Understanding Microbial Biofilms in Receiving Waters Impacted by Airport Deicing Activities

DETAILS

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AIRPORT COOPERATIVE RESEARCH PROGRAM

ACRP REPORT 115

Understanding Microbial Biofilms in Receiving Waters Impacted by Airport Deicing Activities

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AIRPORT COOPERATIVE RESEARCH PROGRAM

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FOREWORD

By Joseph D. Navarrete
Staff Officer
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ACRP Report 115: Understanding Microbial Biofilms in Receiving Waters Impacted by Airport Deicing Activities provides an introduction to the factors than can affect biofilm growth sometimes observed in streams that receive airport runoff containing deicers. While much research has been done on biofilm growth in streams in general, there has been limited investigation focused on the relationship between airport activity and biofilm growth. The issue is significant to many airports, since biofilm growth can sometimes reach a level where environmental regulatory action may be taken. The report will be of particular interest to airport environmental practitioners who wish to understand the relationships among key conditions affecting biofilm growth and what future research is needed to help the industry manage biofilm growth in situations where airport activity may be a contributing factor.

Over the last several years, increased attention has been directed toward the occurrence of microbial biofilms at airport stormwater outfalls. The challenge to the aviation community for addressing this issue is significant, because microbial biofilm growth associated with deicing discharges is not currently predictable, the controlling factors are poorly understood, and the costs of treatment controls can be substantial. Airports and regulators need reliable information on what is and is not known about the factors contributing to the occurrence of microbial biofilms as a first step toward identifying measures to control them.

The research, led by CH2M HILL, began with an extensive literature review and the identification of knowledge gaps. Next, four hypotheses were developed to examine the effect of light, phosphorus, physical stream characteristics, and nutrients on biofilm growth. The hypotheses were then tested using a combination of fieldwork, lab work, and model simulations. The research confirmed that readily biodegradable organic matter (i.e., chemical oxygen demand [COD]) and biological oxygen demand [BOD]) is the most influential factor affecting biofilm growth. The study concluded with the contractor identifying areas of future research that could help the industry obtain the information needed to better address biofilm growth around airports.

Chapter 1 summarizes the issues associated with biofilm growth near airports, the research objectives, and report structure. Chapter 2 summarizes the contractor's literature review, focusing on knowledge gaps. The steps taken to develop hypotheses are described in Chapter 3. Chapter 4 describes the research protocol, including field data collection, laboratory studies, and modeling. A description of the research steps taken to test each hypothesis and the results are contained in Chapter 5. Recognizing that additional research is needed on the topic, the contractor provides a work plan for future research in Chapter 6. A listing of references cited in the report is also available. Details about the biofilm model used to support the research are provided in an appendix.

CONTENTS

SUMMARY

Understanding Microbial Biofilms in Receiving Waters Impacted by Airport Deicing Activities

Introduction

Biofilms are complex ecosystems bound by a matrix of extracellular polymeric substances that are produced by algae and other microorganisms (Callow 2000). Biofilm growth dominated by heterotrophic organisms has been observed at and downstream of outfalls associated with airport deicing activities. This growth can reach an abundance level where regulatory actions are triggered, requiring the airport to eliminate its contribution to the condition. Airports in this situation have been hampered in their efforts because of an absence of scientific information. A fundamental understanding of the developmental characteristics, structure, and function of biofilms is needed for identifying the controllable factors that might minimize biofilm growth in flowing water bodies. With that information, engineers and scientists would be in a better position to design management approaches to minimize biofilm proliferation.

This project undertook preliminary investigations to assess potential factors affecting biofilm growth downstream of airport discharges that contain deicers, consisting of sunlight, nutrients, and physical stream characteristics. This research represents the first step toward understanding biofilms at airports.

The objectives of ACRP Project 02-32 are threefold:

1. Provide airports and regulatory agencies with a reference document that summarizes what is known about the occurrence of prolific biofilms in receiving waters near airports
2. Investigate the relationships between key environmental conditions that affect prolific biofilm growth
3. Recommend the next steps needed to address knowledge gaps

Literature Findings

The research team identified and reviewed approximately 160 scientific papers and relevant publications regarding biofilm development in flowing water bodies that receive surface water runoff from airports that conduct deicing operations. In addition, the team gathered information from six airports currently dealing with biofilm growth that presents an environmental regulatory concern, and one additional airport where the U.S. Geological Survey is conducting research on biofilm growth. The following observations came out of a review of the information from these airports:

- Aircraft and pavement deicers contribute to the seasonal occurrence of biofilms.
- Discharge concentrations of 5-day biochemical oxygen demand (BOD_5) do not by themselves serve as reliable predictors of prolific biofilm growth.

- Reduction in biofilm growth has been observed with reduced deicer concentrations at some airports.
- To date, only one airport has been able to successfully reduce biofilm proliferation to acceptable levels, according to the requirements of that airport's National Pollutant Discharge Elimination System (NPDES) permit.

The review of available literature and airport experience, combined with the experience of the research team members in conducting biofilm research, led to the identification of gaps in knowledge and understanding of the following factors that are likely to influence biofilm proliferation in response to stormwater deicing discharges: light availability, nutrient limitation, and the physical characteristics of the receiving stream.

Testable Hypotheses

Four testable hypotheses were developed based on the identified gaps in knowledge, and approved by the ACRP Project 02-32 panel. During data collection, it became apparent that two of the hypotheses were so closely related that a single set of laboratory experiments and model runs would provide the information needed for testing. Consequently, the two hypotheses were combined into Hypothesis 2/4, a single composite addressing nutrient (nitrogen [N] and phosphorus [P]) limitation.

Hypothesis 1—Effect of Light on Biofilm Growth. The amount of biofilm growth that occurs under given conditions of readily biodegradable dissolved organic matter (i.e., soluble BOD_5) is directly proportional to the availability of sunlight.

Hypothesis 2/4—Potential for Nutrient Limitation on Biofilm Growth.

- 2/4a. Stream biofilms will exhibit phosphorus limitation when the concentration of orthophosphate is in the range 0.005 to 0.025 mg/L (as P) or less.
- 2/4b. Ratios of water column substrate (i.e., organic carbon) and N and P concentrations can be used to identify the rate-limiting nutrient for biofilm growth in a stream.

Hypothesis 3—Impact of Physical Stream Characteristics on Biofilm Growth. The physical characteristics of receiving streams influence the extent of biofilm accumulation for water chemistry conditions as follows:

- Shallow (i.e., ≤1 cm depth), turbulent, well-mixed stream channels promote biofilm growth and require ambient BOD_5 concentrations less than 50 mg/L to avoid prolific biofilm growth.
- Relatively straight stream channels with moderate depth (i.e., 1.3 to 4 cm) and flow promote moderate biofilm growth and require ambient BOD_5 concentrations be maintained in the range of 50 to 100 mg/L or lower to avoid prolific biofilm growth.
- Deep (i.e., ≥10 cm), slow-moving stream channels deter biofilm growth, and can experience ambient BOD_5 concentrations greater than 100 mg/L without prolific biofilm growth.

Data Collection

Laboratory and field investigations were conducted to support testing of the hypotheses. Controlled experiments were conducted in biofilm growth chambers at the Montana State University Center for Biofilm Engineering. Field monitoring of sunlight, water quality, and

biofilm characteristics was conducted between December 2012 and May 2013 in streams receiving stormwater discharges containing deicers from General Mitchell International Airport (MKE) in Milwaukee, Wisconsin, and Gerald R. Ford International Airport (GRR) in Grand Rapids, Michigan.

The AQUASIM biofilm model (Reichert 1998) was specified for conditions generally consistent with those observed in the field and laboratory, but the model was not calibrated to the observations or used in a predictive mode. The value of the model to this research was as a diagnostic tool, representing relative responses of biofilm components to the factors considered in the hypothesis testing.

Results and Discussion

The results of the testing are summarized in the following paragraphs. The reader should note that these findings reflect the specific conditions under which the monitoring and testing were conducted.

Hypothesis 1—Effect of Light. This hypothesis was not supported by the results. The amount of biofilm growth that occurs under given conditions of readily biodegradable dissolved organic matter does not appear to be directly proportional to the availability (or intensity) of sunlight. Although the amount of biofilm observed to grow in the field and laboratory experiments did not appear to differ with different light availability, differences in the appearance of the biofilm suggests that light has an influence on the community composition of the biofilm.

Hypothesis 2/4—Nutrient Limitation. Hypothesis 2/4(a) was not supported by the laboratory results; P-limited conditions were evident at P concentrations substantially greater than 0.025 mg/L. Biofilm communities were observed in the laboratory and field to be dominated by filamentous organisms under low P conditions. This observation is consistent with the literature and suggests that efforts to limit P as a means of controlling biofilm may favor more undesirable components of stream biofilm communities.

Hypothesis 2/4(b) was supported by the laboratory observations. Heterotrophic plate count data were positively correlated with increases in the relative N concentrations. Fungal count data were positively correlated with increases in the relative P concentrations. Chlorophyll a (algal) data did not correlate well with nutrient ratio changes, but the results suggest that dominance of filamentous bacteria inhibited algal growth.

Field results indicate that total dissolved N concentrations at both study locations were in the middle range of concentrations tested in the laboratory. Laboratory results suggest these conditions provide sufficient N for biofilm growth in the receiving streams. Orthophosphorus concentrations observed in both receiving streams suggest concentrations were below 0.025 mg-P/L much of the time during low-flow conditions, a range that the literature suggests is associated with phosphorus limitation on bacteria growth in natural systems. Nonetheless, biofilms grew prolifically at all monitoring locations. It was not possible to conclude from these data whether the biofilms were unresponsive to limiting P levels or whether they were stimulated and sustained by higher P concentrations associated with higher flows during runoff events. Growth was predominantly filamentous in nature, consistent with the laboratory results.

The biofilm model results were consistent with laboratory and field data. Interestingly, the model suggests a threshold between N-limited and non-N-limited conditions when

the carbon-to-nitrogen ratio is about 57; substantially greater than the generally accepted Redfield Ratio of about 6.6:1. This observation suggests that biofilm is capable of maintaining a steady-state biomass with less N than that which would be generally expected. These observations are supported by previous research that reported biofilms can grow with fewer macronutrients in the water column than might be expected based on the Redfield Ratio because of the recycling of nutrients released by decaying bacteria and other microorganisms in the biofilm structure.

Hypothesis 3—Impact of Physical Stream Characteristics. Hypothesis 3 was not supported by the field results. Over the range of stream depths studied, depth did not have an apparent effect on biofilm growth. This study focused on smaller streams that are representative of the majority of airport receiving water systems, and the results do not preclude the possibility that depth may influence biofilm growth with increasing stream size and increasing depth beyond that examined.

Although quantitative differences in biofilm growth were not observed at different depths, qualitative differences were noted. The structure of the biofilm appeared compacted in shallower areas with high velocities; biofilms in deeper, slower sections of stream tended to be less densely packed. Additionally, in natural (i.e., non-concrete lined) sections of stream, the deepest parts of the streambed typically consisted of smaller grain particles (i.e., sand and finer). Biofilm was able to grow on these particles, but was more ephemeral than biofilm growing on nearby rocks, presumably due to the movement of such particles during higher flows, dislodging the attached biofilm.

Concluding Observations

Previous research has shown readily biodegradable organic matter (i.e., chemical oxygen demand [COD] and biological oxygen demand [BOD]), to be the most influential factor affecting biofilm growth (Boualam et al. 2002; Characklis and Marshall 1990). The potential influence of this factor was investigated using data collected by the U.S. Geological Survey at the Kinnickinnic River near 11th Street (location KK), 3.2 km downstream from the confluence with Wilson Park Creek at MKE. This site has larger streamflows, but is otherwise similar in depth, velocity, and light availability to the monitoring locations upstream on Wilson Park Creek. Observed COD concentrations at KK followed the same general temporal trends as those at the Wilson Park Creek monitoring locations, but concentrations at KK were consistently lower, ranging from less than 8 to 65 mg/L, with a median concentration of 19.1 mg/L. COD concentrations were next lowest upstream at 13th Street, where COD concentrations ranged from less than 8 to 180 mg/L, with a median concentration of 32 mg/L. No biofilms were visually observed at KK, whereas prolific biofilm growth was observed at 13th Street. It is worth noting that analysis of data from other concurrent water quality studies occurring at MKE show COD concentrations varying substantially within short time periods, with much larger fluctuations in concentration than the monthly samples collected during biofilm sampling for this investigation.

Future Research

The research team developed suggestions for future research to build on the information and insights gained through the current investigations. These suggestions reflect the research team's opinions of knowledge and information gaps that need to be addressed in

the future, and are not presented with any assumptions as to which specific research organization or entity would be most appropriate to undertake the work.

Field Investigations

Research is needed that documents the change in biofilm community diversity as organic carbon (C) levels in the water column are increased and decreased. A specific question of interest is whether the time between C inputs is long enough for the populations to shift back and forth. This would be airport- and season-specific. The results of this research would provide a better basis upon which to predict the response of stream biofilms to reduced deicer inputs.

It is suggested that additional field surveys be conducted during transition periods when benthic communities are changing to and from a condition of heterotrophic and copiotrophic (i.e., organisms that tend to grow in organic-rich environments) dominance (Upton and Nedwell 1989). Monitoring during these periods will provide insight into potential threshold concentrations influencing biofilm community composition. Sites should be sampled to expand the existing data set and support evaluation of community changes associated with changing in-stream COD concentrations on both spatial (i.e., upstream to downstream) and temporal (i.e., transition to and from heterotrophic and copiotrophic biofilm dominance) scales. Conducting these investigations at the existing streams at MKE and GRR would be advantageous because data collected during those studies would be available to support the research.

The results of the field investigations should be analyzed to characterize the extent and magnitude of biofilm communities that develop at each of the stream stations under different seasonal COD conditions. To the extent possible, tests for statistically significant differences between the stations should be conducted. Additional suggestions for further research should be developed, as appropriate.

The suggested research could be completed over a 1-year period, beginning in September to ensure capturing a full deicing season. The estimated cost for this research is $137,000. This cost includes data analysis and preparation of a simple technical memorandum.

Laboratory Studies

Further laboratory work is needed to better understand the connection between P availability and biofilm composition. Suggested future laboratory research to address this need consists of experiments run in a series of continuous stirred tank biofilm reactors, similar to those used in this study. The experiments should be conducted with inoculum derived from airport runoff streams and C and N concentrations maintained at levels that are adequate for microbial growth, but with P concentrations set at increments between 2.5 and 25 μg/L. The experiments should be run for 6 weeks, with weekly biofilm sampling. Biofilm assays should include those performed in this study (heterotrophic plate count, fungi, and chlorophyll a) as well as molecular analyses to differentiate between bacterial species.

The results of the laboratory experiments should be analyzed to characterize the microbial communities that develop under each of the conditions of P availability, with tests for significant differences between the levels.

The research could be conducted over a period of approximately 5 months, at an estimated cost of $50,000.

Modeling Tool Refinement and Application

There are several suggested research activities related to the biofilm modeling tool. The model applied during this study simulated one profile of heterotrophic organism that is primarily responsible for the degradation of readily biodegradable organic material. The model can be expanded to account for two or more heterotrophic organism profiles to evaluate the competition between organisms that results in the observed differences in biofilm appearance.

Research is also suggested to define relevant processes and state variables, kinetic expressions, conversion factors, stoichiometric relationships, and diffusivity coefficients specific to the conditions under which biofilms in streams associated with airport deicing discharges grow. The following steps should be pursued to accomplish this:

- Conceptualize processes and state variables (e.g., process 1 consumes readily biodegradable organic matter, oxygen, and macronutrients and produces heterotrophic organisms).
- Develop kinetic expressions by operating batch-scale reactors to verify the rate of substrate conversion. This might be performed in conjunction with the suggested laboratory research described above.
- Develop stoichiometric relationships through an energetic analysis.
- Calculate diffusivity coefficients for relevant materials in clean water.

It is also suggested that future studies include parameters that will support biofilm model calibration and validation. These would include chemical analyses (e.g., COD, total N, ammonia as nitrogen, nitrite as nitrogen, nitrate as nitrogen, soluble reactive phosphorus, and total P) of bioreactor influent and effluent streams; and analytical methods, such as florescent in situ hybridization and quantitative polymerase chain reaction to evaluate biofilm mass and identify the genera and relative abundance of bacteria inside a biofilm.

The research could be conducted over a period of 1 year, at an estimated cost of $100,000.

CHAPTER 1

Introduction

This ACRP project was initiated to research factors that affect the growth and proliferation of biofilms in streams receiving stormwater runoff from airports that conduct deicing operations. This research represents the first step towards understanding biofilms at airports. This report presents the findings of the research and presents suggestions for future investigations on this topic.

1.1 Nature of the Problem

Biofilms are complex ecosystems bound by a matrix of extracellular polymeric substances (EPSs) that are produced by algae and other microorganisms[1] (Callow 2000). Biofilms dominated by heterotrophic organisms have been observed growing on the bed material in surface waters downstream of stormwater outfalls from some airports that conduct aircraft and airfield deicing operations. The biofilms can reach an abundance level where regulatory agencies view them as a "nuisance" condition in the context of narrative water quality criteria. When this happens, it may trigger regulatory actions requiring the airport to eliminate its contribution to the condition.

Airports attempting to address their contribution to the growth of problematic biofilms in receiving streams have been hampered by an absence of scientific information. The mechanisms that lead to prolific biofilm growth have not been adequately defined and the environmental conditions that promote or deter the development of biofilms near airport outfalls are poorly understood. Therefore, water quality, hydrologic, and physical conditions that contribute to biofilm growth in the receiving stream cannot be predicted with confidence. As a result, engineering design of mitigation strategies such as source reduction, enhanced containment of deicing runoff, and stormwater treatment have relied principally on professional judgment. To date, the few programs that have successfully eliminated biofilm conditions that presented regulatory compliance problems have been costly and involved an iterative trial-and-error approach.

[1]For the purpose of this report, microorganisms have been operationally defined, based on energy utilization, into three basic categories: heterotrophs or bacteria (organisms that derive energy from organic sources), phototrophs or algae (organisms that derive energy from light), and autotrophs (organisms that derive energy from inorganic sources). Such groups have not been taxonomically defined, and in many cases, likely contain a complex group of organisms (e.g., heterotrophs or bacterial assemblages may include both bacterial and fungal taxonomic members; phototrophic or algal assemblages may contain both algal and photosynthetic bacterial taxonomic members).

1.2 Nomenclature

The research team searched for a term that would capture the idea of vigorous biofilm growth beyond what might be expected under stream conditions in the absence of the influence of significant anthropogenic organic loading. The team considered various options, and settled on "prolific" as an appropriate word to express this concept without implying quantitative or regulatory criteria. Various synonyms could be substituted while retaining the basic concept.

1.3 Research Objectives

The objectives of ACRP Project 02-32 are threefold:

1. Provide airports and regulatory agencies with a reference document that summarizes what is known about the occurrence of prolific biofilms in receiving waters near airports.
2. Investigate the relationships between key environmental conditions that affect prolific biofilm growth.
3. Suggest next steps to address knowledge gaps.

1.4 Structure of This Report

The contents of this report are presented as follows:

Chapter 2	A summary of literature reviewed in terms of key information and knowledge gaps
Chapter 3	Descriptions of four testable hypotheses developed to address key information and knowledge gaps, data requirements, and the recommended testing approaches
Chapter 4	Materials and methods used to gather data to support testing the hypotheses
Chapter 5	Results of the data collection and hypothesis testing
Chapter 6	Suggestions for further research on this topic
References	References cited in this report
Appendix A	Acronyms and abbreviations used in this report
Appendix B	Biofilm model information

CHAPTER 2

Summary of Literature Findings

This chapter presents a summary of literature addressing prolific biofilm development in flowing water bodies that receive surface water runoff from airports conducting deicing operations. Research on the mechanics of key processes related to this topic is limited, as is the existing information that can be used for identifying and evaluating potential controlling factors. This chapter includes references to studies that describe basic biofilm mechanisms that may be related to the prolific growth of biofilm and that form the basis of hypotheses presented by this research team. This chapter is based on information obtained from a comprehensive literature review. This review has been compiled into an annotated bibliography, which is available on the ACRP Project 02-32 webpage on the www.trb.org website.

2.1 Overview

A review of available literature was performed as it pertains to biofilm development in flowing water bodies that receive surface water runoff from airports that conduct deicing operations. Key concepts in the reviewed literature were identified, evaluated, and synthesized according to practical importance and the extent to which they lend themselves to further investigation. The research team reviewed approximately 160 scientific papers and relevant publications. The process of reviewing and applying existing reports and data consisted of the following primary components.

- Available relevant published and unpublished technical papers, reports, data, and other information related to the proliferation of biofilms in flowing water bodies that receive surface water runoff from airports that conduct deicing operations were compiled, organized, and evaluated.
- A systematic evaluation of available information was performed to identify gaps in technical understanding and scientific information that may be applied to support the environmental impact and assessment needs of airports.
- Biofilm mechanisms were identified and prioritized according to their potential for being essential to the evaluation of prolific biofilm growth. Documented studies related to biofilm mechanics were compiled, organized, and evaluated for their potential to fill key information gaps in the investigation of prolific biofilm growth and to lend themselves to further investigation.
- Studies pertaining to airport deicing activities and prolific biofilm growth, and relevant research (according to the research team) conducted on basic mechanisms of biofilm growth in flowing water bodies were synthesized for the development of four hypotheses. The hypotheses are supported by a description of suggested mechanisms for prolific biofilm growth, are quantified, and have defined practical implications associated with their resolution.

Limited information was found regarding the occurrence of prolific biofilms in flowing water bodies that receive surface water runoff containing aircraft and airfield deicers. For the purpose of this project, "prolific" reflects a level of biofilm growth and accumulation that is substantially greater in abundance than would be expected in the absence of such anthropogenic organic inputs.

At least six airports are known to be currently dealing with biofilm growth as an environmental regulatory concern: the Cincinnati/Northern Kentucky International Airport (CVG) in Kentucky; Pittsburgh International Airport (PIT) in Pennsylvania; T. F. Green Airport (PVD) in Rhode Island; Gerald R. Ford International Airport (GRR) in Michigan; Bishop International Airport (FNT) in Michigan; and Des Moines International Airport (DSM) in Iowa. In addition, USGS is conducting research on biofilm growth resulting from airport deicers at General Mitchell International Airport (MKE) in Wisconsin. Review of available material from these airports provided the following information:

- Aircraft and pavement deicers in surface water runoff contribute to the seasonal occurrence of prolific biofilms in flowing water bodies downstream of airport surface water discharges.
- Discharge concentrations of 5-day biochemical oxygen demand (BOD_5) do not by themselves serve as reliable predictors of prolific biofilm growth. This leads to the conclusion that variables such as other water quality constituents, environmental conditions, and stream physical characteristics may influence the response of biofilms in receiving waters.
- Reduction in prolific biofilm growth has been observed with reduced deicer concentrations in some airport discharges.
- Only CVG has been able to successfully reduce biofilm proliferation. That facility discharges a BOD_5 concentration to the receiving stream that is consistently less than 50 mg/L. Since implementing controls that maintain concentrations below that level, biofilm growth has remained acceptable according to the requirements of the airport's National Pollutant Discharge Elimination System (NPDES) permit (Chapman 2010).

To date, none of the airports besides CVG have identified a specific water quality threshold below which biofilms do not develop to the point of being a regulatory compliance problem.

A fundamental understanding of the developmental characteristics, structure, and function of biofilms is needed for identifying the controllable factors that might minimize biofilm growth in flowing water bodies. With that information, engineers and scientists would be in a better position to design technological approaches to minimize biofilm proliferation.

2.2 Key Gaps in Information and Knowledge

The information in the surveyed literature, combined with the experience of the research team members in conducting various aspects of biofilm research, was evaluated in light of the research objectives. That evaluation led to the identification of gaps in knowledge that are key to understanding factors controlling biofilm proliferation in response to stormwater deicing discharges. These gaps are discussed in the following subsections.

2.2.1 Influence of Light on Biofilm Growth

Photosynthetic algae are the primary means of carbon fixation in aquatic ecosystems, providing a critical food source for primary consumers (such as invertebrates and some fish) and the basis of the food chain for higher-level consumers (including many fish and higher animals). Biofilms are complex ecosystems bound by a matrix of EPSs that are produced by algae and other microorganisms (Callow 2000). Algal biofilms will develop on any surface provided moisture,

ultraviolet (UV) light, carbon dioxide, and macronutrients are available. The primary factors likely to limit growth in highly enriched algal systems are inorganic carbon and light (Brune and Novak 1981). A mechanistic understanding of algal biofilms describes the primary chemical and biochemical conversions (Wolf, Picioreanu, and van Loosdrecht 2007; Vazquez-Burney et al. 2009). Other notable works presenting such mechanistic descriptions of algal biofilms have been presented by Liehr, Suidan, and Eheart (1988, 1989, 1990) and Flora et al. (1994, 1995).

Romani and Sabater (1999) observed the accumulation of heterotrophic (bacterial) and phototrophic (algal) populations on growth coupons (that is, artificial surfaces) placed in a shallow, clear, oligotrophic (low organic carbon) stream in northern Spain. The effect of sunlight on those populations was determined by placing coupons in either open areas exposed to sunlight, or within polyvinyl chloride pipes open to streamflow but that prevented light penetration. The results indicated approximately 1.5 times greater bacterial accumulation on sunlight-exposed coupons than on those incubated in the dark, and approximately 6 times greater algal accumulation on sunlight-exposed coupons. This work suggests that in low organic carbon streams, sunlight exposure leads to greater algal biomass accumulation, which then provides more food for heterotrophic organisms.

Lear, Turner, and Lewis (2009) reported on the activity of stream-derived heterotrophic and phototrophic populations in laboratory microcosms under conditions of darkness, natural sunlight, and enhanced (1.5 times) sunlight. Bacterial activity was assessed by acetate degradation, which did not vary significantly between different light conditions. Biofilm structure was observed to vary between treatments, but with the most microbial diversity (bacterial and algal) appearing in the microcosms incubated in the dark, with bacteria dominating the biofilm. In ambient (natural sunlight) incubated microcosms, algae dominated and lower bacterial counts were observed.

The effects of sunlight, when combined with inputs of organic carbon, are not fully understood. The two studies cited offer conflicting conclusions on the effect of sunlight on bacterial accumulation in natural stream waters. The literature also suggests that incidental sunlight is an important factor in biomass accumulation. Knowledge of these interactions would lead to an understanding of the influence of stream shading on biofilm growth, and possibly management alternatives affecting stream light intensity.

2.2.2 Potential for Phosphorus Limitation of Biofilm Growth

Investigations of the importance of bacteria in aquatic systems have focused on free-living bacteria rather than biofilms, in part because of methodological constraints (Mohamed, Lawrence, and Robarts 1998). In many environments, biofilms have proven dominant in terms of bacterial numbers and production (Schallenberg and Kalff 1993, Tibbles et al. 1992). It is recognized that bacterial abundance can be limited by several factors. Bacterial populations in aquatic systems generally are considered to be limited by the bioavailability of organic carbon (Kirchman 1994, Pomeroy 1974) or temperature (Felip, Pace, and Cole 1996; White et al. 1991). In several water bodies, however planktonic bacterial production was limited by inorganic nutrients, primarily phosphorus (P) (Coveney and Wetzel 1992; Morris and Lewis 1992; Toolan, Wehr, and Findlay 1991; Wang, Miller, and Priscu 1992).

Phosphorus is widely regarded as both a necessary macronutrient and one that frequently limits growth in many microbial systems. Additions of phosphorus to otherwise P-limited systems frequently have been observed to lead to enhanced microbial activity, often to the point of stream eutrophication (Correll 1999). Phosphorus measurements generally are expressed as either total phosphorus or orthophosphate (PO_4), the latter of which is the bioavailable state. Correll (1999) suggests that a dynamic equilibrium exists between total P and orthophosphate,

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and that both measurements are important in considering the potential for microbial (bacterial, algal, and fungal) growth. In studying freshwater algae, Grover (1989) observed that orthophosphate levels as low as 0.015 mg/L were sufficient to maximize inorganic carbon assimilation (photosynthetic growth). It is therefore important that strategies to control stream biofilm growth be developed with an understanding of the influence of low levels of phosphorus on bacterial and algal biomass growth rates, as well as organic carbon consumption rates.

A question that arises is "under what conditions does phosphorus become rate-limiting in natural systems with biofilm exposed to low macronutrient concentrations?" Nordeidet, Rusten, and Ødegaard (1994) conducted tests that demonstrated a nitrifying biofilm was phosphorus limited when the PO_4 concentration was less than approximately 0.15 mg P/L (as PO_4-P). Hultman, Jonsson, and Plaza (1994), Jonsson, Plaza, and Hultman (1997), and Jonsson (1998) investigated simultaneous phosphorus precipitation and biological denitrification with heterotrophic microorganisms in controlled systems to examine the impact of phosphorus availability on denitrification. The researchers estimated that 0.1 mg P/L (as PO_4-P) was adequate for biological denitrification while denitrification was adversely impacted when the bulk-phase PO_4-P concentration was less than 0.03 mg/L. Sagberg, Ryrfors, and Berg (2006) reported that P availability impacted nitrification and denitrification in a controlled system. deBarbadillo et al. (2006) investigated PO_4-P requirements for the simultaneous removal of total phosphorus and total nitrogen to very low concentrations in a controlled system and found that oxidized nitrogen (NO_X-N) concentration remaining in the effluent stream increased when the PO_4-P: NO_X-N concentration ratio was less than approximately 0.01. Similarly, Gray (2006) and Gray et al. (2008) observed that the effluent NO_X-N increased when the influent PO_4-P concentration dropped below 0.25 mg/L, corresponding to a condition where the PO_4-P:NO_X-N concentration ratio was less than approximately 0.01.

Husband and Becker (2007) determined that denitrification occurred under average operating conditions without the addition of supplemental phosphorus and PO_4-P:NO_X-N concentration in the range 0.01 to 0.02. Scherrenberg et al. (2008) estimated that adequate denitrification could be maintained when PO_4-P:NO_X-N was 0.005. Scherrenberg et al. (2009) evaluated phosphorus rate limitation and demonstrated that phosphorus rate-limiting conditions prevailed when PO_4-P:NO_X-N was 0.006, resulting in the accumulation of nitrite.

Andersson et al. (1998) observed that NO_X-N removal from a controlled system with PO_4-P = 0.1 mg P/L was about 70 percent of that removed when PO_4-P = 1.0 mg P/L. Peric et al. (2009) evaluated the effect of temperature and transient operating conditions on phosphorus rate-limited operations in a controlled system. At lower wastewater temperatures (13 degrees Celsius [°C]) the average effluent PO_4-P and NO_X-N were less than 0.01 mg P/L and greater than 4.4 mg N/L, respectively. As a result, the average PO_4-P:NO_X-N was 0.002, which is lower than the aforementioned 0.01 threshold for phosphorus rate-limited operation. Boltz et al. (2012) observed that NO_X-N concentrations began to increase when PO_4-P:NO_X-N was less than approximately 0.01.

These studies illustrate both the potential importance of phosphorus as a growth-limiting nutrient and the interplay between phosphorus and other essential nutrients, particularly nitrogen. Phosphorus concentrations in natural systems have been shown to be limiting for bacteria in the range of 0.005-0.025 mg/L (Correll 1999).

2.2.3 Influence of Stream Physical Characteristics on Biofilm Growth

Biofilms are ubiquitous in nature, including flowing water bodies, but the response of biofilm growth in streams affected by aircraft and airfield pavement deicing is poorly understood. This is, in part, because of the complexity and variability of stream geomorphology, hydrodynamics, and ecological features. The ubiquity of biofilms in natural, industrial, and environmental systems

has led to an extensive body of experimental and observational literature examining biofilm growth characteristics and (in some instances) biofilm control mechanisms. Although little work has specifically evaluated the role of deicer fluids on biofilm growth, the experimental systems, processes, and outcomes of previous studies conducted on biofilms in flowing water bodies can provide insight into experimental designs to identify physical control options.

Biofilms are generally categorized by two predominant components: microorganisms and extracellular polymeric substances. The key biofilm processes are recognized as the initial colonization of bacterial cells, their exponential growth, detachment of bacterial cells from the biofilm matrix, and propagation of the detached biofilm fragments to establish biofilms in other locations. The literature suggests that physical characteristics and environmental conditions such as water depth, volumetric flow rate, and geomorphology are reasonably expected to influence key biofilm processes. However, little information exists specifically describing how these variables influence biofilm growth in surface waters exposed to runoff from airport deicing operations. Anecdotal observations report decreased biofilm abundance in areas of deep, slow-moving water.

2.2.4 Determining the Nutrient Limiting Biofilm Growth

In addition to phosphorus, nitrogen may also limit the rate of biochemical transformation processes. Morris and Lewis (1992) observed that the addition of nitrogen, in addition to phosphorus, increased biofilm growth in a natural aquatic system beyond the addition of phosphorus alone. Vrede et al. (1999) reported periods of bacterioplankton being co-limited by phosphorus and nitrogen availability. Similarly, Rier and Stevenson (2006) observed nutrient limitation of biological growth and peak algal accrual was observed in both low phosphorus and low nitrogen conditions. Nitrogen limitations may exist as any one of the common aquatic nitrogenous compounds including ammonium and nitrate. However, nitrogen limitations are commonly expressed as nitrogen. Redfield (1958) developed an empirical ratio that called for a carbon-to-nitrogen-to-phosphorus ratio (C:N:P) of 106:16:1. Kelly, Bothwell, and Schindler (2003) observed that stream biofilms were limited by nitrogen when the C:N ratio was less than approximately 7:1. Given the importance of nitrogen availability to bacterial growth in natural aquatic systems, the identification of phosphorus and/or nitrogen as the rate-limiting soluble material is of importance to evaluating the potential for biofilm proliferation in streams subject to receiving stormwater laden with deicer runoff from airfields.

CHAPTER 3

Testable Hypotheses

The findings of the review of available information led to the definition of four testable hypotheses that were proposed to and approved by the ACRP Project 02-32 panel. In this chapter, each hypothesis is described as a hypothesis statement, a discussion of the knowledge gap to be addressed through the testing, the suggested mechanism underlying biofilm growth response in the context of the hypothesis, the practical implications of the hypothesis, the approach to testing the hypothesis, and the data required to accomplish testing. Subsequent chapters of this report present the materials and methods used to collect data (Chapter 4) and the results and conclusions of the testing (Chapter 5).

3.1 Hypothesis 1—Effect of Light on Biofilm Growth

3.1.1 Hypothesis Statement

The amount of biofilm growth that occurs under given conditions of readily biodegradable dissolved organic matter (i.e., soluble BOD_5) is directly proportional to the availability of sunlight.

3.1.2 Knowledge Gap Addressed

Reports from airports with prolific biofilm conditions anecdotally report different levels of biofilm accumulation in otherwise apparently similar stream segments that experience different levels of light intensity or shading. Algae and bacteria are always components of stream biofilm communities. Photosynthetic algae are generally positively influenced by increasing light, and many bacteria can be adversely affected by light, which suggests that two opposing factors might be involved. This suggests that light may be an important controlling factor on stream biofilm growth. The limited information available in the literature is inconclusive with respect to this phenomenon.

3.1.3 Suggested Mechanism

Biofilm mats contain algae, fungi, water, bacteria, extracellular biopolymers, and particulate matter, including inert biomass and organic particulate matter. Phototrophic organisms, such as algae, use sunlight for energy (by photosynthesis). Biofilm mats typically have a biomass depth that allows development of multiple zones of oxygen reduction, in which substantial anaerobic bacteria may exist. The presence of photosynthesis and anaerobic transformation processes promotes a condition that may be described in one of two ways:

- Bacterial films will require more readily biodegradable organic matter than algal biofilms do to produce equivalent biological mass.

- Bacterial films will produce less biomass than algal mats when biochemically transforming equivalent readily biodegradable organic matter.

In other words, ambient UV light may promote the development of biofilm mats that have more biomass and extent than would be the case under poor UV light conditions, under which the influence of algae is limited by light availability.

3.1.4 Practical Implications

Streambeds that are protected from sunlight (by deep or turbid waters, or shading) may tolerate surface water runoff with a higher BOD_5 concentration without exhibiting prolific biofilm growth.

3.1.5 Approach to Testing the Hypothesis

The approach to testing involves field, laboratory, and modeling components that address the question: Is the amount of biofilm present under a given set of environmental and nutritional conditions proportional to the availability of sunlight?

3.1.6 Data Requirements

Data required to address this hypothesis consist of measurements of biofilm growth under a variety of light conditions. Because organic carbon and UV light are likely rate-limiting factors in the growth of biofilms, availability of BOD_5 and a gross indicator of sunlight availability are necessary. A measure of the amount of biofilm is required to ascertain relative abundance. Preferably, quantitative biofilm data will be available to support testing for a statistically significant positive correlation between growth and sunlight exposure. However, qualitative indicators may provide insight in the absence of quantitative measurements.

Field

The field component of the testing approach requires identifying stream reaches that have the following:

- Similar water quality characteristics
- Similar physical characteristics
- A stormwater runoff component at airports with deicing operations
- Evidence of prolific biofilm growth in association with the deicing season
- A segment exposed to direct sunlight that displays biofilm growth
- One or more segments that are protected from exposure to direct sunlight by natural (e.g., tree canopy) or manmade (e.g., culvert) obstructions and display biofilm growth

In practical terms, consecutive reaches in the same stream are most likely to satisfy these criteria.

Required field data include water quality, light intensity, and biological parameters throughout the deicing season and afterward until the biofilm accumulations have died off.

Laboratory

The required laboratory data consists of measurements of biofilm growth and relative composition by holding water quality, water chemistry, and environmental conditions constant, while varying light intensity within the range (400–700 nanometers) of photosynthetically active radiation (PAR).

Modeling

Modeling analyses will be used to complement and add a depth of understanding to observations made in the field and laboratory by providing a systematic, mathematical reasoning to the observations. The modeled biofilm will consist of the following defined categories: heterotrophic organisms (which consume chemical oxygen demand [COD] and oxygen and are designated as X_H), phototrophic organisms (which consume inorganic carbon and oxygen and are designated as X_P), autotrophic organisms (which consume ammonia and oxygen and are designated as X_N), and inert biomass. The modeling tool will allow the impact that light and dark conditions, and varying concentrations of COD (S_S) has on the active biofilm mass (i.e., $X_H + X_P + X_N$) to be evaluated. The results will then be compared with field and laboratory observations.

3.2 Hypothesis 2—Potential for Phosphorus to Limit Biofilm Growth

3.2.1 Hypothesis Statement

Stream biofilms will exhibit phosphorus limitation when the concentration of PO_4 is in the range 0.005 to 0.025 mg/L or less.

3.2.2 Knowledge Gap Addressed

Phosphorus commonly limits algal and bacterial abundance in aquatic systems, but there is no information available on the potential for phosphorus to limit the growth of biofilms in streams receiving deicer runoff.

3.2.3 Suggested Mechanism

Mohamed et al. (1998) conducted experiments to determine the limiting nutrient in association with high soluble BOD_5 loads from pulp and paper mill effluent in the Fraser River, British Columbia, Canada. In phosphorus-limited systems, it was determined adding phosphorus increased biofilm growth and reduced the amount of extracellular polymeric substances in the biofilm. A high concentration of extracellular polymeric substances is indicative of nutrient-deficient biofilms (Romani and Sabater 2000). The threshold of phosphorus availability is dependent on water conditions. Lock and John (1979) and Blenkinsopp and Lock (1994) found that biofilms were influenced by phosphorus availability under flow pattern and storm flow conditions, respectively.

As a result, a phosphorus-deficient flowing water body may have a different biofilm response to a given BOD_5 concentration than a similar system that has excess phosphorus. Approximate macronutrient requirements of 12 grams (g) nitrogen and 2.3 g phosphorus are needed per 100 g of cell biomass to avoid being the rate-limiting macronutrient (Metcalf and Eddy 2003). Thus, extent of cell colonization on stream surfaces may affect the condition of phosphorus limitation (i.e., as organic carbon is added to a stream system and cell growth occurs, phosphorus may transition from a non-limiting state to a limiting state as demand increases).

3.2.4 Practical Implications

Phosphorus deficient streams may exhibit less prolific biofilm accumulations under a given BOD loading than streams in which biofilm growth is limited by organic carbon or some other factor. Therefore, biofilm suppression may be a side benefit of stormwater P-reduction efforts, or other factors that promote P deficiency.

3.2.5 Approach to Testing the Hypothesis

The approach to testing focuses on laboratory and modeling components. Field investigations are not practical because they require stream segments that are similar in terms of physical and hydrological characteristics, deicer loading and concentrations, and other environmental factors; but differ in terms of being phosphorus limited, or having excessive phosphorus. It is unlikely that such segments can be identified.

The laboratory experimental design examines the response of biofilms to phosphorus limitation in controlled systems designed to simulate related stream conditions and exposed to variable phosphorus and organic carbon (amongst other variables) levels. Existing mathematical models will then be used to confirm and provide insights into the laboratory observations.

The resulting data will support testing for correlation between growth and PO_4 concentration.

3.2.6 Data Requirements

The primary data requirement for testing this hypothesis is measurement of biofilm growth in the presence and absence of phosphorus limiting conditions. Preferably, the biofilm data will be quantitative and support testing for a statistical significance of apparent limitation. However, qualitative indicators may provide insight in the absence of quantitative expressions.

Laboratory

Laboratory data are required to provide an understanding of the bacterial capability to alter their stoichiometric phosphorus requirement, and a basis for evaluating the potential for phosphorus to be a rate-limiting macronutrient against a substantial population shift. The required data consist of measurements of biofilm growth and relative composition in laboratory reactors under conditions that generally replicate field conditions, but with phosphorus present in varying concentrations.

Modeling

Similar to the model applied in testing Hypothesis 1, modeling analyses will be conducted to complement and add a depth of understanding to observations made in the laboratory by providing a systematic, mathematical reasoning to the observations. The modeling tool will allow the impact that varying PO_4 concentrations on the active biofilm mass (i.e., $X_H + X_P + X_N$) to be evaluated. The evaluation will then be compared with field and laboratory observations.

3.3 Hypothesis 3—Impact of Physical Stream Characteristics on Biofilm Growth

3.3.1 Hypothesis Statement

The physical characteristics of receiving streams influence the extent of biofilm accumulation for a given water chemistry condition as follows:

- Category 1: Shallow, turbulent, well-mixed channels promote biofilm growth and require ambient BOD_5 concentrations less than 50 mg/L to avoid prolific biofilm growth.
  - streambed surface area (A_{BED}) to bulk liquid volume (V_B) ratio (A_{BED}:V_B) greater than 100 square meters per cubic meter (m^2/m^3) (1 cm depth)

- Category 2: Relatively straight channels with moderate depth and flow promote moderate biofilm growth and require ambient BOD_5 concentrations be maintained in the range 50 to 100 mg/L or lower to avoid prolific biofilm growth.
  - streambed surface area (A_{BED}) to bulk liquid volume (V_B) ratio (A_{BED}:V_B) in the range 25 to 75 m^2/m^3 (1.3 to 4 cm depth)
- Category 3: Deep, slow-moving channels deter biofilm growth, and can experience ambient BOD_5 concentrations greater than 100 mg/L without prolific biofilm growth.
  - streambed surface area (A_{BED}) to bulk liquid volume (V_B) ratio (A_{BED}:V_B) less than 10 m^2/m^3 (10 cm depth)

3.3.2 Knowledge Gap Addressed

There are anecdotal reports from airports that biofilm growth associated with deicing runoff varies with the physical characteristics within affected stream segments, but there is no clear understanding of the relationship between stream geomorphology and susceptibility to prolific biofilm growth.

3.3.3 Suggested Mechanism

Available literature and understanding of biofilm processes suggest that several stream physical characteristics have the potential to influence biofilm proliferation within a given level of substrate[2] and macronutrient availability. Shallow, fast-moving areas will tend to be well-oxygenated, have a low cross-sectional area to perimeter ratio which exposes attached biofilms to a large proportion of the dissolved nutrients in the water column, and causes higher shear stresses on the biofilm. Increased shear has been shown to influence biofilm structure, resulting in denser and more tenaciously attached biofilms (Buckingham-Meyer, Goeres, and Hamilton, 2007). In contrast, deeper, slow-moving stream segments tend to be less well-oxygenated, have a smaller proportion of the water column in close contact with the stream bottom, and are associated with less dense biofilms with a thicker mass transfer boundary layer above the biofilm, resulting in slower diffusive transport of nutrients into the biofilm (Battin et al. 2003).

3.3.4 Practical Implications

Knowledge of how physical stream channel factors affect the abundance or absence of prolific biofilm accumulations will give airport operators insight as to why prolific biofilms occur in some stream segments and not others, as well as in designing stormwater conveyance channels and other infrastructure with features less likely to promote prolific biofilm growth.

3.3.5 Approach to Testing the Hypothesis

Testing of this hypothesis relies primarily on collection and analysis of field data, supplemented by use of modeling tools to examine relative influence of factors through sensitivity analyses. Preferably, the biofilm data will be quantitative and support testing for a statistical significance of stream channel factors. However, qualitative indicators may provide insight in the absence of quantitative expressions.

[2]For the purposes of this research project, the following biochemical definition of substrate is used: the substance acted upon by an enzyme.

3.3.6 Data Requirements

Required data consists of biofilm growth measurements in adjacent stream segments with similar deicer and other pollutant loading characteristics but with different physical configurations.

3.4 Hypothesis 4—Identifying Nutrients Potentially Limiting to Biofilm Growth

3.4.1 Hypothesis Statement

Ratios of bulk-phase substrate and macronutrient concentrations can be used to identify the rate-limiting substrate for biofilm growth in a stream.

3.4.2 Knowledge Gap Addressed

There is currently no general indicator of a stream's biochemical susceptibility to prolific biofilm growth as a function of limiting macronutrient (i.e., phosphorus and nitrogen) availability. This gap may be filled by evaluating the growth of biofilms under different conditions of relative abundance of substrate and macronutrient availability to determine which of these factors is likely to limit biofilm growth.

3.4.3 Suggested Mechanism

Biofilm growth in flowing stream environments is limited by a single terminal substrate (the electron donor or electron acceptor) or a macronutrient. The idea of a limiting nutrient or substrate is well established in the biodegradation literature. In systems with low levels of organic carbon, the organic carbon electron donor is often the limiting factor. Where organic carbon is more abundant, another factor, such as one of the macronutrients (N or P), may be limiting. Streams receiving deicer fluid runoff are periodically dosed with higher levels of organic carbon and therefore may alternate between conditions of organic carbon limitation versus limitation by some other nutrient.

3.4.4 Practical Implications

Identifying the rate-limiting substrate or macronutrient under different relative conditions will provide a basis for the airport operator to judge a stream's likely predisposition to exhibit more or less prolific biofilm growth under a given set of physical conditions and BOD loading. This information will provide an understanding of why biofilms are more prolific in one stream than in another, and insight into nutrient control strategies for affecting the potential for biofilm growth.

3.4.5 Approach to Testing the Hypothesis

The effect of macronutrient availability will be evaluated through laboratory growth experiments, observing biofilm growth under various conditions of relative availability of phosphorus and nitrogen. Field collection of phosphorus samples in conjunction with monitoring to support Hypothesis 2 will provide an indication of nutrient limiting conditions in streams receiving deicing runoff and experiencing various levels of biofilm growth.

Similar to the model used for testing Hypothesis 2, modeling analyses will be used to complement and add a depth of understanding to observations made in the field and laboratory. The

modeling tool will allow the impact that varying ammonium concentrations has on the active biofilm mass (i.e., $X_H + X_P + X_N$) to be evaluated. The results will then be compared with field and laboratory observations.

3.4.6 Data Requirements

Data requirements for testing this hypothesis consist of laboratory experiments and application of modeling tools. Laboratory experiments are required to assess the interactions of organic carbon and essential macronutrients. Changes in measured biofilm thickness allow assessment of the effects of varying each of the macronutrient parameters individually.

CHAPTER 4

Materials and Methods

This chapter describes the field, laboratory, and computer modeling methods used to develop and evaluate data for testing the hypotheses.

4.1 Field Data Collection

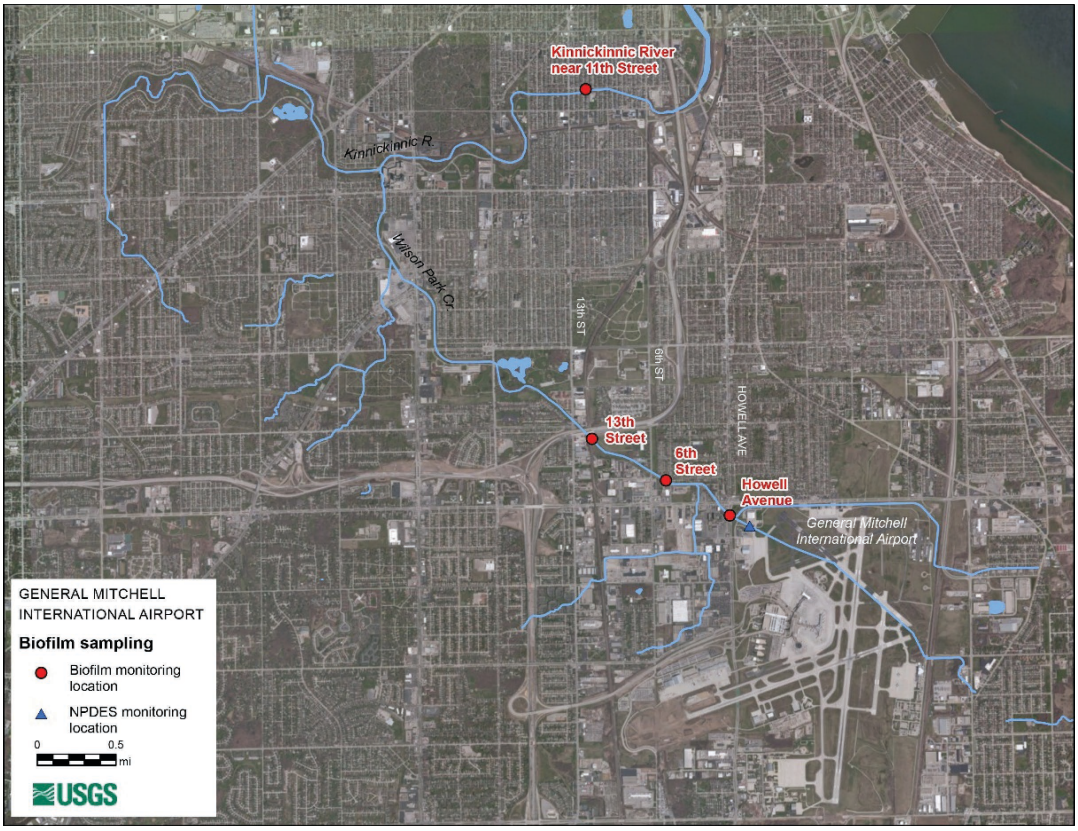
The methods used to collect field information to support evaluation of Hypotheses 1 and 3 are described in this chapter. Data collection efforts were conducted on streams receiving stormwater containing aircraft deicing/anti-icing fluids from General Mitchell International Airport (MKE) in Milwaukee, Wisconsin, and at Gerald Ford International Airport (GRR) in Grand Rapids, Michigan.

4.1.1 Site Selection

Near each airport, sites were selected, representing system-available contrasts in light availability and depth-to-velocity ratios. At both airports, all sites were located downstream of stormwater outfalls receiving deicing runoff, with contrasting sites located as closely together as possible to minimize confounding effects resulting from water quality differences. At MKE, most monitoring locations were located on Wilson Park Creek, which is a tributary to the Kinnickinnic River; an additional downstream site was included on the Kinnickinnic River. The monitoring locations are shown in Figures 4-1A and 4-1B. MKE sites were located on Wilson Park Creek near and beneath Howell Avenue (Howell-light and Howell-dark, respectively), near 6th Street (6th), and near 13th Street (13th), as well as on the Kinnickinnic River near 11th Street (KK). At GRR, all monitoring locations were on an unnamed tributary to the Thornapple River: at 36th Street (36th), Thornapple River Drive (TRD), and Tricklewood Drive (TWD). A summary of the approximate distances from the airport and drainage areas of each of the monitoring locations is contained in Table 4-1.

4.1.2 Biofilm Sampling

Biofilm surveys were conducted at each monitoring location three times (February, March, and May) during the deicing season using methodology adapted from U.S. Environmental Protection Agency (U.S. EPA) periphyton rapid bioassessment procedures. The methodology is a modification of an updated version of the field-based rapid periphyton survey (RPS) cited in U.S. EPA's *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish* (Barbour et al. 1999; Stevenson and Bahls 1999). This updated RPS was developed by Stevenson and Rollins (2007).



(a)

(b)

Figure 4-1. (a) Monitoring locations at MKE in Milwaukee, Wisconsin. (b) Monitoring locations at GRR in Grand Rapids, Michigan. (Biofilm monitoring locations are shown as red circles.)

Table 4-1. Summary of biofilm monitoring location characteristics, as related to Hypotheses 1 and 3.

Monitoring location code	Monitoring location type	Drainage area (km^2)	Distance downstream of airport (km)	Hypothesis 1: Light Availability	Hypothesis 3: Depth-to-Velocity Ratios
Howell-light	Secondary	10.5	0.00	Medium	Low
Howell-dark	Primary	10.5	0.04	Low	Low
6th	Primary	15.5	0.84	High	Low
13th	Primary	16.8	1.72	High	High
KK	Secondary	51.9	8.94	High	Low
36th	Primary	3.5	0.48	High	Variable
TRD	Primary	5.6	1.30	Medium	Variable
TWD	Primary	5.9	1.99	Low	Variable

Primary monitoring locations (Table 4-1) were locations where biofilm community measurements were collected at 10 sample points on each of five transects. Secondary monitoring locations were locations where biofilm community measurements were collected at 10 sample points on a single transect. Figure 4-2 shows the general elements of a sampling reach at a primary sampling location, in this case TRD.

Monitoring cross sections were selected based on the presence of bed material larger than 2 centimeters (cm) in diameter (in the longest dimension). Areas with such coarse streambed material were used because they provide a stable surface for biofilm accumulation and support

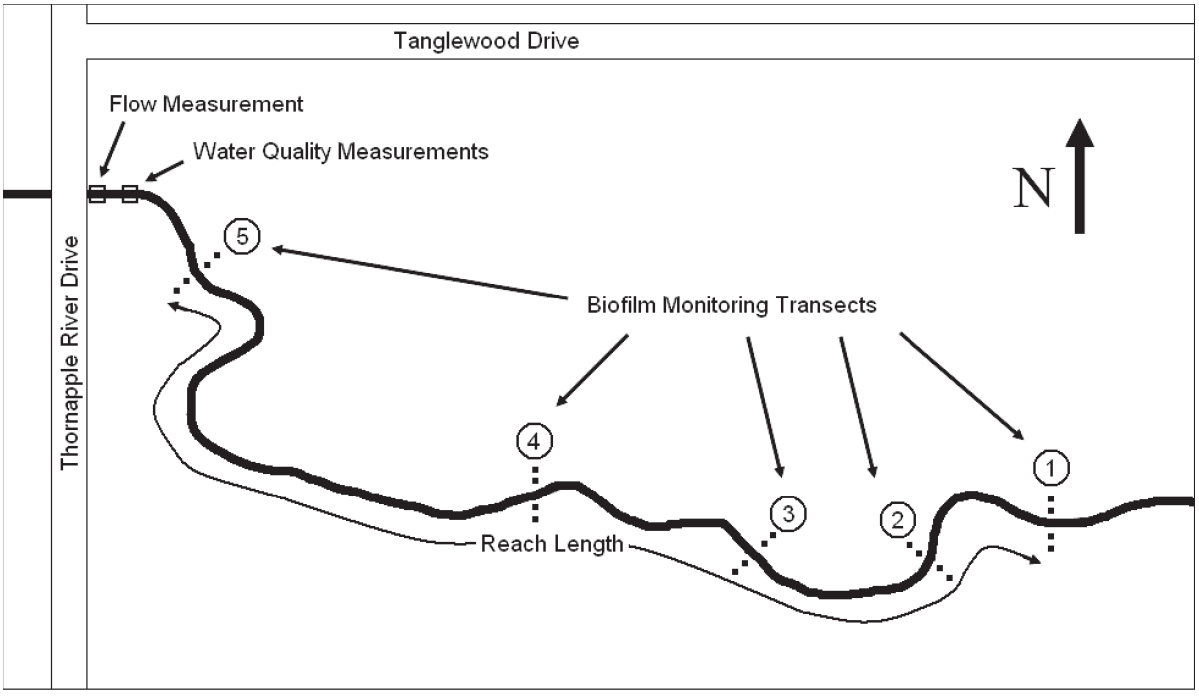


Figure 4-2. Elements of a biofilm monitoring reach.

Table 4-2. Biofilm coverage (density) measurement classes.

Coverage Class	Coverage (percent)
0	0
1	<5
2	5–25
3	25–50
4	50–75
5	75–100

Adapted from Stevenson and Rollins 2007.

establishment of repeatable and reliable long-term monitoring locations (Stevenson and Rollins 2007). Several of the MKE monitoring locations were concrete-lined, and in these settings monitoring transects were established at regularly spaced intervals. Measurements were collected for three general community types—moss, macroalgae, and heterotrophic biofilms[3]. Heterotrophic biofilms, as operationally defined here, describe an assemblage of organisms without visible algal representation. Such biofilms were distinguished from algal-dominated biofilms on the basis of coloration and morphology.

Heterotrophic biofilm coverage (extent) and thickness (magnitude) measurements were recorded at randomly selected transect sampling points. At each of these same locations, heterotrophic biofilm density was measured using a 1 square foot viewing bucket with a 50 point grid. The number of dots overlying heterotrophic bacteria were recorded and converted to a percentage class (Table 4-2).

Biofilm thickness was measured by randomly selecting a bed material particle at each transect point; for particles larger than 2 cm, the biofilm thickness on the particle was measured with a ruler and assigned to a thickness class (Table 4-3).

Measurements and observations for reach-scale and individual transects were recorded on data sheets in the field for subsequent entry into the project database. The final variables used were as follows:

Reach-scale biofilm community metrics

- The biofilm community extent metric is calculated by summing the number of observations where biofilms were present and dividing by the total number of observations.
- The biofilm magnitude metric is calculated by multiplying the coverage class number by the number of biofilm observations in that class and dividing by the total number of observations. These normalization methods provide comparable information and support the evaluation of relative changes in the biofilm community between surveys.
- The biofilm community density metric is simply the percentage of total grid points occupied by heterotrophic biofilm.

[3] In the updated RPS protocol, this category is referred to as "microalgal biofilm." This category was retooled for the current project to measure heterotrophic biofilms.

Table 4-3. Biofilm thickness (magnitude) measurement classes.

Thickness Class	Thickness (mm)	Thickness Characteristics
0	0	Rough surface
1	<0.5	Slimy; visible evidence of biofilm absent
2	0.5–1	Biofilm visible
3	1–5	Measures within this thickness range
4	5–20	Measures within this thickness range
5	>20	Measures within this thickness range

Adapted from Stevenson and Rollins 2007.

Individual transect metric

- Biofilm thickness is used for Hypothesis 3.

These three reach-scale biofilm community metrics were calculated at each monitoring location using all transects (i.e., reach scale) for the evaluation of Hypothesis 1.

4.1.3 Water Quality Sampling and Analysis

Water quality samples were collected at monitoring locations on an approximately monthly[4] basis. Samples were collected from the most upstream and downstream primary monitoring locations in an effort to characterize differences in water quality across the geographic range of sites at each airport; additional samples were collected from KK and TRD to yield better resolution of changes in COD concentrations within each system. Samples were collected either as grab or equal-width-increment (EWI) samples (USGS 2006) and sent to the Wisconsin State Laboratory of Hygiene (WSLH) for analysis of a suite of parameters relevant to biofilm growth. In situ measurements of water temperature, specific conductance, pH, and dissolved oxygen were collected during each biofilm sampling event using a YSI® (Yellow Springs, Ohio) multiparameter sonde calibrated and operated in accordance with standard USGS procedures (Wilde, variously dated).

A summary of laboratory analytical parameters and methods organized by monitoring location is shown in Table 4-4.

Samples were collected in two sequentially collected bottles. Samples were collected either in clean plastic quart (946-milliliter [mL]) bottles provided by WSLH or in clean plastic 1-liter bottles using a US DH-81 setup (with or without nozzle, as allowed by stream depth) (Wilde 2004). In general, sampling was conducted as a single vertical grab sample at sites known to be well-mixed, and as an equal-width-increment (or, where too shallow, a multiple vertical grab sample) at sites that were poorly mixed or had unknown mixing (USGS 2006). The water from one bottle was analyzed for total suspended solids (TSS) and the water from the other bottle was split as follows for multiple analyses: 250 mL were acidified (to pH <2) with sulfuric acid then analyzed for unfiltered COD and total phosphorus; 250 mL were filtered (0.45-micrometer

[4] At both airports, conditions did not permit for data collection on an exact monthly basis, however sampling intervals were spaced throughout the monitoring period on an approximately monthly basis, as conditions allowed.

Table 4-4. Laboratory analytical parameters for monthly[4] water quality samples.

			Location							
Parameter	Analytical Method	Typical Level of Detection (mg/L)	Howell-light	Howell-dark	6th	13th	KK	36th	TRD	TWD
COD (filtered)	ASTM D1252-95(B)	8.5		X		X		X	X	X
COD (unfiltered)	ASTM D1252-95(B)	8.5		X		X	X	X	X	X
Total Phosphorus (unfiltered)	U.S. EPA 365.1	0.005		X		X		X		X
Orthophosphate (filtered)	SM 4500PE	0.002		X		X		X		X
Nitrate+Nitrite (filtered)	U.S. EPA 353.2	0.019		X		X		X		X
Kjeldahl Nitrogen (filtered)	U.S. EPA 351.2	0.14		X		X		X		X
Ammonia (filtered)	U.S. EPA 350.1	0.015		X		X		X		X
TSS (unfiltered)	SM 2540D-Modified	2		X		X		X		X

[μm] pore size) and acidified (to pH <2) with sulfuric acid, then analyzed for nitrate and nitrite, Kjeldahl nitrogen, ammonia, and COD; 60 mL were filtered (0.45-μm pore size) then analyzed for PO_4. All samples were stored on wet ice or in a refrigerator and were submitted to WSLH for analysis within 36 hours of collection.

Multiple precautions were taken to ensure accurate water quality results. Clean sample handling techniques were used to minimize the potential for sample contamination and cross contamination and to ensure personal protection of sampling staff. Quality control samples (one replicate and one blank sample) were collected at each airport to verify precision and accuracy of the full suite of water quality results. Certified inorganic blank water (Ricca, ACS Reagent Grade Water, 9150-1) was used for nutrient analyses, and Milli-Q® (EMD Millipore®; Billerica, Massachusetts) water from the Wisconsin Mercury Research Laboratory was used for TSS and COD analyses.

In situ measurements and laboratory analytical results received from WSLH were entered into the project database for use in comparison with biofilm community characteristics.

4.1.4 Surface Light Availability

Light availability at the monitoring locations was assessed using a Solar Pathfinder™ (Linden, Tennessee) to support the Hypothesis 1 evaluation. These data provided the percent of available solar radiation reaching the stream for each month biofilm samples were collected. Measurements were taken at each site once during leaf-off and once during budding conditions to normalize the observations and account for the temporal differences in deciduous tree cover

in the riparian area. Half-hour periods shaded by deciduous trees were assigned a half value in accordance with manufacturer recommendations for leaf-off periods. The result of these measurements indicated the percent of available light reaching the stream surface during the sampling period.

Direct measurements of PAR were also collected, using an Onset® PAR sensor (Bourne, Massachusetts). Multiple measurements were logged over a short, defined time period. For each time period, the median of these logged values was calculated and was used to provide a range of values observed in nature and at the sampling sites.

Measurements for Solar Pathfinder™ data and for PAR measurements were entered into the project database for use in comparison to biofilm community characteristics.

Both types of light assessments performed for this study were measured above the water surface, and as such do not necessarily reflect the light reaching biofilms on the stream bottom. Light penetration through the water column could be inhibited by turbidity and ice cover. Further, temperature effects associated with greater light availability could potentially enhance growth through temperature effects. As such, water quality samples were analyzed for TSS to measure the amount of suspended particulate matter in the stream. Approximately biweekly, photographs were taken at each primary monitoring location to document ice cover conditions. Further, in situ water temperature measurements were collected during each biofilm trip, as noted above.

4.1.5 Channel Geometry and Water Velocity Measurements

The effect of physical stream characteristics on biofilm accumulation (Hypothesis 3) was evaluated by collecting measurements of stream width, depth, and velocity during biofilm surveys. Wetted width was measured at every transect studied. Depth and velocity measurements were collected at paired transects at selected monitoring locations (i.e., 13th, 36th, TRD, and TWD) to facilitate comparisons between contrasting transects. At measured transects, water depth and velocity were recorded at each of the 10 biofilm density measurement points. Stream channel measurements were recorded on the field sheet and entered into the project database for use in comparison to biofilm community metrics.

4.2 Laboratory Studies

Laboratory tests were undertaken at the Center for Biofilm Engineering (CBE) to assess the factors that could influence the growth of biofilms in streams receiving runoff from airports that use deicer fluids. These factors are expressed as hypotheses in Chapter 3 and include the effects of sunlight on stream biofilms, the effect of available phosphorus on stream biofilms, the effects of receiving stream physical characteristics on stream biofilms, and the importance of the relative concentrations of bulk-phase dissolved organic carbon and macronutrients on stream biofilms.

Laboratory tests were divided into two phases that addressed Hypotheses 1, 2, and 4. Phase 1 was directed at Hypothesis 1, while Phase 2 covered Hypotheses 2 and 4. The goals of each laboratory testing phase are outlined in the following paragraphs.

Hypothesis 1 postulates that incident light is an important factor in stream biofilm growth and accumulation because of the conversion of sunlight to biomass by phototrophic organisms. Phase 1 testing therefore assessed the effects of variations in incident light (simulated sunlight) on the growth rate, overall accumulation, and composition of simulated airport stream runoff biofilms.

Hypothesis 2 postulates that available phosphorus is an important factor in stream biofilm growth because of the unique position of phosphorus as a limiting nutrient in many environmental systems. Hypothesis 4 postulates that biofilm proliferation can be predicted through an understanding of the bulk fluid ratio of readily biodegradable dissolved organic carbon and macronutrients, such as nitrogen and phosphorus. Phase 2 experiments covered both Hypotheses 2 and 4 in a consolidated approach, and determined the effects of varying levels of phosphorus and nitrogen on biofilm growth rate, overall accumulation, and species composition of simulated airport stream runoff biofilms.

4.2.1 Reactor System

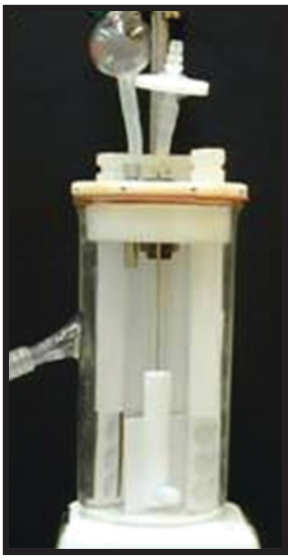


Figure 4-3. CDC biofilm reactor.

The Centers for Disease Control and Prevention (CDC) reactor system (Figure 4-3) was used for these studies (BioSurface Technologies, Bozeman, Montana). The CDC reactor is a continuous stirred tank reactor (CSTR) with a liquid volume of approximately 400 mL and a headspace volume of approximately 600 mL. An overflow spout maintains the liquid volume at a constant level. The reactor features eight removable rods, each of which houses three biofilm growth coupons. These coupons can be removed individually and the attached biofilm subject to imaging via microscopy or to growth-based assays. The operation of the CDC reactor has been standardized into an American Society for Testing and Materials (ASTM) method (ASTM E2562), and portions of this standard method were used for this experimentation. For these tests, the CDC reactor was operated with a constant influent flow rate, simulating the environment of a section of stream exposed to natural flora and levels of organic carbon that mimic stream conditions when deicer fluid runoff is present.

4.2.2 Inoculum

The inoculum used for these experiments was derived from field samples collected from airport runoff streams that experience robust biofilm growth. Biofilm samples were collected from field sites at MKE and GRR and sent to CBE. These samples were homogenized and used to inoculate each reactor system.

4.2.3 Phase 1 Experiments

Experiments to assess the importance of incident sunlight on biofilm were performed by exposing the CDC reactor to periodic simulated sunlight generated by a grow lamp (six 4-foot-long T5 fluorescent Accupro® AFL 28W bulbs).

Previous research in the growth response of natural stream algae to varying levels of incident light indicates that growth saturation occurs at approximately 100 µmol/m^2/s. This corresponds to the light intensity on an overcast day. Full, direct sunlight can vary in intensity from 32,000 to over 100,000 lux (lx) (600–1800 µmol/m^2/s). Therefore, stream algae have been shown to be capable of growing at maximum rate with only a fraction of full sunlight. In order to discern differences in the importance of algal growth to stream biofilms, one reactor was kept in the dark, and the other two were exposed to light at 12 percent and 25 percent full sunlight as determined with an Ocean Optics® spectroradiometer (Figure 4-4). Reactors with partial sunlight were operated on a 12-hour on, 12-hour off cycle to mimic natural photoperiods.

Influent media for the Phase 1 experiments consisted of dechlorinated tap water amended with minimal salts medium and 50 mg/L propylene glycol as the carbon source. The minimal salts medium included supplemental nitrogen, phosphorus, and potassium in the form of potassium nitrate (KNO_3) (25 mg/L) and monopotassium phosphate (KH_2PO_4) (2 mg/L) providing

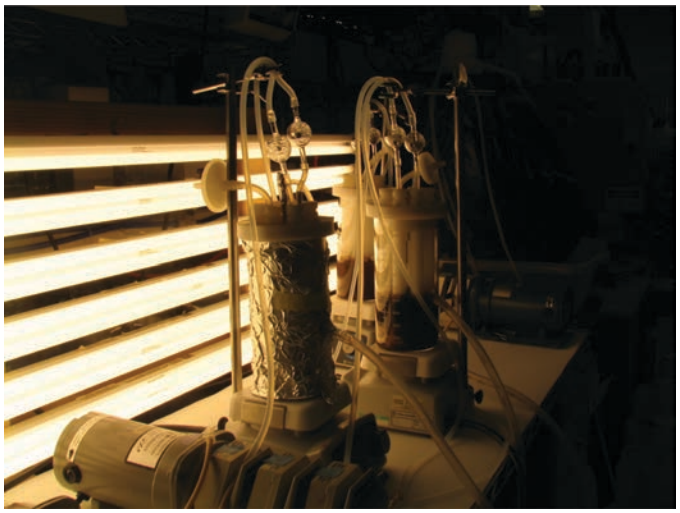


Figure 4-4. Biofilm growth reactors in dark, at 12 percent full sunlight, and at 25 percent full sunlight.

C:N and C:P ratios of approximately 10:1 and 100:1, respectively. The growth media (minimal salts + carbon) recipe was as follows:

- 50 mg/L propylene glycol ($C_3H_8O_2$): $C_3H_8O_2$ contains 47.4 percent C resulting in a dose of 23.7 mg/L as C
- KNO_3 added to achieve a target a C:N ratio of 10:1. KNO_3 contains 13.8 percent N resulting in a dose of 17.3 mg/L as KNO_3
- KH_2PO_4 added to achieve a target C:P ratio of 100:1. KH_2PO_4 contains 23 percent P resulting in a dose of 1.05 mg/L as KH_2PO_4

Reactors were inoculated with the field-derived consortium, which included both heterotrophic and phototrophic organisms. Influent media for the experiments was intended to mimic conditions found in natural streams receiving airport deicer fluid runoff. The influent media was a combination of effluent from a biologically activated carbon (BAC) column system and nutrient amendments described above. BAC water is Bozeman municipal water, which has passed through a spent granular activated carbon filter, which de-chlorinates the water and enhances the microbiological loading.

The reactors were inoculated, filled with growth media, and operated in batch mode (no influent or effluent) for a period of 48 hours to facilitate attachment of inoculated cells. After this attachment period, the reactors were switched to continuous flow mode, with a reactor residence time of 60 minutes. Reactors that experienced half or full sunlight were operated on a 12-hour on/12-hour off cycle to mimic natural photoperiods.

Reactors were operated for a total of 4 weeks. Twice weekly, samples were taken in triplicate from each reactor. Coupons were placed in sterile, buffered dilution water and sonicated/vortexed to disaggregate the attached biofilm. These samples were serially diluted and spread plated on R2A agar and Sabouraud Dextrose Agar with 1.5 percent tellurite to determine viable cell densities of bacterial heterotrophs and fungi, respectively. Chlorophyll *a* concentrations of phototrophs were determined following the method of Ördög et al. (2012).

4.2.4 Phase 2 Experiments

Phase 2 testing work also utilized the CDC reactor system described above. Biofilms were also established as above, using stream inocula and nutrient amended dechlorinated tap water as

Table 4-5. Phase 2 schedule of nutrient additions.

Nitrogen Varied						Phosphorus Varied					
Day	C – as $C_3H_8O_2$ (mg/L)	N – as KNO_3 (mg/L)	P – as KH_2PO_4 (mg/L)	C:N Ratio	C:P Ratio	Day	C – as $C_3H_8O_2$ (mg/L)	N – as KNO_3 (mg/L)	P – as KH_2PO_4 (mg/L)	C:N Ratio	C:P Ratio
0-14	23.7	0.24	0.239	100:1	100:1	0-14	23.7	2.40	0.005	10:1	5,000:1
14-28	23.7	0.48	0.239	50:1	100:1	14-28	23.7	2.40	0.024	10:1	1,000:1
28-42	23.7	1.21	0.239	20:1	100:1	28-42	23.7	2.40	0.048	10:1	500:1
42-56	23.7	2.40	0.239	10:1	100:1	42-56	23.7	2.40	0.239	10:1	100:1

Notes: Initial system operation and sampling procedures were identical to Phase 1.

feed. For Phase 2 experiments, the relative concentrations of organic carbon (as propylene glycol), available nitrogen, and phosphorus (as PO_4) in the feed media were systematically varied to assess the effects of these variations on attached biomass growth. Two reactors were operated. In reactor 1, initial conditions used 23.7 mg/L organic carbon, phosphorus in excess, and nitrogen was the varied compound. The concentration of available nitrogen started at a C:N ratio of 100:1 and was varied in stepwise fashion at bi-weekly intervals to 50:1, 20:1, and 10:1. The experiment ran 8 weeks, with sampling on a weekly basis. In reactor 2, organic carbon was again set at 23.7 mg/L, with nitrogen in excess, and phosphorus was varied, beginning at a C:P ratio of 5000:1, followed by 1000:1, 500:1 and 100:1. Biofilm sampling occurred on a biweekly basis, as above. Both reactors experienced 25 percent full sunlight conditions and were operated on a 12-hour on, 12-hour off cycle to mimic natural photoperiods.

Influent media for the Phase 2 experiments was dechlorinated tap water amended with minimal salts medium and 50 mg/L propylene glycol as the carbon source. The minimal salts medium included supplemental nitrogen, phosphorus, and potassium. Concentrations of additive compounds and the schedule of variation are shown in Table 4-5.

4.3 Biofilm and Kinetic Model

A one-dimensional biofilm model was implemented using AQUASIM (Reichert 1998) to provide a tool to support the hypotheses testing. The process, kinetic, and stoichiometric model is based on that described by Wolf, Picioreanu, and van Loosdrecht (2007), and was expanded to include a mathematical description of the fate of soluble reactive phosphorus in a representative hypothetical receiving water. A complete list of state variables, stoichiometric parameters, kinetic parameters, biofilm parameters, and transformation rate expressions are presented in Appendix B.

Biofilm detachment was modeled using the rate of detachment, k_{det}, ($r_{det,1} = k_{det} \cdot L_F$). The modeling approach maintained a constant biofilm thickness, L_F. The rate of biofilm detachment may change depending on the assumed biofilm biomass distribution since the rate of growth and loss (in this case, by endogenous respiration) is dependent on local substrate availability and environmental conditions. Biofilm fragments were assumed to detach from the biofilm surface; therefore, preferential detachment is considered for the heterogeneous (layered) biofilm biomass distribution (i.e., bacteria growing at the biofilm-liquid interface detach from the biofilm surface and enter the bulk of the liquid). Substrate concentration gradients external to the biofilm were modeled as a mass transfer resistance using the concept of a mass-transfer boundary layer (MTBL) with thickness L_L.

4.3.1 Assumptions

The following three key assumptions were made in the modeling analyses:

- The processes, kinetics, stoichiometric relationships, and one-dimensional biofilm model provide a representative mechanistic description of the physical systems investigated as a part of ACRP Project 02-32.
- The conditions summarized in Table 4-6 are representative of the streams investigated in this study and provide a basis for bounding model simulations to gain insights into observed field and laboratory results.
- The hypothetical stream segment is modeled as a single continuous flow stirred tank reactor (a CFSTR, or a single completely mixed reactor) with no concentration gradients.

Table 4-6. Water characteristics for the simulated stream scenarios.

Parameter	Number of Observations	Minimum	Maximum	Average	Values Used in Modeling
Water Quality					
COD (mg/L)	15	27	2,233	241	1 – 100
Sunlight intensity ($\mu mol/m^2/s$)			No Data		0 and 25 (dark and light)
Light period					16 h per 24 h
Dissolved oxygen (mg/L)	67	4.16	14.92	8.86	14
Carbon dioxide, or bicarbonate ($mol\ m^{-3}$)			No Data		0.0015
NH_3-N (mg/L)	24	0.09	2.20	0.15	0.01 – 2.2
Nitrate (mg/L)	24	0.01	0.82	0.60	0 – 0.8
Total Kjeldahl nitrogen (mg/L)	24	0.25	2.20	0.73	0.01 – 2.2
Total phosphorus (mg/L)	24	0.002	0.050	0.003	0 – 0.24
Soluble reactive phosphorus (mg/L)	24	0.002	0.050	0.003	0 – 0.24
Selected Model Parameters					
Biofilm thickness (μm)			No Data		5,000
Density, biofilm ($kg\ m^{-3}$)			No Data		170
Temperature (°C)	67	1.6	17.7	6.8	19.5
pH	67	6.75	8.81	7.27	7.5
Stream flow rate ($m^3\ d^{-1}$)			Undefined		100
Biofilm area of differential section (m^2)			Undefined		0.15

4.3.2 Simulations

The following simulations were executed:

1. Model runs related to Hypothesis 1 evaluated the impact of light intensity (as irradiance, I) on the distribution of active biofilm mass for a range of readily biodegradable COD concentrations.
   a. Dark was assumed to have an I of 0 μmol/m^2/s.
   b. The sunlight condition was assumed to be I=25 μmol/m^2/s, comparable to the highest light intensity used in the laboratory experiments.
   c. The readily biodegradable COD concentration was set at 100 mg/L.
   d. The dissolved oxygen concentration was set at 14 mg O_2/L.
2. Model runs related to Hypotheses 2 and 4 evaluated the impact that variable macronutrient concentrations, specifically phosphorus and nitrogen, had on biofilm biomass.
   a. The readily biodegradable COD concentration was set at 100 mg/L.
   b. The dissolved oxygen concentration was set at 14 mg O_2/L.
   c. The soluble reactive phosphorus concentration ranged from 0 to 0.24 mg P/L as orthophosphate (PO_4-P).
   d. The ammonia concentration ranged from 0 to 2.2 mg N/L as ammonia (NH_4).
   e. Biofilm thickness (L_F) was set at 5 millimeters (mm), to be representative of in-stream biofilms.

The model was configured to simulate a single CFSTR with a water volume of 0.003 cubic meters (m^3). This volume resulted in a flow rate through the CFSTR that does not result in a concentration reduction, similar to in-stream conditions in the immediate area of a biofilm growth. The water characteristics were developed based on field observations from streams investigated as a part of this study and are described in Table 4-6.

It should be recognized that while the model was specified for conditions generally consistent with those observed in the field and laboratory, the model was not calibrated to these observations. The value of the model to this research is in its representation of relative responses of biofilm components to the factors considered in the hypothesis testing. In no case should any of the model projected responses be considered as precise quantitative predictions.

CHAPTER 5

Hypothesis Testing Results and Conclusions

This chapter describes the results and conclusions of hypothesis testing. The reader should note that these findings reflect the specific conditions under which the monitoring and testing were conducted.

5.1 Hypothesis 1—Effect of Light on Biofilm Growth

Hypothesis Statement

The amount of biofilm growth that occurs under given conditions of readily biodegradable dissolved organic matter (i.e., soluble BOD_5) is directly proportional to the availability of sunlight.

5.1.1 Field Investigations

Background

The investigative plan called for comparisons between two monitoring locations at MKE and between three monitoring locations at GRR. At MKE biofilm growth in a dark culvert (Howell-dark) was to be compared with growth at a largely unshaded monitoring location downstream (6th St) having similar depth and velocity. Concerns about potential water quality changes between the MKE sites led to adding a secondary monitoring location (i.e., Howell-light) to facilitate comparisons with the nearby Howell-dark site.

The monthly COD results from upstream and downstream biofilm monitoring locations on Wilson Park Creek and from all three monitoring locations on the unnamed tributary were used in addressing this hypothesis. Solar Pathfinder™ measurements were used to assess riparian cover, and the percent of available light reaching the water surface in each stream reach, and PAR readings were used for assessing synoptic light-intensity at the water surface in each stream reach.

Results and Conclusions

Water quality data collected during the biofilm monitoring period indicated that readily biodegradable organic matter (as measured by COD) was present in concentrations ranging from 31 to 550 mg/L at MKE (in Wilson Park Creek) and 20.3 to 586 mg/L at GRR (in the unnamed tributary). Additional COD samples were collected before and after the biofilm monitoring period. At MKE, sampling commenced 69 days before the biofilm monitoring period and concluded 11 days after; COD concentrations for this full time period ranged from less than 8 to 550 mg/L in Wilson Park Creek. At GRR, sampling commenced 54 days before the biofilm monitoring period and concluded 26 days after; COD concentrations for this full time period ranged from 12.8 to 586 mg/L in the unnamed tributary. Heterotrophic biofilm magnitude at monitoring locations tended to increase or stay steady in value from February to May, regardless of whether light

availability increased, decreased, or stayed the same (Figure 5-1). Photos showing the appearance of biofilms during the May sampling event are shown in Figure 5-2. At each location, the biofilm was of a filamentous form and varied in color from rust-orange, to yellow-brown, to beige.

A corollary question for this hypothesis was "is the quality or intensity of sunlight more important relative to simple availability as a driver of heterotrophic biofilm growth?" This is a particularly relevant question given that the time period of the study coincides with a time of increasing light intensity in the northern hemisphere. PAR measurements were taken to assist in answering this question. Most notably, heterotrophic biofilm magnitude at the Howell-dark site increased between February and May (Figure 5-1[b]) despite the fact that daytime PAR readings taken (during March and May; no measurement was taken in February) consistently registered below a measurable level. Biofilm magnitude was initially less than that observed at the adjacent Howell-light location, but reached a similar level by the end of the monitoring period. Any confounding issues related to turbidity, ice cover, and temperature were thought to be negligible. Given the proximity (40 meters) of these two monitoring locations, water quality (i.e., turbidity and temperature) was assumed to be the same at each. Further, ice cover percentages measured during biofilm sampling events were identical at each monitoring location.

5.1.2 Laboratory Experiments

Background

The goal of the Hypothesis 1 laboratory testing was to assess the effects of variations in incident light on the growth, overall accumulation, and composition of laboratory-grown airport stream runoff biofilms. Within each reactor, no substantial difference in biofilm colonization was observed between the side closest to and farthest from the light, as measured by the tests described here.

Results and Conclusions

Biofilm Appearance. After 4 days of operation, all reactors were visibly turbid, indicating robust microbial growth (Figure 5-3). There were no discernible differences between the light and dark reactors at this time.

After 17 days of operation, differences were evident between the reactors incubated in the light versus the dark reactor (Figure 5-4). The light reactors showed the presence of both darker brown cell accumulations (5-4b) and green algal accumulations (5-4a).

At the conclusion of the experiment (Day 28), visual differences between the biomass accumulated in the reactors were obvious (Figure 5-5). Both reactors incubated under light conditions contained dark brown biofilms while the reactor incubated in the dark reactor appeared beige.

Under closer examination, biofilm from the 25 percent full sunlight reactor had obvious green patches (Figure 5-6a) and appeared darker than the 12 percent full sunlight biofilm (Figure 5-6b).

Heterotrophic Organisms. Heterotrophic plate counts (HPC) were quantified at twice-weekly intervals during the 28 days of operation of the reactors for all 3 light conditions (Figure 5-7). With the exception of a slight decrease in colonization at Day 6 to ~10^7 colony-forming units per square centimeter (CFU/cm^2), HPC levels in all reactors varied from approximately log 7.5 to 8.5 CFU/cm^2. While the more intense light condition (25 percent full sunlight) showed the highest levels of HPC from Days 10 through 28, HPC levels in the reactor incubated in darkness were almost equally abundant, suggesting that light was not an important determining factor in HPC counts in this test. Heterotrophic organisms utilize organic carbon to produce cell mass and energy. In these experiments, organic carbon (as well as other inorganic nutrients) was present in abundance, providing a robust growth environment for heterotrophs under all light conditions.

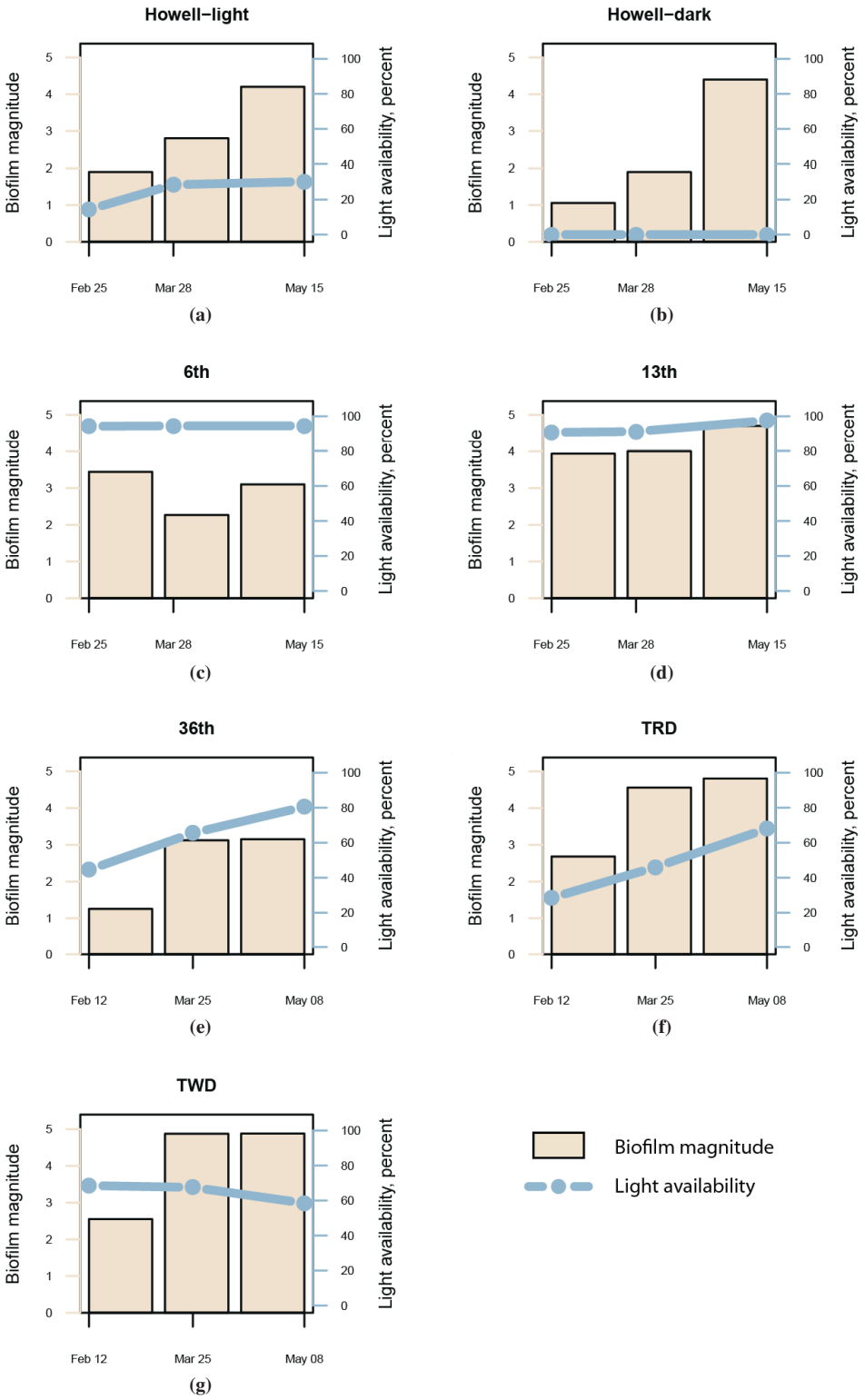
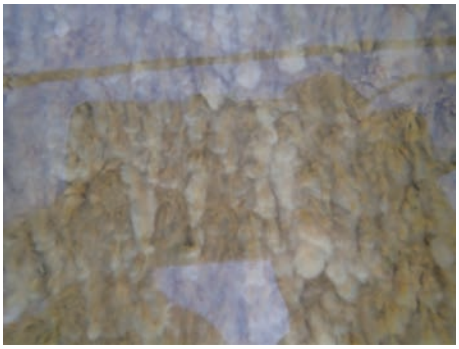


Figure 5-1. Heterotrophic biofilm magnitude and solar Pathfinder™ measurements of light availability. (Monitoring locations: [a] Howell-light, [b] Howell-dark, [c] 6th, [d] 13th at MKE, and [e] 36th, [f] TRD, and [g] TWD at GRR.)

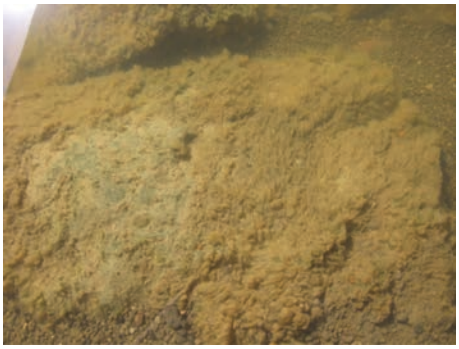
(a)

(b)



(c)

(d)



(e)

(f)

(g)

Figure 5-2. Photographs of biofilms from May 2013. (Monitoring locations: [a] Howell-light, [b] Howell-dark, [c] 6th, [d] 13th at MKE, and [e] 36th, [f] TRD, and [g] TWD at GRR.)

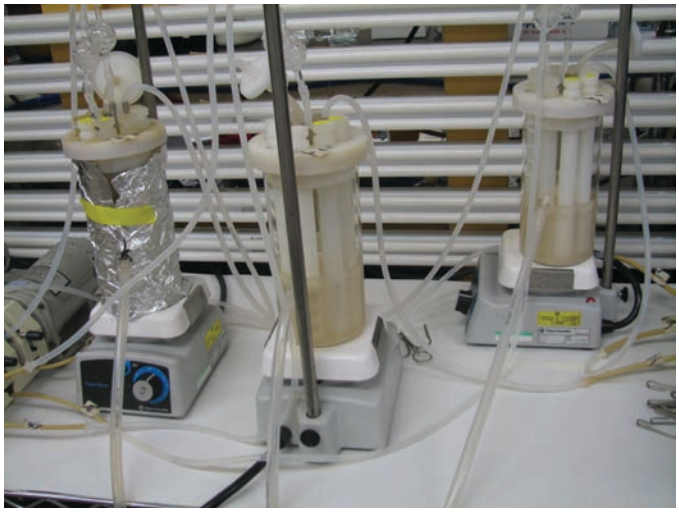


Figure 5-3. Reactor appearance after 4 days of operation.

(a) (b) (c)

Figure 5-4. Reactor appearance after 17 days of operation. ([a] incubated in 25 percent full sunlight, [b] incubated in 12 percent full sunlight, and [c] incubated in darkness.)

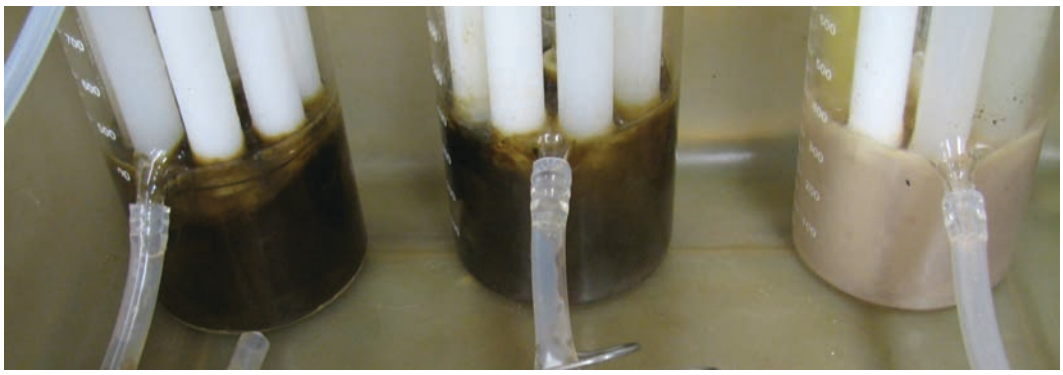


Figure 5-5. Reactors at the conclusion of the experiment on Day 28. (From left to right, 25 percent full sunlight, 12 percent full sunlight, dark.)

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(a) (b) (c)

Figure 5-6. Close-up views of reactor rods at the conclusion of the experiment, Day 28. ([a] 25 percent full sunlight, [b] 12 percent full sunlight, and [c] dark.)

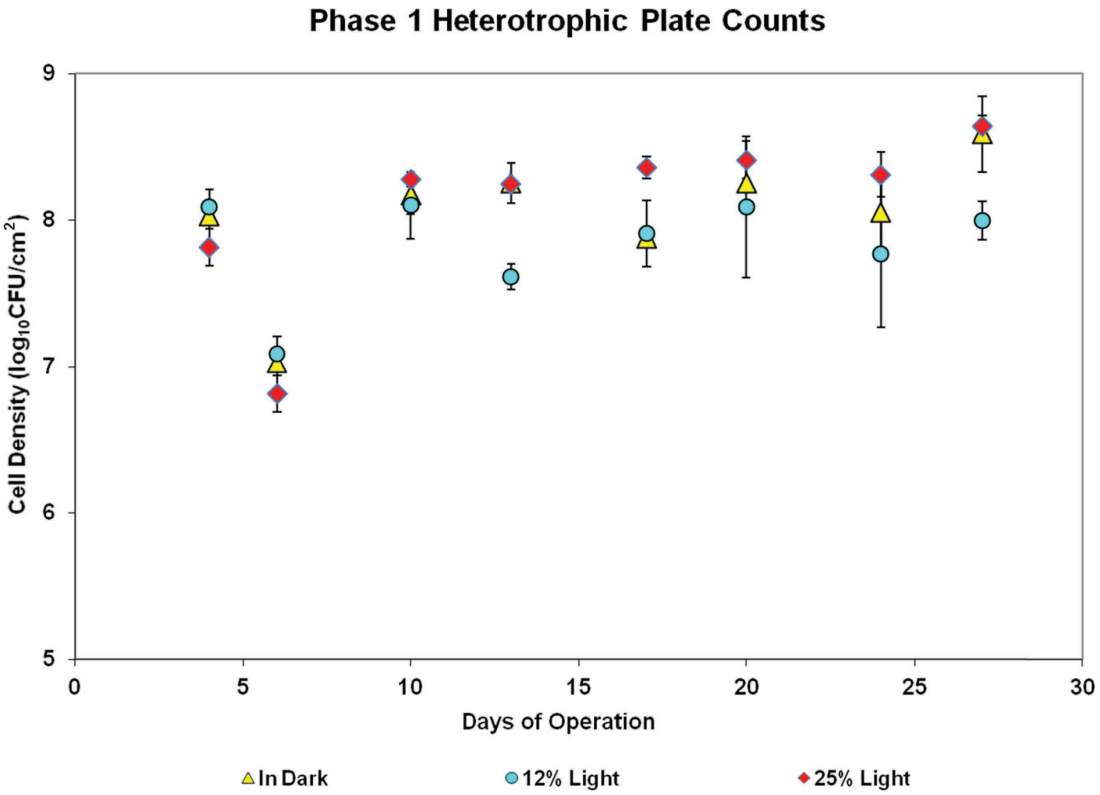

Figure 5-7. Heterotrophic plate counts from biofilm accumulated on coupons in reactors incubated in darkness, 12 percent full sunlight, and 25 percent full sunlight. (Error bars represent ±1 standard deviation from the mean.)

The levels of HPC observed, approximately 10^8 CFU/cm^2, are typical for relatively high nutrient conditions. This level of biomass accumulation represents a biofilm approximately 50 to 100 µm in average thickness, a condition that would likely lead to the development of discrete anaerobic zones near the biofilm-substratum interface. Such anaerobic zones are common in biofilms >50 µm thick because aerobic heterotrophs consume oxygen as it diffuses into the biofilm from the bulk fluid. At the base of thicker biofilms, anaerobic organisms utilizing (in order of decreasing energy) nitrate, iron (II), manganese (II), or sulfate as electron acceptors may proliferate. Anaerobic organisms were not quantified as part of this study.

Fungal Organisms. Fungal organisms showed lower levels of colonization and more temporal variability than did HPC organisms (Figure 5-8). Fungal counts were 2 to 4 orders of magnitude lower than HPC in all test reactors, regardless of light intensity.

Algal Organisms. Algae were quantified through the measurement of chlorophyll *a*. Relative levels of chlorophyll *a* for all light conditions during the experiment are shown in Figure 5-9. None of the reactors had measureable levels of chlorophyll *a* until the Day 20 sample, where biofilms in both the 12 percent and 25 percent full sunlight reactors developed measurable quantities. At the conclusion of the experiment, the biofilm from the 25 percent full sunlight reactor had approximately 3 mg/L chlorophyll *a*, while the 12 percent full sunlight reactor had approximately 1 mg/L. As would be expected, biofilm in the reactor incubated in darkness accumulated no measureable chlorophyll *a*.

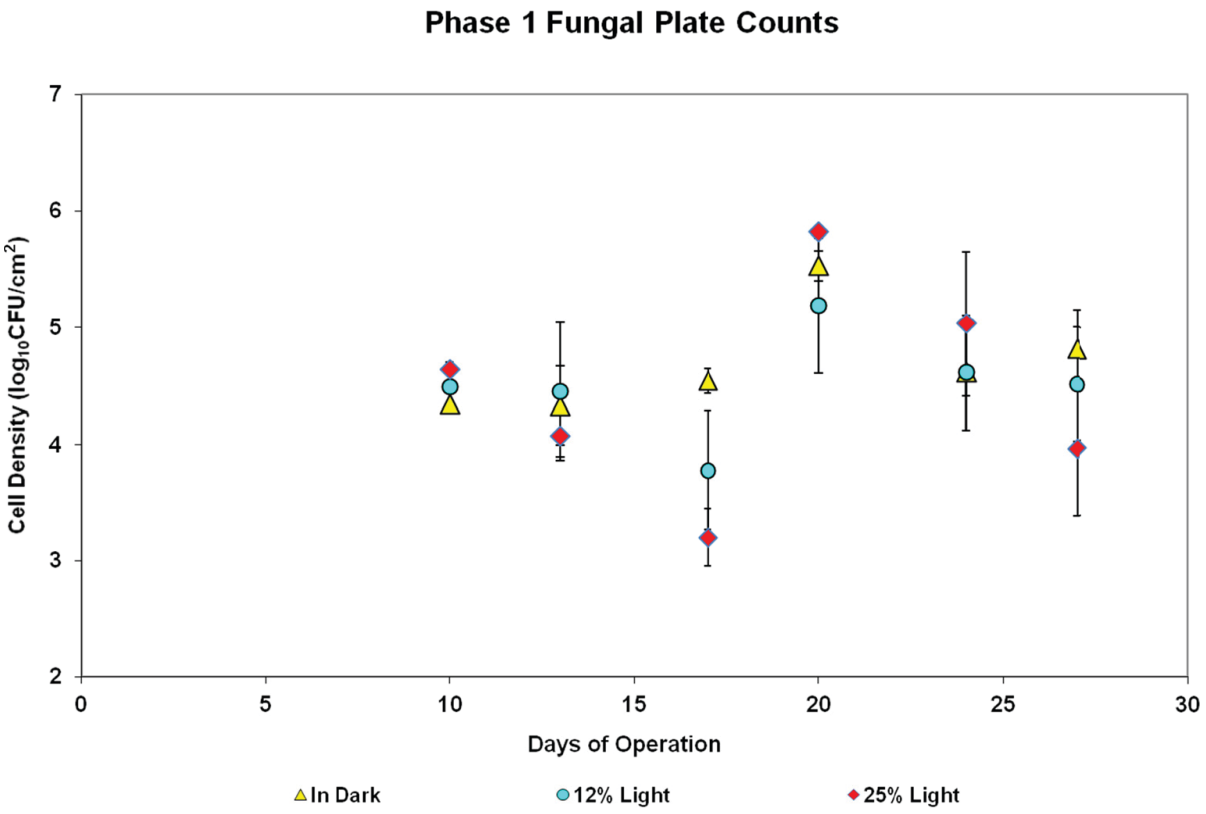

Figure 5-8. Fungal plate counts from biofilm accumulated on coupons in reactors incubated in darkness, 12 percent full sunlight, and 25 percent full sunlight. (Error bars represent ±1 standard deviation from the mean.)

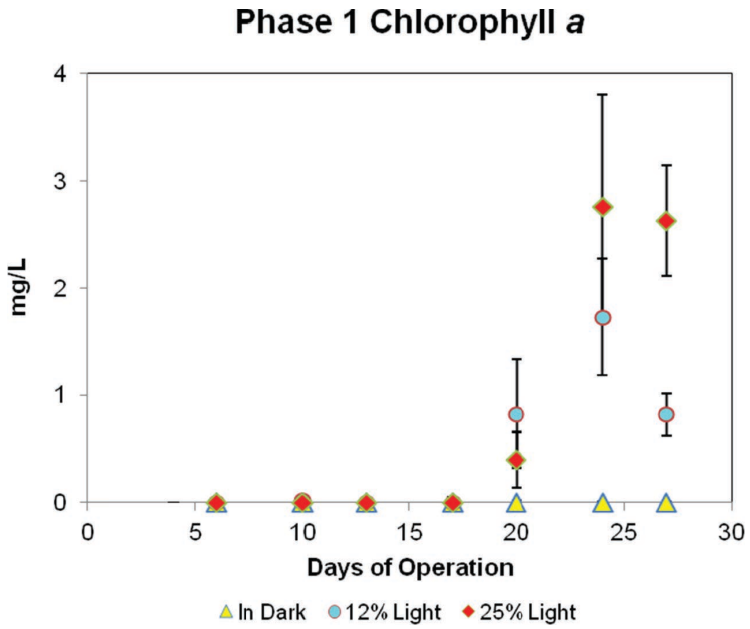

Figure 5-9. Chlorophyll a from biofilm accumulated on coupons in reactors incubated in darkness, 12 percent full sunlight, and 25 percent full sunlight. (Error bars represent ±1 standard deviation from the mean.)

5.1.3 Modeling Evaluations

The amount of phototrophic organisms (i.e., algae) comprising the active biofilm biomass (i.e., the sum of heterotrophic [X_H], phototrophic [X_P], and nitrifying [X_N] organism categories) was evaluated as a function of readily biodegradable COD under light (i.e., 25 percent full sunlight) and dark conditions. Figures 5-10 and 5-11 present the model estimated active fraction of phototrophic organisms (defined as X_P / [$X_H + X_P + X_N$]) under a range of COD concentrations in the presence and absence of sunlight, respectively.

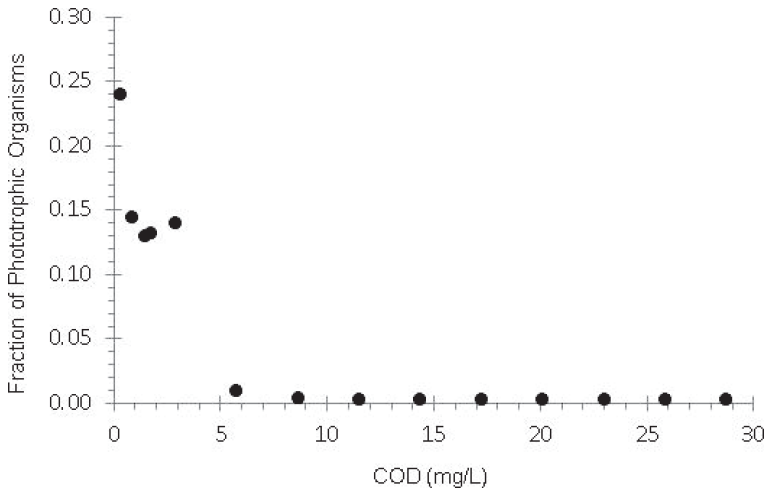

Figure 5-10. Model projected active fraction of phototrophic organisms over range of COD concentrations with sunlight.

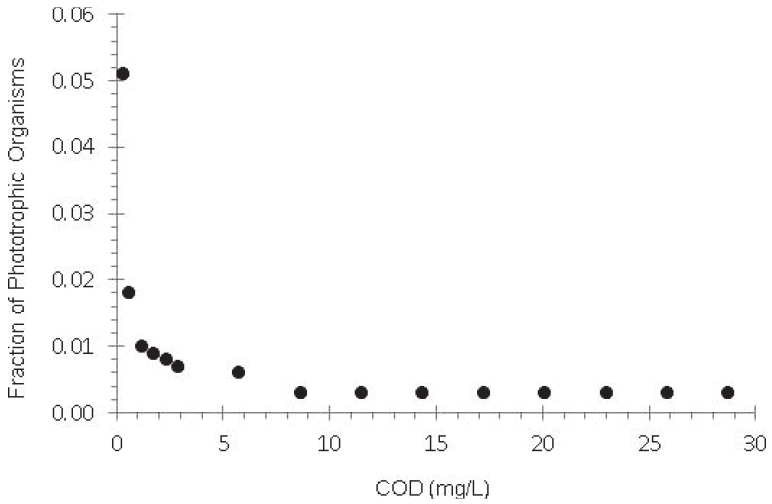

Figure 5-11. Model projected active fraction of phototrophic organisms over range of COD concentrations without sunlight.

Under low concentrations of COD (i.e., COD = 1 mg/L), phototrophic organisms compose approximately 5 percent of the projected active biofilm mass for the dark condition and approximately 25 percent of the active biofilm mass for the sunlight condition. In both cases, increasing the COD results in a reduced amount of phototrophic biomass when compared with the total active biomass; the phototrophic fraction was reduced to 1 percent at COD concentrations greater than 40 mg/L. This result indicates that, even under sunlight conditions, increasing COD concentrations promote the development of biofilms that are composed of predominantly heterotrophic organisms. Consideration of the processes represented in the model suggests that this dominance is the result of heterotrophic organisms having a higher growth rate and biomass yield than phototrophic organisms under elevated COD concentrations. Consequently, the heterotrophs outcompete the phototrophs for space inside the biofilm, independent of the presence or absence of sunlight. The results and interpretation are consistent with the field and laboratory observations.

5.1.4 Discussion and Conclusions

The field and laboratory results do not agree with the observations by Romani and Sabater (1999) of substantially greater bacterial and algal accumulation on sunlight-exposed coupons compared with those incubated in the dark. However, the literature observations were in low organic carbon streams, while this study involved relatively high organic carbon (i.e., COD) systems. The observed differences between the literature and this study are likely to be attributable to the competitive advantage that heterotrophic organisms have over phototrophic organisms in systems with relatively elevated concentrations of COD. While phototrophic organisms in the biofilm are affected by the presence or absence of sunlight, they compose such a small fraction of the total active biomass that their response is insignificant to overall biomass proliferation.

Bacterial activity was not described in the field monitoring or the laboratory experiments, so direct comparison with the report by Lear, Turner, and Lewis (2009) that bacterial activity did not vary substantially between different light conditions is not possible. However, those authors also observed that biofilm structure varied with light condition, which is consistent with the observed differences in bioreactor color under different light conditions.

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Hypothesis 1 is not supported by the results. The amount of biofilm growth that occurs under given conditions of readily biodegradable dissolved organic matter does not appear to be directly proportional to the availability (or intensity) of sunlight.

5.2 Hypothesis 2/4—Nutrient Limitation on Biofilm Growth

During the implementation of data collection for Hypotheses 2 and 4, it became apparent to the research team that the two hypotheses are so closely related that a single set of laboratory experiments and model runs would provide the information needed for testing. Consequently, it also made sense to combine the data results and testing of the two hypotheses into the unified discussion on nutrient limitation presented in this subsection.

Hypotheses Statements

The two components of combined Hypothesis 2/4 are as follows:

a. Stream biofilms will exhibit phosphorus limitation when the concentration of PO_4 is in the range 0.005 to 0.025 mg/L (as P) or less.
b. Ratios of water column substrate (i.e., organic carbon) and nitrogen and phosphorus concentrations can be used to identify the rate-limiting nutrient for biofilm growth in a stream.

5.2.1 Laboratory Experiments

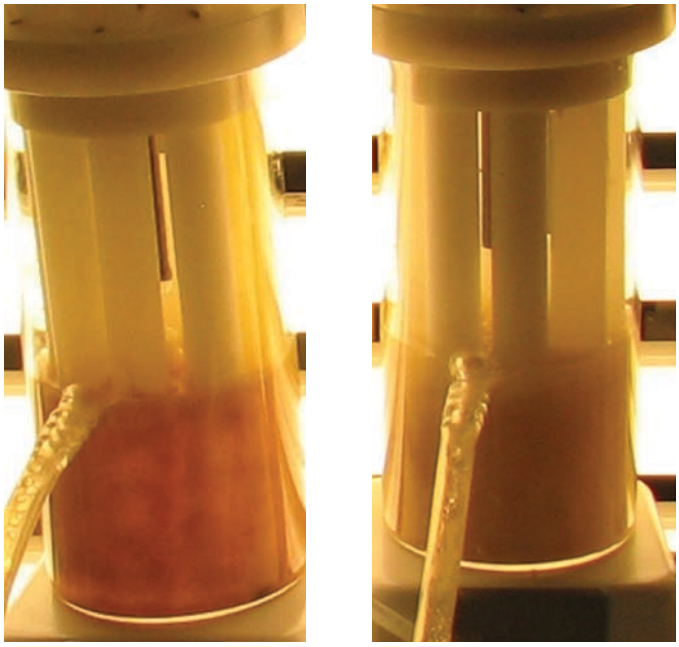
Background

Phase 2 experiments were designed to assess the importance of both Nitrogen (N) and Phosphorus (P) as potentially limiting nutrients in stream biofilm development. N and P were tested in separate reactors by starting at very low concentrations of available N and P and stepping these levels up over time while monitoring biofilm biomass growth. Two reactors were operated: one where Carbon (C) and P were maintained in excess and N was incrementally varied over a C:N range of 100:1 to 10:1; and the other where C and N were maintained in excess while P was varied over a C:P range of 5000:1 to 100:1 (Table 4-5). The resulting data reflects the effects of independently varying levels of P and N on biofilm accumulation and composition.

Results

Biofilm Appearance. After inoculation, both reactors became turbid at approximately Day 5 of operation, although levels of biofilm remained relatively low during this time (HPC data are presented in the next section). The reactor turbidity masked the appearance of the biofilm itself; however, by Day 28 of operation, the reactors had a distinctly different appearance. The N-varied reactor appeared more reddish-brown while the P-varied reactor appeared grey-brown (Figure 5-12). A color and morphological difference was noted between HPC colonies from each reactor during the first week of the experiment. Colonies from the N-varied reactor appeared yellow or pink in color and were circular in morphology while colonies from the P-varied reactor were white, asymmetrical, and appeared filamentous (Figure 5-13). Although HPC colonies were not identified to the species level, the appearance of filamentous colonies is consistent with the growth of organisms such as *Sphaerotilus natans*, a filamentous bacterium associated with organic-rich wastewater discharges whose appearance has been noted in airport deicer fluid runoff streams. The differences in colony morphology are consistent with the overall reactor appearance throughout the experiment, with the N-varied reactor appearing more red-brown and the P-varied reactor more grey-brown (Figure 5-14).

Also of note in Figure 5-14(a) is the appearance of green algal growth at the air-water interface and at the bottom of the reactor. No algal growth was observed in the P-varied reactor (Figure 5-14b). These color differences persisted until the end of the experiment, with algal growth



(a) (b)

Figure 5-12. Reactor appearance at Day 28. ([a] Nitrogen-varied reactor and [b] Phosphorus-varied reactor.)

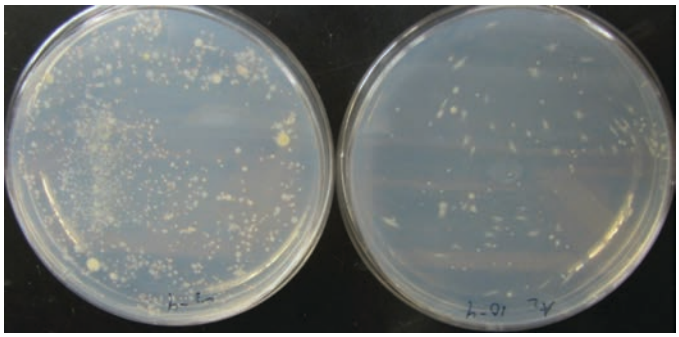
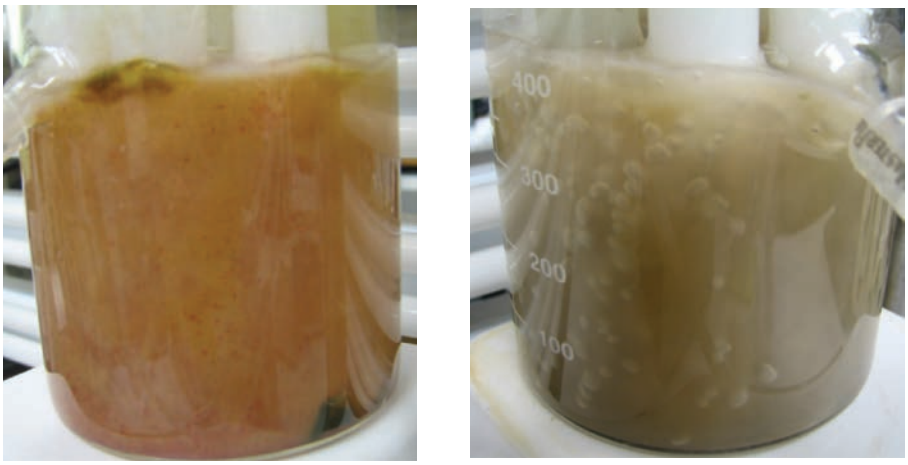


Figure 5-13. Colony appearance after 2 weeks of operation. (Colonies on the left plate were from the N-varied reactor and colonies on the right plate were from the P-varied reactor.)



(a) (b)

Figure 5-14. Reactor appearance at Day 35 of operation. ([a] N-varied reactor and [b] P-varied reactor.)

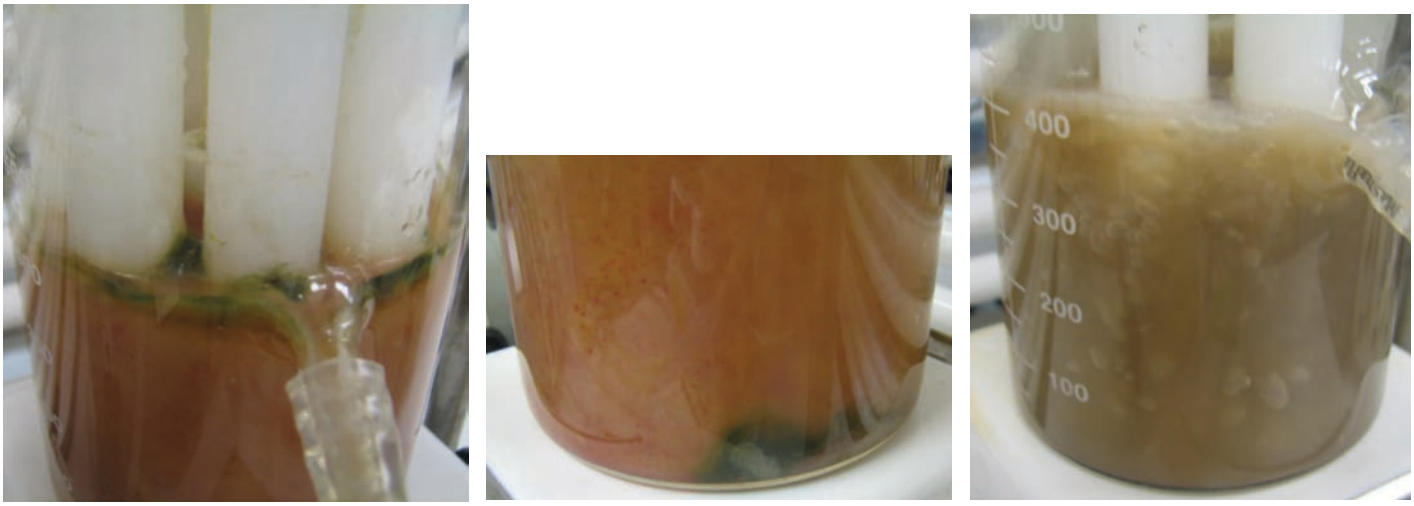
increasing in the N-varied reactor and remaining apparently absent from the P-varied reactor (Figure 5-15).

It is evident from the appearance of the two reactors that, despite starting with the same inoculum, different populations became dominant over the course of the experiment as a result of the nutrient limitations imposed during the first weeks of operation. The effects of phosphorus limitation in promoting the growth of filamentous bacteria, specifically *Sphaerotilus natans*, have been noted for decades (Gaudy and Wolfe 1961). These experiments confirm this effect in the context of propylene glycol as the carbon source. Furthermore, this work shows that (over the time span of this study) the initially dominant population, once established, is resistant to change despite the alleviation of the conditions that allowed it to grow to prominence.

Heterotrophic Organisms. HPC were quantified on a weekly basis throughout the experiment. During the first 2 weeks of operation, the level of P in the P-varied reactor was 0.005 mg/L, a level which has been shown to limit microbial and algal growth (Correll 1999). During this initial period, biofilm in the P-varied reactor was measured at 10^6–10^7 CFU/cm^2 (Figure 5-16). This level of biofilm is approximately tenfold less than was measured at the same time points in Phase 1 experiments where P was not limiting. A fivefold increase in P to 0.025 mg/L resulted in an increase in HPC to >10^8 CFU/cm^2, a level similar to the steady-state biofilm in Phase 1 experiments. Growth continued to increase at concentrations greater than 0.025 mg-P/L, indicating that P was still limiting at these higher concentrations.

The N-varied reactor started with 0.24 mg/L N, a C:N ratio of 100:1. While this C:N ratio should have limited growth, a log 7.5-8 CFU/cm^2 biofilm was measured during this period, similar to that measured in Phase 1 experiments. A two-fold increase in N to 0.48 mg/L did not have a substantial effect on HPC in the N-varied reactor, as HPC dropped slightly (difference not statistically significant) during the Day 14 through 28-dose period. Under these conditions, it is likely that not all the organic carbon was consumed during the experiment, yet considerable heterotrophic growth did occur with the available nutrients.

It is important to note that the HPC data do not provide insight as to the types of organisms present in the biofilms, beyond the fact that they are aerobic heterotrophs. If only HPC had been collected, without the visual evidence presented in the reactor photos above, the substantial differences in the composition of the biofilms between the two reactors would not have been noted.



(a) (b) (c)

Figure 5-15. Reactor appearance at Day 47. ([a] air-water interface of N-varied reactor, [b] bottom of N-varied reactor, and [c] P-varied reactor.)

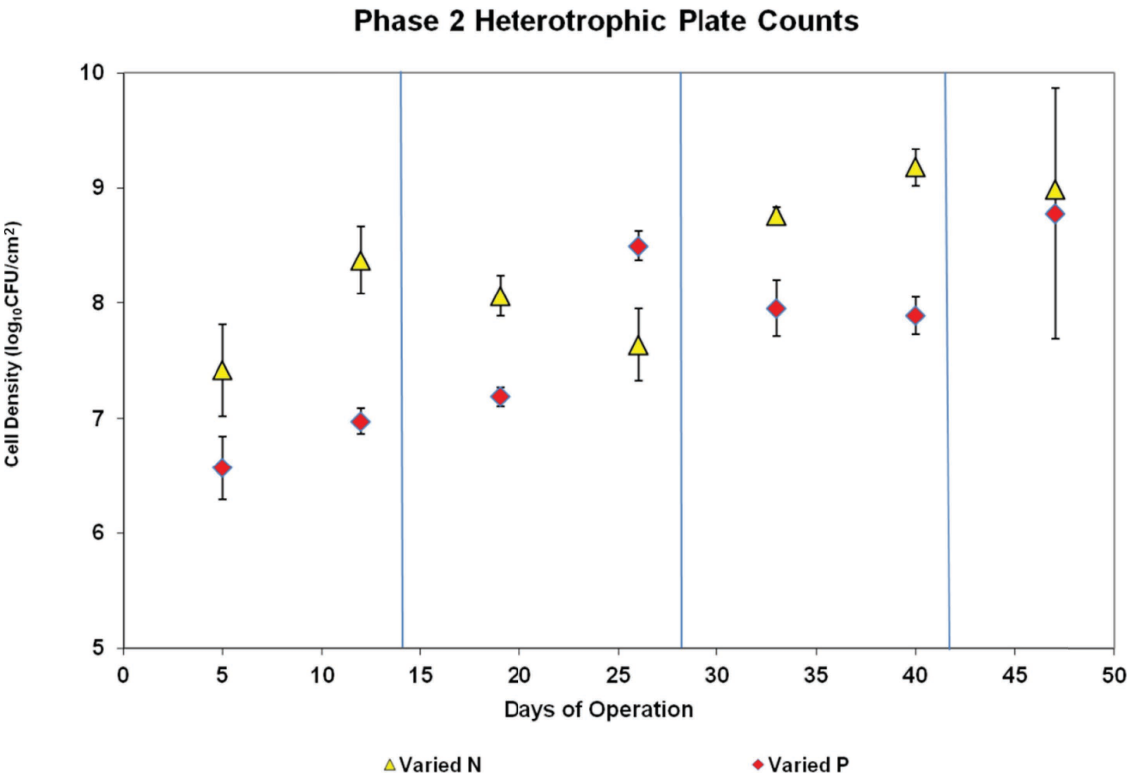


Figure 5-16. Heterotrophic plate counts from biofilm accumulated on coupons in reactors. (Error bars represent ±1 standard deviation from the mean. Vertical lines denote times when the varied nutrient concentration was increased [see Table 4-5]).

This is an important point to the extent that some biofilm morphologies may be considered less objectionable in the context of water quality in streams receiving airport runoff.

Fungal Organisms. As in Phase 1 experiments, fungal organisms showed lower levels of colonization and more temporal variability than did HPC (Figure 5-17). Fungal counts in both reactors remained at $10^3 - 10^4$ CFU/cm^2 for the duration of the experiment, with the exception of the Day 47 count in the N-varied reactor. Higher levels of fungal growth in streams are generally correlated with recalcitrant biomass such as leaf litter or other cellulosic material (Gessner 1997).

Algal Organisms. Algae were quantified through the measurement of chlorophyll *a*. Relative levels of chlorophyll *a* for both reactors are shown in Figure 5-18. Virtually no chlorophyll was recovered from any of the biofilm samples from either the N- or the P-varied reactors. Photographs of the N-varied reactor (Figures 5-14a, 5-15a, and 5-15b) clearly show algal accumulations at the air-water interface and at the glass-water interface. It is likely that discrete areas of algal growth occurred in the reactor, but that these were not present on the coupons, probably because light could not readily penetrate the highly turbid reactors. In any case, algae were not a major component of the biofilm under the nutrient-varied conditions. This finding is consistent with the Hypothesis 1 results.

5.2.2 Field Observations

Background

Because the site selection process limited available sites to locations along two streams, it was not possible to identify and monitor sites with sufficiently dissimilar nutrient concentrations to support direct testing of the nutrient limitation hypotheses. However, water quality samples

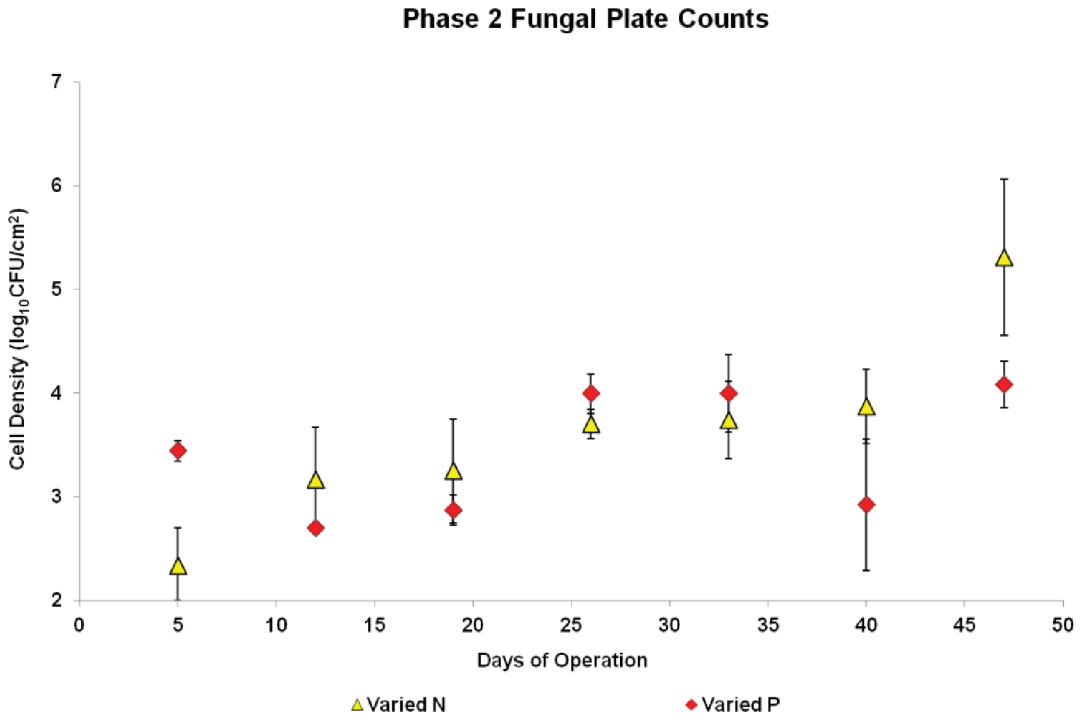


Figure 5-17. Fungal plate counts from biofilm accumulated on coupons. (Error bars represent ±1 standard deviation from the mean.)

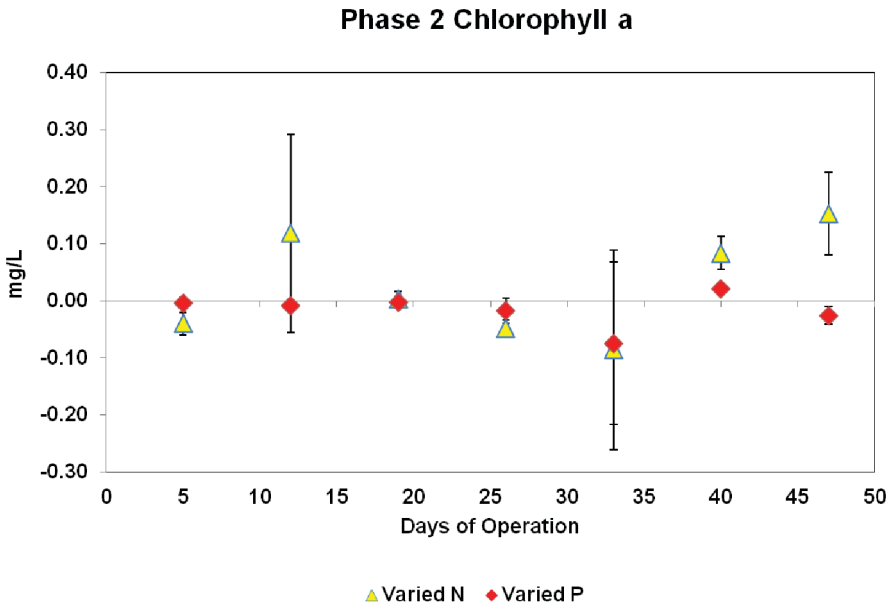


Figure 5-18. Chlorophyll a from biofilm accumulated on coupons in reactors. (Error bars represent ±1 standard deviation from the mean.)

from the upstream- and downstream-most monitoring locations on Wilson Park Creek and the unnamed tributary were analyzed for available N (as dissolved total N) and P (as ortho-phosphorus), allowing for characterization of receiving water nutrient concentrations and comparison with the laboratory testing effort.

Results

Total dissolved N values for each water quality sample were calculated by summing values for dissolved Kjeldahl nitrogen and nitrate (and nitrite) N, both of which were reported in mg/L as N. Total dissolved N concentrations fluctuated, but generally fell within the middle range of concentrations tested in the lab (Figure 5-19[a]). Overall, concentrations of total dissolved N at MKE ranged from 0.38 and 1.3 mg/L as N (median of 0.77 mg/L as N); concentrations at GRR fluctuated between 0.20 and 2.2 mg/L as N (median of 0.73 mg/L as N).

The majority of water quality samples were collected during low-flow conditions. The exceptions were the December 20, 2012, GRR samples and the February 25, 2013, MKE sample at Howell-dark. The December 20 GRR samples were unavoidably collected following a period of heavy rainfall (approximately 0.69 inch during preceding 8 hours). The February 25, 2013, MKE sample at Howell-dark was collected late in the day, after substantial snowmelt had occurred resulting in elevated flows. Across all samples, concentrations at MKE ranged from less than 0.002 to 0.027 mg/L as P (median of 0.003 mg/L as P) (Figure 5-19[b]). Concentrations at GRR

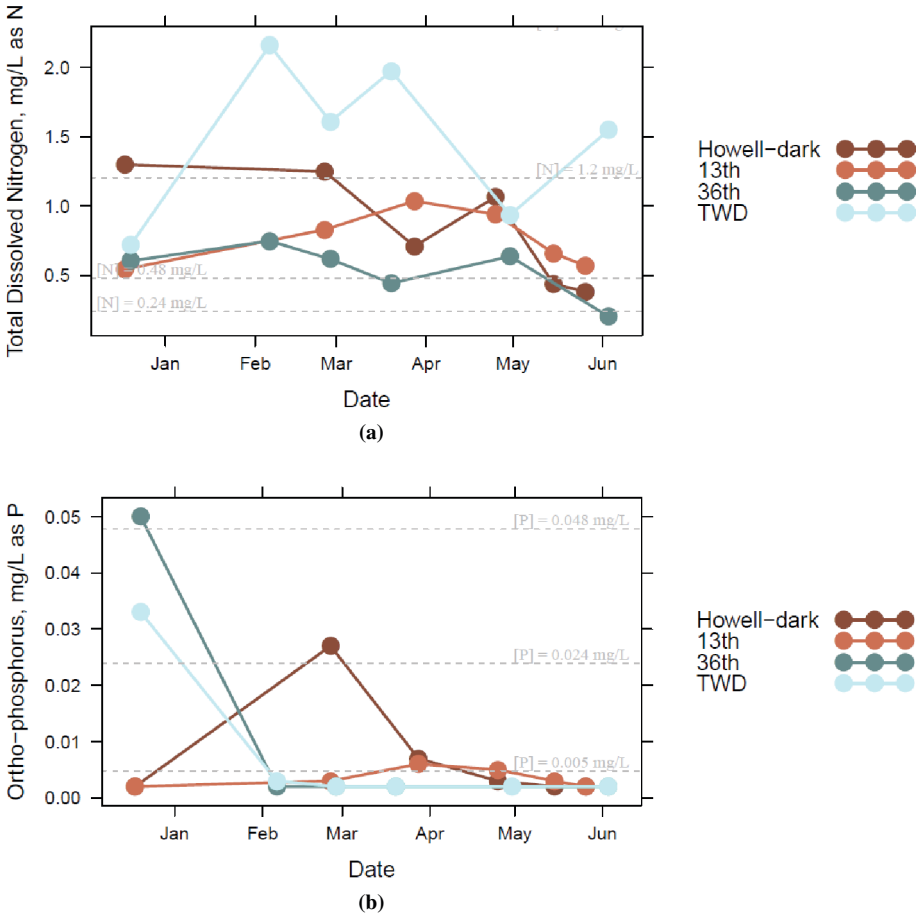


Figure 5-19. Measured total dissolved nitrogen (a) and ortho-phosphorus as P (b) concentrations at water-quality monitoring locations (with lines showing concentrations and ratios used in laboratory tests).

ranged from less than 0.002 to 0.05 mg/L as P (median of < 0.002 mg/L as P). The samples collected during the elevated flow events had higher P concentrations.

5.2.3 Modeling Evaluations

The model was used to investigate the relative influence of macronutrients by considering the projected response of heterotrophic organisms, which have been shown to dominate the biofilms, under a fixed condition of relatively abundant COD and varying concentrations of P and N. The estimated concentration of heterotrophic organisms per unit area of the modeled biofilm model was used as an indicator of biofilm biomass.

A COD concentration of 100 mg/L was used in all model runs. The ranges of P and N concentrations investigated were 0 to 0.240 mg-P/L and 0 to 2.2 mg-N/L, respectively. For the P-varied model runs, N was set at 2.2 mg-N/L. The N-varied runs were conducted with P at 0.240 mg-P/L. Ratios of C:P and C:N were calculated from the input values. Figures 5-20 and 5-21 present the results.

The format of the x-axis in Figures 5-20 and 5-21 is such that the relative availability of the varied nutrient is reduced as the value of the C:P or C:N ratio increases. The ratio at which biofilm concentrations begin to decrease with increasing ratio is where limitation by the varied nutrient is indicated. No such threshold is apparent for P in Figure 5-20, indicating that P was limiting across the entire range of P concentrations and C:P ratios examined.

In contrast, Figure 5-21 has two distinct regions. The first is defined by C:N values less than about 57 where biomass does not change with decreasing C:N (i.e., increasing relative availability of N), while the second is where C:N values are greater than about 57 and biomass

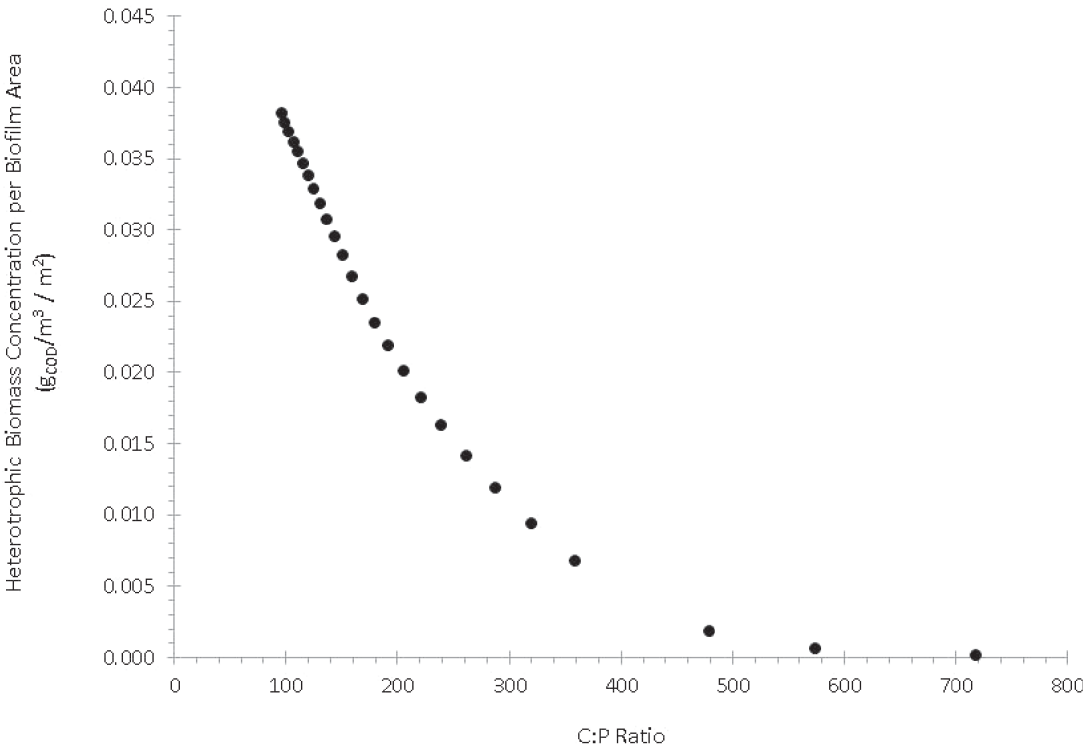


Figure 5-20. Heterotrophic organism concentration per unit area in the modeled biofilm as a function of C:P ratio.

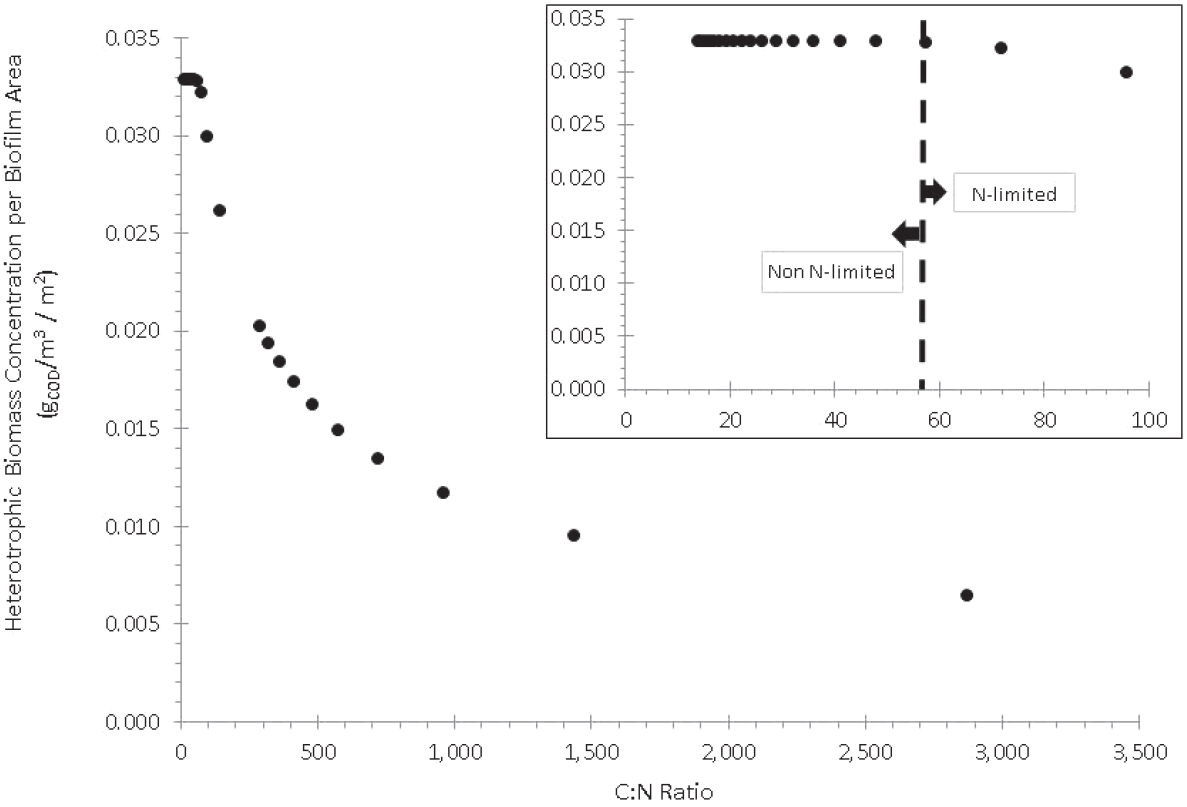


Figure 5-21. Heterotrophic organism concentration per unit area in the modeled biofilm as a function of C:N ratio. (Inset shows the lowest portion of the range.)

decreases with increasing C:N (i.e., decreasing relative availability of N). Thus, the indicated threshold between nitrogen limited and non-nitrogen limited conditions is at a C:N of about 57:1.

The Redfield Ratio (Redfield 1958) represents a generally accepted stoichiometric ratio of carbon-to-nitrogen-to-phosphorus (C:N:P = 106:16:1 C:N:P) that will meet the macronutrient requirements of bacteria while converting organic carbon into biomass and energy. This ratio can be used to evaluate the results of the modeling, and potentially provide insights into the laboratory results.

The threshold between N limited and non-N limited conditions indicated by the modeling is substantially higher than suggested by the Redfield Ratio (i.e., 106:16 or 6.6:1). This difference suggests that biofilm growth is less sensitive to the availability of water column N than would be expected from the Redfield Ratio. Boltz et al. (2012) investigated controlled systems having low levels of N and P (i.e., either macronutrient may be the limiting material) and reported that biofilm growth was able to maintain a steady-state biomass with a carbon: macronutrient ratio (in this case N) far greater than predicted by the Redfield Ratio. The laboratory reactors used in this study were saturated with macronutrients to develop a robust biofilm. Then, the amounts of N and P in the feed water were increased over time, starting with relatively high C:P and C:N ratios. The literature and model suggest that robust biofilms contain a substantial amount of biomass in a state of decay. The decaying biomass releases its stored macronutrients, of which N is in the greater amount. This "reservoir" of macronutrients supplements what is available from the water column, resulting in laboratory results showing that biofilm can grow on a substantially reduced amount of N than expected. In reality, the true available N consists of the sum

of concentrations in the ambient water and N that has accumulated in the biofilm structure over the duration of the experiments.

5.2.4 Discussion and Conclusions

Hypothesis 2/4(a) was not supported by the laboratory results; phosphorus limited conditions were evident at P concentrations substantially greater than 0.025 mg/L. However, the laboratory and field results also suggest that the dominant biofilm organisms under low P conditions were filamentous bacteria, similar to *Sphaerotilus natans* in colony morphology and overall biofilm appearance. This is consistent with the previously noted work by Gaudy and Wolfe (1961) and suggests that efforts to limit P as a means of controlling biofilm may favor *S. natans*, a more undesirable component of stream biofilms than other biofilm organisms.

Hypothesis 2/4(b) was confirmed for HPC and fungal count data, both of which were positively correlated with increases in the relative nitrogen and phosphorus concentrations, respectively. Although the chlorophyll *a* (algal) data did not correlate well with nutrient ratio changes, higher levels of algal growth were observed in the N-varied reactors as N was increased. Interestingly, the dominance of the P-varied reactor by filamentous bacteria evidently inhibited algal growth. Algae were likely either outcompeted by bacteria early in the experiment, or they did not survive the initial weeks of P limitation and were not present to re-grow as conditions became more favorable.

The field data collected provide actual receiving stream context for comparison with the laboratory studies. As noted above, total dissolved N concentrations observed at both airports generally fell in the middle range of concentrations tested in the laboratory. Given that laboratory results showed prolific growth at all N concentrations tested, such concentrations would be expected to provide sufficient N for biofilm growth in the receiving streams. Phosphorus concentrations in natural systems have been shown to be limiting for bacteria in the range of 0.005-0.025 mg/L (Correll 1999). Ortho-phosphorus concentrations observed in both receiving streams suggest concentrations were below 0.025 mg-P/L much of the time during low-flow conditions. Biofilms grew prolifically at all monitoring locations on Wilson Park Creek and the unnamed tributary (Figure 5-1). It is not possible to determine from these data whether the biofilms were unresponsive to limiting P levels or whether they were stimulated and sustained by higher P concentrations associated with higher flows during runoff events. As can be seen in the photos in Figure 5-2, growth was predominantly filamentous in nature, a characteristic that laboratory results suggest is favored by P limiting conditions.

The biofilm model results were consistent with laboratory and field data, and provide insights into the underlying mechanisms. The laboratory system showed evidence of P limitation throughout the range of P concentrations and C:P ratios examined. This observation is consistent with the model output, which suggests P limitation for the entire range of conditions modeled. What is even more interesting is that the model suggests a threshold between N limited and non-N limited conditions when C:N is about 57. This value is substantially greater than the generally accepted Redfield Ratio, suggesting that biofilm is capable of maintaining a steady-state biomass with less N than that which would be generally expected. These observations are supported by previous research that reported biofilms can grow with fewer macronutrients in the water column than might be expected based on the Redfield Ratio because of the recycling of stored nutrients released by decaying bacteria in the biofilm structure.

5.3 Hypothesis 3—Impact of Physical Stream Characteristics on Biofilm Growth

Hypothesis Statement

The physical characteristics of receiving streams influence the extent of biofilm accumulation for a given water chemistry condition as follows:

- Category 1: Shallow, turbulent, well-mixed channels promote biofilm growth and require ambient BOD_5 concentrations less than 50 mg/L to avoid prolific biofilm growth.
  - streambed surface area (A_{BED}) to bulk liquid volume (V_B) ratio (A_{BED}:V_B) greater than 100 m^2/m^3 (i.e., a depth less than 0.01 meter)
- Category 2: Relatively straight channels with moderate depth and flow promote moderate biofilm growth and require ambient BOD_5 concentrations be maintained in the range 50 to 100 mg/L or lower to avoid prolific biofilm growth.
  - streambed surface area (A_{BED}) to bulk liquid volume (V_B) ratio (A_{BED}:V_B) in the range 25 to 75 m^2/m^3 (i.e., depths between 0.013 and 0.04 meters)
- Category 3: Deep, slow-moving channels deter biofilm growth, and can experience ambient BOD_5 concentrations greater than 100 mg/L without prolific biofilm growth.
  - streambed surface area (A_{BED}) to bulk liquid volume (V_B) ratio (A_{BED}:V_B) less than 10 m^2/m^3 (i.e., a depth greater than 0.10 meter)

5.3.1 Field Investigations

Background

Initial plans for investigations into biofilm growth called for comparisons between biofilm growth at two monitoring locations (one deep, one shallow) at MKE and between two transects (one deeper, one shallower) at each of three monitoring locations at GRR. However, monitoring data revealed substantial water quality differences between the monitoring locations at MKE which could have confounded the interpretation of observed differences in biofilm growth. As a result, the strategy at MKE changed to drawing comparisons between two transects with different depth characteristics at one of the monitoring locations (13th). The strategy at GRR remained unchanged.

Results

Biofilm grew prolifically at all transects studied for Hypothesis 3, regardless of depth (Figure 5-22). Biofilm growth at compared transects were frequently similar or identical in thickness. In instances where notable differences between transects were observed, biofilms tended to be thicker at shallower transects; however, there was an overall absence of consistent pattern.

Average depths at the studied stream transects ranged from 0.06 to 0.36 meters, with a median value of 0.15 meters. Thus, all the transects fell into Category 3 of the hypothesis statement; stream conditions did not allow for sampling of transects representing the depth characteristics in Categories 1 and 2 of the hypothesis statement. Measured discharges on Wilson Park Creek at MKE and the unnamed tributary at GRR during sampling trips ranged from 0.22 to 10 cfs, with a median value of 2.4 cfs. Longer term flow records are also available for sites on these streams. On Wilson Park Creek at MKE, long-term annual mean streamflow values at nearby gaged sites range from 2.93 (0.23 km upstream of the biofilm monitoring locations) to 14.1 cfs (4.1 km downstream of the biofilm monitoring locations). On the unnamed tributary at GRR, average measured flow rates during low-flow conditions during a 2011 study ranged from approximately 0.8 to 2.4 to 3.5 cfs at 36th, TRD, and TWD, respectively (Table 5-1).

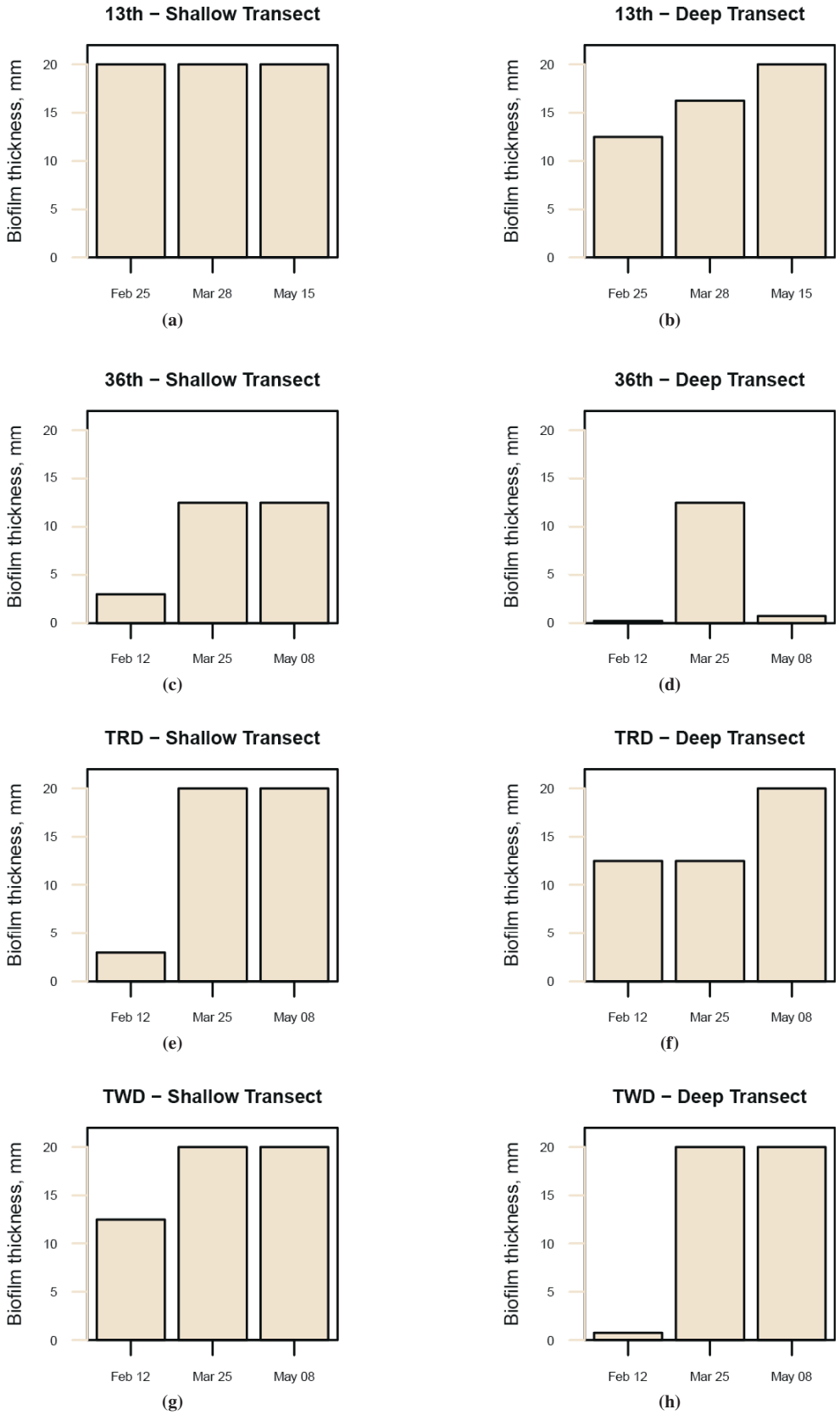


Figure 5-22. Median biofilm thickness at transects studied for Hypothesis 3.

Table 5-1. Long-term streamflow from studied streams.

Airport	Stream	Monitoring location short name	Relative location	Long-term streamflow (cfs)	Water-years*
MKE	Wilson Park Creek	Outfall 7	0.23 kilometer upstream of Howell-dark	2.93†	1997-2012
MKE	Wilson Park Creek	St. Lukes	4.1 kilometers downstream of 13th	14.1†	1997-2012
MKE	Kinnickinnic River	KK	N/A	25.1†	1983-2012
GRR	unnamed tributary	36th	N/A	0.8‡	2011-2012
GRR	unnamed tributary	TRD	N/A	2.4‡	2011-2012
GRR	unnamed tributary	TWD	N/A	3.5‡	2011-2012

*A water year is a 12-month period starting on October 1 of a given year and running through September 30 of the following year.
†Long-term annual mean streamflow from each site (USGS 2013).
‡Average of low-flow measurements collected during 2011–2012 study (LimnoTech, Inc., 2013).

Although the streams investigated in this study did not cover the full range of physical conditions described in the hypothesis statement, they were representative of conditions reported for typical airport receiving streams; according to a U.S. EPA survey of airports, the majority of initial receiving waters draining airports have flows less than 20 cfs (U.S. EPA 2012) (Figure 5-23).

Transects from within the same reach were selected for comparison in an effort to control for water quality differences. Historical relationships between winter COD and BOD_5 concentrations at each airport were used to estimate in-stream BOD_5 during the study period to support hypothesis evaluation. Estimated BOD_5 values ranged from less than 3.1 to 285.7 mg/L (median of 22.5 mg/L) in Wilson Park Creek at MKE, and 7.8 to 346 mg/L (median of 39.7 mg/L) in the unnamed tributary at GRR (Figure 5-24). Typical Wisconsin urban runoff BOD_5 concentrations have been reported as 9.4 mg/L (Bannerman, Legg, and Greb 1996). As expected, concentrations in these streams during winter months were elevated above typical urban runoff concentrations, and concentrations decreased with increasing distance from the airport. It should be noted that these streams are subject to substantial fluctuation in oxygen demand (Figure 5-24), and that the BOD results presented here represent instantaneous snapshots of concentration that characterize overall trends but are not likely reflective of the absolute minima and maxima in oxygen demand to which stream biota were exposed over the time period.

5.3.2 Discussion and Conclusions

Hypothesis 3 was rejected by the field results, for the range of depths sampled. The lack of a consistent pattern in biofilm growth differences between deeper and shallower transects implied that, over the range of stream depths studied, depth did not have an apparent effect on biofilm growth. It should be noted that this study focused on smaller streams that are representative of the majority of airport receiving water systems. These findings do not preclude the possibility

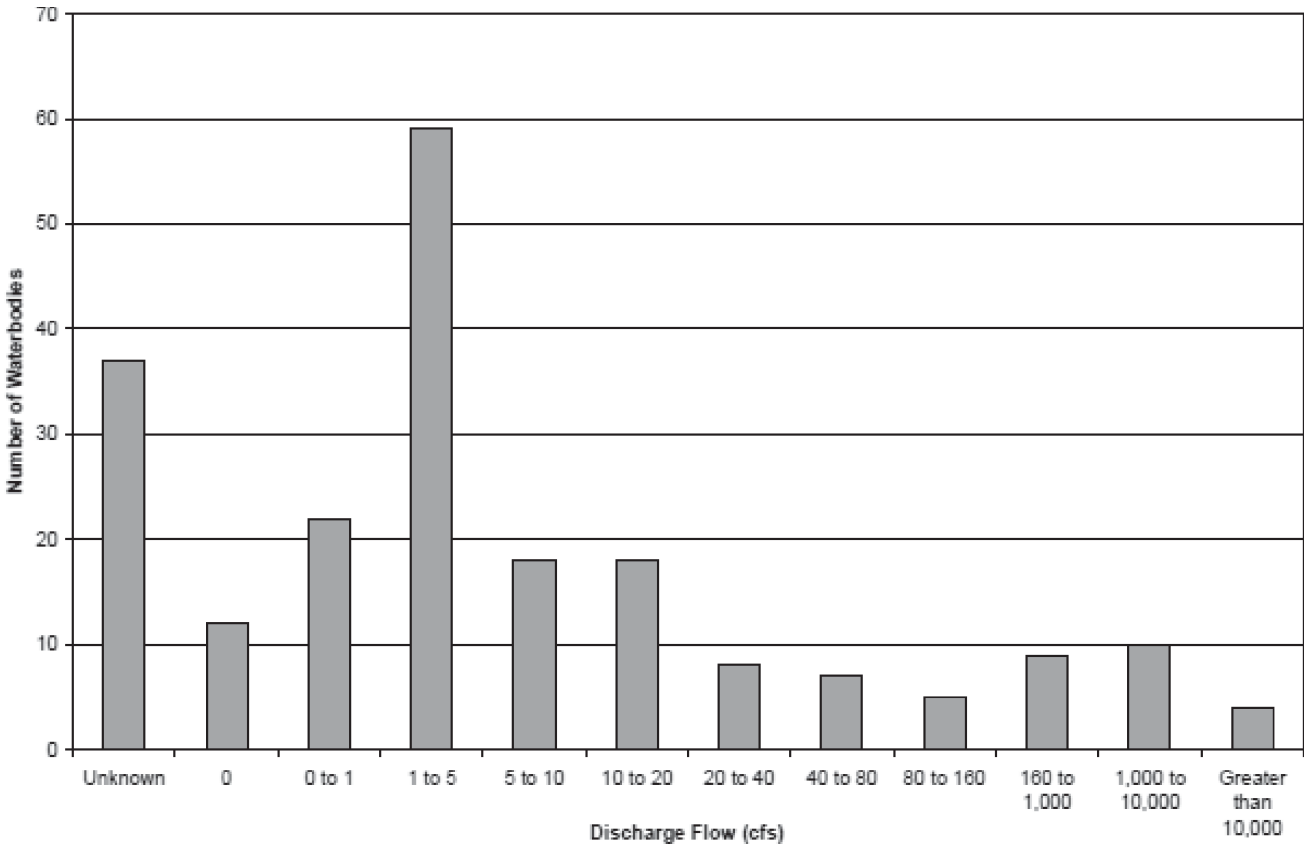


Figure 5-23. Initial receiving water discharge flows at U.S. EPA surveyed airports. (Figure 3-2 in U.S. EPA 2012.)

that depth may influence biofilm growth with increasing stream size and increasing depth beyond that examined.

Transects from the same reach were used in this comparison to control for substantial differences in water quality. BOD concentration trends in these two streams indicated concentrations were elevated above background urban runoff concentrations throughout most of the study period (U.S. EPA 1983). BOD concentrations were sufficient to sustain prolific biofilm growth

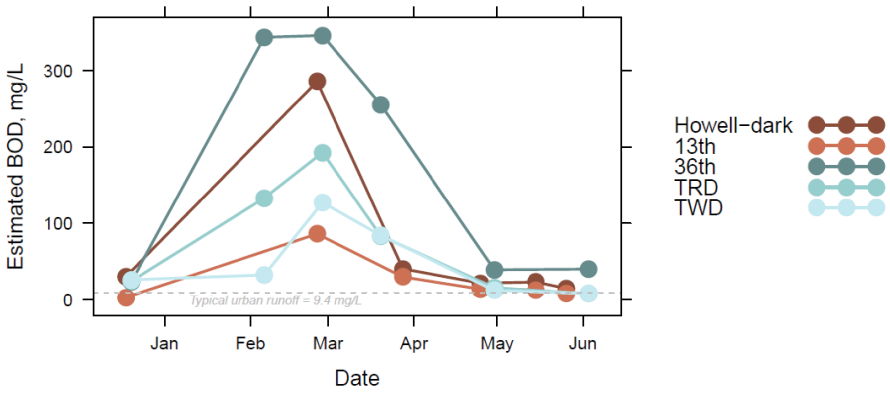


Figure 5-24. Estimated BOD concentrations in collected water quality samples.

at these transects (Figure 5-22) and at all biofilm sites on Wilson Park Creek and the unnamed tributary (Figure 5-1), in general.

Although there did not appear to be quantitative differences in biofilm growth at different depths, anecdotal differences were noted during sampling. For example, the structure of the biofilm appeared compacted in shallower areas with high velocities; biofilms in deeper, slower sections of streams tended to be less densely packed. This is consistent with observations made by Buckingham-Meyer et al. (2007). Additionally, in natural (i.e., non-concrete lined) sections of streams, the deepest parts of the streambed were typically composed of smaller grain particles (i.e., sand and finer). Biofilm was able to grow on these particles, but was more ephemeral than biofilm growing on nearby rocks. This was presumably due to the tendency of such particles to move during higher flows, dislodging the attached biofilm.

5.4 Summary of Hypothesis Testing Results

The conclusions of the hypothesis testing are summarized as follows:

- Hypothesis 1 was rejected. Biofilm growth was not noticeably affected by the availability or intensity of sunlight.
- Hypothesis 2/4 (a) was rejected while (b) was accepted. Heterotrophic biofilm growth did not exhibit P limitation at concentrations in the range 0.005 to 0.025 mg-P/L or less, but growth was observed to be correlated with C:P and C:N ratios.
- Hypothesis 3 was rejected. Biofilm growth was not noticeably affected by stream depth, within the range studied.

Although the results indicate no notable effect of light and phosphorus on biofilm accumulation, they do provide evidence that biofilm community composition is affected by light and relative availability of P. With regard to the underlying issue of biofilms growing in streams that receive airport runoff containing deicers, it is especially noteworthy that P limiting conditions appear to favor the growth of more noxious filamentous forms of biofilm. Unfortunately, neither the laboratory results nor the biofilm model can describe how variable substrate and macronutrient availability might result in different types of heterotrophic bacteria (particularly filamentous bacteria) dominating the biofilm community.

The laboratory results and biofilm model also suggest that biofilms are less subject to N limitation due to the recycling of nitrogen from release during decay of bacteria inside the biofilm. This observation may explain the prolific growth of stream biofilms under apparently nutrient limited conditions.

5.5 Concluding Observations

Previous research has shown readily biodegradable organic matter, as reflected in COD and BOD concentrations, to be the most influential factor affecting biofilm growth (Boualam et al. 2002; Characklis and Marshall 1990). The potential influence of this factor was investigated using additional data collected by the USGS at a secondary monitoring location (KK) established on the Kinnickinnic River, 3.6 km downstream from the confluence with Wilson Park Creek (Figure 4-1[a]). This site has larger streamflows (Table 5-1), but the channel is otherwise similar in depth, velocity, and light availability to the monitoring locations upstream on Wilson Park Creek. Biofilm measurements were collected along a single transect at this site during the final two biofilm monitoring trips, and no heterotrophic biofilms were observed during either trip. Observed COD concentrations at this location followed the same general temporal trends as concentrations at the Wilson Park Creek monitoring locations, but concentrations at KK were

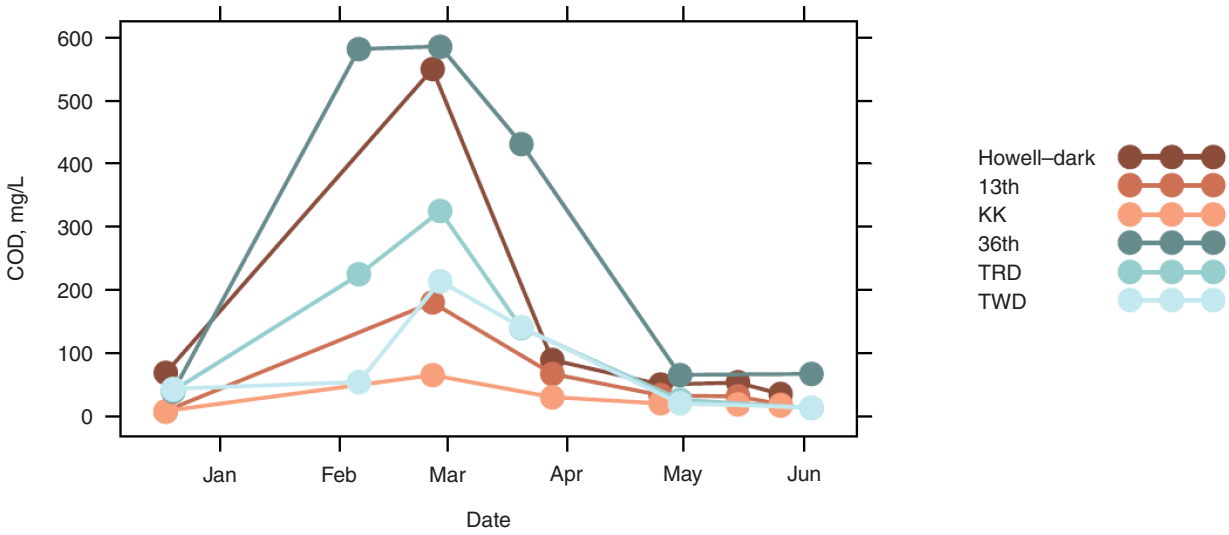


Figure 5-25. COD concentrations in collected water quality samples.

consistently lower. COD concentrations at KK ranged from less than 8 to 65, with a median concentration of 19.1 mg/L (Figure 5-25). COD concentrations were next lowest upstream at 13th, where COD concentrations ranged from less than 8 to 180, with a median concentration of 32 mg/L. Prolific biofilm growth was observed at 13th (Figure 5-1).

As part of other concurrent water quality studies occurring at MKE, flow-weighted composite samples for COD were collected by the USGS throughout the 2012–2013 deicing season at the Outfall 7 site (Table 5-1), yielding a quasi-continuous record of COD concentrations. These flow-composite data show COD concentrations varying substantially within short time periods (Figure 5-26[a]). As noted above, the point samples collected for this study simply yield a snapshot of COD concentrations. To get a more complete understanding of COD concentrations, available flow-composite COD concentrations were used, together with knowledge of flow and relative drainage area differences between monitoring locations, to estimate COD concentrations at downstream biofilm monitoring locations (Figure 5-26). Medians of estimated COD

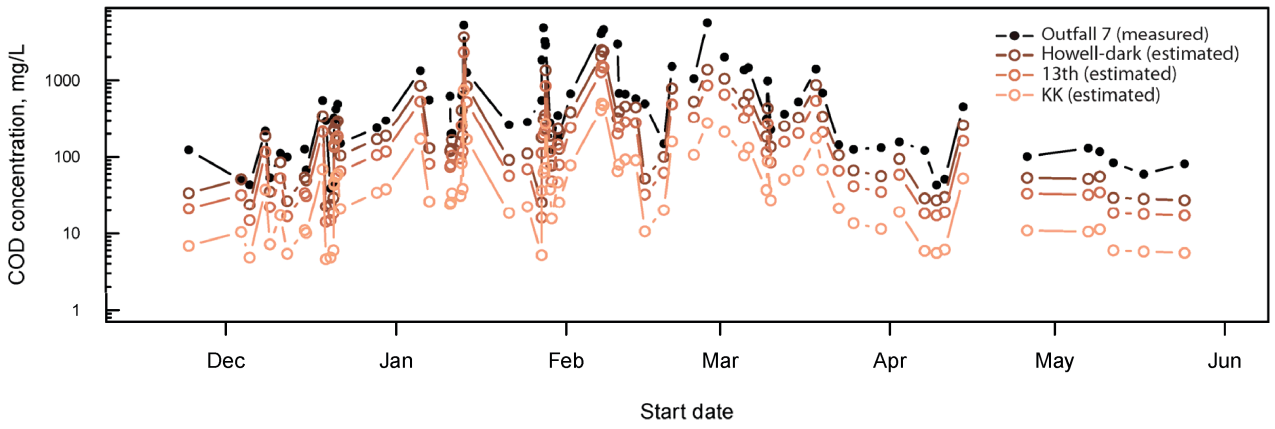


Figure 5-26. Flow-composite COD concentrations from Outfall 7, with estimated flow-composite COD concentrations at downstream monitoring locations. ([a] Flow-composite COD concentrations at Outfall 7 and estimated flow-composite COD concentrations based on measured concentrations in point samples at [b] Howell-dark, [c] 13th, and [d] KK.)

(continued on next page)

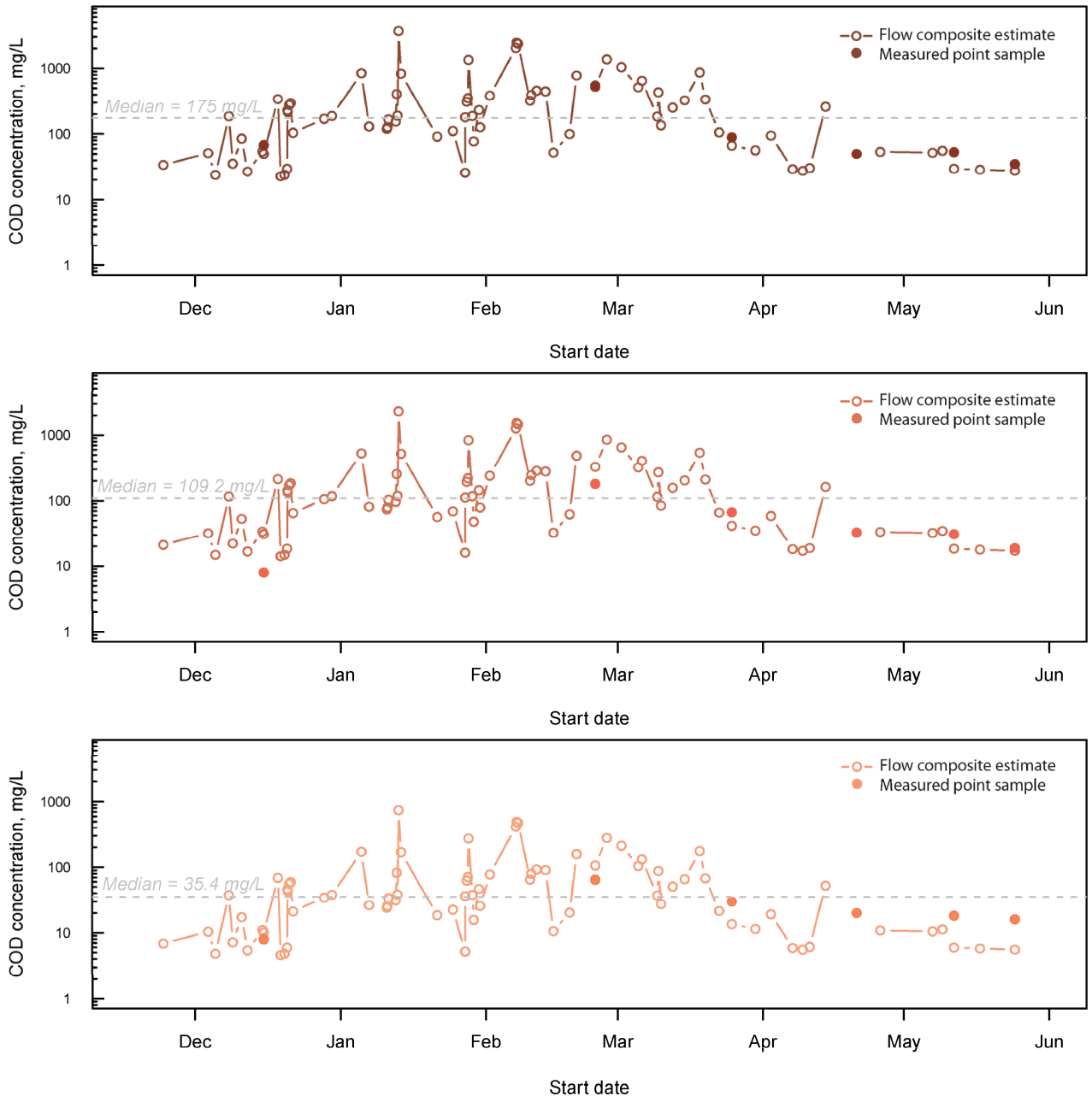


Figure 5-26. (Continued).

concentrations at downstream monitoring locations decreased from upstream to downstream from 175.0 to 109.2 to 35.4 mg/L at Howell-dark, 13th, and KK, respectively. Results of the flow-composite samples at the Outfall 7 site and estimated concentrations at the downstream sites indicate much larger fluctuations in concentration than the monthly samples collected during biofilm sampling. Given that the samples collected during biofilm sampling were grab samples at one point in time, and the high-frequency concentration estimates are based on longer term composite samples (up to 1 week), a precise comparison is not possible. Nonetheless, results from samples collected during biofilm sampling (solid circles in Figure 5-26) indicate that the estimation of high-frequency concentrations (open circles) provides reasonable results.

CHAPTER 6

Work Plan for Future Research

This chapter presents the research team's suggestions for future research to build on the information and insights gained through the current investigations. The suggestions include scopes of work and estimated costs for research in the field and laboratory, as well as further modeling tool development and application.

It should be noted that these suggestions are not presented with any assumptions of appropriateness for ACRP or any other specific research organization or entity. Instead, they reflect the research team's opinions of knowledge and information gaps that need to be addressed in the future.

6.1 Field Investigations

In-stream concentrations of readily biodegradable organic material, as reflected in COD or BOD, appear to be the dominant factor for biofilm proliferation and accumulation. Quantitative understanding of this relationship is currently lacking. This lack of understanding prevents the establishment of confident design criteria for deicing runoff systems where controlling or eliminating problematic conditions associated with stream biofilms is a requirement.

The field and laboratory results of this study suggest shifts in biofilm community structure with changing nutrient conditions. The literature supports the idea that prolific biofilms that arise following an input of high levels of organic C via deicer fluids represent an ecological shift to copiotrophic (that is, organisms that tend to grow in organic-rich environments) conditions (Upton and Nedwell 1989). What is less clear is how those copiotrophs respond when levels of organic C are reduced to background levels, which under most surface water conditions would be considered oligotrophic. As noted, there is evidence of biofilms storing nutrients, including C. Thus, research is needed that documents the change in bacterial diversity as organic C levels in the water column are increased and decreased. A specific question of interest is whether the time between C inputs is long enough for the populations to shift back and forth. This shift would be airport and season specific. The results of this research would provide a better basis upon which to predict the response of stream biofilms to reduced deicer inputs.

To better understand the relationship between COD concentrations and biofilm development, it is suggested that additional field surveys be conducted during transition periods when benthic communities are changing to and from a condition of heterotrophic and copiotrophic dominance. Monitoring during these periods will provide insight into potential threshold concentrations influencing biofilm community composition. Selected sites should be sampled to expand the existing data set and support evaluation of community changes associated with changing in-stream COD concentrations on both spatial (i.e., upstream to downstream) and temporal (i.e., transition to and from heterotrophic and copiotrophic biofilm dominance) scales.

Suggested field research consists of biofilm surveys and the collection of water quality samples, as follows:

- Biofilm surveys should be conducted at three monitoring locations in two receiving streams during the deicing season. Surveys should be conducted at each site every other week over two 5-week monitoring periods: one in the late fall/early deicing season and one in the early spring/late deicing season, for a total of six biofilm surveys at each site.
- Water quality samples should be collected at the upstream and downstream sites on each stream. A total of eight sets of samples should be collected: one set during each of the biofilm monitoring trips as well as an additional set 2 weeks before each biofilm monitoring period. In addition, two field quality control samples (one blank and one replicate) should be collected at each airport. Water quality constituents should include COD, total phosphorus, orthophosphorus, nitrate + nitrite, Kjeldahl nitrogen, and ammonia.
- Conducting these investigations at the existing streams at MKE and GRR would be advantageous because data collected during those studies would be available to support the research.
- The results of the field investigations should be analyzed to characterize the extent and magnitude of biofilm communities that develop at each of the stream stations under different seasonal COD conditions. To the extent possible, tests for statistically significant differences between the stations should be conducted. A technical memorandum should be prepared describing how the experiments were conducted, the results of the data analysis, and the conclusions drawn. Suggestions for further research should be included, as appropriate.
- Estimated cost for this research is $137,000. This cost includes data analysis and preparation of a simple technical memorandum.
- The research could be completed over a 1-year period, beginning in September to ensure capturing a full deicing season.

6.2 Laboratory Studies

Task 5 laboratory experimental work determined that phosphorus availability may have a unique ability to influence not only the extent of biofilm accumulation in streams receiving airport runoff, but also the bacterial composition of these biofilms. In Task 5 laboratory work, the organisms that became dominant under low P condition were filamentous bacteria, similar to *Sphaerotilus natans* in colony morphology and overall biofilm appearance. Efforts to limit P as a means of controlling biofilm should take this into consideration as *S. natans* is often perceived to be an undesirable component of stream biofilms. Further laboratory work is therefore needed to better understand the connection between P availability and biofilm composition.

Suggested future laboratory research consists of experiments run in 4 continuous stirred tank biofilm reactors, similar to those used in Task 5. The experiments could be conducted as follows:

- Each reactor should be inoculated with a culture derived from airport runoff streams, similar to the culture used in Task 5.
- C and N in the reactors should be maintained at levels that are adequate for microbial growth, but P levels in each of the four reactors should be set at 2.5 μg/L, 5 μg/L, 10 μg/L, and 25 μg/L.
- The experiments should be run for 6 weeks, with biofilm sampling performed weekly. Biofilm assays should include those performed in Task 5 (HPC, fungi, and chlorophyll *a*) as well as molecular analyses to differentiate between bacterial species.

The results of the laboratory experiments should be analyzed to characterize the microbial communities that develop under each of the conditions of P availability, with tests for significant differences between the four levels. A technical memorandum should be prepared describing

how the experiments were conducted, the results of the data analysis, and the conclusions drawn. Suggestions for further research should be included, as appropriate.

The research could be conducted over a period of approximately 5 months, at an estimated cost of $50,000.

6.3 Modeling Tool Refinement and Application

A change in biofilm appearance was observed in the laboratory experiments with fluctuating availability of readily biodegradable organic material. The biofilm model applied during this study considers one type (i.e., profile) of heterotrophic organism that is primarily responsible for the degradation of readily biodegradable organic material. The model can be expanded to account for two or more types of carbon-degrading organisms. In doing so, the competition between organisms that result in the apparent difference in biofilm structure may be evaluated. Future research is suggested to expand the model to define relevant processes and state variables, kinetic expressions, conversion factors, stoichiometric relationships, and diffusivity coefficients. The following steps could be pursued to accomplish this:

- Conceptualize processes and state variables (e.g., process 1 consumes readily biodegradable organic matter [S_S], oxygen [S_{O2}], and macronutrients, and produces heterotrophic organisms [X_H]).
- Develop kinetic expressions by operating batch-scale reactors to verify the rate of substrate conversion. This might be done in conjunction with the work described under Section 6.2.
- Develop stoichiometric relationships through an energetic analysis (i.e., counting electrons).
- Calculate diffusivity coefficients for relevant materials in clean water.

Biofilm model calibration and validation requires bulk-liquid chemical analyses. It is suggested that future studies include chemical analyses (e.g., COD, TN, NH_3-N, NO_2-N, NO_3-N, SRP, and TP) of bioreactor influent and effluent streams. More sophisticated analytical methods, such as florescent in situ hybridization (FISH) and quantitative polymerase chain reaction (qPCR) can be applied to evaluate biofilm mass, and identify the genera and relative abundance of bacteria inside a biofilm. It is suggested that these methods be used to quantify biofilm biomass in terms of type of bacteria inside the biofilm, and their relative location and abundance. With this information, the model may be used to provide a quantitative description of biofilm response to water quality conditions.

The suggested future research affiliated with modeling includes the following:

1. Conceptualize an expanded model that accounts for two types of competing heterotrophic organisms that thrive or are suppressed under varying organic loads typical of steams that receive deicer-laden runoff from airfields.
2. Conduct chemical analyses on all laboratory experiments (e.g., COD, TN, NH_3-N, NO_2-N, NO_3-N, SRP, and TP).
3. Evaluate all biofilm samples using FISH and qPCR.

The research could be conducted over a period of 1 year, at an estimated cost of $100,000.

References

Andersson, B., H. Aspegren, U. Nyberg, J. la Cour Jansen, and H. Ødegaard. 1998. Increasing the capacity of an extended nutrient removal plant by using different techniques. *Water Science and Technology*, 37(9). 175–183.

Bannerman, R. T., Legg, A. D., Greb, S. R. (1996). Quality of Wisconsin stormwater, 1989–94. OFR 96-458. U.S. Geological Survey, Reston, VA, pp. 10–16.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and Stribling, J. B. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers—Periphyton, Benthic Macroinvertebrates, and Fish, 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Battin, T. J., Kaplan, L. A., Denis Newbold, J., Cheng, X., Hansen, C. 2003. Effects of current velocity on the nascent architecture of stream microbial biofilms. *App. Environ. Microbiol.* (69)9. 5443–5452.

Blenkinsopp, S. A., and Lock, M. A. (1994). The impact of storm-flow on river biofilm architecture. *J. Phycol.* (30). 807–818.

Boltz, J. P., Morgenroth, E., Daigger, G. T., deBarbadillo, C., Murthy, S., Sørensen, K. H., and Stinson, B. 2012. Method to identify potential phosphorus rate-limiting conditions in post-denitrification biofilm reactors within systems designed for simultaneous low-level effluent nitrogen and phosphorus concentrations. *Water Research.* 46(19). 6228–38.

Boualam, Marc, Fabienne Quilès, Laurence Mathieu, and Jean-Claude Block. 2002. Monitoring the Effect of Organic Matter on Biofilm Growth in Low Nutritive Waters by ATR/FT-IR Spectroscopy, *Biofouling: The Journal of Bioadhesion and Biofilm Research.* 18:1, 73–81.

Brune, D. E., and Novak, J. T. 1981. The use of carbonate equilibrium chemistry in quantifying algal carbon uptake kinetics. *J. App. Microbiol. and Biotechnol.* (13) 71–76.

Buckingham-Meyer, K., Goeres, D. M., and Hamilton, M. A. 2007. Comparative evaluation of biofilm efficacy tests. *Journal of Microbiological Methods.* 70(2), 236–244.

Callow, M. E. 2000. Algal Biofilms. In *Biofilms: Recent Advances in Their Study and Control.* Amsterdam: Harwood Academic. pp. 196–218.

Chapman, D. 2010. Don Chapman, Environmental/Safety Manager, Cincinnati Northern Kentucky International Airport. Personal communications.

Characklis, W. G., and Marshall, K. C. 1990. *Biofilms.* Wiley, New York, NY.

Correll, D. L. 1999. Phosphorus: a rate-limiting nutrient in surface waters. *Poultry Science.* (78) pp. 674–682.

Coveney, M. F., and Wetzel, R. G. 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. *App. Environ. Microbiol.* (58) 150–156.

deBarbadillo. C., Rectanus, R., Canham, R., and Schauer, P. 2006. Tertiary denitrification and very low phosphorus limits: a practical look at phosphorus initiations on denitrification filters. *Proc., Water Environment Federation Technical Exhibition and Conference* (WEFTEC), Dallas, Texas, Water Environmental Federation: Alexandria, VA.

Felip, M., Pace, M. L., and Cole, J. J. (1996) Regulation of planktonic bacteria; growth rates: the effects of temperature and resources. *Microb. Ecol.* (31) 15–28.

Flora, J. R., Suidan, M. T., Biswas, P., and Sayles, G. D. 1994. Modeling Substrate Transport into Biofilms: Role of Multiple Ions and pH Effects. *ASCE JEE.* 119(5). 908–930.

Flora, J. R., Suidan, M. T., Biswas, P., and Sayles, G. D. 1995. Modeling Algal Biofilms: Role of Carbon, Light, Cell Surface Charge, and Ionic Species. *Wat. Env. Res.* 67. 87–94.

Gaudy, E., and R. S. Wolfe. 1961. Factors affecting filamentous growth of *Sphaerotilus natans*, *Appl Microbiol.*, 9(6):580–584.

Gessner, M. O. 1997. Fungal biomass, production, and sporulation associated with particulate organic matter in streams. *Limnetica*, 13(2):33–44.

Gray, R. 2006. Personal communication. Truckee Meadows WWTP Denitrification Process Operating Issues. May 31 and June 6.

Gray, R., Brisbin, M., Jones, S., Hutchinson, G., Howell, D., and Bruketta, D. 2008. Manager's Perspective on Separate Stage Fixed Film Nitrification and Denitrification Processes at the Truckee Meadows Water Reclamation Facility. *Proc., Water Environment Federation Technical Exhibition and Conference (WEFTEC) Workshop 101: WEF/WERF Demonstrated Processes for Limit of Technology Nutrient Removal—Achievable Limits and Statistical Reliability*, Chicago, Illinois. Water Environment Federation: Alexandria, VA.

Grover, J. P. 1989. Phosphorus-dependent growth kinetics of 11 species of freshwater algae. *Limnol. Oceanogr.* (34) 341–348.

Hill, W. R. and S. E. Fanta. 2009. Quantifying phosphorus and light effects in stream algae, *Limnol. Oceanogr.* 54(1) 368–380.

Hultman, B., Jonsson, K., and Plaza, E. 1994. Combined nitrogen and phosphorus removal in a full-scale continuous upflow sand filter. *Water Sci. Technol.*, 29 (10–11), 127–134.

Husband, J., and Becker, E. 2007. Demonstration testing of denitrification effluent filters to achieve limit of technology for total nitrogen and phosphorus. *Proc., Nutrient Removal Specialty Conference*, International Water Association, Baltimore, Maryland. Water Environment Federation: Alexandria, VA.

Jonsson, K. 1998. Experiences of nitrogen and phosphorus removal in deep-bed filters at Hendriksdal Sewage Works in Stockholm. *Water Sci. Technol.*, 37, 193–200.

Jonsson, K., Plaza, E., and Hultman, B. 1997. Experiences of nitrogen and phosphorus removal in deep-bed filters in the Stockholm area. *Water Sci. Technol.*, 36, 183–190.

Kelly, D. J., Bothwell, M. L., and Schindler, D. W. 2003. Effects of solar ultraviolet radiation on stream benthic communities. *Ecology.* 84(10). 2724–2740.

Kirchman, D. L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. *Microbial Ecology.* (28) 255–271.

Lear, G., S. J. Turner, and G. D. Lewis. 2009. Effect of light regimes on the utilization of an exogenous carbon source by freshwater biofilm bacterial communities. *Aquat. Ecol.* 43. 207–220.

Liehr, S. K., J. W. Eheart, and M. T. Suidan. 1988. A Modeling Study of the Effect of pH on Carbon Limited Algal Biofilms. *Water Research.* 22(8): 1033–41. DOI:10.1016/0043-1354(88)90151-0.

Liehr, S. K., M. T. Suidan, and J. W. Eheart. 1989. Effect of Concentration Boundary Layer on Carbon Limited Algal Biofilms. *Journal of Environmental Engineering.* 115(2): 320. DOI:10.1061/(ASCE)0733-9372(1989)115:2(320).

Liehr, S. K., M. T. Suidan, and J. W. Eheart. 1990. A Modeling Study of Carbon and Light Limitation in Algal Biofilms. *Biotechnology and Bioengineering.* 35(3): 233–43. DOI:10.1002/bit.260350304.

LimnoTech, Inc. 2013. "Draft December 2012 Updated Evaluation of Relative Contribution of Dry Weather Flow from GFIA to the Unnamed Tributary of the Thornapple River and Assessment of Potential Impacts of Discharge Removal." LimnoTech, Ann Arbor, MI.

Lock, M. A., and John, P. H. 1979. The effect of flow patterns on uptake of phosphorus by river periphyton. *Limnol. Oceanogr.* (24)2. pp. 376–383.

Metcalf and Eddy. 2003. *Wastewater Engineering: Treatment and Reuse*, Tchobanoglous, G., Burton, F. L., Stensel, H. D. (Eds.). McGraw-Hill. New York, NY.

Mohamed, M. N., J. R. Lawrence, and R. D. Robarts. 1998. Phosphorus Limitation of Heterotrophic Biofilms from the Fraser River, British Columbia, and the Effect of Pulp Mill Effluent. *Microb. Ecol.* 37: 121–30.

Morris, D. P., and Lewis, Jr., W. M. 1992. Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. *Limno. Oceanogr.* 37(6). 1179–1192.

Nordeidet, B., Rusten, R., and Ødegaard, H. 1994. Phosphorus requirements for tertiary nitrification in a biofilm. *Wat. Sci. Technol.* (29) 10-11. pp. 77–82.

Ördög, V., W. A. Stirk, P. Balint, J. van Staden, and C. Lovasz. 2012. Changes in lipid, protein and pigment concentrations in nitrogen-stressed *Chlorella minutissima* cultures, *J. Appl. Phycol.*, 24(4). 907–914.

Peric, M., Neupane, D., Stinson, B., Locke, E., Kharkar, S., Passarelli, N., Sultan, M., Shih, G., Murthy, S., Bailey, W., Carr, J., and Der Minassian, R. 2009. Phosphorus requirements in a post-denitrification MBBR at a combined limit of technology nitrogen and phosphorus plant. *Proc., Nutrient Conference of Water Environment Federation*, 2009, Washington, D.C. Water Environment Federation: Alexandria, VA.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. *Bio. Sci.* (24) 499–504.

Redfield, A. C. 1958. The Biological Control of Chemical Factors in the Environment, *American Scientist* 46(3). 205–221.

Reichert, P. 1998. Aquasim 2.0–User Manual. Computer program for the identification and simulation of aquatic systems. Swiss Federal Institute for Environmental Science and Technology (EAWAG). CH 8600 Dübendorf, Switzerland.

Rier, S. T., and R. J. Stevenson. 2006. Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia* 561:131–147.

Romani, A. M., and Sabater, S. 1999. Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology.* 41. 729–736.

Romani, A. M., and Sabater, S. 2000. Influence of algal biomass on extracellular enzyme activity in river biofilms. *Microbial Ecology.* (41) pp. 16–24.

Sagberg, P., Ryrfors, P., and Berg, K. G. 2006. 10 years of operation of an integrated nutrient removal treatment plant: ups and downs, background and water treatment. *Water Sci. Technol.*, 53 (12), 83–90.

Schallenberg, M., and Kalff, J. 1993. Sediment bacteria in lakes and other aquatic systems. *Ecology.* (74) 919–934.

Scherrenberg, S. M., Menkveld, H. W. H., Bechger, M., and van der Graaf, J. H. J. M. 2009. Phosphorus and nitrogen profile measurements to locate phosphorus limitation in a fixed bed filter. *Water Sci. Technol.*, 60(10). 2537–2544.

Scherrenberg, S. M., van Nieuwenhuijzen, A. F., den Elzen, J. J. M., van den Berg van Saparoea, F. H., Malsch, A., and van der Graaf, J. H. J. M. 2008. Aiming at complete nitrogen and phosphorus removal from WWTP effluent—the limits of technology. *Proc., 81st Annual Water Environment Federation Technical Exposition and Conference*, Chicago, Illinois. Water Environment Federation: Alexandria, VA.

Steele, J. H. 1962. Environmental control of photosynthesis in the sea. *Limnol. Oceanogr.* 7:137–150.

Stevenson, R. J., and L. L. Bahls. 1999. Periphyton Protocols. In Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish (M. T. Barbour et al. eds.), 2nd ed. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C., p. 6-1–6-22.

Stevenson, R. J., and S. L. Rollins. 2007. Ecological Assessments with Benthic Algae. In *Methods in Stream Ecology* (F. R. Hauer and G. A. Lamberti, eds.), 2nd ed. Academic Press, Burlington, MA. pp. 785–803.

Tibbles, B. J., Davis, C. L., Harris, J. M., and Lucas, M. I. 1992. Estimates of bacterial productivity in marine sediments and water from a temperate salt marsh lagoon. *Microb. Ecol.* (23) 195–209.

Toolan, T., Wehr, J. D., and Findlay, S. 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-autotrophic lake. *Appl. Environ. Microbiol.* (57) 2074–2078.

Upton A. C., and Nedwell, D. B. 1989. Nutritional flexibility of oligotrophic and copiotrophic Antarctic bacteria with respect to organic substrates. *Fems Microbiology Ecology.* 62(1): 1–6.

U.S. Environmental Protection Agency (EPA). 1983. Results of the Nationwide Urban Runoff Program: Volume 1—Final Report. EPA-832-R-83-112. U.S. Environmental Protection Agency, Washington, D.C.

U.S. Environmental Protection Agency (EPA). 2012. Environmental Impact and Benefit Assessment for the Final Effluent Limitation Guidelines and Standards for the Airport Deicing Category. EPA-821-R-12-003. U.S. Environmental Protection Agency, Washington, D.C. pp. 3-11–3-13.

U.S. Geological Survey (USGS). 2006. Collection of Water Samples (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A4, September 2006, http://pubs.water.usgs.gov/twri9A4/ (Accessed December 12, 2012).

U.S. Geological Survey. 2013. Water-resources data for the United States, Water Year 2012. U.S. Geological Survey Water-Data Report WDR-US-2012, sites 040871475, 040871488, and 04087159, accessed through http://wdr.water.usgs.gov/wy2012/search.jsp.

Vazquez-Burney, R., Johnson, B. R., Daigger, G. T., Bays, J., and Boltz, J. P. 2009. Variables, processes, and transformation-rate expressions describing periphytic algae-based biofilms: development of the algal biofilm model (ABM). *Proc., 82nd Annual Water Environment Federation Technical Exhibition and Conference* (WEFTEC), Water Environmental Federation, Orlando, FL.

Vrede, K., T. Vrede, A. Isaksson, and A. Karlsson. 1999. Effects of nutrients (phosphorous, nitrogen, and carbon) and zooplankton on bacterioplankton and phytoplankton—a seasonal study. *Limnol. Oceanogr.*, 44(7). 1616–1624.

Wang, L., Miller, T. D., and Priscu, J. C. 1992. Bacterioplankton nutrient deficiency in a eutrophic lake. *Arch. Hydrobiol.* (125) 423–439.

White, P. A., Kalff, J., Rasmussen, J. B., and Gasol, J. M. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. *Microb. Ecol.* (21). 99–118.

Wilde, F. D., ed. 2004. Cleaning of Equipment for Water Sampling (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A3, April 2004, http://pubs.water.usgs.gov/twri9A3/ (Accessed December 12, 2012).

Wilde, F. D., ed. Variously dated. Field Measurements: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A6, with Sec. 6.0–6.8, http://pubs.water.usgs.gov/twri9A6 (Accessed December 12, 2012).

Wolf, G., C. Picioreanu, and M. C. M. van Loosdrecht. 2007. Kinetic Modeling of Phototrophic Biofilms: the PHOBIA Model. *Biotechnology and Bioengineering.* 97(5): 1064–79. DOI:10.1002/bit.21306.

APPENDIX A

Acronyms and Abbreviations

°C	degrees Celsius
µg/L	microgram(s) per liter
µm	micrometer
µmol/m^2/s	micromole(s) per square meter per second
6th	6th Street
13th	13th Street
36th	36th Street
ACRP	Airport Cooperative Research Program
ASTM	American Society for Testing and Materials
BAC	biologically activated carbon
BOD	biochemical oxygen demand
BOD_5	5-day biochemical oxygen demand
C	organic carbon
CBE	Center for Biofilm Engineering
CDC	Centers for Disease Control and Prevention
cfs	cubic feet per second
CFSTR	continuous flow stirred tank reactor
CFU/cm^2	colony-forming units per square centimeter(s)
cm	centimeter
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
CVG	Cincinnati/Northern Kentucky International Airport
DSM	Des Moines International Airport
EPS	extracellular polymeric substance
EWI	equal-width-increment
FISH	fluorescent *in situ* hybridization
FNT	Bishop International Airport
g	gram(s)
g/mol	gram(s) per mole
GRR	Gerald R. Ford International Airport
HPC	heterotrophic plate count
I	irradiance
KH_2PO_4	monopotassium phosphate
KK	Kinnickinnic River near 11th Street
km	kilometer(s)
km^2	square kilometer(s)
kmol/m^3	kilomole(s) per cubic meter
KNO_3	potassium nitrate

lx	lux
m^2/m^3	square meter(s) per cubic meter
m^3	cubic meter(s)
mm	millimeter(s)
mg/L	milligram(s) per liter (parts per million)
µg/L	microgram(s) per liter (parts per billion)
MKE	General Mitchell International Airport
mL	milliliter(s)
mol/kmol	mole(s) per kilomole
MTBL	mass-transfer boundary layer
N	nitrogen
NH_3-N	ammonia as nitrogen
NO_2-N	nitrite as nitrogen
NO_3-N	nitrate as nitrogen
NO_X-N	oxidized nitrogen
NPDES	National Pollutant Discharge Elimination System
P	phosphorus
PAR	photosynthetically active radiation
PIT	Pittsburgh International Airport
PO_4	orthophosphate
PO_4-P	orthophosphate as phosphorus
PVD	T. F. Green Airport
®	registered trademark
qPCR	quantitative polymerase chain reaction
RPS	rapid periphyton survey
SRP	soluble reactive phosphorus
™	trade mark
TN	total nitrogen
TP	total phosphorus
TRD	Thornapple River Drive
TSS	total suspended solids
TWD	Tricklewood Drive
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
UV	ultraviolet
WSLH	Wisconsin State Laboratory of Hygiene
YSI®	Yellow Springs, Ohio

APPENDIX B

Biofilm Model Information

Mass Transfer Boundary Layer Thickness

The MTBL thickness, L_L, was estimated from fluid dynamics using a method similar to that described by Boltz, Morgenroth, and Sen (2010):

$$L_L = \frac{L_c}{Sh} \tag{1}$$

where L_c is a characteristic length and *Sh* is the non-dimensional Sherwood number. The following empirical correlation described by Rowe et al. (1965) was used to calculate the Sherwood number:

$$Sh = A + B \cdot Re^m \cdot Sc^n \tag{2}$$

The following empirical parameter values and relationships were applied to estimate L_L.

A = 2.0 (value by Rowe, Claxton, and Lewis [1965] for spherical particles)
B = 0.8 (value by Rowe, Claxton, and Lewis [1965] for spherical particles)
m = ½ (value by Rowe, Claxton, and Lewis [1965] for spherical particles)
n = ⅓ (value by Rowe, Claxton, and Lewis [1965] for spherical particles)
Re = Reynolds number = $(U \cdot L_c)/\nu$
U = water velocity in vicinity of biofilm surface
ν = kinematic viscosity of water = 1.0×10^{-6} m²/s
Sc = Schmidt number = $\nu/D_{W,i}$
$D_{W,i}$ = diffusion coefficient of substance i in water (m² per day)

Temperature dependency of diffusion coefficients was accounted for according to the following:

$$D(T) = D(20°\text{C}) \cdot \frac{273 + T}{273 + 20°\text{C}} \cdot \frac{\mu(20°\text{C})}{\mu(T)} \tag{3}$$

where D is the diffusion coefficient, T the temperature in degrees Celsius (°C), and μ the dynamic viscosity of water in N m^{-2} s.

Biochemical Rate Expressions

The biochemical rate expressions are multiplicative Monod-type expressions except for the simple first-order lysis rate(s). Wolf, Picioreanu, and van Loosdrecht (2007) selected a maximum value of each saturation function under the claim that simulated activity was too low when using multiplicative Monod-type equations. Light limitation is characterized by saturation-type kinetics

at low-light intensities and by inhibition kinetics at high-light intensities. The most commonly used model describing photoinhibition is the Steele relationship (Steele 1962), Equation (1).

$$f_L = \frac{I}{I_s} e^{\left(I - \frac{I}{I_s}\right)} \tag{4}$$

Here, f_L is the fractional reduction in phototrophic biomass growth rate due to light limitation, I_S is the saturation light intensity ($\mu E\ L^{-2}\ T^{-1}$), and I is the light intensity ($\mu E\ L^{-2}\ T^{-1}$). Wolf, Picioreanu, and van Loosdrecht (2007) used the Eilers-Peeters relationship to describe light dependency in an algae biofilm. Light attenuation across the biofilm was modeled using the Beer-Lambert law, Equation (5).

$$I = I_0 \cdot e^{-\gamma \cdot z} \tag{5}$$

Here, I_0 is the light intensity at the surface of the biofilm ($\mu E\ L^{-2}\ T^{-1}$), γ is the extinction coefficient (L^{-1}), and z is the spatial dimension in the algae biofilm normal to the growth medium.

Chemical Processes

Chemical conversion processes in the modified PHOBIA model include acid-base equilibria to calculate biofilm pH profiles, speciation, and to determine inorganic carbon availability for the growth of phototrophic and chemoautotrophic organisms. Table B-1 (after Wolf, Picioreanu, and van Loosdrecht 2007) is the chemical processes, stoichiometric, and rate-expression matrix. The charge balance used to calculate the concentration of protons was also presented by Wolf, Picioreanu, and van Loosdrecht (2007).

The equations are summarized as follows. Equation (6) is the charge balance.

$$S_H + S_{NH_4} + \underbrace{S_{cat}}_{S_{Na^+}, S_{Mg^{2+}}} - S_{HCO_3} - S_{NO_3} - 2 \cdot S_{CO_3} - S_{OH} - \underbrace{S_{an}}_{S_{Cl^-}, S_{SO_4^{2-}}} = 0 \tag{6}$$

Equation (6) can be rearranged as Equation (7).

$$\left(S_{NH_4} + \underbrace{S_{cat}}_{S_{Na^+}, S_{Mg^{2+}}} - S_{HCO_3} - S_{NO_3} - 2 \cdot S_{CO_3} - \underbrace{S_{an}}_{S_{Cl^-}, S_{SO_4^{2-}}}\right) = \sum_i S_{i,\,ion} \tag{7}$$

The pH is calculated with Equation (8).

$$S_{OH} = \frac{K_{a,H_2O}}{S_H} \tag{8}$$

Substitute Equations (7) and (8) into Equation (6) and rearrange results in Equation (9) for the calculation of pH.

$$S_H = 0.5 \cdot \left(\sqrt{\left(\sum_i S_{i,ion} \right)^2 + 4 \cdot K_{a,H_2O}} - \sum_i S_{i,ion} \right) \tag{9}$$

Model Variables and Parameters

A complete list of state variables, stoichiometric parameters, kinetic parameters, biofilm parameters, and transformation rate expressions used in the model is provided in Tables B-2 through B-8.

Table B-1. Chemical processes, stoichiometric, and rate-expression matrix (after Wolf et al. 2007).

Index i	Soluble Ions					Rate
	S_{NH3} mol m^{-3}	S_{NH4} mol m^{-3}	S_{CO2} mol m^{-3}	S_{HCO3} mol m^{-3}	S_{CO3} mol m^{-3}	
Dissociation HCO_3^-				-1	1	$k_{AB,HCO_3/CO_3} \cdot \left(S_{HCO_3} - S_H \cdot \frac{S_{CO_3}}{K_{a,HCO_3/CO_3}} \right)$
Hydration CO_2			-1	1		$k_{AB,CO_2/H_2O} \cdot \left(S_{CO_2} - S_{HCO_3} \cdot \frac{S_H}{K_{a,CO_2/H_2O}} \right)$
Hydration CO_2 (relevant for pH >10)			-1	1		$k_{AB,CO_2/OH} \cdot \left(S_{CO_2} \cdot \frac{K_{a,H_2O}}{S_H} - \frac{S_{HCO_3}}{K_{a,CO_2/OH}} \right)$
Dissociation NH_3	1	-1				$k_{AB,NH_3/NH_4} \cdot \left(S_{NH_4} - S_H \cdot \frac{S_{NH_3}}{K_{a,NH_3/NH_4}} \right)$

Table B-2. State variables for the modified PHOBIA without storage. Unless otherwise noted, values are from Wolf et al. (2007).

Index i	Variable	Definition	Units
		Soluble Components	
1	S_{CO2}	Carbon Dioxide (ion)	mol m^{-3}
2	S_{HCO3}	Bicarbonate (ion)	mol m^{-3}
3	S_{CO3}	Carbonate (ion)	mol m^{-3}
4	S_{NH4}	Ammonium (ion)	mol m^{-3}
5	S_{NH3}	Ammonia	g COD m^{-3}
6	S_{NO3}	Nitrate (ion)	mol m^{-3}
7	S_H	Hydrogen (ion)	mol m^{-3}
8	S_{an}	Anions	mol m^{-3}
9	S_{cat}	Cations	mol m^{-3}
10	S_{O2}	Dissolved Oxygen	g COD m^{-3}
11	S_{PO4}	Soluble Reactive Phosphorus	(mol or g COD) m^{-3}
12	S_S	Readily Biodegradable Organic Compounds	g COD m^{-3}
13	S_I	Inert Soluble Organic Compounds	g COD m^{-3}
14	$S_{I,PS}$	Inert Soluble Organic Compounds from Inactivation of Phototrophs	g COD m^{-3}
		Particulate Components	
15	X_S	Slowly Biodegradable Substrates	g COD m^{-3}
16	X_H	Heterotrophic Biomass	g COD m^{-3}
17	X_A	Autotrophic Nitrifying Biomass	g COD m^{-3}
18	X_{PS}	Phototrophic Biomass	g COD m^{-3}
19	X_I	Inert Organic Compounds	g COD m^{-3}
20	X_{EPS}	Extracellular Polymeric Substances	g COD m^{-3}
21	X_{EPSI}	Inert Extracellular Polymeric Substances	g COD m^{-3}
22	X_{PG}	Internally Stored Polyglucose	g COD m^{-3}

Table B-3. Stoichiometric parameter values for the modified PHOBIA. Unless otherwise noted, values are from Wolf et al. (2007).

Symbol	Description	Value	Unit
	Conversion factors		
Nitrogen:			
	Soluble Material		
i_{NSI}	Nitrogen content of inert soluble COD, S_I	0.01	g N g^{-1} COD
i_{NSS}	Nitrogen content of readily biodegradable substrate, S_S	0.03	g N g^{-1} COD
Particulate Material			
i_{NXI}	Nitrogen content of inert particulate COD, X_I	0.02	g N g^{-1} COD
i_{NXS}	Nitrogen content of slowly biodegradable substrate, X_S	0.04	g N g^{-1} COD
i_{NBM}	Nitrogen content of biomass, X_H, X_A, X_{M1}, X_{M2}	0.07	g N g^{-1} COD
Total Suspended Solids:			
i_{TSSXI}	TSS to COD ratio for X_I	0.75	g TSS g^{-1} COD
i_{TSSXS}	TSS to COD ratio for X_S	0.75	g TSS g^{-1} COD
i_{TSSBM}	TSS to COD ratio for biomass, X_H, X_A, X_{M1}, X_{M2}	0.90	g TSS g^{-1} COD
	Stoichiometric parameters		
Hydrolysis			
f_{SI}	Production of S_I in hydrolysis	0.1	g COD g^{-1} COD
Heterotrophic biomass			
$Y_{H,O2}$	Yield of heterotrophs using oxygen	0.63	g COD g^{-1} COD
$Y_{H,NO}$	Yield of heterotrophs using nitrate	0.54	g COD g^{-1} COD
f_{XI}	Production of X_I in endogenous respiration	0.2	g COD g^{-1} COD
Autotrophic biomass			
Y_A	Yield of autotrophs	0.24	g COD g^{-1} COD
f_{XI}	Production of X_I in endogenous respiration	0.2	g COD g^{-1} COD
Phototrophic biomass			
$Y_{O2/e-}$	Yield of oxygen produced per electron transferred	DEFINE	g COD g^{-1} COD
f_{XI}	Production of X_I in endogenous respiration	0.2	g COD g^{-1} COD

Table B-4. Kinetic parameter values (at 20°C) for the modified PHOBIA. Unless otherwise noted, values are from Wolf et al. (2007).

Symbol	Description	Value	Unit	θ
Hydrolysis of particulate substrates: X_S				
k_h	Hydrolysis rate constant	3.00	d^{-1}	1.041
K_X	Hydrolysis saturation constant	1.00	g X_S g^{-1} X_H	-
Heterotrophic organisms: X_H				
μ_H	Maximum growth rate on substrate	6.00	d^{-1}	1.072
$\eta_{NO3,H}$	Reduction factor for denitrification	0.80	-	-
$b_{H,O2}$	Aerobic endogenous respiration rate of X_H	0.20	d^{-1}	1.072
$b_{H,NO}$	Anoxic endogenous respiration rate of X_H	0.10	d^{-1}	1.072
$K_{O2,H}$	Saturation/inhibition coefficient for oxygen	0.10	g O_2 m^{-3}	-
K_S	Saturation coefficient for growth on S_S	4.00	g COD m^{-3}	-
$K_{NO3,H}$	Saturation/inhibition coefficient for nitrate	0.14	g N m^{-3}	-
$K_{NH4,H}$	Saturation coefficient for ammonium (nutrient)	0.01	g N m^{-3}	-
$K_{ALK,H}$	Saturation coefficient for alkalinity (HCO_3^-)	0.10	mole HCO_3^- m^{-3}	-
Nitrifying (autotrophic) organisms: X_A				
μ_A	Maximum growth rate of X_A	1.00	d^{-1}	1.111
$b_{A,O2}$	Aerobic endogenous respiration rate of X_A	0.15	d^{-1}	1.116
$b_{A,NO}$	Anoxic endogenous respiration rate of X_A	0.05	d^{-1}	1.116
$K_{O2,A}$	Saturation coefficient for oxygen	0.80	g O_2 m^{-3}	-
$K_{NH4,A}$	Saturation coefficient for ammonium (substrate)	0.70	g N m^{-3}	-
$K_{NO3,A}$	Saturation/inhibition coefficient for nitrate	0.14	g N m^{-3}	-
$K_{ALK,A}$	Saturation coefficient for alkalinity (HCO_3^-)	0.40	mole HCO_3^- m^{-3}	-
Phototrophic organisms: X_{PS}				
μ_{PS}	Maximum growth rate on substrate	2.56	d^{-1}	1.13
$\eta_{NO3,PS}$	Reduction factor respiration when light deprived	0.20	-	-
b_{PS}	Endogenous respiration rate of X_{PS}	0.03	d^{-1}	1.029
$K_{CO2,PS}$	Saturation/inhibition coefficient for carbon dioxide	0.50	g O_2 m^{-3}	-
$K_{PO4,PS}$	Saturation coefficient for soluble reactive phosphorus	0.50	g COD m^{-3}	-
$K_{NO3,PS}$	Saturation/inhibition coefficient for nitrate	0.80	g N m^{-3}	-
$K_{NH4,PS}$	Saturation coefficient for ammonium (nutrient)	0.005	g N m^{-3}	-
$K_{HCO3,PS}$	Saturation coefficient for bicarbonate	0.10	mole HCO_3^- m^{-3}	-
$K_{I,PS}$	Inhibition coefficient for light	8×10^{-5}	mole e^- m^{-3}	-
$K_{PG,PS}$	Half-saturation coefficient for internal PG use	0.005	g COD m^{-3}	-
Chemical				
$k_{AB,HCO3/CO3}$	Rate constant for bicarbonate dissociation	10^{12}	d^{-1}	-
$pK_{a,HCO3/CO3}$	Dissociation constant for bicarbonate protolysis	10.33	-	-
$k_{AB,CO2/H2O}$	Rate constant for carbon dioxide hydrolysis	2,221	d^{-1}	-
$pK_{a,CO2/H2O}$	Dissociation constant for carbon dioxide hydrolysis	6.36	-	-
$k_{AB,CO2/OH}$	Rate constant carbon dioxide hydrolysis, high pH	7.19×10^8	d^{-1}	-
$pK_{a,CO2/OH}$	Dissociation const. carbon dioxide hyd., high pH	-7.64	-	-
$k_{AB,NH3/NH4}$	Rate constant for ammonia dissociation	10^{12}	d^{-1}	-
$pK_{a,NH3/NH4}$	Dissociation constant for ammonia	9.68	-	-

Table B-5. Biofilm parameters and diffusion coefficients.

Symbol	Description	Value	Unit
Diffusion coefficients in water			
D_S	Readily biodegradable substrate	1.0×10^{-4}	$m^2\ d^{-1}$
D_{O2}	Oxygen	1.73×10^{-4}	$m^2\ d^{-1}$
D_{NH4}	Ammonium	1.7×10^{-4}	$m^2\ d^{-1}$
D_{NO3}	Nitrate	1.47×10^{-4}	$m^2\ d^{-1}$
D_{NH3}	Ammonia	1.7×10^{-4}	$m^2\ d^{-1}$
D_{HCO3}	Bicarbonate	1.02×10^{-4}	$m^2\ d^{-1}$
D_{CO3}	Carbonate	7.9×10^{-5}	$m^2\ d^{-1}$
D_H	Hydrogen	1.0×10^{-20}	$m^2\ d^{-1}$
D_{CO2}	Carbon dioxide	1.65×10^{-4}	$m^2\ d^{-1}$
Biofilm parameters			
D_F/D	Ratio of diffusion in biofilm to diffusion in water	0.8	-
ε_ℓ	Fraction of the liquid volume in the biofilm	0.8	-
ρ_{EPS}	EPS density in the biofilm	ρ_X / 6	$g\ COD_X/m^3$
ρ_X	Biomass density in the biofilm	170,000	$g\ COD_X/m^3$
ρ_{PG}	Polyglucose density in the biofilm	$\rho_X \times 20$	$g\ COD_X/m^3$
L_L	External mass transfer layer thickness	100	μm

Table B-6. Stoichiometric matrix of soluble state variables for the modified PHOBIA.

Index i	S_{CO2}	S_{HCO3}	S_{NH3}	S_{NO3}	S_{N2}	S_{O2}	S_{PO4}	S_F	S_A	S_I	X_S	X_H	X_A	X_{PS}	X_{TSS}	X_I	X_{daa}	X_{PG}	Rate
1.			$v_{1,NH3}$				$v_{1,PO4}$	$1-f_{SI}$		f_{SI}	-1				$-i_{TSSXS}$				r1
2.			$v_{2,NH3}$				$v_{2,PO4}$	$1-f_{SI}$		f_{SI}	-1				$-i_{TSSXS}$				r2
3.			$v_{3,NH3}$				$v_{3,PO4}$	$1-f_{SI}$		f_{SI}	-1				$-i_{TSSXS}$				r3
4.	$v_{4,CO2}$		$v_{4,NH3}$			$v_{4,O2}$	$v_{4,PO4}$							1	$v_{4,TSS}$			$v_{4,PG}$	r4
5.	$v_{5,CO2}$			$v_{5,NO3}$		$v_{5,O2}$	$v_{5,PO4}$							1	$v_{5,TSS}$			$v_{5,PG}$	r5
6.		$v_{6,HCO3}$	$v_{6,NH3}$			$v_{6,O2}$	$v_{6,PO4}$							1	$v_{6,TSS}$			$v_{6,PG}$	r6
7.		$v_{7,HCO3}$		$v_{7,NO3}$		$v_{7,O2}$	$v_{7,PO4}$							1	$v_{7,TSS}$			$v_{7,PG}$	r7
8.	$v_{8,CO2}$		$v_{8,NH3}$			$v_{8,O2}$	$v_{8,PO4}$	$v_{8,F}$						-1	$v_{8,TSS}$			$v_{8,PG}$	r8
9.			$v_{9,NH3}$				$v_{9,PO4}$				f_{XI}			-1	$v_{9,TSS}$	f_{XI}	f_{Xdaa}		r9
10.	$v_{10,CO2}$		$v_{10,NH3}$			$v_{10,O2}$	$v_{10,PO4}$	$v_{10,F}$				1			$v_{10,TSS}$				r10
11.	$v_{11,CO2}$		$v_{11,NH3}$			$v_{11,O2}$	$v_{11,PO4}$		$v_{11,A}$			1			$v_{11,TSS}$				r11
12.	$v_{12,CO2}$		$v_{12,NH3}$	$v_{12,NO3}$	$v_{12,N2}$		$v_{12,PO4}$	$v_{12,F}$				1			$v_{12,TSS}$				r12
13.	$v_{13,CO2}$		$v_{13,NH3}$	$v_{13,NO3}$	$v_{13,N2}$		$v_{13,PO4}$		$v_{13,A}$			1			$v_{13,TSS}$				r13
14.			$v_{14,NH3}$				$v_{14,PO4}$	-1	1										r14
15.			$v_{15,NH3}$								f_{XI}	-1			$v_{15,TSS}$	f_{XI}	f_{Xdaa}		r15
16.	$v_{17,CO2}$		$v_{16,NH3}$	$v_{16,NO3}$		$v_{16,O2}$							1		$v_{16,TSS}$				r16
17.			$v_{17,NH3}$								f_{XI}		-1		$v_{17,TSS}$	f_{XI}	f_{Xdaa}		r17
18.			$v_{18,NH3}$				$v_{18,PO4}$				1				$v_{18,TSS}$		-1		r18

X_{TSS} was calculated from the particulate state variables:

$$X_{TSS,bulk} = X_{TSS,inorganic,in} + i_{TSBM} \cdot \left(X_{A,bulk} + X_{H,bulk} + X_{M1,bulk} + X_{M2,bulk} \right) + i_{TSXS} \cdot X_{S,bulk} + i_{TSXI} \cdot X_{I,bulk}$$

$$X_{TSS,biofilm} = i_{TSBM} \cdot \left(X_{A,biofilm} + X_{H,biofilm} + X_{M1,bulk} + X_{M2,bulk} \right) + i_{TSXS} \cdot X_{S,biofilm} + i_{TSXI} \cdot X_{I,biofilm}$$

where $X_{TSS,inorganic,in}$ is the amount of TSS that is not accounted for by influent concentrations of X_S, X_H, X_A, X_{PS}, and X_I.

Table B-7. Process descriptions.

Index i	Process Description
Hydrolysis	
1.	Aerobic
2.	Anoxic
3.	Anaerobic
Phototrophic Organisms: X_{PS}	
4.	Growth on Carbon Dioxide and Ammonia, S_{CO2}
5.	Growth on Carbon Dioxide and Nitrate, S_{CO2}
6.	Growth on Bicarbonate and Ammonia, S_{HCO3}
7.	Growth on Bicarbonate and Nitrate, S_{HCO3}
8.	Respiration
9.	Lysis of X_{PS}
Heterotrophic Organisms: X_H	
10.	Growth on Fermentable Substrate, S_F
11.	Growth on Fermentation Products, S_A
12.	Denitrification with Fermentable Substances, S_F
13.	Denitrification with Fermentation Products, S_A
14.	Fermentation
15.	Lysis of X_H
Chemoautotrophic (Nitrifying) Organism: X_A	
16.	Growth on Oxygen, S_{NH3}
17.	Lysis of X_A
Microbial Decay Products: X_{daa}	
18.	Anaerobic Hydrolysis of Decay Products

Table B-8. Process rate equations for the modified PHOBIA.

Rate	Rate Equation
r1.	$K_H \cdot \frac{S_{O2}}{K_{O2,Y} + S_{O2}} \cdot \frac{X_S/X_H}{K_X + X_S/X_H} \cdot X_H$
r2.	$K_H \cdot \eta_{NO3,Y} \cdot \frac{K_{O2,Y}}{K_{O2,Y} + S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3,Y} + S_{NO3}} \cdot \frac{X_S/X_H}{K_X + X_S/X_H} \cdot X_H$
r3.	$K_H \cdot \eta_{fe} \cdot \frac{K_{O2,Y}}{K_{O2,Y} + S_{O2}} \cdot \frac{K_{NO3Y}}{K_{NO3,Y} + S_{NO3}} \cdot \frac{X_S/X_H}{K_X + X_S/X_H} \cdot X_H$
r4.	$\mu_{PS} \cdot f_L \cdot \frac{S_{CO2}}{K_{CO2} + S_{CO2}} \cdot \frac{S_{NH3}}{K_{NH3,PS} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,PS} + S_{PO4}} \cdot X_{PS}$
r5.	$\mu_{PS} \cdot f_L \cdot \frac{S_{CO2}}{K_{CO2} + S_{CO2}} \cdot \frac{S_{NO3}}{K_{NO3,PS} + S_{NO3}} \cdot \frac{K_{NH3,PS}}{K_{NH3,PS} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,PS} + S_{PO4}} \cdot X_{PS}$
r6.	$\mu_{PS} \cdot f_L \cdot \frac{S_{HCO3}}{K_{HCO3,PS} + S_{HCO3}} \cdot \frac{K_{CO2,PS}}{K_{CO2,PS} + S_{CO2}} \cdot \frac{S_{NH3}}{K_{NH3,PS} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,PS} + S_{PO4}} \cdot X_{PS}$
r7.	$\mu_{PS} \cdot f_L \cdot \frac{S_{HCO3}}{K_{HCO3,PS} + S_{HCO3}} \cdot \frac{K_{CO2,PS}}{K_{CO2,PS} + S_{CO2}} \cdot \frac{S_{NO3}}{K_{NO3,PS} + S_{NO3}} \cdot \frac{K_{NH3,PS}}{K_{NH3,PS} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,PS} + S_{PO4}} \cdot X_{PS}$
r8.	$\mu_{PS} \cdot \eta_{L,PS} \cdot \frac{S_{O2}}{K_{O2,PS} + S_{O2}} \cdot \frac{K_{I,PS}}{K_{I,PS} + I} \cdot \frac{X_{PG}}{K_{PG,PS} + X_{PG}} \cdot X_{PS}$
r9.	$b_{PS} \cdot X_{PS}$
r10.	$\mu_H \cdot \frac{S_{O2}}{K_{O2,H} + S_{O2}} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_F + S_A} \cdot \frac{S_{NH3}}{K_{NH3} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4} + S_{PO4}} \cdot X_H$
r11.	$\mu_H \cdot \frac{S_{O2}}{K_{O2,H} + S_{O2}} \cdot \frac{S_A}{K_{A,H} + S_A} \cdot \frac{S_A}{S_F + S_A} \cdot \frac{S_{NH3}}{K_{NH3,H} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,H} + S_{PO4}} \cdot X_H$
r12.	$\mu_H \cdot \eta_{NO3H} \cdot \frac{K_{O2,H}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3,H} + S_{NO3}} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_F + S_A} \cdot \frac{S_{NH3}}{K_{NH3,H} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,H} + S_{PO4}} \cdot X_H$
r13.	$\mu_H \cdot \eta_{NO3H} \cdot \frac{K_{O2,H}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3,H} + S_{NO3}} \cdot \frac{S_A}{K_{A,H} + S_A} \cdot \frac{S_A}{S_F + S_A} \cdot \frac{S_{NH3}}{K_{NH3,H} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,H} + S_{PO4}} \cdot X_H$
r14.	$q_{fer} \cdot \frac{K_{O2,H}}{K_{O2,H} + S_{O2}} \cdot \frac{K_{NO3,H}}{K_{NO3,H} + S_{NO3}} \cdot \frac{S_F}{K_F + S_F} \cdot X_H$
r15.	$b_H \cdot X_H$
r16.	$\mu_H \cdot \frac{S_{O2}}{K_{O2,A} + S_{O2}} \cdot \frac{S_{CO2}}{K_{CO2,A} + S_{CO2}} \cdot \frac{S_{NH3}}{K_{NH3,H} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{PO4,A} + S_{PO4}} \cdot X_A$
r17.	$b_A \cdot X_A$
r18.	$K_{H,daa} \cdot \frac{K_{O2,Y}}{K_{O2,Y} + S_{O2}} \cdot \frac{K_{NO3,Y}}{K_{NO3,Y} + S_{NO3}} \cdot \frac{X_{daa}/X_H}{K_X + X_{daa}/X_H} \cdot X_H$

References

Boltz, J. P., Morgenroth, E., and Sen, D. 2010. Mathematical modeling of biofilms and biofilm reactors for engineering design. *Water Sci. Technol.*, 62(8), 1821–1836.

Rowe, P. N., Claxton, K. T., and Lewis, J. B. 1965. Heat and Mass Transfer from a Single Sphere in an Extensive Flowing Fluid. *Trans. Instn. Chem. Engrs.* 43. 14–31.

Wolf, G., C. Picioreanu, and M. C. M. van Loosdrecht. 2007. Kinetic Modeling of Phototrophic Biofilms: the PHOBIA Model. *Biotechnology and Bioengineering.* 97(5): 1064–79.

Abbreviations and acronyms used without definitions in TRB publications:

A4A	Airlines for America
AAAE	American Association of Airport Executives
AASHO	American Association of State Highway Officials
AASHTO	American Association of State Highway and Transportation Officials
ACI–NA	Airports Council International–North America
ACRP	Airport Cooperative Research Program
ADA	Americans with Disabilities Act
APTA	American Public Transportation Association
ASCE	American Society of Civil Engineers
ASME	American Society of Mechanical Engineers
ASTM	American Society for Testing and Materials
ATA	American Trucking Associations
CTAA	Community Transportation Association of America
CTBSSP	Commercial Truck and Bus Safety Synthesis Program
DHS	Department of Homeland Security
DOE	Department of Energy
EPA	Environmental Protection Agency
FAA	Federal Aviation Administration
FHWA	Federal Highway Administration
FMCSA	Federal Motor Carrier Safety Administration
FRA	Federal Railroad Administration
FTA	Federal Transit Administration
HMCRP	Hazardous Materials Cooperative Research Program
IEEE	Institute of Electrical and Electronics Engineers
ISTEA	Intermodal Surface Transportation Efficiency Act of 1991
ITE	Institute of Transportation Engineers
MAP-21	Moving Ahead for Progress in the 21st Century Act (2012)
NASA	National Aeronautics and Space Administration
NASAO	National Association of State Aviation Officials
NCFRP	National Cooperative Freight Research Program
NCHRP	National Cooperative Highway Research Program
NHTSA	National Highway Traffic Safety Administration
NTSB	National Transportation Safety Board
PHMSA	Pipeline and Hazardous Materials Safety Administration
RITA	Research and Innovative Technology Administration
SAE	Society of Automotive Engineers
SAFETEA-LU	Safe, Accountable, Flexible, Efficient Transportation Equity Act: A Legacy for Users (2005)
TCRP	Transit Cooperative Research Program
TEA-21	Transportation Equity Act for the 21st Century (1998)
TRB	Transportation Research Board
TSA	Transportation Security Administration
U.S.DOT	United States Department of Transportation