiveness for TCE remediation. Most wetland plants (like *Phragmites*) should be cut well above the water line to prevent drowning. TCE is not known to bioaccumulate in the plant material, and the composted plants should be non-toxic.

Contaminants other than TCE can also be modeled with the important assumption that toxic effects and non-competitive inhibition are cumulative. Competitive inhibition may become a remediation factor at low TCE concentrations since TCE degrades more rapidly than cDCE and VC at those levels. More research into contaminants other than TCE is essential (see below).

With any changes made to a wetland treatment system, it must be remembered that steady-state performance is never instantaneous. Microbial levels took over three months of simulation time to arrive at a steady-state, though microbial migration may help to affect a faster steady-state. Provided TCE concentration is not increased, variations in loading (flow) rate should not adversely affect microbial performance unless the water has a high CBOD or high copper concentration. Pretreatment of high CBOD water may maintain a higher level of TCE remediation performance.

#### **4. What are the influential factors of oxygen transport in a wetland plant?**

The most influential factors of a plant's ability to deliver oxygen to the rhizosphere are the plant's capacity for HIC and the permeability of the root structure to radial oxygen loss. HIC is controlled by leaf stomata density, pore size, and venting path resistance. Stomata density is likely a plant characteristic. Pore size can be adjusted by the plant to minimize water vapor loss and enhance CO<sub>2</sub> exchange for photosynthesis leading to changing remediation conditions throughout the day. Venting path resistance may change seasonally, but plants with higher aerenchymal percentages and numerous standing culms will likely have the lowest resistance. HIC is optimum at low humidity and high sunlight, but substantial HIC is maintained even at high humidity (80%) and low light levels. Data on leaf heating effects from radiation would result in a more accurate assessment of HIC effects.

Root permeability is a function of plant characteristics and the nature of the soil. A moderately toxic soil may invoke a higher radial oxygen loss to raise the eH of the soil and oxidize ferrous metals. Very high levels of metals, though, may result in development of root lignification which will impede oxygen loss and lower oxygen levels in the soil.

Though this model did not show that phloem flow contributed directly to raised rhizosphere oxygen concentrations, photosynthesis and phloem transport helped to raise oxygen levels throughout the plant, potentially creating a buffering effect from oxygen stored for night time use. Low CO<sub>2</sub> concentrations limited the plant's ability to produce more oxygen, suggesting that plant recirculation of CO<sub>2</sub> and increased CO<sub>2</sub> levels could increase plant oxygen output.

## **5. How is oxygen level in the root zone affected seasonally?**

This model did not account for seasonal effects directly. Many atmospheric conditions are seasonally driven and those weather conditions are a driving influence in root zone oxygen levels and oxygen levels throughout the plant itself. Even at high humidity conditions, HIC can significantly raise oxygen levels in the root system. HIC, however, is an active process that requires functioning leaves; when the plant senesces and loses leaf function, Bernoulli effects of wind and diffusion become more significant factors for air movement inside the plant. In cold weather, lower HIC is offset by lower bacterial activity; consequently, oxygen levels may remain high even though the oxygen flux from the roots will be much lower. In the winter, wetland treatment systems may retain some treatment capacity, but they will be more sensitive to high TCE concentrations and will have a lower capacity to recover from toxic effects. The higher radiation levels in the summer will also contribute to higher sugar and oxygen levels throughout the plant, both helping to maintain aerobic rhizospheres at night.

### **Application**

As suggested by Amon *et al.* (2007), plant characteristics should be an engineering consideration in wetland treatment processes. The capacity of plants to deliver oxygen into saturated soil conditions has a significant effect on the populations that live there and the ability of methanotrophs to recover from the toxic effects that accompany TCE bioremediation. As a conservative measure, TCE concentrations should not exceed 100 ppb in order to maintain a healthy population of methanotrophs in the treatment system. Soil conditions should be determined in order to determine a treatment

rate, and hydraulic flow can then be adjusted to optimize treatment system efficiency. This model can be used to enter plant and soil conditions and then iterate a flow rate solution.

#### Model strengths.

The numerical integration used by this model is a powerful tool, allowing simultaneous consideration of numerous variables that would not be subject to an analytical solution. The model has substantial room to grow in order to accommodate other substrates, other contaminants, other bacteria populations, more rhizosphere levels, and other root sections. It can also be broken down into components so that the soil/microbe model can be used independently. The model also makes adjustments for sMMO/pMMO expression depending on environmental conditions. This results in a more realistic dynamic effect from TCE toxicity.

#### Model Limitations.

The finite time step allowed by the program used limits the ability to run the model for all components (plant, soil, microbe) at the same time. The soil and microbe portions are not as sensitive to computation error as the plant sections where small volumes require small time steps. Difficulties establishing steady state concentrations throughout the system may detract from the model's accuracy; fundamental model behaviors, though, should still remain intact. The model does not portray leaf heating in the HIC, and does not display diurnal variation, though a time step input could adjust light and heat levels cyclically. As modeled, TCE is the only contaminant in the wetland,

but daughter-products of metabolism are also significant factors that are ignored. Though copper and oxygen are the primary considerations in MMO expression, other factors like soil eH, pH, and bacterial species may also play roles in MMO expression. The diffusion of carbon sources from outside sources may also be an artificiality. In reality, the plant has a significant impact upon rhizosphere carbon concentrations that this model (for lack of data) does not include. The relationships between high and low carbon levels and their effects on microbes and remediation, however, are still valid. Finally, nitrogen limitation may be an important factor in bacterial growth and recovery, but it is not included in the model and may explain het:meth ratios that are smaller than those in natural conditions. Incorporating other factors not covered by the model is highly encouraged for future research.

### **Future research**

Wetland plants have only recently become a popular research focus. Even the most popularly studied ones like *Phragmites australis*, however, lack many empirical values that would aid the modeling process. Better characterization of wetland plant physiology would help to refine models and make their results more accurate. Since *Phragmites australis* grows well in uniform stands, it makes sense to model it as such. This model could also be applied using another plant species, or with global parameters representative of a wetland plant consortium.

Knowledge of bacteria that reside in the rhizosphere is lacking; full characterization is still not known, let alone the specific behaviors of each species. A minority species may prove to be extremely influential in rhizosphere dynamics. Since

heterotrophic populations are the driving force in oxygen levels throughout the rhizosphere, a detailed study of their characteristics may greatly aid modeling accuracy. More bacterial types could be added to the model, as well as considerations for arbuscular mycorrhizal fungi or predatorial species.

The characterization of methanotrophs by MMO expression is questionable. MMO expression may be a strict function of oxygen and copper levels; certain species are simply more adept at living with low oxygen concentrations, and sMMO expression is more common at those low oxygen levels. Hence, methanotroph typing should be tied to aerobic capability and not MMO expression. Past testing and modeling for methanotrophs has often assumed oxygen to be a non-limiting substrate. This results in underestimating transformation capacities (since pure sMMO will have a very high  $T_c$ ), and overestimating growth capacities (since pMMO growth maximizes methanotroph return from methane consumption). More experiments should be done with methanotrophs using controlled oxygen conditions.

For good reason, TCE is one of the most widely studied contaminants. Modeling parameters for other contaminants are less well defined. More research into the affinities, transformation capacities and consumption rates of microbial groups for other contaminants would permit broader application of this model. Incorporation of multiple contaminants into this model could further clarify the dynamic that exists in the rhizosphere.

## Appendix A. Model Parameters

Table X. Thompson Model Parameters

Parameter	Value	Units	Source	Comment
<b>Atmospheric</b>				
Atmospheric Pressure	101325	Pa		
Gas Constant	0.0821	L*atm/mol K		
Viscosity of air (20 deg C)	1.82E-05	N s/ m <sup>2</sup>	(Clark, 1996:552)	
Diffusivity of Water Vapor in Air	Variable	m <sup>2</sup> /s	Wastewater Treatment	D <sub>w</sub> = 2.4e-5 m <sup>2</sup> /s (Nobel, 1991: 398)
O <sub>2</sub> fraction	0.21	-		
CO <sub>2</sub> fraction	0.001	-		
N <sub>2</sub> fraction	0.78	-		
Other gas fraction	0.009	-		
CO <sub>2</sub> Henry Constant	29.81	L*atm/mol		
O <sub>2</sub> Henry Constant	779.67	L*atm/mol		
<b>Plant</b>				
Aboveground Mass	1500	g	(Asaeda, 2001)	approx 1500/m <sup>2</sup> , 567-1678 g/m <sup>2</sup> (Soetaert, 2003)
Belowground Mass	3000	g	approx 3000 (Asaeda, 2001)	2800-3346 (Soetaert, 2003)
Plant Area density	300	-	(Soetaert, 2003)	126-587 shoots/m <sup>2</sup>
Plant Tissue O <sub>2</sub> Usage Rate	0.004	g/g/d	(Asaeda, 2002: 229)	.005 g/g/day (Asaeda et al., 2002) but plant averaged approx .004
Leaf Area Index	7	-	(Asaeda, 2000: 310)	
Number of leaves	-	-		
Fractional Porosity	0.027%	%	(Beckett, 2001: 277)	
Leaf Thickness	0.3	um	(Nobel, 1991)	
Leaf Void Percent	30	%	(Nobel, 1991: 347)	A leaf is often 30% air by volume.
Leaf Pore Diameter	0.2	um	(Armstrong, 1996: 183)	
Rvp	6.90E+09	Pa*s/m <sup>3</sup>		
Stem height	200	cm		
Stem radius	0.5	cm		
Stem Aerenchymal Percent	60	%		
Stem Phloem percent	5	%		
Stem Xylem percent	5	%		
Stem Tissue percent	30	%		
Hair Zone Length	3	cm	Armstrong	
Root depth	20	cm	likely much deeper in mature stands	
Root cap Length	0.3	cm		
Root tip radius	0.5	mm		
Number of roots	10	-		
Root magnitude	4	-		
Tap Root Aerenchyma Percent	60	%	(Armstrong, 1992: 203)	60% aerenchyma in rhizome
Tap Root Phloem Percent	5	%		
Tap Root Xylem Percent	5	%		
Tap Root Tissue Percent	30	%		

Parameter	Value	Units	Source	Comment
Lat Root Aerenchyma Percent	43	%	(Colmer, 2003: 19; Justin and Armstrong, 1987)	52% in O2 deficient soil
Lat Root Phloem Percent	5	%		
Lat Root Xylem Percent	5	%		
Lat Root Tissue Percent	42	%		
Lat Root Stele Percent	5	%		
Root Hair Aerenchyma Percent	0	%		
Root Hair Phloem Percent	5	%		
Root Hair Xylem Percent	5	%		
Root Hair Tissue Percent	85	%		
Root Hair Stele Percent	5	%		
<b>Soil</b>				
Rhizo Increment	0.5	mm		
D O2 in Soil	0.09612	cm <sup>2</sup> /hr	(Somell, 2000: 677)	2.3 cm <sup>2</sup> /day= .09612 cm <sup>2</sup> /hr
D Methane in Soil	0.05375	cm <sup>2</sup> /hr	(Noguera, 2000; Cussler, 1994)	1.29 cm <sup>2</sup> /day= .05375 cm <sup>2</sup> /hr
D Acetate in Soil	0.021	cm <sup>2</sup> /hr		.5 cm <sup>2</sup> /day?
D TCE in Soil	0.0325	cm <sup>2</sup> /hr	(Anderson, 1994; Wilke and Chang, 1955)	.78 cm <sup>2</sup> /day= .0325 cm <sup>2</sup> /hr
Soil Saturation	1	-		100% saturation in wetland
Soil porosity	0.4	-		average value for wetland soil
<b>Microbial</b>				
<b>Methanotrophs:</b>				
k methane	0.0917	g/g/h	(Smith, L. H., 2000)	2.2 g CH <sub>4</sub> / g cells/day methanotrophic mixed-culture 95% CI 2.0-2.4
Ks Methane	0.07	mg/L	(Smith, L. H., 2000)	.07 mg methane/L, 95% confidence interval .02-.11 mg/L
KsO <sub>2</sub>	0.05	mg/L	(Tartakovsky, 2006: 80)	.05 mg/L
Meth Yield	0.7	g/g	(Anderson, 1994; Alvarez-Cohen and McCarty, 1991)	.7 mg methanotrophs/ mg methane
Meth Decay Coeff bd	0.00417	/hr	(Anderson, 1994: 388)	.1/day
<b>Heterotrophs:</b>				
KsO <sub>2</sub> Het	0.0003	g/L	(van Bodegom, 2001:3591)	.0003g/L +/- .000762 published pure heterotrophic culture values
Ks Carbon Het	0.0348	g/L	(van Bodegom, 2001)	.0348g/L +/- .0234 from published pure heterotrophic culture values
Vmax Het	0.0625	/hr	(Tartakovsky, 2006: 80)	1.5/day = .0625/h
Het Decay Coeff	0.0021	/hr		.05/day
Het Yield	0.67	g/g		
<b>Bioremediation</b>				
k TCE	0.4	g/g/h		9.6 g/g/d is highest value found for a mixed methanotroph culture (Alvarez-Cohen, 1996)
Tc	0.21	g/g	(Noguera, 2000; Smith, 1997)	.21 mg TCE/ mg methanotrophs, 95% CI .18-.24 (Smith, 2000)
kl TCE	0.005	g/L	(Tartakovsky, 2006: 80)	5 mg/L
K TCE half sat *	0.007	g/L	(Alvarez-Cohen, 2001:114)	7.0 mg/L average value found (Alvarez-Cohen, 2001:114)

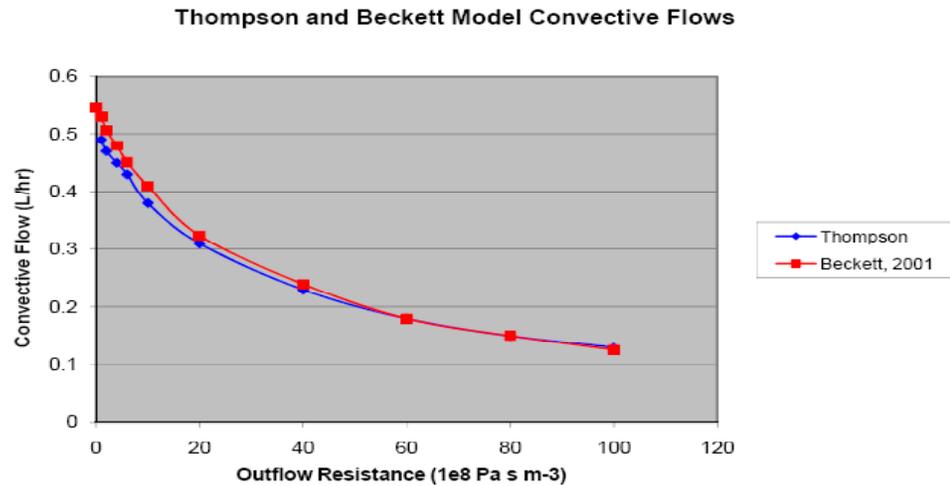
\* SMMO has a slightly lower Km for TCE of 35uM or .0046 g/L (Field, 2004: 31) that is not used in this model

## Appendix B. Venting Resistance Data

Rvp	1e8 Pa	HIC (L/hr)	Beckett flow	Potential pressure delta *	Static Pressure (Pa)	delta **	Dyn Pressure (Pa) ***	Beckett dyn pressure	Comment
0	0		0.5457						No resistance - max flow
1.00E+08	1	0.49	0.53	220	14	206	539	360	+
2.00E+08	2	0.47	0.5064	235	26	209	524	330	
4.00E+08	4	0.45	0.48	266	50	216	493	310	
6.00E+08	6	0.43	0.4518	289	71	218	470	290	
1.00E+09	10	0.38	0.41	327	105	222	432	260	
2.00E+09	20	0.31	0.324	410	173	237	349	200	
4.00E+09	40	0.23	0.24	509	254	255	250	130	
6.00E+09	60	0.18	0.18	562	298	264	197	90	
8.00E+09	80	0.15	0.15	592	325	267	167	70	
1.00E+10	100	0.13	0.126	623	348	275	136	55	
1.00E+12	10000	0.002 (0)		759	466	293	0 (0)		++ Blocked flow condition

\* Testing used a max pressure delta of 759 at 32% RH vice 750 Pa for the Beckett model.  
 \*\* Potential - static pressure  
 \*\*\* Thompson model does not calculate dynamic pressure: 759 - Potential pressure delta  
 + Thompson model includes limit on water flux from plant; flow may be more representative than Beckett model at low venting resistance.  
 ++ Beckett blocked-flow pressure was 462 Pa.

Venting Resistance Profile vs Beckett, 2001

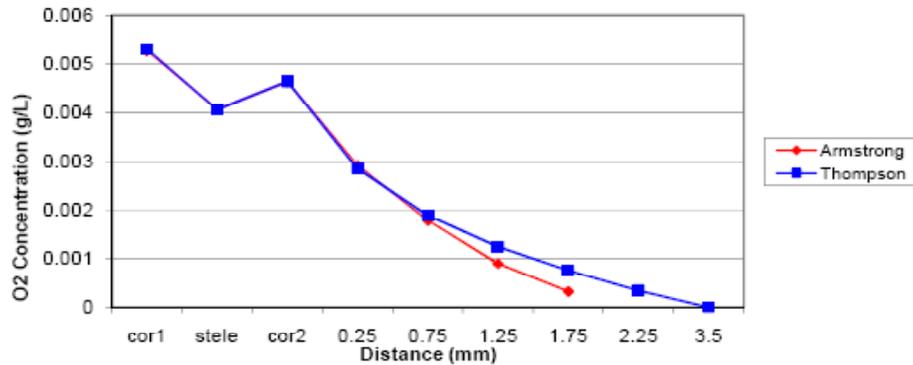


## Appendix C. Rhizosphere Profile Comparison Data

Aerenchymal flow velocity	0.77214036
Aerenchymal flow rate	0.03638626
kTCE Rhizo 1	0.01764857
kTCE Rhizo 2	0.02190756
kTCE Rhizo 3	0.02619537
kTCE Rhizo 4	0.03083619
kTCE Rhizo 5	0.03608297
O <sub>2</sub> Concentration in Stele RHZ	0.00404405
O <sub>2</sub> Conc in Cortex RHZ	0.00462548
O <sub>2</sub> Conc RHZ Rhizo 1	0.00284672
O <sub>2</sub> Conc RHZ Rhizo 2	0.00188599
O <sub>2</sub> Conc RHZ Rhizo 3	1.24E-03
O <sub>2</sub> Conc RHZ Rhizo 4	7.45E-04
O <sub>2</sub> Conc RHZ Rhizo 5	3.41E-04
Photosynthesis of Shoots	0.00631914
Real pressure differential	69.7403262
RmP	1.1556E+10
Percent ambient O <sub>2</sub>	75.4715176
Relative Humidity %	94
Global radiation level	200

	lat root cortex	RHZ stele	RHZ cortex	Rhizo 1	Rhizo 2	Rhizo 3	Rhizo 4	Rhizo 5	Rhizo Sump*
	cor1	steele	cor2	0.25	0.75	1.25	1.75	2.25	3.5
Armstrong	0.00526	0.00405	0.00462	0.00292	0.00178	0.00089	0.00032		
Thompson	0.00529	0.0040441	0.004625478	0.002847	0.001886	1.24E-03	7.45E-04	3.41E-04	0

Rhizosphere Profile Comparison



\* Thompson oxygen sump was at a fixed distance of 3.5 mm from the root.

## Appendix D. Sensitivity Testing Data

Adjustments	Value	Sensitivity					Comments	Sensitivity			
		-0.5	-0.1	ORIG **	0.1	2X		High	Med	Low	Other
								>30%	10-30%	<10%	
Humidity *	50	0.003864	0.003864	0.003864	0.003846	0.002218	(10, 40, 50, 60, 98)	X			high sensitive at high humidity
Global radiation level	1500	0.003671		0.00366		0.003674	(200, 1000, 1500, 2000, 3000)			X	
Temperature	298	0.00366		0.00366	0.003519	0.00265	(278, 295, 298, 301, 318)		X		
Atmospheric Pressure	101325	0.00304	0.00337	0.00366		0.003791			X		
Pm (Photosynthetic capacity)	0.21	0.00366		0.00366		0.00366				X	
K CO2	0.00044	0.00366		0.00366		0.00366				X	
Leaf Area Index	7	0.0036		0.00366		0.0037				X	
CS Diffusion Transfer Coefficient	0.025	0.00366	0.00366	0.00366	0.00366	0.00366				X	
O2 RHZ Diffusion Coeff	0.45	0.00279	0.00352	0.00366	0.00379	0.00439			X		
Lat root air transfer coeff	0.41	0.00317	0.00361	0.00366		0.00394			X		
RZ1 phloem rate coefficient	0.005	0.00366		0.00366		0.00366				X	
Rvp	6.90E+09	0.00374	0.00367	0.00366	0.00366	0.00352				X	
Leaf Tissue Percent	70		0.00367	0.00366	0.00367					X	
Leaf Pore Diameter	0.2	0.00368	0.00367	0.00366	0.00366	0.00364				X	
Fractional Porosity	0.00027	0.0036	0.00366	0.00366		0.00371				X	
Plant Tissue O2 UsageRate	0.004	0.00398		0.00366		0.00304			X		
Leaf CO2 Transfer Coeff	5	0.00367		0.00366		0.00367				X	
Phloem bulk flow rate	0.004	0.00366		0.00366		0.00367				X	
Xylem bulk flow rate	0.004	0.00369		0.00366		0.00363				X	
Number of Roots	10	0.00408	0.00374	0.00366	0.0036	0.0031			X		
Root Magnitude	4	0.00408		0.00366		0.0031			X		
Root tip radius	0.5	0.00328		0.00366		0.00363	(.25, .5, .8)			X	
Hair Zone Length	3	0.00408		0.00366		0.0031			X		
Plant area density	300	0.00295	0.00357	0.00366		0.00394			X		
Rhizome mass fraction	0.85		0.00348	0.00366	0.00388				X		
Above/Below Area Mass	1500/3000	0.00396		0.00366	0.00361	0.00308			X		varied total plant mass
Wind Speed	0.056		0.00369	0.00366	0.00364	0.00364	(.01, .056, 5, 15)			X	
CO2 fraction	0.001	0.0367		0.00366		0.00367	(.0005, .001, .01)			X	
EH dermal thickness RHZ	0.09	0.0044		0.00366	0.00353	0.00275		X			

\* Humidity was set at 80% for remainder of sensitivity testing.

\*\* Plant parameter sensitivity testing measured output in Rhizo 1.

No microbes/advection were active during plant sensitivity testing.

## Appendix E. HIC and Radiation Output Data

Global radiation level	Relative Humidity %	HIC (L/hr)	Percent ambient O2	O2 Conc in Stele RHZ (g/L)	O2 Conc in Cortex	O2 Conc RHZ Rhizo 1	O2 Conc RHZ Rhizo 2	O2 Conc RHZ Rhizo 3	O2 Conc RHZ Rhizo 4	O2 Conc RHZ Rhizo 5	
				Stele	Cortex	1	2	3	4	5	
3000	10	0.5325	99.22	0.005631452	0.006342044	0.003856	0.002514	0.001624	0.000962	0.000436	
	20										
	30										
	40										
	50	0.2991	97.81	0.005609122	0.006318898	0.003833	0.002492	0.001607	0.00095	0.00043	
	60										
	70	0.1803	96.36	0.005469748	0.006167878	0.003737	0.002428	0.001564	0.000924	0.000419	
	80	0.1204	94.60	0.00534257	0.006028257	0.003674	0.0024	0.001554	0.000922	0.000419	
	90	0.0601	89.73	0.005002293	0.00565892	0.003453	0.002259	0.001465	0.00087	0.000395	
1500	10	0.5325	99.62	0.005633578	0.006344213	0.003864	0.002523	0.001632	0.000968	0.000439	
	20	0.4747	99.40	0.005633578	0.006344213	0.003864	0.002523	0.001632	0.000968	0.000439	
	30	0.4165	99.18	0.005633578	0.006344213	0.003864	0.002523	0.001632	0.000968	0.000439	
	40	0.3580	98.94	0.005633578	0.006344213	0.003864	0.002523	0.001632	0.000968	0.000439	
	50	0.2991	98.67	0.005633578	0.006344213	0.003864	0.002523	0.001632	0.000968	0.000439	
	60	0.2399	97.90	0.005603287	0.006310945	0.003846	0.002512	0.001626	0.000964	0.000438	
	70	0.1803	95.72	0.005436715	0.006131006	0.003739	0.002445	0.001585	0.000941	0.000427	
	80	0.1204	94.08	0.005312191	0.005994835	0.003664	0.0024	0.001558	0.000926	0.000421	
	90	0.0601	90.42	0.005058419	0.005717453	0.003502	0.002298	0.001493	0.000888	0.000404	
		96	0.0237	77.88	0.004227917	0.004814779	0.002965	0.001956	0.001277	0.000763	0.000348
		98	0.0116	60.38	0.003080152	0.003567618	0.002218	0.001476	0.000972	0.000584	0.000267
500	10										
same	20										
	30										
	40										
	50										
	60										
	70	0.1803	95.72	0.005436718	0.006131009	0.003739	0.002445	0.001585	0.000941	0.000427	
	80										
	90										
200	80	0.1204	94.56	0.005339657	0.006025276	0.003671	0.002398	0.001553	0.000921	0.000418	
same	90	0.0601	87.30	0.004843469	0.005486234	0.00335	0.002193	0.001423	0.000846	0.000384	
	96	0.0238	74.25	0.003977869	0.004545178	0.00278	0.001823	0.001184	0.000704	0.00032	
	98	0.0116	53.97	0.002642218	0.003094435	0.001894	0.001242	0.000807	0.00048	0.000218	
20	98	0.0118	53.48	0.002608646	0.003058239	0.00187	0.001226	0.000796	0.000474	0.000215	

## Appendix F. Soil Variable Testing Data

Test	HIC	Carbon	Methane	Copper	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	kTCE1	kTCE2	kTCE3	kTCE4	kTCE5
	O2 (g/L)	C (g/L)	CH4 (mg/L)	Cu (ug/L)												
1	Low	0.01	0	0	0.002382108	0.002811694	0.00117061	5.59E-04	2.96E-04	1.64E-04	7.45E-05	0.2638	0.3135	0.3490	0.3700	0.3858
2		0.0025		15												
3				100												
4				200												
5			0.07	0	0.002373931	0.0028028	0.0011433	5.27E-04	2.62E-04	1.26E-04	4.08E-05	0.2660	0.3174	0.3541	0.3765	0.3921
6				15	0.002373931	0.0028028	0.0011433	5.27E-04	2.62E-04	1.26E-04	4.08E-05	0.1333	0.1654	0.1845	0.1962	0.2043
7				100	0.002373931	0.0028028	0.0011433	5.27E-04	2.62E-04	1.26E-04	4.08E-05	0.0375	0.0465	0.0519	0.0562	0.0575
8				200	0.002373931	0.0028028	0.0011433	5.27E-04	2.62E-04	1.26E-04	4.08E-05	0.0213	0.0265	0.0295	0.0314	0.0327
9			0.16	0	0.002363774	0.002791753	0.00110963	4.90E-04	2.23E-04	8.23E-05	2.15E-05	0.2687	0.3221	0.3603	0.3843	0.3958
10				15	0.002363774	0.002791753	0.00110963	4.90E-04	2.23E-04	8.23E-05	2.15E-05	0.1348	0.1678	0.1877	0.2002	0.2062
11				100	0.002363774	0.002791753	0.00110963	4.90E-04	2.23E-04	8.23E-05	2.15E-05	0.0379	0.0472	0.0528	0.0563	0.0580
12				200	0.002363774	0.002791753	0.00110963	4.90E-04	2.23E-04	8.23E-05	2.15E-05	0.0216	0.0268	0.0300	0.0320	0.0330
13			0.5	0	0.002329637	0.002754624	0.00099589	3.68E-04	8.54E-05	2.12E-05	6.93E-06	0.2684	0.3384	0.3838	0.3958	0.3986
14				15	0.002329637	0.002754624	0.00099589	3.68E-04	8.54E-05	2.12E-05	6.93E-06	0.1398	0.1763	0.1999	0.2062	0.2077
15				100	0.002329637	0.002754624	0.00099589	3.68E-04	8.54E-05	2.12E-05	6.93E-06	0.0393	0.0496	0.0562	0.0580	0.0584
16				200	0.002329637	0.002754624	0.00099589	3.68E-04	8.54E-05	2.12E-05	6.93E-06	0.0224	0.0282	0.0320	0.0330	0.0332
17		0.035	0	0	0.002148748	0.002557971	0.00036606	1.13E-04	5.21E-05	2.81E-05	1.28E-05	0.3345	0.3788	0.3899	0.3945	0.3975
18				15	0.002148748	0.002557971	0.00036606	1.13E-04	5.21E-05	2.81E-05	1.28E-05	0.1743	0.1973	0.2031	0.2065	0.2071
19				100	0.002148748	0.002557971	0.00036606	1.13E-04	5.21E-05	2.81E-05	1.28E-05	0.0490	0.0555	0.0571	0.0578	0.0583
20				200	0.002148748	0.002557971	0.00036606	1.13E-04	5.21E-05	2.81E-05	1.28E-05	0.0279	0.0316	0.0325	0.0329	0.0331
21	Scenario 1		0.07	0	0.002145183	0.002554092	0.00038415	9.79E-05	3.67E-05	1.63E-05	6.55E-06	0.3362	0.3815	0.3929	0.3968	0.3987
22				15	0.002145183	0.002554092	0.00038415	9.79E-05	3.67E-05	1.63E-05	6.55E-06	0.1751	0.1988	0.2047	0.2067	0.2077
23				100	0.002145183	0.002554092	0.00038415	9.79E-05	3.67E-05	1.63E-05	6.55E-06	0.0493	0.0559	0.0576	0.0582	0.0584
24				200	0.002145183	0.002554092	0.00038415	9.79E-05	3.67E-05	1.63E-05	6.55E-06	0.0280	0.0318	0.0327	0.0331	0.0332
25			0.16	0	0.002139709	0.002548137	0.00036686	7.59E-05	2.02E-05	7.81E-06	3.22E-06	0.3388	0.3855	0.3960	0.3985	0.3994
25B				0	0.002139958	0.002548408	0.00036687	7.69E-05	2.08E-05	7.96E-06	3.20E-06	0.3386	0.3853	0.3959	0.3984	0.3994
26	Scenario 4A			15	0.002139958	0.002548408	0.00036687	7.69E-05	2.08E-05	7.96E-06	3.20E-06	0.1764	0.2008	0.2063	0.2070	0.2081
27				100	0.002139958	0.002548408	0.00036687	7.69E-05	2.08E-05	7.96E-06	3.20E-06	0.0496	0.0565	0.0580	0.0584	0.0585
28				200	0.002139958	0.002548408	0.00036687	7.69E-05	2.08E-05	7.96E-06	3.20E-06	0.0282	0.0321	0.0330	0.0332	0.0333
29			0.5	0	0.002128614	0.002536078	0.00032913	2.27E-05	4.26E-06	1.49E-06	6.17E-07	0.3440	0.3955	0.3992	0.3997	0.3999
30				15	0.002128614	0.002536078	0.00032913	2.27E-05	4.26E-06	1.49E-06	6.17E-07	0.1792	0.2061	0.2079	0.2082	0.2083
31				100	0.002128614	0.002536078	0.00032913	2.27E-05	4.26E-06	1.49E-06	6.17E-07	0.0504	0.0560	0.0565	0.0566	0.0566
32				200	0.002128614	0.002536078	0.00032913	2.27E-05	4.26E-06	1.49E-06	6.17E-07	0.0287	0.0330	0.0333	0.0333	0.0333
33		0.06	0	0	0.00211909	0.002525736	0.00029777	6.95E-05	3.01E-05	1.60E-05	7.30E-06	0.3487	0.3867	0.3941	0.3968	0.3986
34				15												
35				100												
36				200												
37			0.07	0	0.002045055	0.002445739	0.00027862	5.51E-05	1.79E-05	8.22E-06	3.52E-06	0.3516	0.3894	0.3965	0.3984	0.3993
38				15	0.002045055	0.002445739	0.00027862	5.51E-05	1.79E-05	8.22E-06	3.52E-06	0.1832	0.2029	0.2066	0.2075	0.2080
39				100	0.002045055	0.002445739	0.00027862	5.51E-05	1.79E-05	8.22E-06	3.52E-06	0.0515	0.0571	0.0581	0.0584	0.0585
40				200	0.002045055	0.002445739	0.00027862	5.51E-05	1.79E-05	8.22E-06	3.52E-06	0.0293	0.0324	0.0330	0.0332	0.0333
41			0.16	0	0.002012398	0.002410611	0.00026205	3.76E-05	8.28E-06	3.35E-06	1.42E-06	0.3541	0.3927	0.3984	0.3993	0.3997
42				15	0.002012398	0.002410611	0.00026205	3.76E-05	8.28E-06	3.35E-06	1.42E-06	0.1845	0.2046	0.2075	0.2080	0.2082
43				100	0.002012398	0.002410611	0.00026205	3.76E-05	8.28E-06	3.35E-06	1.42E-06	0.0519	0.0575	0.0584	0.0585	0.0586
44				200	0.002012398	0.002410611	0.00026205	3.76E-05	8.28E-06	3.35E-06	1.42E-06	0.0295	0.0327	0.0332	0.0333	0.0333
45			0.5	0	0.001979521	0.002374811	0.00024609	1.19E-05	1.83E-06	6.52E-07	2.73E-07	0.3566	0.3977	0.3996	0.3999	0.3999
46				15	0.001979521	0.002374811	0.00024609	1.19E-05	1.83E-06	6.52E-07	2.73E-07	0.1858	0.2072	0.2082	0.2083	0.2084
47				100	0.001979521	0.002374811	0.00024609	1.19E-05	1.83E-06	6.52E-07	2.73E-07	0.0523	0.0583	0.0586	0.0586	0.0586
48				200	0.001979521	0.002374811	0.00024609	1.19E-05	1.83E-06	6.52E-07	2.73E-07	0.0297	0.0331	0.0333	0.0333	0.0333

Test	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	Tot Het	Tot Meth	% sMMO	Het to meth	Comments
1	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	1.307E-02	1.008E-02	6.049E-03	1.766E-03	1.338E-06	0.030906611	0	#DIV/0!	#DIV/0!	
2											0	0	#DIV/0!	#DIV/0!	
3											0	0	#DIV/0!	#DIV/0!	
4											0	0	#DIV/0!	#DIV/0!	
5	3.988E-06	5.466E-06	5.592E-05	4.841E-04	1.324E-03	1.326E-02	1.024E-02	5.673E-03	1.270E-03	4.237E-08	0.030439159	0.00187332	96.80422	16.24880479	
6	3.988E-06	5.466E-06	5.592E-05	4.841E-04	1.324E-03	1.326E-02	1.024E-02	5.673E-03	1.270E-03	4.237E-08	0.030439159	0.00187332	50.32766	16.24880479	
7	3.988E-06	5.466E-06	5.592E-05	4.841E-04	1.324E-03	1.326E-02	1.024E-02	5.673E-03	1.270E-03	4.237E-08	0.030439159	0.00187332	14.15752	16.24880479	
8	3.988E-06	5.466E-06	5.592E-05	4.841E-04	1.324E-03	1.326E-02	1.024E-02	5.673E-03	1.270E-03	4.237E-08	0.030439159	0.00187332	8.050352	16.24880479	
9	6.429E-06	1.640E-05	2.566E-04	1.820E-03	1.256E-03	1.366E-02	1.018E-02	4.882E-03	3.238E-04	8.424E-10	0.029043404	0.00335551	96.56274	8.655426727	
10	6.429E-06	1.640E-05	2.566E-04	1.820E-03	1.256E-03	1.366E-02	1.018E-02	4.882E-03	3.238E-04	8.424E-10	0.029043404	0.00335551	50.30607	8.655426727	
11	6.429E-06	1.640E-05	2.566E-04	1.820E-03	1.256E-03	1.366E-02	1.018E-02	4.882E-03	3.238E-04	8.424E-10	0.029043404	0.00335551	14.15144	8.655426727	
12	6.429E-06	1.640E-05	2.566E-04	1.820E-03	1.256E-03	1.366E-02	1.018E-02	4.882E-03	3.238E-04	8.424E-10	0.029043404	0.00335551	8.046895	8.655426727	
13	9.241E-07	4.439E-06	4.174E-03	1.468E-03	2.449E-04	1.520E-02	9.501E-03	5.847E-04	1.189E-06	1.032E-12	0.025288619	0.00589172	96.83866	4.292233792	
14	9.241E-07	4.439E-06	4.174E-03	1.468E-03	2.449E-04	1.520E-02	9.501E-03	5.847E-04	1.189E-06	1.032E-12	0.025288619	0.00589172	50.44982	4.292233792	
15	9.241E-07	4.439E-06	4.174E-03	1.468E-03	2.449E-04	1.520E-02	9.501E-03	5.847E-04	1.189E-06	1.032E-12	0.025288619	0.00589172	14.19187	4.292233792	
16	9.241E-07	4.439E-06	4.174E-03	1.468E-03	2.449E-04	1.520E-02	9.501E-03	5.847E-04	1.189E-06	1.032E-12	0.025288619	0.00589172	8.069888	4.292233792	
17	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	4.019E-02	1.205E-02	2.930E-03	7.148E-04	9.469E-06	0.055897416	0	#DIV/0!	#DIV/0!	
18	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	4.019E-02	1.205E-02	2.930E-03	7.148E-04	9.469E-06	0.055897416	0	#DIV/0!	#DIV/0!	
19	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	4.019E-02	1.205E-02	2.930E-03	7.148E-04	9.469E-06	0.055897416	0	#DIV/0!	#DIV/0!	
20	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	4.019E-02	1.205E-02	2.930E-03	7.148E-04	9.469E-06	0.055897416	0	#DIV/0!	#DIV/0!	
21	2.755E-06	9.204E-05	3.149E-04	3.510E-04	1.142E-04	4.015E-02	1.211E-02	2.158E-03	1.521E-09	1.599E-18	0.054414476	0.00087494	98.45821	62.19231263	sMMO, low HIC, low methane
22	2.755E-06	9.204E-05	3.149E-04	3.510E-04	1.142E-04	4.015E-02	1.211E-02	2.158E-03	1.521E-09	1.599E-18	0.054414476	0.00087494	51.29355	62.19231263	
23	2.755E-06	9.204E-05	3.149E-04	3.510E-04	1.142E-04	4.015E-02	1.211E-02	2.158E-03	1.521E-09	1.599E-18	0.054414476	0.00087494	14.42922	62.19231263	
24	2.755E-06	9.204E-05	3.149E-04	3.510E-04	1.142E-04	4.015E-02	1.211E-02	2.158E-03	1.521E-09	1.599E-18	0.054414476	0.00087494	8.204851	62.19231263	
25	2.988E-05	3.261E-04	9.201E-04	3.536E-04	5.261E-05	4.007E-02	1.204E-02	1.268E-03	8.194E-05	1.886E-05	0.053479513	0.00168114	98.40748	31.81142462	all hets .01 meths .01 11X320hrs
26B	2.988E-05	3.103E-04	9.209E-04	3.808E-04	7.145E-05	4.000E-02	1.195E-02	1.160E-03	6.697E-06	6.088E-08	0.053118526	0.00171309	98.42839	31.00750977	all hets .00001 all meths .05 12X320
26	2.988E-05	3.103E-04	9.209E-04	3.808E-04	7.145E-05	4.000E-02	1.195E-02	1.160E-03	6.697E-06	6.088E-08	0.053118526	0.00171309	51.27802	31.00750977	
27	2.988E-05	3.103E-04	9.209E-04	3.808E-04	7.145E-05	4.000E-02	1.195E-02	1.160E-03	6.697E-06	6.088E-08	0.053118526	0.00171309	14.42485	31.00750977	
28	2.988E-05	3.103E-04	9.209E-04	3.808E-04	7.145E-05	4.000E-02	1.195E-02	1.160E-03	6.697E-06	6.088E-08	0.053118526	0.00171309	8.202366	31.00750977	
29	2.022E-06	2.209E-03	1.604E-03	3.420E-04	3.820E-05	4.009E-02	1.203E-02	1.905E-04	3.084E-09	3.866E-16	0.052315533	0.00419564	99.32027	12.46902507	
30	2.022E-06	2.209E-03	1.604E-03	3.420E-04	3.820E-05	4.009E-02	1.203E-02	1.905E-04	3.084E-09	3.866E-16	0.052315533	0.00419564	51.74266	12.46902507	
31	2.022E-06	2.209E-03	1.604E-03	3.420E-04	3.820E-05	4.009E-02	1.203E-02	1.905E-04	3.084E-09	3.866E-16	0.052315533	0.00419564	14.55568	12.46902507	
32	2.022E-06	2.209E-03	1.604E-03	3.420E-04	3.820E-05	4.009E-02	1.203E-02	1.905E-04	3.084E-09	3.866E-16	0.052315533	0.00419564	8.27669	12.46902507	
33	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	4.299E-02	1.377E-02	2.798E-03	6.003E-04	4.070E-06	0.060159745	0	#DIV/0!	#DIV/0!	
34											0	0	#DIV/0!	#DIV/0!	
35											0	0	#DIV/0!	#DIV/0!	
36											0	0	#DIV/0!	#DIV/0!	
37	2.395E-05	1.145E-04	3.305E-04	2.342E-04	4.401E-05	4.187E-02	1.358E-02	2.145E-03	3.269E-06	7.923E-10	0.05759996	0.00074717	98.67954	77.09050283	
38	2.395E-05	1.145E-04	3.305E-04	2.342E-04	4.401E-05	4.187E-02	1.358E-02	2.145E-03	3.269E-06	7.923E-10	0.05759996	0.00074717	51.40866	77.09050283	
39	2.395E-05	1.145E-04	3.305E-04	2.342E-04	4.401E-05	4.187E-02	1.358E-02	2.145E-03	3.269E-06	7.923E-10	0.05759996	0.00074717	14.46166	77.09050283	
40	2.395E-05	1.145E-04	3.305E-04	2.342E-04	4.401E-05	4.187E-02	1.358E-02	2.145E-03	3.269E-06	7.923E-10	0.05759996	0.00074717	8.223295	77.09050283	
41	3.303E-05	3.873E-04	8.695E-04	2.688E-04	3.563E-05	4.125E-02	1.333E-02	1.575E-03	5.404E-07	3.797E-11	0.056150824	0.00159425	99.06598	35.22087916	
42	3.303E-05	3.873E-04	8.695E-04	2.688E-04	3.563E-05	4.125E-02	1.333E-02	1.575E-03	5.404E-07	3.797E-11	0.056150824	0.00159425	51.61016	35.22087916	
43	3.303E-05	3.873E-04	8.695E-04	2.688E-04	3.563E-05	4.125E-02	1.333E-02	1.575E-03	5.404E-07	3.797E-11	0.056150824	0.00159425	14.51829	35.22087916	
44	3.303E-05	3.873E-04	8.695E-04	2.688E-04	3.563E-05	4.125E-02	1.333E-02	1.575E-03	5.404E-07	3.797E-11	0.056150824	0.00159425	8.255496	35.22087916	
45	2.052E-06	2.291E-03	1.486E-03	3.081E-04	3.183E-05	4.054E-02	1.305E-02	1.057E-03	4.268E-08	8.980E-13	0.054655414	0.00411851	99.83465	13.27066868	2nd highest sMMO
46	2.052E-06	2.291E-03	1.486E-03	3.081E-04	3.183E-05	4.054E-02	1.305E-02	1.057E-03	4.268E-08	8.980E-13	0.054655414	0.00411851	51.96644	13.27066868	
47	2.052E-06	2.291E-03	1.486E-03	3.081E-04	3.183E-05	4.054E-02	1.305E-02	1.057E-03	4.268E-08	8.980E-13	0.054655414	0.00411851	14.60163	13.27066868	
48	2.052E-06	2.291E-03	1.486E-03	3.081E-04	3.183E-05	4.054E-02	1.305E-02	1.057E-03	4.268E-08	8.980E-13	0.054655414	0.00411851	8.302888	13.27066868	

Test	O2 (g/L)	C (g/L)	CH4 (mg/L)	Cu (ug/L)	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	kTCE1	kTCE2	kTCE3	kTCE4	kTCE5
49	MED	0.01	0	0	0.00386131	0.004418748	0.00206691	9.97E-04	4.89E-04	2.46E-04	1.12E-04	0.1988	0.2683	0.3222	0.3668	0.3790
50	0.004			15												
51				100												
52				200												
53			0.07	0	0.00385328	0.004409995	0.00203828	9.86E-04	4.59E-04	2.17E-04	7.40E-05	0.2001	0.2710	0.3260	0.3613	0.3859
54				15	0.00385328	0.004409995	0.00203828	9.86E-04	4.59E-04	2.17E-04	7.40E-05	0.1043	0.1412	0.1699	0.1882	0.2010
55	Scenario 2			100	0.00385328	0.004409995	0.00203828	9.86E-04	4.59E-04	2.17E-04	7.40E-05	0.0293	0.0397	0.0478	0.0529	0.0585
56				200	0.00385328	0.004409995	0.00203828	9.86E-04	4.59E-04	2.17E-04	7.40E-05	0.0187	0.0228	0.0272	0.0301	0.0322
57			0.16	0	0.003843406	0.004399279	0.00200432	9.24E-04	4.13E-04	1.62E-04	3.68E-05	0.2018	0.2749	0.3322	0.3703	0.3928
58				15												
59				100												
60	Scenario 3			200	0.003843406	0.004399279	0.00200432	9.24E-04	4.13E-04	1.62E-04	3.68E-05	0.0168	0.0229	0.0277	0.0309	0.0327
61			0.5	0	0.003803451	0.004355833	0.00186686	7.65E-04	2.50E-04	4.43E-05	9.33E-06	0.2088	0.2904	0.3559	0.3914	0.3982
62				15	0.003803451	0.004355833	0.00186686	7.65E-04	2.50E-04	4.43E-05	9.33E-06	0.1088	0.1513	0.1854	0.2039	0.2074
63				100	0.003803451	0.004355833	0.00186686	7.65E-04	2.50E-04	4.43E-05	9.33E-06	0.0306	0.0428	0.0522	0.0574	0.0584
64				200	0.003803451	0.004355833	0.00186686	7.65E-04	2.50E-04	4.43E-05	9.33E-06	0.0174	0.0242	0.0297	0.0326	0.0332
65		0.035	0	0	0.00344651	0.003967825	0.00064203	1.83E-04	6.78E-05	3.48E-05	1.58E-05	0.3038	0.3702	0.3870	0.3932	0.3989
66				15												
67				100												
68				200												
69			0.07	0	0.003438193	0.003958873	0.00062741	0.000147722	5.12E-05	2.11E-05	8.04E-06	0.3054	0.3728	0.3901	0.3959	0.3984
70				15	0.003438193	0.003958873	0.00062741	0.000147722	5.12E-05	2.11E-05	8.04E-06	0.1591	0.1942	0.2032	0.2062	0.2076
71				100	0.003438193	0.003958873	0.00062741	0.000147722	5.12E-05	2.11E-05	8.04E-06	0.0448	0.0548	0.0572	0.0580	0.0584
72				200	0.003438193	0.003958873	0.00062741	0.000147722	5.12E-05	2.11E-05	8.04E-06	0.0255	0.0311	0.0325	0.0330	0.0332
73			0.16	0	0.003433124	0.003953361	0.00060994	0.000127089	3.10E-05	1.06E-05	3.97E-06	0.3075	0.3763	0.3940	0.3979	0.3992
74	Scenario 4			15	0.003433124	0.003953361	0.00060994	0.000127089	3.10E-05	1.06E-05	3.97E-06	0.1602	0.1961	0.2052	0.2073	0.2080
75				100	0.003433124	0.003953361	0.00060994	0.000127089	3.10E-05	1.06E-05	3.97E-06	0.0451	0.0552	0.0577	0.0583	0.0585
76				200	0.003433124	0.003953361	0.00060994	0.000127089	3.10E-05	1.06E-05	3.97E-06	0.0256	0.0314	0.0328	0.0332	0.0333
77			0.5	0	0.003416563	0.00393535	0.00055283	5.25E-05	6.39E-06	1.84E-06	6.88E-07	0.3142	0.3899	0.3987	0.3996	0.3999
78				15	0.003416563	0.00393535	0.00055283	5.25E-05	6.39E-06	1.84E-06	6.88E-07	0.1637	0.2031	0.2077	0.2082	0.2083
79				100	0.003416563	0.00393535	0.00055283	5.25E-05	6.39E-06	1.84E-06	6.88E-07	0.0461	0.0571	0.0584	0.0586	0.0586
80				200	0.003416563	0.00393535	0.00055283	5.25E-05	6.39E-06	1.84E-06	6.88E-07	0.0461	0.0571	0.0584	0.0586	0.0586
81		0.06	0	0	0.003379684	0.003895294	0.00042675	8.64E-05	3.68E-05	2.18E-05	9.90E-06	0.3304	0.3836	0.3928	0.3957	0.3980
82				15												
83				100												
84				200												
85			0.07	0	0.003374764	0.003889943	0.00040984	6.93E-05	1.87E-05	7.71E-06	2.99E-06	0.3327	0.3867	0.3963	0.3985	0.3994
86				15	0.003374764	0.003889943	0.00040984	6.93E-05	1.87E-05	7.71E-06	2.99E-06	0.1733	0.2015	0.2065	0.2076	0.2081
87	Scenario 2A			100	0.003374764	0.003889943	0.00040984	6.93E-05	1.87E-05	7.71E-06	2.99E-06	0.0488	0.0567	0.0581	0.0584	0.0585
88				200	0.003374764	0.003889943	0.00040984	6.93E-05	1.87E-05	7.71E-06	2.99E-06	0.0277	0.0322	0.0330	0.0332	0.0333
89			0.16	0	0.003371131	0.003885991	0.0003973	5.40E-05	6.72E-06	3.07E-06	1.19E-06	0.3344	0.3898	0.3963	0.3984	0.3998
90				15												
91				100												
92				200	0.003371131	0.003885991	0.0003973	5.40E-05	6.72E-06	3.07E-06	1.19E-06	0.0279	0.0325	0.0332	0.0333	0.0333
93			0.5	0	0.003364494	0.00387878	0.00037468	1.89E-05	1.67E-06	4.78E-07	1.78E-07	0.3375	0.3963	0.3997	0.3999	0.4000
94				15	0.003364494	0.00387878	0.00037468	1.89E-05	1.67E-06	4.78E-07	1.78E-07	0.1758	0.2065	0.2082	0.2083	0.2084
95				100	0.003364494	0.00387878	0.00037468	1.89E-05	1.67E-06	4.78E-07	1.78E-07	0.0495	0.0581	0.0586	0.0586	0.0586
96				200	0.003364494	0.00387878	0.00037468	1.89E-05	1.67E-06	4.78E-07	1.78E-07	0.0281	0.0330	0.0333	0.0333	0.0333

Test	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	Tot Het	Tot Meth	% sMMO	Het to meth	Comments
49	2.072E-14	2.073E-14	2.073E-14	2.070E-14	2.063E-14	9.637E-03	1.432E-02	1.288E-02	6.797E-03	3.408E-20	0.043635256	1.0351E-13	76.23092	4.2155E+11	
50											0	0	#DIV/0!	#DIV/0!	
51											0	0	#DIV/0!	#DIV/0!	
52											0	0	#DIV/0!	#DIV/0!	
53	2.166E-08	6.392E-06	1.982E-06	2.587E-04	1.978E-03	9.996E-03	1.455E-02	1.276E-02	4.790E-03	2.002E-18	0.04209267	0.0022606	95.54961	18.62012916	approx 20 iterations X 320 hours = 267 days
54	2.166E-08	6.392E-06	1.982E-06	2.587E-04	1.978E-03	9.996E-03	1.455E-02	1.276E-02	4.790E-03	2.002E-18	0.04209267	0.0022606	49.77827	18.62012916	
55	2.166E-08	6.392E-06	1.982E-06	2.587E-04	1.978E-03	9.996E-03	1.455E-02	1.276E-02	4.790E-03	2.002E-18	0.04209267	0.0022606	14.00296	18.62012916	MMO mix. med HIC. low methane
56	2.166E-08	6.392E-06	1.982E-06	2.587E-04	1.978E-03	9.996E-03	1.455E-02	1.276E-02	4.790E-03	2.002E-18	0.04209267	0.0022606	7.962468	18.62012916	
57	3.871E-09	9.744E-07	3.191E-06	1.400E-03	3.052E-03	1.012E-02	1.464E-02	1.236E-02	4.298E-03	4.454E-19	0.041414596	0.00445652	96.42	9.293040984	
58											0	0	#DIV/0!	#DIV/0!	
59											0	0	#DIV/0!	#DIV/0!	
60	3.871E-09	9.744E-07	3.191E-06	1.400E-03	3.052E-03	1.012E-02	1.464E-02	1.236E-02	4.298E-03	4.454E-19	0.041414596	0.00445652	5.035	9.293040984	pMMO, med HIC, med methane
61	4.967E-08	5.291E-06	1.991E-03	4.990E-03	1.383E-03	1.137E-02	1.535E-02	9.759E-03	5.103E-04	4.666E-21	0.036988463	0.0083687	96.00771	4.4198583	
62	4.967E-08	5.291E-06	1.991E-03	4.990E-03	1.383E-03	1.137E-02	1.535E-02	9.759E-03	5.103E-04	4.666E-21	0.036988463	0.0083687	50.01692	4.4198583	
63	4.967E-08	5.291E-06	1.991E-03	4.990E-03	1.383E-03	1.137E-02	1.535E-02	9.759E-03	5.103E-04	4.666E-21	0.036988463	0.0083687	14.07009	4.4198583	
64	4.967E-08	5.291E-06	1.991E-03	4.990E-03	1.383E-03	1.137E-02	1.535E-02	9.759E-03	5.103E-04	4.666E-21	0.036988463	0.0083687	8.000642	4.4198583	
65	3.355E-20	3.356E-20	3.356E-20	3.352E-20	3.341E-20	6.038E-02	2.062E-02	5.028E-03	1.277E-03	2.135E-21	0.087306591	1.676E-19	92.54672	5.20922E+17	
66											0	0	#DIV/0!	#DIV/0!	
67											0	0	#DIV/0!	#DIV/0!	
68											0	0	#DIV/0!	#DIV/0!	
69	1.224E-05	5.030E-05	2.815E-04	4.677E-04	1.946E-04	6.043E-02	2.061E-02	4.418E-03	2.779E-04	6.235E-12	0.085737417	0.00100635	96.12354	85.1966322	
70	1.224E-05	5.030E-05	2.815E-04	4.677E-04	1.946E-04	6.043E-02	2.061E-02	4.418E-03	2.779E-04	6.235E-12	0.085737417	0.00100635	51.1192	85.1966322	
71	1.224E-05	5.030E-05	2.815E-04	4.677E-04	1.946E-04	6.043E-02	2.061E-02	4.418E-03	2.779E-04	6.235E-12	0.085737417	0.00100635	14.38017	85.1966322	
72	1.224E-05	5.030E-05	2.815E-04	4.677E-04	1.946E-04	6.043E-02	2.061E-02	4.418E-03	2.779E-04	6.235E-12	0.085737417	0.00100635	8.176962	85.1966322	
73	1.758E-05	1.614E-04	1.026E-03	6.108E-04	1.383E-04	6.024E-02	2.043E-02	3.223E-03	4.713E-05	2.851E-13	0.083941858	0.00195452	98.33317	42.94763256	
74	1.758E-05	1.614E-04	1.026E-03	6.108E-04	1.383E-04	6.024E-02	2.043E-02	3.223E-03	4.713E-05	2.851E-13	0.083941858	0.00195452	51.22841	42.94763256	MMO mix. med HIC, med methane
75	1.758E-05	1.614E-04	1.026E-03	6.108E-04	1.383E-04	6.024E-02	2.043E-02	3.223E-03	4.713E-05	2.851E-13	0.083941858	0.00195452	14.4109	42.94763256	
76	1.758E-05	1.614E-04	1.026E-03	6.108E-04	1.383E-04	6.024E-02	2.043E-02	3.223E-03	4.713E-05	2.851E-13	0.083941858	0.00195452	8.194431	42.94763256	
77	2.016E-06	1.518E-03	2.568E-03	6.516E-04	9.628E-05	5.969E-02	2.017E-02	2.260E-03	3.215E-06	5.981E-15	0.082114537	0.00483553	99.01531	16.98149145	
78	2.016E-06	1.518E-03	2.568E-03	6.516E-04	9.628E-05	5.969E-02	2.017E-02	2.260E-03	3.215E-06	5.981E-15	0.082114537	0.00483553	51.58378	16.98149145	
79	2.016E-06	1.518E-03	2.568E-03	6.516E-04	9.628E-05	5.969E-02	2.017E-02	2.260E-03	3.215E-06	5.981E-15	0.082114537	0.00483553	14.51086	16.98149145	
80	2.016E-06	1.518E-03	2.568E-03	6.516E-04	9.628E-05	5.969E-02	2.017E-02	2.260E-03	3.215E-06	5.981E-15	0.082114537	0.00483553	14.51086	16.98149145	reran same numbers
81	1.855E-07	1.852E-07	1.848E-07	1.841E-07	1.833E-07	6.584E-02	2.277E-02	3.969E-03	2.600E-05	9.405E-15	0.092626863	9.2288E-07	95.01006	100367.7085	
82											0	0	#DIV/0!	#DIV/0!	
83											0	0	#DIV/0!	#DIV/0!	
84											0	0	#DIV/0!	#DIV/0!	
85	1.756E-05	7.116E-05	3.122E-04	3.714E-04	1.422E-04	6.683E-02	2.332E-02	4.771E-03	3.439E-05	1.501E-15	0.094951488	0.0009145	98.92795	103.8288095	
86	1.756E-05	7.116E-05	3.122E-04	3.714E-04	1.422E-04	6.683E-02	2.332E-02	4.771E-03	3.439E-05	1.501E-15	0.094951488	0.0009145	51.53827	103.8288095	
87	1.756E-05	7.116E-05	3.122E-04	3.714E-04	1.422E-04	6.683E-02	2.332E-02	4.771E-03	3.439E-05	1.501E-15	0.094951488	0.0009145	14.49806	103.8288095	
88	1.756E-05	7.116E-05	3.122E-04	3.714E-04	1.422E-04	6.683E-02	2.332E-02	4.771E-03	3.439E-05	1.501E-15	0.094951488	0.0009145	8.243995	103.8288095	
89	1.986E-05	2.008E-04	1.066E-03	4.521E-04	9.521E-05	6.672E-02	2.330E-02	4.272E-03	1.188E-05	9.297E-17	0.094311094	0.00183436	99.24726	51.41368052	
90											0	0	#DIV/0!	#DIV/0!	
91											0	0	#DIV/0!	#DIV/0!	
92	1.986E-05	2.008E-04	1.066E-03	4.521E-04	9.521E-05	6.672E-02	2.330E-02	4.272E-03	1.188E-05	9.297E-17	0.094311094	0.00183436	8.270605	51.41368052	
93	2.068E-06	1.540E-03	2.534E-03	6.391E-04	9.502E-05	6.672E-02	2.330E-02	4.166E-03	2.895E-06	7.071E-18	0.094190259	0.00481037	99.65027	19.58068561	max sMMO at high meth and high C
94	2.068E-06	1.540E-03	2.534E-03	6.391E-04	9.502E-05	6.672E-02	2.330E-02	4.166E-03	2.895E-06	7.071E-18	0.094190259	0.00481037	51.91458	19.58068561	
95	2.068E-06	1.540E-03	2.534E-03	6.391E-04	9.502E-05	6.672E-02	2.330E-02	4.166E-03	2.895E-06	7.071E-18	0.094190259	0.00481037	14.60392	19.58068561	
96	2.068E-06	1.540E-03	2.534E-03	6.391E-04	9.502E-05	6.672E-02	2.330E-02	4.166E-03	2.895E-06	7.071E-18	0.094190259	0.00481037	8.304189	19.58068561	

Test	O2 (g/L)	C (g/L)	CH4 (mg/L)	Cu (ug/L)	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	kTCE1	kTCE2	kTCE3	kTCE4	kTCE5
97	HIGH	0.01	0	0	0.005433646	0.00612669	0.00307894	1.56E-03	7.46E-04	3.43E-04	1.40E-04	0.1599	0.2266	0.2924	0.3420	0.3740
98	0.006			15												
99				100												
100				200												
101			0.07	0	0.005425975	0.006118354	0.0030493	1.53E-03	7.19E-04	3.21E-04	1.19E-04	0.1609	0.2286	0.2953	0.3453	0.3778
102				15	0.005425975	0.006118354	0.0030493	1.53E-03	7.19E-04	3.21E-04	1.19E-04	0.0838	0.1191	0.1538	0.1799	0.1968
103				100	0.005425975	0.006118354	0.0030493	1.53E-03	7.19E-04	3.21E-04	1.19E-04	0.0236	0.0335	0.0433	0.0506	0.0554
104				200	0.005425975	0.006118354	0.0030493	1.53E-03	7.19E-04	3.21E-04	1.19E-04	0.0134	0.0190	0.0246	0.0288	0.0315
105			0.16	0	0.005417075	0.006108685	0.00301445	1.48E-03	6.67E-04	2.65E-04	6.46E-05	0.1619	0.2317	0.3010	0.3537	0.3876
106				15	0.005417075	0.006108685	0.00301445	1.48E-03	6.67E-04	2.65E-04	6.46E-05	0.0844	0.1207	0.1568	0.1843	0.2019
107				100	0.005417075	0.006108685	0.00301445	1.48E-03	6.67E-04	2.65E-04	6.46E-05	0.0237	0.0340	0.0441	0.0518	0.0568
108				200	0.005417075	0.006108685	0.00301445	1.48E-03	6.67E-04	2.65E-04	6.46E-05	0.0135	0.0193	0.0251	0.0295	0.0323
109			0.5	0	0.005383816	0.00607254	0.00288416	1.32E-03	5.15E-04	1.11E-04	1.92E-05	0.1662	0.2426	0.3190	0.3792	0.3962
110				15	0.005383816	0.00607254	0.00288416	1.32E-03	5.15E-04	1.11E-04	1.92E-05	0.0866	0.1264	0.1662	0.1976	0.2064
111	Scenario 7			100	0.005383816	0.00607254	0.00288416	1.32E-03	5.15E-04	1.11E-04	1.92E-05	0.0244	0.0356	0.0467	0.0556	0.0581
112				200	0.005383816	0.00607254	0.00288416	1.32E-03	5.15E-04	1.11E-04	1.92E-05	0.0138	0.0202	0.0266	0.0316	0.0330
113		0.035	0	0	0.004933522	0.005583284	0.00112079	2.82E-04	1.08E-04	5.24E-05	2.33E-05	0.2578	0.3510	0.3796	0.3899	0.3954
114				15												
115				100												
116				200												
117			0.07	0	0.004929744	0.005579178	0.00110599	2.73E-04	9.76E-05	4.29E-05	1.51E-05	0.2590	0.3525	0.3816	0.3917	0.3970
118				15	0.004929744	0.005579178	0.00110599	2.73E-04	9.76E-05	4.29E-05	1.51E-05	0.1349	0.1836	0.1988	0.2041	0.2068
119				100	0.004929744	0.005579178	0.00110599	2.73E-04	9.76E-05	4.29E-05	1.51E-05	0.0380	0.0517	0.0559	0.0574	0.0582
120				200	0.004929744	0.005579178	0.00110599	2.73E-04	9.76E-05	4.29E-05	1.51E-05	0.0216	0.0294	0.0318	0.0326	0.0331
121			0.16	0	0.004923395	0.005572273	0.00108109	2.46E-04	6.80E-05	2.24E-05	7.69E-06	0.2811	0.3567	0.3870	0.3966	0.3985
122				15	0.004923395	0.005572273	0.00108109	2.46E-04	6.80E-05	2.24E-05	7.69E-06	0.1360	0.1856	0.2016	0.2061	0.2076
123				100	0.004923395	0.005572273	0.00108109	2.46E-04	6.80E-05	2.24E-05	7.69E-06	0.0383	0.0523	0.0567	0.0580	0.0584
124				200	0.004923395	0.005572273	0.00108109	2.46E-04	6.80E-05	2.24E-05	7.69E-06	0.0218	0.0297	0.0322	0.0330	0.0332
125			0.5	0	0.00490281	0.005549874	0.00100029	1.51E-04	1.47E-05	3.62E-06	1.25E-06	0.2680	0.3722	0.3971	0.3993	0.3998
126				15	0.00490281	0.005549874	0.00100029	1.51E-04	1.47E-05	3.62E-06	1.25E-06	0.1396	0.1939	0.2069	0.2080	0.2083
127				100	0.00490281	0.005549874	0.00100029	1.51E-04	1.47E-05	3.62E-06	1.25E-06	0.0393	0.0545	0.0582	0.0585	0.0586
128				200	0.00490281	0.005549874	0.00100029	1.51E-04	1.47E-05	3.62E-06	1.25E-06	0.0223	0.0310	0.0331	0.0333	0.0333
129		0.06	0	0	0.004797812	0.005435632	0.00058934	1.00E-04	3.26E-05	1.55E-05	6.97E-06	0.3099	0.3811	0.3936	0.3970	0.3986
130				15												
131				100												
132				200												
133			0.07	0	0.004795783	0.005433625	0.00058139	9.02E-05	2.12E-05	7.26E-06	2.79E-06	0.3108	0.3829	0.3959	0.3986	0.3994
134				15	0.004795783	0.005433625	0.00058139	9.02E-05	2.12E-05	7.26E-06	2.79E-06	0.1619	0.1995	0.2082	0.2076	0.2081
135				100	0.004795783	0.005433625	0.00058139	9.02E-05	2.12E-05	7.26E-06	2.79E-06	0.0456	0.0561	0.0580	0.0584	0.0585
136				200	0.004795783	0.005433625	0.00058139	9.02E-05	2.12E-05	7.26E-06	2.79E-06	0.0259	0.0319	0.0330	0.0332	0.0333
137			0.16	0	0.004792699	0.005430271	0.00056929	7.64E-05	1.06E-05	3.07E-06	1.12E-06	0.3123	0.3854	0.3979	0.3994	0.3998
138				15	0.004792699	0.005430271	0.00056929	7.64E-05	1.06E-05	3.07E-06	1.12E-06	0.1627	0.2008	0.2073	0.2081	0.2083
139				100	0.004792699	0.005430271	0.00056929	7.64E-05	1.06E-05	3.07E-06	1.12E-06	0.0458	0.0565	0.0583	0.0585	0.0586
140				200	0.004792699	0.005430271	0.00056929	7.64E-05	1.06E-05	3.07E-06	1.12E-06	0.0260	0.0321	0.0332	0.0333	0.0333
141	Scenario 5		0.5	0	0.004784777	0.00542166	0.00053825	3.32E-05	2.16E-06	5.19E-07	1.80E-07	0.3160	0.3935	0.3996	0.3999	0.4000
142				15	0.004784777	0.00542166	0.00053825	3.32E-05	2.16E-06	5.19E-07	1.80E-07	0.1646	0.2050	0.2082	0.2083	0.2084
143				100	0.004784777	0.00542166	0.00053825	3.32E-05	2.16E-06	5.19E-07	1.80E-07	0.0463	0.0577	0.0586	0.0586	0.0586
144	Scenario 6			200	0.004784777	0.00542166	0.00053825	3.32E-05	2.16E-06	5.19E-07	1.80E-07	0.0263	0.0326	0.0333	0.0333	0.0333

Test	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	Tot Het	Tot Meth	% sMMO	Het to meth	Comments
97	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.057E-03	1.414E-02	1.926E-02	1.388E-02	4.020E-03	0.057350335	0	0	#DIV/0!	#DIV/0!
98											0	0	0	#DIV/0!	#DIV/0!
99											0	0	0	#DIV/0!	#DIV/0!
100											0	0	0	#DIV/0!	#DIV/0!
101	1.106E-08	3.170E-06	1.587E-05	2.380E-04	2.062E-03	6.628E-03	1.449E-02	1.927E-02	1.269E-02	1.805E-07	0.05307624	0.00231944	93.42568	22.88321597	18X320 hrs to steady state
102	1.106E-08	3.170E-06	1.587E-05	2.380E-04	2.062E-03	6.628E-03	1.449E-02	1.927E-02	1.269E-02	1.805E-07	0.05307624	0.00231944	48.87187	22.88321597	
103	1.106E-08	3.170E-06	1.587E-05	2.380E-04	2.062E-03	6.628E-03	1.449E-02	1.927E-02	1.269E-02	1.805E-07	0.05307624	0.00231944	13.69172	22.88321597	
104	1.106E-08	3.170E-06	1.587E-05	2.380E-04	2.062E-03	6.628E-03	1.449E-02	1.927E-02	1.269E-02	1.805E-07	0.05307624	0.00231944	7.78549	22.88321597	max pMMO at high O2 low C low meth
105	2.597E-08	6.013E-06	3.184E-05	7.870E-04	4.475E-03	6.547E-03	1.475E-02	1.918E-02	1.181E-02	6.097E-08	0.052281823	0.00529981	95.46888	9.864857573	
106	2.597E-08	6.013E-06	3.184E-05	7.870E-04	4.475E-03	6.547E-03	1.475E-02	1.918E-02	1.181E-02	6.097E-08	0.052281823	0.00529981	49.73621	9.864857573	
107	2.597E-08	6.013E-06	3.184E-05	7.870E-04	4.475E-03	6.547E-03	1.475E-02	1.918E-02	1.181E-02	6.097E-08	0.052281823	0.00529981	13.99113	9.864857573	
108	2.597E-08	6.013E-06	3.184E-05	7.870E-04	4.475E-03	6.547E-03	1.475E-02	1.918E-02	1.181E-02	6.097E-08	0.052281823	0.00529981	7.95574	9.864857573	
109	3.742E-08	3.581E-06	6.433E-06	7.026E-03	2.978E-03	7.512E-03	1.653E-02	1.863E-02	3.538E-03	2.701E-10	0.04621642	0.01001318	96.04777	4.615560294	highest meth population at high meth low C
110	3.742E-08	3.581E-06	6.433E-06	7.026E-03	2.978E-03	7.512E-03	1.653E-02	1.863E-02	3.538E-03	2.701E-10	0.04621642	0.01001318	50.03779	4.615560294	
111	3.742E-08	3.581E-06	6.433E-06	7.026E-03	2.978E-03	7.512E-03	1.653E-02	1.863E-02	3.538E-03	2.701E-10	0.04621642	0.01001318	14.07597	4.615560294	
112	3.742E-08	3.581E-06	6.433E-06	7.026E-03	2.978E-03	7.512E-03	1.653E-02	1.863E-02	3.538E-03	2.701E-10	0.04621642	0.01001318	8.003981	4.615560294	
113	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	7.280E-02	2.940E-02	7.886E-03	2.133E-03	2.107E-04	0.112432535	0	0	#DIV/0!	#DIV/0!
114											0	0	0	#DIV/0!	#DIV/0!
115											0	0	0	#DIV/0!	#DIV/0!
116											0	0	0	#DIV/0!	#DIV/0!
117	1.281E-06	3.366E-05	2.023E-04	5.797E-04	4.022E-04	7.327E-02	2.853E-02	7.145E-03	1.141E-04	5.751E-16	0.109065583	0.00121911	97.63753	89.463505	
118	1.281E-06	3.366E-05	2.023E-04	5.797E-04	4.022E-04	7.327E-02	2.853E-02	7.145E-03	1.141E-04	5.751E-16	0.109065583	0.00121911	50.86601	89.463505	
119	1.281E-06	3.366E-05	2.023E-04	5.797E-04	4.022E-04	7.327E-02	2.853E-02	7.145E-03	1.141E-04	5.751E-16	0.109065583	0.00121911	14.30895	89.463505	
120	1.281E-06	3.366E-05	2.023E-04	5.797E-04	4.022E-04	7.327E-02	2.853E-02	7.145E-03	1.141E-04	5.751E-16	0.109065583	0.00121911	8.136461	89.463505	
121	1.025E-06	9.275E-05	9.298E-04	9.461E-04	2.578E-04	7.353E-02	2.875E-02	5.932E-03	2.017E-05	7.903E-15	0.108225348	0.0022274	97.66522	48.58812129	
122	1.025E-06	9.275E-05	9.298E-04	9.461E-04	2.578E-04	7.353E-02	2.875E-02	5.932E-03	2.017E-05	7.903E-15	0.108225348	0.0022274	50.88043	48.58812129	
123	1.025E-06	9.275E-05	9.298E-04	9.461E-04	2.578E-04	7.353E-02	2.875E-02	5.932E-03	2.017E-05	7.903E-15	0.108225348	0.0022274	14.31301	48.58812129	
124	1.025E-06	9.275E-05	9.298E-04	9.461E-04	2.578E-04	7.353E-02	2.875E-02	5.932E-03	2.017E-05	7.903E-15	0.108225348	0.0022274	8.138768	48.58812129	
125	2.007E-06	8.359E-04	3.470E-03	1.036E-03	1.562E-04	7.320E-02	2.890E-02	5.589E-03	8.875E-05	7.504E-13	0.107685355	0.00549968	98.44001	19.58028672	hets maxed on C
126	2.007E-06	8.359E-04	3.470E-03	1.036E-03	1.562E-04	7.320E-02	2.890E-02	5.589E-03	8.875E-05	7.504E-13	0.107685355	0.00549968	51.28407	19.58028672	
127	2.007E-06	8.359E-04	3.470E-03	1.036E-03	1.562E-04	7.320E-02	2.890E-02	5.589E-03	8.875E-05	7.504E-13	0.107685355	0.00549968	14.42655	19.58028672	
128	2.007E-06	8.359E-04	3.470E-03	1.036E-03	1.562E-04	7.320E-02	2.890E-02	5.589E-03	8.875E-05	7.504E-13	0.107685355	0.00549968	8.203334	19.58028672	
129	0.000E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00	9.248E-02	3.375E-02	8.147E-03	1.768E-03	5.241E-05	0.136195261	0	0	#DIV/0!	#DIV/0!
130											0	0	0	#DIV/0!	#DIV/0!
131											0	0	0	#DIV/0!	#DIV/0!
132											0	0	0	#DIV/0!	#DIV/0!
133	1.441E-05	4.753E-05	2.965E-04	4.636E-04	1.561E-04	9.186E-02	3.345E-02	7.999E-03	8.865E-04	2.082E-06	0.134190399	0.00097815	98.95807	137.1882155	
134	1.441E-05	4.753E-05	2.965E-04	4.636E-04	1.561E-04	9.186E-02	3.345E-02	7.999E-03	8.865E-04	2.082E-06	0.134190399	0.00097815	51.55396	137.1882155	
135	1.441E-05	4.753E-05	2.965E-04	4.636E-04	1.561E-04	9.186E-02	3.345E-02	7.999E-03	8.865E-04	2.082E-06	0.134190399	0.00097815	14.50248	137.1882155	
136	1.441E-05	4.753E-05	2.965E-04	4.636E-04	1.561E-04	9.186E-02	3.345E-02	7.999E-03	8.865E-04	2.082E-06	0.134190399	0.00097815	8.246506	137.1882155	
137	1.439E-05	1.147E-04	1.045E-03	7.108E-04	1.481E-04	9.188E-02	3.345E-02	7.661E-03	3.401E-04	1.505E-07	0.133329474	0.00203304	99.31407	65.58148018	
138	1.439E-05	1.147E-04	1.045E-03	7.108E-04	1.481E-04	9.188E-02	3.345E-02	7.661E-03	3.401E-04	1.505E-07	0.133329474	0.00203304	51.73943	65.58148018	
139	1.439E-05	1.147E-04	1.045E-03	7.108E-04	1.481E-04	9.188E-02	3.345E-02	7.661E-03	3.401E-04	1.505E-07	0.133329474	0.00203304	14.55465	65.58148018	
140	1.439E-05	1.147E-04	1.045E-03	7.108E-04	1.481E-04	9.188E-02	3.345E-02	7.661E-03	3.401E-04	1.505E-07	0.133329474	0.00203304	8.276172	65.58148018	
141	2.063E-06	8.868E-04	3.403E-03	1.000E-03	1.499E-04	9.188E-02	3.345E-02	7.620E-03	9.424E-05	8.049E-09	0.133048379	0.0054418	99.6565	24.44933092	sMMO, high HIC, high methane
142	2.063E-06	8.868E-04	3.403E-03	1.000E-03	1.499E-04	9.188E-02	3.345E-02	7.620E-03	9.424E-05	8.049E-09	0.133048379	0.0054418	51.91782	24.44933092	
143	2.063E-06	8.868E-04	3.403E-03	1.000E-03	1.499E-04	9.188E-02	3.345E-02	7.620E-03	9.424E-05	8.049E-09	0.133048379	0.0054418	14.80483	24.44933092	
144	2.063E-06	8.868E-04	3.403E-03	1.000E-03	1.499E-04	9.188E-02	3.345E-02	7.620E-03	9.424E-05	8.049E-09	0.133048379	0.0054418	8.304709	24.44933092	pMMO, high HIC, high methane

## Appendix G. TCE Testing Data

Test	Scenario	Loading (L/m <sup>2</sup> /hr)	TCE (mg/L)	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	kTCE1	kTCE2	kTCE3	kTCE4	kTCE5	
1A			0.01	0.00208109	0.00248495	3.70E-04	9.50E-05	3.67E-05	1.75E-05	7.63E-06	3.38E-01	3.82E-01	0.39286238	0.39656772	0.39849579	
1	1	0.68	0.05	0.00210363	0.0025092	3.84E-04	1.04E-04	4.55E-05	2.42E-05	1.09E-05	3.36E-01	3.80E-01	0.39119087	0.39526031	0.39785651	
2	sMMO		0.1	0.00208348	0.00248755	3.78E-04	1.09E-04	5.15E-05	2.83E-05	1.29E-05	3.37E-01	3.79E-01	0.39005992	0.39446848	0.39746915	
3	low HIC		0.3	0.00208962	0.00249445	4.00E-04	1.19E-04	5.67E-05	3.13E-05	1.42E-05	3.34E-01	3.78E-01	0.38909068	0.39389452	0.39720234	
4	low CH4		0.5													
5	med C		3													
5A			0.005	0.00208123	0.00248511	3.71E-04	9.51E-05	3.62E-05	1.68E-05	7.21E-06	3.38E-01	3.82E-01	0.39296576	0.39670046	0.39857773	
5B			0.01	0.00208085	0.00248469	3.70E-04	9.48E-05	3.67E-05	1.76E-05	7.69E-06	3.38E-01	3.82E-01	0.39286111	0.39654761	0.39848271	
6		2.4	0.05	0.00208217	0.00248613	3.74E-04	1.02E-04	4.68E-05	2.65E-05	1.21E-05	3.38E-01	3.81E-01	0.39095448	0.39481513	0.39762577	
7			0.1	0.00208382	0.00248792	3.80E-04	1.11E-04	5.36E-05	3.10E-05	1.41E-05	3.37E-01	3.79E-01	0.38967125	0.39395586	0.39722875	
8			0.3	0.00209	0.00249465	4.00E-04	1.20E-04	5.88E-05	3.40E-05	1.55E-05	3.34E-01	3.78E-01	0.38868792	0.39338334	0.39696401	
9			0.5													
10			3													
11A			0.005	0.00208076	0.0024846	3.69E-04	9.43E-05	3.57E-05	1.65E-05	6.98E-06	3.38E-01	3.82E-01	0.39306231	0.39675421	0.39862366	
11B			0.01	0.00208068	0.0024845	3.69E-04	9.47E-05	3.68E-05	1.78E-05	7.69E-06	3.38E-01	3.82E-01	0.39283676	0.39650984	0.3984823	
11		4.1	0.05	0.00208148	0.00248537	3.72E-04	1.01E-04	4.62E-05	2.63E-05	1.20E-05	3.38E-01	3.81E-01	0.39106148	0.39485694	0.39764572	
12			0.1	0.0020832	0.00248725	3.78E-04	1.09E-04	5.32E-05	3.11E-05	1.41E-05	3.37E-01	3.79E-01	0.38973666	0.39394175	0.39722301	
13			0.3	0.00208936	0.00249394	3.98E-04	1.20E-04	5.87E-05	3.42E-05	1.56E-05	3.34E-01	3.78E-01	0.38871494	0.39333976	0.39694468	
14			0.5													
15			3													
16		2	0.68	0.05	0.00344497	0.00396621	1.79E-03	8.43E-04	4.01E-04	1.85E-04	6.38E-05	3.12E-02	4.14E-02	0.04892718	0.05371783	0.05682599
17	MMO mix		0.1	0.00344536	0.00396663	1.79E-03	8.44E-04	4.04E-04	1.90E-04	6.91E-05	3.12E-02	4.14E-02	0.04886162	0.05359782	0.05668289	
18	med HIC		0.3	0.00344923	0.00397084	1.80E-03	8.61E-04	4.21E-04	2.06E-04	8.41E-05	3.11E-02	4.12E-02	0.04851957	0.05320766	0.05627866	
19	low CH4		0.5	0.00345335	0.00397532	1.82E-03	8.80E-04	4.40E-04	2.22E-04	9.68E-05	3.10E-02	4.09E-02	0.04816511	0.05282943	0.05594197	
19A			1.5	0.00343969	0.00396119	1.87E-03	9.47E-04	5.00E-04	2.67E-04	1.21E-04	3.06E-02	4.00E-02	0.04702032	0.05177935	0.05529831	
20	low C		3	0.00348183	0.00400659	1.96E-03	1.04E-03	5.76E-04	3.16E-04	1.44E-04	2.99E-02	3.88E-02	0.04563682	0.05069781	0.05473364	
21		2.4	0.05	0.00342597	0.00394586	1.77E-03	8.30E-04	3.96E-04	1.83E-04	6.35E-05	3.14E-02	4.16E-02	0.04902712	0.05374656	0.05683485	
22			0.1	0.00342633	0.00394625	1.77E-03	8.29E-04	3.97E-04	1.86E-04	6.79E-05	3.14E-02	4.16E-02	0.04901059	0.05367435	0.05671557	
23			0.3	0.00343037	0.00395064	1.78E-03	8.46E-04	4.13E-04	2.02E-04	8.27E-05	3.13E-02	4.14E-02	0.04867857	0.05329604	0.05631709	
24			0.5	0.0034346	0.00395524	1.80E-03	8.64E-04	4.31E-04	2.18E-04	9.52E-05	3.12E-02	4.11E-02	0.04832633	0.05292362	0.05598341	
25			3	0.00347636	0.00400065	1.94E-03	1.02E-03	5.64E-04	3.09E-04	1.40E-04	3.01E-02	3.90E-02	0.04595007	0.05084933	0.05481371	
26		4.1	0.05	0.00342238	0.00394196	1.75E-03	8.16E-04	3.84E-04	1.73E-04	5.82E-05	3.15E-02	4.18E-02	0.04928229	0.05399222	0.05697933	
27			0.1	0.00342309	0.00394273	1.76E-03	8.18E-04	3.89E-04	1.81E-04	6.55E-05	3.15E-02	4.18E-02	0.0491642	0.05379291	0.05678149	
28			0.3	0.00342695	0.00394694	1.77E-03	8.36E-04	4.08E-04	1.99E-04	8.10E-05	3.14E-02	4.15E-02	0.04879253	0.05337106	0.05636066	
29			0.5	0.00343119	0.00395154	1.78E-03	8.55E-04	4.26E-04	2.15E-04	9.38E-05	3.13E-02	4.12E-02	0.04842577	0.05298204	0.05601995	
30			3	0.00347785	0.00400226	1.94E-03	1.03E-03	5.74E-04	3.16E-04	1.43E-04	3.00E-02	3.88E-02	0.04567455	0.05070163	0.05473971	
16A	2 ALT	0.68	0.05	0.00300395	0.00348747	3.72E-04	6.54E-05	1.95E-05	9.03E-06	3.85E-06	4.95E-02	5.88E-02	0.05806055	0.05835998	0.05850908	
17A	MMO mix		0.1	0.00300531	0.00348895	3.76E-04	6.81E-05	2.19E-05	1.07E-05	4.76E-06	4.94E-02	5.67E-02	0.0579925	0.05831143	0.05848294	
18A	med HIC		0.3	0.00300834	0.00349225	3.87E-04	7.56E-05	2.91E-05	1.58E-05	7.18E-06	4.92E-02	5.65E-02	0.05778958	0.05816579	0.05841297	
19A	low CH4		0.5	0.00301157	0.00349576	3.98E-04	8.38E-05	3.48E-05	1.88E-05	8.53E-06	4.90E-02	5.63E-02	0.05762874	0.05808164	0.05837436	
			1.5	0.00302928	0.00351502	4.59E-04	1.10E-04	4.78E-05	2.60E-05	1.18E-05	4.78E-02	5.56E-02	0.05726598	0.05787612	0.05827981	
20A	high C		3	0.00305092	0.00353854	5.33E-04	1.45E-04	6.69E-05	3.69E-05	1.68E-05	4.64E-02	5.47E-02	0.0567431	0.05757047	0.05813851	

Test	M1	M2	M3	M4	M5	Tot Meth (g)	Tot Hets (g)	TCE Cons Rate	TCE Trtmnt Eff	% sMMO	Comments
1A	2.45E-05	7.97E-05	2.23E-04	1.86E-04	3.23E-05	5.46E-04	5.381E-02	1.57E-07	100.00	97.56	for %sMMO calculation: kmax = 0.40
1	3.76E-05	4.77E-05	4.12E-05	7.86E-06	1.54E-08	1.34E-04	5.304E-02	2.17E-07	76.52	93.00	
2	3.86E-05	4.90E-06	1.08E-07	7.51E-11	6.98E-18	4.36E-05	5.413E-02	1.32E-07	23.23	85.41	
3	4.85E-08	1.92E-11	1.21E-14	4.26E-19	1.05E-27	4.89E-08	5.384E-02	3.40E-10	0.02	82.97	
4											
5											
5A	2.28E-05	8.33E-05	2.72E-04	2.49E-04	4.64E-05	6.74E-04	5.310E-02	9.31E-08	93.93	97.84	
5B	2.50E-05	8.04E-05	2.23E-04	1.87E-04	2.97E-05	5.45E-04	5.354E-02	1.57E-07	79.23	97.54	
6	3.74E-05	4.24E-05	4.42E-05	1.93E-05	1.39E-07	1.43E-04	5.320E-02	2.30E-07	23.20	93.57	
7	3.65E-05	7.09E-06	1.13E-06	4.45E-08	1.27E-12	4.48E-05	5.329E-02	1.35E-07	6.82	86.17	
8	7.27E-07	9.76E-09	3.24E-10	3.56E-12	1.71E-17	7.42E-07	5.306E-02	5.20E-09	0.09	83.07	
9											
10											
11A	2.40E-05	8.60E-05	2.70E-04	2.44E-04	6.05E-05	6.84E-04	5.316E-02	9.47E-08	55.70	97.86	high flow conditions result in reduced treatment eff
11B	2.50E-05	7.88E-05	2.23E-04	1.93E-04	3.65E-05	5.58E-04	5.333E-02	1.61E-07	47.32	97.59	but consumption rate remains relatively constant
11	3.77E-05	4.28E-05	4.42E-05	1.93E-05	1.32E-07	1.44E-04	5.347E-02	2.31E-07	13.61	93.60	
12	3.50E-05	8.51E-06	2.25E-06	2.05E-07	5.91E-11	4.60E-05	5.350E-02	1.39E-07	4.09	86.88	
13	7.16E-07	1.18E-08	6.60E-10	1.71E-11	8.23E-16	7.34E-07	5.329E-02	5.15E-09	0.05	83.17	
14											
15											
16	6.17E-06	8.66E-06	2.91E-05	2.69E-04	1.16E-03	1.47E-03	3.968E-02	4.30E-07	100.00	13.98	
17	8.64E-06	9.11E-06	2.77E-05	2.29E-04	8.15E-04	1.09E-03	3.979E-02	6.18E-07	100.00	13.88	
18	1.86E-05	1.14E-05	2.60E-05	1.27E-04	2.74E-04	4.57E-04	3.925E-02	6.82E-07	40.08	13.39	
19	2.55E-05	1.21E-05	2.34E-05	7.29E-05	8.70E-05	2.21E-04	3.870E-02	4.79E-07	16.89	12.60	
19A	1.20E-05	1.80E-06	5.64E-12	1.75E-13	5.04E-16	1.21E-05	3.599E-02	3.66E-08	0.45	7.65	
20	3.80E-11	9.59E-15	4.30E-17	1.04E-18	8.46E-21	3.83E-11	3.309E-02	2.01E-13	0.00	7.42	
21	6.61E-06	9.14E-06	3.01E-05	2.72E-04	1.16E-03	1.47E-03	3.956E-02	4.31E-07	43.46	13.97	
22	9.28E-06	9.71E-06	2.90E-05	2.32E-04	8.09E-04	1.09E-03	3.981E-02	6.17E-07	31.13	13.88	
23	1.91E-05	1.17E-05	2.75E-05	1.29E-04	2.68E-04	4.55E-04	3.931E-02	6.78E-07	11.39	13.39	
24	2.55E-05	1.22E-05	2.48E-05	7.35E-05	8.21E-05	2.18E-04	3.877E-02	4.73E-07	4.77	12.59	
25	2.44E-09	7.49E-12	2.73E-13	3.36E-14	1.46E-15	2.47E-09	3.433E-02	1.30E-11	0.00	7.46	
26	7.78E-06	1.09E-05	3.45E-05	2.96E-04	1.10E-03	1.45E-03	4.142E-02	4.21E-07	24.77	13.98	
27	1.01E-05	1.05E-05	3.08E-05	2.38E-04	7.94E-04	1.08E-03	4.056E-02	6.14E-07	18.05	13.88	
28	1.94E-05	1.22E-05	2.80E-05	1.30E-04	2.63E-04	4.53E-04	3.972E-02	6.73E-07	6.60	13.39	
29	2.60E-05	1.26E-05	2.46E-05	7.31E-05	8.14E-05	2.18E-04	3.909E-02	4.72E-07	2.77	12.58	
30	3.97E-13	8.01E-18	5.06E-21	2.41E-23	3.39E-26	4.01E-13	3.253E-02	2.10E-15	0.00	7.44	
16A	2.40E-05	7.44E-05	2.24E-04	1.93E-04	4.67E-05	5.63E-04	8.549E-02	1.49E-07	52.67	14.40	
17A	2.83E-05	6.85E-05	1.59E-04	1.24E-04	1.64E-05	3.97E-04	8.443E-02	2.11E-07	37.17	14.30	
18A	4.18E-05	4.50E-05	4.10E-05	1.40E-05	3.17E-08	1.42E-04	8.392E-02	2.17E-07	12.77	13.71	
19A	4.88E-05	1.58E-05	2.02E-06	3.16E-08	7.64E-14	6.67E-05	8.353E-02	1.57E-07	5.55	12.73	
	1.38E-06	4.04E-09	3.74E-11	7.14E-14	1.23E-20	1.39E-06	8.168E-02	6.46E-09	0.08	11.91	
20A	2.20E-13	6.45E-19	2.85E-22	6.36E-26	9.13E-34	2.23E-13	7.856E-02	1.80E-15	0.00	11.41	

Test	Scenario	Loading	TCE (mg/L)	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	kTCE1	kTCE2	kTCE3	kTCE4	kTCE5	
31		3	0.68	0.05	0.00341893	0.00393821	1.74E-03	7.95E-04	3.60E-04	1.40E-04	3.43E-05	1.80E-02	2.39E-02	0.02830428	0.03117541	0.03277732
32	pMMO			0.1	0.00341865	0.00393791	1.74E-03	7.95E-04	3.62E-04	1.47E-04	3.80E-05	1.80E-02	2.39E-02	0.02828052	0.03108937	0.03271806
33	med HIC			0.3	0.00342251	0.00394211	1.75E-03	8.11E-04	3.76E-04	1.60E-04	4.75E-05	1.79E-02	2.38E-02	0.02811363	0.03088674	0.03256707
34	med CH4			0.5	0.00342629	0.00394621	1.77E-03	8.27E-04	3.90E-04	1.73E-04	5.64E-05	1.79E-02	2.37E-02	0.02794661	0.03070702	0.03242769
34A				1.5												
35	low C			3	0.00347666	0.00400097	1.94E-03	1.04E-03	5.88E-04	3.29E-04	1.50E-04	1.71E-02	2.20E-02	0.02583578	0.02868958	0.03103629
36		2.4		0.05	0.00341525	0.00393421	1.73E-03	7.85E-04	3.53E-04	1.38E-04	3.36E-05	1.80E-02	2.40E-02	0.02838011	0.03120794	0.03278734
37				0.1	0.00341551	0.00393449	1.73E-03	7.86E-04	3.56E-04	1.44E-04	3.72E-05	1.80E-02	2.40E-02	0.02834235	0.03111481	0.03273114
38				0.3	0.00341915	0.00393845	1.74E-03	8.02E-04	3.70E-04	1.57E-04	4.67E-05	1.80E-02	2.39E-02	0.02817928	0.03092494	0.03258043
39				0.5	0.00342297	0.0039426	1.76E-03	8.18E-04	3.85E-04	1.70E-04	5.55E-05	1.79E-02	2.38E-02	0.02801077	0.0307444	0.03244215
40				3	0.00347206	0.00399597	1.92E-03	1.03E-03	5.75E-04	3.23E-04	1.47E-04	1.72E-02	2.21E-02	0.02596058	0.02874715	0.03107828
41A				0.01	0.00341118	0.00392978	1.71E-03	7.74E-04	3.48E-04	1.36E-04	3.18E-05	1.81E-02	2.41E-02	0.02844395	0.03123574	0.03281744
41		4.1		0.05	0.00341152	0.00393016	1.72E-03	7.75E-04	3.49E-04	1.38E-04	3.38E-05	1.81E-02	2.41E-02	0.02842813	0.03120498	0.03278517
42				0.1	0.00341204	0.00393072	1.72E-03	7.78E-04	3.53E-04	1.43E-04	3.70E-05	1.81E-02	2.41E-02	0.02838722	0.03112563	0.03273375
43				0.3	0.00341573	0.00393473	1.73E-03	7.93E-04	3.66E-04	1.56E-04	4.64E-05	1.80E-02	2.40E-02	0.0282262	0.03094071	0.03258502
44				0.5	0.00341951	0.00393884	1.74E-03	8.09E-04	3.80E-04	1.69E-04	5.51E-05	1.80E-02	2.38E-02	0.02806124	0.03076306	0.03244859
45				3	0.00347107	0.00399489	1.92E-03	1.03E-03	5.77E-04	3.23E-04	1.47E-04	1.72E-02	2.21E-02	0.0259452	0.02874136	0.03107566
46		4	0.68	0.05	0.00306579	0.00355433	5.30E-04	1.11E-04	3.27E-05	1.40E-05	5.89E-06	1.65E-01	1.98E-01	0.20507063	0.20695672	0.20778133
47	MMO mix			0.1	0.00306757	0.00355627	5.36E-04	1.18E-04	3.99E-05	1.90E-05	6.34E-06	1.65E-01	1.97E-01	0.20435574	0.20644586	0.20753023
48	med HIC			0.3	0.00307239	0.00356151	5.52E-04	1.39E-04	6.01E-05	3.40E-05	1.55E-05	1.64E-01	1.95E-01	0.20237234	0.20493626	0.20680277
49	med CH4			0.5	0.00307827	0.00356791	5.72E-04	1.59E-04	7.23E-05	4.05E-05	1.84E-05	1.62E-01	1.93E-01	0.20119148	0.20428825	0.20650216
50	med C			3	0.00313694	0.00363168	7.74E-04	2.73E-04	1.37E-04	7.77E-05	3.53E-05	1.51E-01	1.84E-01	0.19516317	0.20066976	0.20480477
51		2.4		0.05	0.00306453	0.00355297	5.25E-04	1.09E-04	3.20E-05	1.36E-05	5.74E-06	1.65E-01	1.98E-01	0.20513884	0.20699108	0.2077972
52				0.1	0.00306629	0.00355488	5.31E-04	1.16E-04	3.91E-05	1.86E-05	6.18E-06	1.65E-01	1.97E-01	0.20442953	0.20648418	0.20754634
53				0.3	0.00307138	0.00356041	5.49E-04	1.37E-04	5.95E-05	3.37E-05	1.53E-05	1.64E-01	1.95E-01	0.20242504	0.2049719	0.20681987
54				0.5	0.00307762	0.0035672	5.70E-04	1.58E-04	7.25E-05	4.09E-05	1.86E-05	1.63E-01	1.93E-01	0.20117285	0.20425611	0.20648793
55				3	0.00313514	0.00362973	7.68E-04	2.70E-04	1.35E-04	7.68E-05	3.49E-05	1.51E-01	1.84E-01	0.19533298	0.20075685	0.2048469
56		4.1		0.05	0.00306419	0.00355259	5.24E-04	1.10E-04	3.23E-05	1.35E-05	5.61E-06	1.66E-01	1.98E-01	0.20510877	0.20700681	0.20781005
57				0.1	0.00306513	0.00355362	5.27E-04	1.14E-04	3.85E-05	1.82E-05	8.00E-06	1.65E-01	1.97E-01	0.20449008	0.20652703	0.20756488
58				0.3	0.00307011	0.00355903	5.45E-04	1.35E-04	5.84E-05	3.28E-05	1.49E-05	1.64E-01	1.95E-01	0.20253006	0.20506603	0.20686153
59				0.5	0.00307627	0.00356573	5.66E-04	1.56E-04	7.10E-05	3.97E-05	1.80E-05	1.63E-01	1.93E-01	0.20131085	0.20437559	0.2065441
60				3	0.00313254	0.0036269	7.59E-04	2.64E-04	1.32E-04	7.47E-05	3.39E-05	1.52E-01	1.84E-01	0.19562835	0.20095914	0.20494364
46A	4 ALT	0.68		0.05	0.00164831	0.00201485	2.92E-04	6.53E-05	2.37E-05	1.12E-05	5.00E-06	1.82E-01	2.02E-01	0.20597555	0.20724115	0.20787297
47A	MMO mix			0.1	0.00164954	0.00201619	2.96E-04	7.17E-05	2.97E-05	1.52E-05	6.90E-06	1.82E-01	2.01E-01	0.20536838	0.20682848	0.20767742
48A	LOW HIC			0.3	0.00165349	0.00202048	3.10E-04	9.25E-05	4.73E-05	2.80E-05	1.27E-05	1.81E-01	1.99E-01	0.20361808	0.20554099	0.20708219
49A	med CH4			0.5	0.00165881	0.00202626	3.27E-04	1.09E-04	5.68E-05	3.38E-05	1.54E-05	1.79E-01	1.98E-01	0.20269409	0.2049585	0.2068129
50A	med C			3	0.0016971	0.0020679	4.55E-04	1.84E-04	1.03E-04	6.12E-05	2.78E-05	1.70E-01	1.91E-01	0.19829745	0.20225926	0.20555381

Test	M1	M2	M3	M4	M5	Tot Meth	Tot Het	TCE Cons Rate	TCE Trtmnt Eff	% sMMO	Comments
31	2.72E-06	2.42E-06	3.90E-06	1.40E-03	2.09E-03	3.50E-03	3.794E-02	4.82E-07	100.00	8.03	
32	2.08E-05	2.96E-05	1.09E-04	1.07E-03	1.84E-03	3.06E-03	3.753E-02	8.31E-07	100.00	7.95	
33	1.39E-05	1.69E-05	8.73E-05	6.83E-04	1.16E-03	1.96E-03	3.702E-02	1.49E-06	87.70	7.90	
34	1.92E-05	1.68E-05	8.70E-05	4.91E-04	8.13E-04	1.43E-03	3.649E-02	1.69E-06	59.60	7.82	
34A											
35	7.34E-05	1.29E-05	5.20E-06	1.20E-06	3.25E-09	9.28E-05	2.965E-02	2.68E-07	1.58	4.60	
36	5.58E-06	5.60E-06	1.30E-05	1.39E-03	2.10E-03	3.52E-03	3.824E-02	4.83E-07	48.75	8.03	
37	1.19E-05	1.82E-05	8.15E-05	1.12E-03	1.81E-03	3.04E-03	3.787E-02	8.25E-07	41.60	7.98	
38	1.50E-05	1.77E-05	9.01E-05	6.88E-04	1.14E-03	1.96E-03	3.735E-02	1.49E-06	25.03	7.90	
39	2.01E-05	1.76E-05	8.95E-05	4.93E-04	8.06E-04	1.43E-03	3.681E-02	1.69E-06	17.02	7.82	
40	7.28E-05	1.15E-05	7.38E-06	4.27E-06	1.64E-07	9.61E-05	3.092E-02	2.82E-07	0.47	4.74	
41A	4.09E-06	5.69E-06	9.76E-06	1.59E-03	2.39E-03	4.00E-03	3.833E-02	1.11E-07	32.61	8.04	
41	1.49E-05	2.30E-05	4.86E-05	1.36E-03	2.07E-03	3.52E-03	3.819E-02	4.84E-07	28.46	8.00	
42	1.21E-05	2.22E-05	8.61E-05	1.12E-03	1.79E-03	3.04E-03	3.796E-02	8.25E-07	24.28	7.97	
43	1.54E-05	2.01E-05	9.35E-05	6.92E-04	1.13E-03	1.95E-03	3.746E-02	1.49E-06	14.59	7.90	
44	2.04E-05	1.97E-05	9.12E-05	4.96E-04	7.99E-04	1.43E-03	3.699E-02	1.69E-06	9.92	7.81	
45	7.40E-05	1.35E-05	5.17E-06	1.22E-06	3.24E-09	9.39E-05	3.042E-02	2.73E-07	0.27	4.63	
46	3.27E-05	1.65E-04	4.42E-04	2.33E-04	4.16E-05	9.14E-04	7.618E-02	5.81E-07	100.00	50.69	
47	4.07E-05	1.24E-04	2.32E-04	1.35E-04	1.87E-05	5.51E-04	7.532E-02	7.27E-07	100.00	50.06	
48	5.76E-05	4.69E-05	3.50E-05	1.12E-05	5.73E-09	1.51E-04	7.554E-02	5.53E-07	32.52	46.33	good demo test
49	5.86E-05	3.22E-06	1.13E-07	5.65E-10	2.08E-17	6.20E-05	7.572E-02	3.20E-07	11.30	40.99	
50	1.82E-40	3.30E-53	1.06E-59	2.45E-65	1.10E-76	1.89E-40	6.800E-02	3.17E-42	0.00	36.29	
51	3.41E-05	1.68E-04	4.43E-04	2.30E-04	4.17E-05	9.17E-04	7.634E-02	5.83E-07	58.75	50.69	
52	4.16E-05	1.26E-04	2.33E-04	1.33E-04	1.78E-05	5.51E-04	7.551E-02	7.28E-07	36.70	50.06	
53	5.82E-05	4.74E-05	3.46E-05	1.05E-05	4.72E-09	1.51E-04	7.537E-02	5.53E-07	9.30	46.32	screen capture of TCE induced pMMO assertion
54	5.79E-05	4.11E-06	2.62E-07	3.13E-09	7.13E-16	6.23E-05	7.509E-02	3.21E-07	3.24	41.15	
55	2.33E-40	4.98E-53	2.93E-59	1.63E-64	4.34E-75	2.43E-40	6.849E-02	4.07E-42	0.00	36.37	
56	3.56E-05	1.66E-04	4.44E-04	2.37E-04	4.54E-05	9.28E-04	7.569E-02	5.89E-07	34.66	50.69	
57	4.24E-05	1.27E-04	2.33E-04	1.32E-04	1.69E-05	5.52E-04	7.584E-02	7.29E-07	21.45	50.06	
58	5.87E-05	4.79E-05	3.43E-05	9.70E-06	3.71E-09	1.51E-04	7.559E-02	5.54E-07	5.43	46.32	12345 strata of meths, reversal due to toxicity
59	5.83E-05	4.10E-06	2.44E-07	2.36E-09	4.56E-16	6.27E-05	7.542E-02	3.24E-07	1.90	41.22	methane recovery from toxic effects
60	1.52E-31	2.20E-41	2.23E-46	6.73E-51	1.52E-60	1.58E-31	6.943E-02	2.66E-33	0.00	36.48	pMMO toxicity threshol < 3 ppm
46A	4.99E-05	2.55E-04	3.18E-04	1.43E-04	8.35E-06	7.72E-04	4.181E-02	4.94E-07	100.00	50.81	
47A	5.56E-05	1.62E-04	1.61E-04	8.09E-05	1.87E-06	4.61E-04	4.138E-02	6.14E-07	100.00	50.30	
48A	6.12E-05	3.99E-05	1.81E-05	3.23E-06	3.05E-10	1.23E-04	4.151E-02	4.54E-07	26.72	47.66	
49A	4.72E-05	1.49E-06	3.17E-08	1.13E-10	4.31E-18	4.87E-05	4.145E-02	2.53E-07	8.93	44.95	
50A	8.41E-37	2.85E-44	9.39E-49	2.47E-53	1.93E-63	8.79E-37	3.750E-02	1.65E-38	0.00	40.66	

Test	Scenario	Loading	TCE (mg/L)	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	KTCE1	KTCE2	KTCE3	KTCE4	KTCE5	
61		5	0.68	0.05	0.00478557	0.00542253	5.41E-04	3.53E-05	2.89E-06	8.51E-07	3.18E-07	3.16E-01	3.93E-01	0.39942763	0.39983157	0.39993702
62	sMMO			0.1	0.00478233	0.005419	5.29E-04	3.94E-05	4.17E-06	1.45E-06	5.76E-07	3.17E-01	3.92E-01	0.39917559	0.39971366	0.39988608
63	high HIC			0.3	0.00478242	0.00541909	5.29E-04	5.50E-05	1.15E-05	5.74E-06	2.59E-06	3.17E-01	3.89E-01	0.39773823	0.39886623	0.39948771
64	high CH4			0.5	0.00478494	0.00542184	5.39E-04	7.16E-05	2.12E-05	1.18E-05	5.36E-06	3.16E-01	3.86E-01	0.39584826	0.3976783	0.39894084
65	high carbon			3	0.00485749	0.00550068	8.23E-04	1.97E-04	8.16E-05	4.31E-05	1.96E-05	2.85E-01	3.64E-01	0.38448407	0.39164045	0.39615556
66		2.4		0.05	0.00478361	0.0054204	5.34E-04	3.40E-05	2.74E-06	7.96E-07	3.57E-07	3.17E-01	3.93E-01	0.39945908	0.39984238	0.39992941
67				0.1	0.00478286	0.00541958	5.31E-04	3.87E-05	4.04E-06	1.36E-06	5.84E-07	3.17E-01	3.92E-01	0.39920199	0.39973015	0.39988449
68				0.3	0.0047816	0.00541821	5.26E-04	5.43E-05	1.11E-05	5.02E-06	2.28E-06	3.18E-01	3.90E-01	0.397807	0.39900779	0.39954862
69				0.5	0.00478389	0.0054207	5.35E-04	6.99E-05	2.02E-05	1.01E-05	4.61E-06	3.16E-01	3.87E-01	0.39603862	0.39800406	0.39908999
70				3	0.00483132	0.00547193	7.19E-04	1.52E-04	5.87E-05	3.08E-05	1.41E-05	2.95E-01	3.72E-01	0.38870257	0.39399625	0.39722705
71		4.1		0.05	0.00478314	0.00541988	5.32E-04	3.40E-05	2.77E-06	7.62E-07	2.92E-07	3.17E-01	3.93E-01	0.39945288	0.39984913	0.39994225
72				0.1	0.00478006	0.00541654	5.20E-04	3.80E-05	3.99E-06	1.33E-06	5.38E-07	3.18E-01	3.93E-01	0.39921136	0.39973678	0.39989357
73				0.3	0.00478038	0.00541688	5.21E-04	5.33E-05	1.10E-05	5.28E-06	2.39E-06	3.18E-01	3.90E-01	0.39783808	0.39895672	0.3995275
74				0.5	0.00478289	0.00541962	5.31E-04	6.92E-05	2.01E-05	1.06E-05	4.81E-06	3.17E-01	3.87E-01	0.39605256	0.39791664	0.39905029
75				3	0.0048545	0.00549744	8.11E-04	1.92E-04	8.02E-05	4.37E-05	1.98E-05	2.86E-01	3.65E-01	0.3847373	0.39154121	0.39611157
76		6	0.68	0.05	0.00478561	0.00542256	5.42E-04	3.46E-05	2.42E-06	5.86E-07	2.07E-07	2.63E-02	3.28E-02	0.03329351	0.03332367	0.03332992
77	pMMO			0.1	0.00478669	0.00542374	5.46E-04	3.61E-05	2.68E-06	6.83E-07	2.43E-07	2.63E-02	3.27E-02	0.03328913	0.03332206	0.03332933
78	high HIC			0.3	0.00479046	0.00542784	5.61E-04	4.14E-05	3.83E-06	1.11E-06	4.04E-07	2.61E-02	3.27E-02	0.03327032	0.03331509	0.03332667
79	high CH4			0.5	0.00479147	0.00542893	5.64E-04	4.66E-05	5.12E-06	1.64E-06	6.17E-07	2.61E-02	3.26E-02	0.03324905	0.03330635	0.03332315
				1.5												
80	high carbon			3	0.00482974	0.00547053	7.14E-04	1.25E-04	4.17E-05	2.23E-05	1.01E-05	2.46E-02	3.14E-02	0.0326586	0.03296901	0.03316768
80A				5	0.00487554	0.0055203	8.94E-04	2.46E-04	1.12E-04	6.56E-05	2.98E-05	2.31E-02	2.97E-02	0.03157729	0.03228492	0.03284837
81		2.4		0.05	0.00478463	0.0054215	5.38E-04	3.42E-05	2.39E-06	6.01E-07	2.14E-07	2.63E-02	3.28E-02	0.03329392	0.03332342	0.03332981
82				0.1	0.00478542	0.00542236	5.41E-04	3.54E-05	2.63E-06	6.81E-07	2.40E-07	2.63E-02	3.28E-02	0.03329002	0.03332211	0.03332937
83				0.3	0.00478926	0.00542653	5.56E-04	4.06E-05	3.74E-06	1.09E-06	3.97E-07	2.62E-02	3.27E-02	0.03327167	0.03331534	0.03332678
84				0.5	0.00478929	0.00542657	5.56E-04	4.59E-05	5.03E-06	1.62E-06	6.08E-07	2.62E-02	3.26E-02	0.03325047	0.03330665	0.0333233
85				3	0.00482925	0.00546999	7.12E-04	1.25E-04	4.21E-05	2.26E-05	1.02E-05	2.47E-02	3.14E-02	0.03265348	0.03296417	0.03316508
85A				5	0.00487084	0.00551519	8.75E-04	2.37E-04	1.09E-04	6.42E-05	2.92E-05	2.33E-02	2.98E-02	0.03163059	0.03230758	0.03285913
86		4.1		0.05	0.00478321	0.00541995	5.32E-04	3.34E-05	2.33E-06	5.87E-07	2.03E-07	2.64E-02	3.28E-02	0.03329488	0.03332365	0.03332998
87				0.1	0.00478411	0.00542094	5.36E-04	3.47E-05	2.57E-06	6.67E-07	2.34E-07	2.64E-02	3.28E-02	0.03329104	0.03332234	0.03332948
88				0.3	0.00478807	0.00542525	5.51E-04	3.99E-05	3.66E-06	1.07E-06	3.88E-07	2.62E-02	3.27E-02	0.03327302	0.03331571	0.03332693
89				0.5	0.00478744	0.00542455	5.49E-04	4.52E-05	4.94E-06	1.59E-06	5.98E-07	2.62E-02	3.26E-02	0.03325196	0.03330708	0.03332347
90				3	0.00482758	0.00546818	7.06E-04	1.23E-04	4.09E-05	2.20E-05	9.96E-06	2.47E-02	3.14E-02	0.03267154	0.03297423	0.03316976
90A				5	0.00486976	0.00551402	8.71E-04	2.37E-04	1.09E-04	6.43E-05	2.92E-05	2.33E-02	2.98E-02	0.03163264	0.03230583	0.03285843

Test	M1	M2	M3	M4	M5	Tot Meth	Tot Het	TCE Cons Rate	TCE Trtmnt Eff	% sMMO	Comments
61	1.43E-06	7.64E-04	2.05E-03	5.73E-04	9.45E-05	3.49E-03	1.328E-01	1.07E-06	100.00	99.37	
62	4.54E-05	5.62E-04	1.16E-03	3.33E-04	5.75E-05	2.16E-03	1.326E-01	1.64E-06	100.00	98.77	
63	9.87E-05	2.47E-04	2.16E-04	5.78E-05	2.93E-06	6.24E-04	1.319E-01	1.95E-06	100.00	95.16	
64	1.32E-04	1.25E-04	6.08E-05	1.50E-05	5.49E-08	3.34E-04	1.313E-01	1.83E-06	64.62	89.84	
65	8.55E-23	1.38E-40	2.85E-50	6.99E-58	1.12E-71	8.76E-23	1.222E-01	1.22E-24	0.00	69.45	
66	4.99E-06	7.81E-04	2.08E-03	5.75E-04	5.49E-06	3.43E-03	1.355E-01	1.05E-06	100.00	99.36	little difference from lower loading rate
67	3.21E-05	5.80E-04	1.17E-03	3.38E-04	2.25E-05	2.15E-03	1.335E-01	1.61E-06	100.00	98.89	high methane levels help sustain viability
68	1.00E-04	2.52E-04	2.15E-04	4.58E-05	3.96E-07	6.15E-04	1.322E-01	1.94E-06	32.57	95.07	
69	1.33E-04	1.30E-04	5.55E-05	5.73E-06	3.11E-10	3.26E-04	1.321E-01	1.81E-06	18.27	89.57	
70	1.72E-18	4.21E-29	8.75E-34	2.78E-37	1.11E-42	1.74E-18	1.237E-01	1.33E-20	0.00	72.76	
71	8.55E-06	7.87E-04	2.04E-03	5.68E-04	7.89E-05	3.49E-03	1.333E-01	1.08E-06	63.82	99.34	
72	5.13E-05	5.71E-04	1.16E-03	3.33E-04	4.85E-05	2.17E-03	1.331E-01	1.65E-06	48.56	98.74	
73	1.01E-04	2.51E-04	2.16E-04	5.39E-05	1.86E-06	6.25E-04	1.325E-01	1.96E-06	19.18	95.16	
74	1.34E-04	1.30E-04	5.71E-05	9.35E-06	3.02E-09	3.31E-04	1.320E-01	1.83E-06	10.76	89.75	
75	2.09E-18	2.26E-29	2.62E-34	7.15E-38	9.66E-45	2.14E-18	1.231E-01	2.99E-20	0.00	69.72	
76	1.86E-06	8.27E-04	2.98E-03	9.16E-04	1.37E-04	4.87E-03	1.330E-01	2.36E-07	83.34	8.29	
77	1.63E-06	7.76E-04	2.64E-03	8.46E-04	1.35E-04	4.40E-03	1.325E-01	4.28E-07	75.55	8.29	
78	1.16E-06	6.48E-04	1.76E-03	6.18E-04	1.17E-04	3.14E-03	1.316E-01	9.22E-07	54.23	8.28	
79	2.85E-05	5.51E-04	1.30E-03	4.69E-04	9.79E-05	2.45E-03	1.308E-01	1.23E-06	43.36	8.24	
80	1.93E-04	2.23E-04	1.54E-04	5.97E-05	3.84E-06	6.34E-04	1.215E-01	1.84E-06	10.80	7.44	
80A	2.61E-04	4.12E-05	1.15E-05	2.70E-06	5.83E-08	3.17E-04	1.109E-01	1.08E-06	3.81	6.09	
81	6.43E-06	8.46E-04	2.98E-03	9.09E-04	1.31E-04	4.88E-03	1.321E-01	2.39E-07	24.08	8.29	
82	5.89E-06	7.87E-04	2.64E-03	8.45E-04	1.39E-04	4.43E-03	1.324E-01	4.32E-07	21.76	8.29	
83	4.04E-06	6.56E-04	1.76E-03	6.17E-04	1.20E-04	3.16E-03	1.317E-01	9.27E-07	15.58	8.28	
84	4.30E-05	5.46E-04	1.30E-03	4.69E-04	1.00E-04	2.46E-03	1.309E-01	1.25E-06	12.58	8.24	
85	1.97E-04	2.22E-04	1.53E-04	5.97E-05	2.22E-06	6.35E-04	1.207E-01	1.84E-06	3.10	7.43	
85A	2.62E-04	4.20E-05	1.31E-05	2.16E-06	1.04E-09	3.19E-04	1.139E-01	1.09E-06	1.10	6.13	
86	1.09E-05	8.56E-04	2.98E-03	9.13E-04	1.54E-04	4.92E-03	1.324E-01	2.41E-07	14.16	8.29	
87	9.75E-06	7.99E-04	2.65E-03	8.45E-04	1.44E-04	4.45E-03	1.326E-01	4.35E-07	12.78	8.29	
88	6.93E-06	6.64E-04	1.76E-03	6.17E-04	1.21E-04	3.18E-03	1.318E-01	9.32E-07	9.14	8.27	
89	5.45E-05	5.43E-04	1.31E-03	4.68E-04	1.01E-04	2.48E-03	1.312E-01	1.26E-06	7.42	8.23	
90	1.98E-04	2.24E-04	1.55E-04	6.06E-05	2.31E-06	6.41E-04	1.216E-01	1.86E-06	1.82	7.44	
90A	2.70E-04	3.62E-05	8.71E-06	7.99E-07	3.30E-11	3.16E-04	1.138E-01	1.09E-06	0.64	6.07	

Test	Scenario	Loading	TCE (mg/L)	O2 Stele	O2 Cortex	O1	O2	O3	O4	O5	kTCE1	kTCE2	kTCE3	kTCE4	kTCE5	
91		7	0.68	0.05	0.00337878	0.00389455	1.60E-03	6.47E-04	2.09E-04	4.21E-05	1.00E-05	3.28E-02	4.44E-02	0.05312562	0.05742435	0.05833067
92	MMO mix			0.1	0.00337968	0.00389554	1.61E-03	6.48E-04	2.10E-04	4.57E-05	1.15E-05	3.28E-02	4.44E-02	0.05311061	0.05732514	0.05828827
93	med HIC			0.3	0.00338174	0.00389779	1.61E-03	6.60E-04	2.30E-04	6.26E-05	1.78E-05	3.27E-02	4.42E-02	0.05264001	0.05685961	0.05810953
94	high CH4			0.5	0.00338563	0.00390201	1.63E-03	6.75E-04	2.48E-04	7.69E-05	2.27E-05	3.26E-02	4.40E-02	0.05222414	0.05647263	0.05797021
94A	low carbon			1	0.00339543	0.00391266	1.66E-03	7.18E-04	2.94E-04	1.13E-04	3.66E-05	3.23E-02	4.33E-02	0.05118109	0.05552464	0.057577
95				3	0.00344167	0.00396293	1.82E-03	9.24E-04	4.93E-04	2.66E-04	1.13E-04	3.10E-02	4.03E-02	0.04713874	0.05179917	0.05551291
95A				5	0.00349212	0.00401778	1.99E-03	1.14E-03	6.85E-04	4.07E-04	1.85E-04	2.97E-02	3.76E-02	0.04381181	0.04880024	0.05370281
96		2.4	0.05													
97				0.1	0.00337745	0.00389311	1.60E-03	6.39E-04	1.96E-04	4.04E-05	1.02E-05	3.28E-02	4.46E-02	0.05343426	0.05747205	0.05832572
98				0.3	0.00337851	0.00389426	1.60E-03	6.50E-04	2.21E-04	5.95E-05	1.71E-05	3.28E-02	4.44E-02	0.05284604	0.05694318	0.05812783
99				0.5	0.00338229	0.00389838	1.62E-03	6.66E-04	2.41E-04	7.46E-05	2.22E-05	3.27E-02	4.41E-02	0.05237177	0.05653428	0.05798431
99A				1	0.00339217	0.00390912	1.65E-03	7.09E-04	2.88E-04	1.10E-04	3.60E-05	3.24E-02	4.34E-02	0.0513049	0.05558951	0.05759545
100				3	0.00343675	0.00395758	1.80E-03	9.09E-04	4.86E-04	2.62E-04	1.12E-04	3.11E-02	4.05E-02	0.04727791	0.05189456	0.05555229
100A				5	0.00348943	0.00401485	1.98E-03	1.13E-03	6.80E-04	4.03E-04	1.83E-04	2.97E-02	3.77E-02	0.0438956	0.0488787	0.05374729
101		4.1	0.05													
102				0.1												
103				0.3	0.00337541	0.0038909	1.59E-03	6.41E-04	2.16E-04	5.78E-05	1.68E-05	3.29E-02	4.45E-02	0.05296114	0.05699029	0.0581381
104				0.5	0.00337898	0.00389478	1.60E-03	6.57E-04	2.37E-04	7.29E-05	2.18E-05	3.28E-02	4.43E-02	0.05247917	0.05657885	0.05799502
104A				1	0.00338921	0.0039059	1.64E-03	7.02E-04	2.84E-04	1.08E-04	3.55E-05	3.25E-02	4.35E-02	0.05139835	0.05563696	0.05760984
105				3	0.00343619	0.00395697	1.80E-03	9.09E-04	4.84E-04	2.61E-04	1.11E-04	3.11E-02	4.05E-02	0.04731061	0.05192568	0.05556688
105A				5	0.00348751	0.00401277	1.98E-03	1.12E-03	6.76E-04	4.00E-04	1.82E-04	2.98E-02	3.77E-02	0.04396677	0.04894265	0.0537837
<b>Scenario 5 Profile Testing</b>																
61A		5	0.68	0.005	0.00478452	0.00542138	5.37E-04	3.31E-05	2.18E-06	5.37E-07	1.86E-07	3.16E-01	3.94E-01	0.39956946	0.39989377	0.39996281
61B	sMMO			0.01	0.00466302	0.00528939	6.16E-05	1.53E-06	3.29E-08	2.93E-09	0.00E+00	3.88E-01	4.00E-01	0.39999349	0.39999942	0.4
61	high HIC			0.05	0.00478557	0.00542253	5.41E-04	3.53E-05	2.89E-06	8.51E-07	3.18E-07	3.16E-01	3.93E-01	0.39942763	0.39983157	0.39993702
62	high CH4			0.1	0.00478233	0.005419	5.29E-04	3.94E-05	4.17E-06	1.45E-06	5.76E-07	3.17E-01	3.92E-01	0.39917559	0.39971366	0.39988608
63	high carbon			0.3	0.00478242	0.00541909	5.29E-04	5.50E-05	1.15E-05	5.74E-06	2.59E-06	3.17E-01	3.89E-01	0.39773823	0.39886623	0.39948771
63A				0.4	0.00478375	0.00542055	5.34E-04	6.25E-05	1.54E-05	7.41E-06	3.37E-06	3.17E-01	3.88E-01	0.39697056	0.39853777	0.39933352
64				0.5	0.00478494	0.00542184	5.39E-04	7.16E-05	2.12E-05	1.18E-05	5.36E-06	3.16E-01	3.86E-01	0.39584826	0.3976783	0.39894084
64A				0.75	0.00479087	0.00542829	5.62E-04	1.00E-04	3.72E-05	2.06E-05	9.35E-06	3.13E-01	3.81E-01	0.39277027	0.39596888	0.39815674
64B				1	0.00480066	0.00543893	6.01E-04	1.16E-04	4.31E-05	2.27E-05	1.03E-05	3.09E-01	3.78E-01	0.39165464	0.3955569	0.39796734
65				3	0.00485749	0.00550068	8.23E-04	1.97E-04	8.16E-05	4.31E-05	1.96E-05	2.85E-01	3.64E-01	0.38448407	0.39164045	0.39615556
65A				5	0.00489459	0.00554099	9.68E-04	2.71E-04	1.20E-04	6.45E-05	2.93E-05	2.71E-01	3.53E-01	0.37750737	0.38762023	0.39427528
65B				10	0.00498825	0.00564276	1.34E-03	4.92E-04	2.46E-04	1.36E-04	6.18E-05	2.41E-01	3.22E-01	0.35666332	0.37478536	0.38812769

Test	M1	M2	M3	M4	M5	Tot Meth	Tot Het	TCE Cons Rate	TCE Trtmt Eff	% sMMO	Comments
91	3.71E-06	8.31E-07	2.18E-03	3.50E-03	9.35E-04	6.62E-03	3.298E-02	9.03E-07	100.00	14.03	
92	3.68E-06	2.68E-07	1.94E-03	2.95E-03	7.95E-04	5.69E-03	3.309E-02	1.59E-06	100.00	14.00	
93	3.29E-05	8.40E-05	1.15E-03	1.82E-03	5.80E-04	3.66E-03	3.248E-02	3.06E-06	100.00	13.81	
94	3.13E-05	1.01E-04	8.14E-04	1.33E-03	5.53E-04	2.83E-03	3.213E-02	3.65E-06	100.00	13.71	
94A	4.73E-05	1.07E-04	4.91E-04	7.71E-04	4.62E-04	1.88E-03	3.125E-02	4.05E-06	71.42	13.40	
95	1.17E-04	9.43E-05	1.56E-04	1.99E-04	1.34E-04	7.01E-04	2.656E-02	2.63E-06	15.49	11.61	
95A	1.69E-04	2.71E-05	5.05E-06	5.40E-07	1.26E-09	2.01E-04	2.228E-02	7.29E-07	2.57	7.78	
96											
97	1.08E-05	2.85E-06	2.03E-03	2.83E-03	7.44E-04	5.61E-03	3.446E-02	1.58E-06	79.55	14.02	
98	3.81E-05	8.70E-05	1.18E-03	1.77E-03	5.42E-04	3.62E-03	3.310E-02	3.04E-06	51.02	13.81	
99	3.35E-05	1.07E-04	8.31E-04	1.30E-03	5.29E-04	2.80E-03	3.252E-02	3.63E-06	36.64	13.70	
99A	4.90E-05	1.11E-04	4.98E-04	7.63E-04	4.48E-04	1.87E-03	3.158E-02	4.03E-06	20.34	13.40	
100	1.19E-04	9.52E-05	1.56E-04	1.94E-04	1.27E-04	6.92E-04	2.746E-02	2.60E-06	4.37	11.59	
100A	1.70E-04	2.66E-05	4.92E-06	5.04E-07	1.10E-09	2.02E-04	2.259E-02	7.30E-07	0.74	7.79	
101											
102											
103	2.82E-05	1.13E-04	1.20E-03	1.74E-03	5.18E-04	3.60E-03	3.338E-02	3.03E-06	29.72	13.81	
104	3.48E-05	1.13E-04	8.44E-04	1.29E-03	5.13E-04	2.79E-03	3.279E-02	3.63E-06	21.33	13.70	
104A	5.04E-05	1.15E-04	5.04E-04	7.57E-04	4.40E-04	1.87E-03	3.173E-02	4.03E-06	11.85	13.40	
105	1.20E-04	9.70E-05	1.57E-04	1.95E-04	1.25E-04	6.94E-04	2.700E-02	2.61E-06	2.56	11.59	
105A	1.70E-04	2.67E-05	4.72E-06	4.60E-07	9.34E-10	2.02E-04	2.270E-02	7.32E-07	0.43	7.80	

Scenario 5 Profile Testing

61A	1.97E-06	8.79E-04	3.25E-03	9.45E-04	1.43E-04	5.23E-03	1.338E-01	1.33E-07	100.00	99.52	TCE degradation limited by TCE avail
61B	1.92E-06	6.90E-04	3.32E-03	1.02E-03	1.40E-04	5.18E-03	1.441E-01	1.31E-07	100.00	99.74	
61	1.43E-06	7.64E-04	2.05E-03	5.73E-04	9.45E-05	3.49E-03	1.328E-01	1.07E-06	100.00	99.37	5X conc but 9X degradation rate
62	4.54E-05	5.62E-04	1.16E-03	3.33E-04	5.75E-05	2.16E-03	1.326E-01	1.64E-06	100.00	98.77	
63	9.87E-05	2.47E-04	2.16E-04	5.78E-05	2.93E-06	6.24E-04	1.319E-01	1.95E-06	100.00	95.16	optimum TCE degradation rate, tox effects accumulate on meths
63A	1.17E-04	1.85E-04	1.13E-04	8.43E-06	1.70E-10	4.26E-04	1.322E-01	1.88E-06	82.94	92.40	
64	1.32E-04	1.25E-04	6.08E-05	1.50E-05	5.49E-08	3.34E-04	1.313E-01	1.83E-06	64.62	89.84	
64A	1.59E-04	2.09E-05	6.44E-07	7.21E-09	3.66E-15	1.81E-04	1.301E-01	1.41E-06	33.18	79.99	
64B	1.25E-04	6.18E-08	2.09E-11	4.64E-15	2.46E-24	1.25E-04	1.295E-01	1.06E-06	18.67	76.81	TCE degradation limited by meth tox
65	8.55E-23	1.38E-40	2.85E-50	6.99E-58	1.12E-71	8.76E-23	1.222E-01	1.22E-24	0.00	69.45	het non-compet inhibition becomes significant
65A	1.65E-54	1.30E-87	2.62E-103	2.38E-114	1.27E-131	1.74E-54	1.158E-01	3.61E-56	0.00	64.41	
65B	4.46E-168	1.92E-241	1.67E-274	3.37E-295	8.201E-321	4.92E-168	1.004E-01	1.61E-169	0.00	0.00	

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## Vita

Ian F. Thompson graduated from Towson High School in Towson, MD and entered the U. S. Naval Academy in July 1992. He graduated in May 1996 with a B.S. in History and was commissioned as a Second Lieutenant in the U. S. Marine Corps. He then attended The Basic School in Quantico, Virginia. After completing TBS in March 1997, he attended flight school at NAS Pensacola. He was designated a Naval Flight Officer in October 1998 and completed Joint Aviation Electronic Warfare School before transferring to VAQ-129 at NAS Whidbey Island. Upon completion of the EA-6B training program in Apr 2000, he was assigned to MCAS Cherry Point, North Carolina with VMAQ-2. He served as the ground training officer and deployed to Iwakuni, Japan Mar-Jun 01. He attended TACP class in Oct 2001 and was assigned to 1<sup>st</sup> Bn 2<sup>d</sup> Marines as a Forward Air Controller Dec 01-Dec 2002. He returned to VMAQ-2 and acted as the Embarkation and Responsible Officer, the Logistics Officer, Assistant Electronic Warfare Officer, and the Administration Officer, serving three tours in support of Operation Iraqi Freedom. He also served as the Second Marine Aircraft Wing Staff Secretary from May-Sep 05. He reported to the Air Force Institute of Technology and entered the Graduate School of Engineering and Management in Aug 2006. Major Thompson's subsequent orders are to Headquarters Marine Corps, Washington, DC.

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<b>14. ABSTRACT</b> This study analyzes rhizosphere conditions that enhance the effective aerobic degradation of TCE in wetland bioremediation systems. A plant model was built using Stella 9.0 modeling software and uses numerical integration evaluation; it addresses movement of oxygen through plant vascular and aerenchymal systems, and into the rhizosphere where oxygen and other substrates influence bacteria. Methanotrophs and heterotrophs are assumed to be influential bacteria groups. Variations of humidity-induced-convection, methane, soil carbon, and copper concentrations are evaluated. Varying concentrations and hydraulic loadings of TCE are assessed with respect to TCE consumption rate and TCE treatment efficiency. Soil conditions most directly affected TCE consumption, and hydraulic conditions most directly influenced treatment efficiencies. The research identified low carbon, low copper, high oxygen, and high methane concentrations as most conducive conditions for remediation. Variations in soil carbon had the highest impact on consumption rates; minimizing organic carbon concentrations of the influent may enhance remediation rates. It is recommended to first optimize soil conditions in a wetland treatment system, and then adjust hydraulic loading to achieve optimal treatment efficiencies. The model developed can be used to determine likely remediation rates and to then optimize efficiency by adjusting flow rates for a wetland bioremediation system.					
<b>15. SUBJECT TERMS</b> TCE, Radial Oxygen Loss, Bioremediation, Methanotroph, Wetland Treatment System, Rhizosphere, Plant Model, Computer Modeling, Phragmites australis, Humidity-Induced-Convection, MMO, HIC, ROL					
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