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Evaluation of a denitrifying bioreactor to improve water quality of tertiary treated water using DGT

A thesis
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Abstract

A proven nitrogen remediation strategy, denitrifying bioreactors are a low cost, edge-of-field approach for removing nitrate from subsurface drainage waters. The methodology to evaluate the performance of denitrifying bioreactors has remained practically unchanged for many years and is largely dependent upon grab sampling to evaluate their performance. In this study, a controlled flow denitrifying bioreactor treating wastewaters was used to evaluate a monitoring technique called Diffusive Gradients in Thin-Films (DGT) in place of grab sampling. Corbett et al., (in press) previously showed that DGT could replace high frequency grab sampling (e.g. hourly to daily) and significantly improve upon traditional low frequency sampling. Importantly, DGT provided NO_3^- concentrations comparable to high frequency grab sampling but require less time on site. In the current study, the bioreactor provided complete removal of nitrate within 4 meters with influent NO_3^- concentrations ranging from 9.7 to 15.9 mg NO_3^- L^{-1} over the four deployments. This study also demonstrated how grab samples can easily overestimate and underestimate NO_3^- removal with low frequency data providing a removal rate of 6.3 to 19.6 g N m^{-3} d^{-1} because of the infrequency of sampling. The time-weighted average concentrations calculated using Purolite AE520 DGT provided nitrate removal rates (NRR) that ranged from 5.7 to 9.4 g N m^{-3} d^{-1}. This removal rate was higher than many other bioreactors most likely because influent wastewater contained dissolved organic carbon that could also support high denitrification. A Q_{10} of 3.9 was calculated using the NRR from this study and Corbett et al., (in press) illustrating that temperature was a determining factor in NO_3^- removal. A survey of redox reactions was also undertaken with oxygen, sulfate, methylmercury and total mercury measured. Sulfate reduction also measured for each deployment (average of 3.6 g S m^{-3} d^{-1}; 1.7 to 5.10 g S m^{-3} d^{-1}) via grab sampling. Methylmercury and total mercury concentrations were consistently below health guidelines. No phosphate reduction was observed. This study showed how DGT can provide more accurate NO_3^- removal data and were easier to use compared to traditional point-in-time grab sampling.

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List of Abbreviations

APA	Agarose cross-linked polyacrylamide
AVG	average
DBL	diffusive boundary layer
DGT	Diffusive Gradients in Thin-films
DO	dissolved oxygen
DOC	dissolved organic carbon
GS	grab sample
HCl	hydrochloric acid
Hg	mercury (total)
LIC	Livestock Improvement Corporation
MDL	material diffusive layer
MeHg	methylmercury
N	nitrogen
NH_4^+	ammonium
NO^{2-}	nitrite
NO_3^-	nitrate
NO_3^--N	Nitrate-nitrogen
NRE	nitrogen removal efficiency
NRR	nitrogen removal efficiency
O_2	oxygen
P	phosphorus
PO_4^{3-}	phosphate
SO_4^{2-}	sulfate
STD	standard deviation

Chapter 1

Introduction

1.1 Background

With the world's population on the rise, there has been an increase in anthropogenic inputs into the environment. One nutrient of concern is excess nitrogen and particularly NO_3^-. Excess NO_3^- has several sources, but the two main ones are the agricultural community's use of fertilizers and municipal wastewater systems. Both industries struggle to control nutrient discharge to sensitive downstream ecosystems. Perhaps the most famous example is the hypoxic zone in the Northern Gulf of Mexico, which stems from agricultural nutrient pollution (Erisman et al., 2013). Though in other areas such as the Chesapeake Bay in the United States polluted runoff (water from streets, parking lots, lawns, and other surfaces that go untreated) and sewage runoff are equally responsible for nutrient pollution (CBF, 2019).

Hypoxic zones are oxygen-depleted areas resulting from high nutrient discharges and freshwater bodies are particularly sensitive to N. Anthropogenic N inputs such as synthetic fertilizers and high-density livestock operations have disrupted the natural N cycle due to the demand for increased output of agricultural products (Erisman et al., 2013). Another concerning anthropogenic source is septic systems, where NO_3^- that is not treated percolates through to groundwater. While N is naturally fixed in soils by bacteria and lightning, excessive amounts of N in the environment are concerning as NO_3^- is highly susceptible to leaching from soils. This leaching of N can lead to eutrophication once N reaches fresh and marine waters (e.g. hypoxic zones) and are also a concern for human health (e.g. "blue-baby" syndrome) and cancers when consumed in excess drinking water.

The consequences of NO_3^- become a larger issue when mole/tile or sub-surface drainage are used to remove excess water from soils in agricultural areas. In these areas, subsurface drainage provides soil water condition that is favorable for crop growth and management (Skaggs et al., 1994). However, this rapid drainage has also created a pathway for easily leached NO_3^- to exit the field more quickly and enter downstream waterways. Several N-specific mitigation strategies have been developed to decrease NO_3^- from agricultural fields including cropland and, in New Zealand, grazed pastures.

Among the several mitigation strategies currently available for landowners are denitrifying bioreactors. Denitrifying bioreactors are a promising solution for those with tiled drained fields who do not wish to remove land from production as bioreactors are considered an edge-of-field practice. Placed on subsurface drainage lines at the edge of a field or receiving inputs from wastewater treatment systems in a pipe flow through them, denitrifying bioreactors are excavated trenches filled with a carbon source through which drainage is routed (Robertson & Cherry, 1995; Schipper & Vojvodić-Vuković, 1998). These bioreactors provide conditions for denitrification to occur and thereby reduce the amount of NO_3^- that would otherwise have left the field. Denitrification is the biological reduction of NO_3^- into nitrogen gases (such as N_2 and N_2O) under anaerobic conditions (Coyne, 2008).

Denitrifying bioreactors treating both agricultural and wastewaters have already been installed in several places throughout the world including New Zealand, Canada, the United States, Australia, and parts of Europe (Addy et al., 2016). Nitrate removal rate is an index typically used to quantify how much NO_3^- is being removed by the denitrifying bioreactors. To quantify removal rates water must first be collected through the bioreactor and analyzed for NO_3^-. Typically, water quality data is collected by grab sampling or taking a sample at a point in time (Christianson et al., 2012b; Rozemeijer et al., 2010; Vrana et al., 2005). While grab sampling is the predominate way most water quality data is collected, automated methods have also been developed (Maxwell et al., 2019). However, both grab sampling and automated methods operate on the principle of one sample collected at a single point of time which does not reflect the "whole picture" of the temporal variation of nutrient concentrations in the water. A major limitation of grab sampling technique is that it is time-consuming requiring significant labor cost, especially high frequency collections such as hourly to daily. Automated sampling methods have high start-up (initial purchase cost) and maintenance costs associated with gathering a complete data set. All of the above sampling techniques also require samples to be analyzed soon after collection to prevent biological transformations within the sample. While this collection method has not changed in many years, new options are being explored.

A novel approach by Zhang & Davison (1994) developed a technique named Diffusive Gradients in Thin-Films or DGT. This technique relies on the simple concept of Fick's First Law of Diffusion (the phenomena where molecules move from areas of high concentration to low concentrations). Diffusive Gradients in Thin-films are simple units made up of five parts; a cap, filter membrane, hydrogel, binding layer, and a base that when assembled are roughly equal in size

to a golf ball (Davison, 2016). These units can be deployed in water systems where the water flow passes the DGT and the selected analyte diffuses inwards and is bound to the binding layer. The DGT is then recovered and the analyte eluted from the binding layer later and analyzed using standard techniques (Zhang & Davison, 1995).

Since being developed, DGT have been used for a variety of applications including chemical speciation in solutions, sediment geochemistry, bioavailability, and dynamic processes in water and soils (Davison & Zhang, 2012). Thus far, DGT have been developed to bind oxyanions, trace metals, inorganic nutrients, sulfide, phosphate, ammonium, and NO_3^- (Davison, 2016). However, only recently have DGT been specifically used to quantify nitrate concentration. Huang et al., (2016b) developed the first NO_3^- specific DGT using a binding layer of Purolite AE520. More recently their DGT technique was modified to monitor NO_3^- removal in a denitrifying bioreactor (Corbett et al., in press).

1.2 Objectives

The aim of this thesis was to further explore the utility of Diffusive Gradients in Thin-films (DGT) as an alternative to traditional grab sampling methods and as a tool to monitor NO_3^- removal within a nitrogen remediation strategy specifically, bioreactors.

Specifically, the objectives were:

1) To determine whether A520E-DGT methods could be used to monitor denitrifying bioreactors more effectively than grab sampling.

2) Evaluate the NO_3^- removal rates as well as sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) and compare removal to previously collected data at the site and against broader bioreactor literature.

This study was conducted at the LIC bioreactor installed in 2013 in Newstead, New Zealand. Prior to conducting this study reported here, a large part of my time was spent on learning the DGT technique. Initial studies were aligned with an ongoing research study and this data was reported in Corbett et al., (in press). Building on this previous study, modifications to the methodology for this present study included; (i) reducing the number of wells sampled, (ii) improving deployment of DGT in the field, (iii) taking additional measurements such as dissolved oxygen to gain greater insight into microbial processes in the bioreactor.

Initial measurements (Corbett et al., in press) were made during summer (a warmer time of the year) and NO_3^- concentration was reduced to zero within three meters. Consequently, I expected lower rates of nitrate removal during this study that was conducted in winter with colder water temperatures. I also expected to see lower sulfate removal than those in our previous findings (Corbett et al., in press).

1.3 Thesis Layout

Following this introduction, Chapter 2 is a two-part review of literature focusing on denitrifying bioreactors and diffusive gradients in thin-films (DGT). In-depth detail on denitrifying bioreactors and how NO_3^- removal is quantified is presented first. This review also summarizes published rates of field scale bioreactors for later comparison with measurements reported here. A short review of the principles of DGT and previous uses is then presented.

Chapter 3 contains the study findings and discussion on the use of DGT to monitor NO_3^- removal within denitrifying bioreactors. Chapter 3 has been written in the format of a paper for potential subsequent submission and consequently, there is some repetition of literature and introductory material for completeness.

Chapter 4 presents a holistic summary and conclusions for the thesis along with future research recommendations.

An appendix contains numerical data collected and presented in this thesis.

Chapter 2

Literature Review

2.1 Introduction

Water quality concerns as a result of nutrient pollution or excess is one of the leading problems faced by many nations including New Zealand. Nutrient pollution is an externality of the agricultural industry that rapidly expanded to meet global demands and is now trying to balance natural resources and sustainably while maintaining current production levels (Godfray et al., 2014). While in non-agricultural settings municipal waste and septic systems are a source of nitrogen into groundwaters. One of the more concerning nutrients seen in excess is N, specifically highly bioavailable nitrate (NO_3^-) and ammonium.

Nitrate has always been present in soils through natural nitrogen cycling processes. With intensified agriculture, nutrient pollution from anthropogenic sources occurs from excess nitrogen fertilizers and leaching from both the field and manure in livestock operations. The addition of nitrogen fertilizer causes alterations of the natural N cycle and accumulation of reactive N compounds (Galloway et al., 2003). As fertilizer is applied, it can be taken up by plants, become bound to soil particles, or gets converted to NO_2^- then to NO_3^- by bacteria. Once in NO_3^- form, this excess nitrogen becomes highly soluble in water and can be leached from fields following rain events and carried through extensive drainage networks to sensitive downstream water bodies which can become impaired through eutrophication and subsequent hypoxia.

To combat both environmental and human health impacts of excess NO_3^-, practical on-farm solutions are needed. There are a variety of nitrogen management practices currently used and can work better when “stacked” or used together (Christianson et al., 2018; Haas et al., 2017; Motsinger et al., 2016). These include nitrogen application management practices, crop rotation, cover crops, tillage practices, perennial/energy crops, controlled drainage, saturated/riparian buffers, wetlands, and denitrifying woodchip bioreactors (Dinnes et al., 2002). Denitrifying woodchip bioreactors specifically, are a promising practice recommended to remove excess NO_3^- as it leaves artificially drained areas, and these are covered in more detail below (Section 2.3).

Cover crops are planted on fallow ground to prevent erosion as well as to reduce NO_3^- loss and to improve soil via organic matter (Reicosky & Forcella, 1998; Dabney et al., 2001; Tonitto et al., 2005). Amending tillage practices by either reducing or changing them entirely can leave greater ground vegetation coverage, reduce compaction, and have potential fuel savings (Dinnes et al., 2002; McLaughlin and Mineau, 1995). In the past decade, the planting of perennials and energy crops have emerged as N remediation strategies as they can reduce nitrogen leaching and typically require less nitrogen fertilizer (Heggenstaller, et al., 2008; Sanderson & Adler, 2008; Smith et al., 2013). Controlled drainage, also known as drainage water management, is the practice of using control structure on tile-drained fields to maintain the water level in fields when drainage may not be needed (Cooke & Verma, 2012; Woli et al., 2010). There is some evidence that controlled drainage enhances in field denitrification reducing NO_3^- leaching. Riparian buffers are vegetated areas located next to streams and waterways that slow down water and nutrient movement by providing a natural barrier that supports plant uptake of NO_3^- in underlying groundwater and denitrification in groundwater as it exits the field (Mayer et al., 2007). Like riparian buffers, saturated buffers are located next to streams to slow, filter water, and remove NO_3^-, but have the addition of tile drainage being routed through them instead of free flow similar to controlled drainage (Chandrasoma et al., 2019; Groh et al., 2018). Finally, wetlands are an area that is flooded with water where oxygen-free processes can take place. Wetlands provide valuable nutrient cycling processes that can transform, sequester, and remove nitrogen and other nutrients as they move through and have proven effective for treating drainage waters (Kadlec & Wallace, 2009; Leverenz et al., 2010; Tanner et al., 2012).

With all of these different nutrient remediation strategies, there is a need to quantify their effectiveness to measure how well they work and determine which mitigation practice to use. As these practices are quite diverse, one way to compare them is to calculate their removal efficiencies. Currently, this is done through the collection of grab samples taken from locations where water enters and exits. A major problem with grab sampling is that these are only a reflection of what is in the system at that point in time and for these practices are generally only collected monthly at best. This can result in poor representation of how much water is treated and furthermore environmental and anthropogenic factors

influencing the systems are poorly quantified. One way to overcome this problem may be to use DGT. Thus far, DGT have been proven as a successful alternative to grab sampling within a nutrient remediation strategy such as denitrifying bioreactors (Corbett et al., in press).

While all of these previously described mitigation strategies play an important role in reducing N inputs into ground and surface waters, this thesis focuses on denitrifying bioreactors and here I review; how they work, their carbon sources, factors controlling NO_3^- removal, design parameters, and how to calculate NO_3^- removal rates. An overview of DGT and their previous uses and the research aims, and outcomes will then be reviewed. This literature review will conclude with key gaps and recommendations for quantifying NO_3^- removal rates.

2.1 Denitrifying bioreactors

2.1.1 General information

First researched in New Zealand and Canada in the 1990s, denitrifying bioreactors are an edge-of-field nitrogen management practice for removing NO_3^- from drainage and wastewaters (Blowes et al., 1994; Robertson & Cherry, 1995; Schipper & Vojvodić-Vuković, 1998). For agricultural purposes, denitrifying bioreactors can be divided into two categories; denitrification walls and denitrification beds (see Schipper et al., 2010 for full terminology). Denitrifying walls treat groundwater as it flows through them, while denitrifying beds have tile drainage systems routing water or receive inputs from wastewater treatment systems in a pipe flow through them. In this study, denitrification beds are being evaluated. These practices are low-tech enhancing the natural process of denitrification (conversion of NO_3^- into nitrogen gases) through the creation of ideal environmental conditions without removing land from production. Four environmental conditions need to come together to create the ideal environment; denitrifying bacteria, a carbon source, NO_3^- or another N oxide, and anoxic conditions (i.e. no oxygen) before denitrification can occur (Seitzinger et al., 2006). Denitrifying bioreactors achieve these conditions by being excavated trenches filled with a solid carbon source (typically woodchips), that treats diverted drainage water before entering surface waters (Figure 2.1).

Anoxic conditions are created by controlling the saturation level within the bioreactor through the inclusion of control structures at the inlet and outlet. Boards or stop logs placed within the control structures enable landowners to control the saturation level within the bioreactor as needed. Sometimes control structures are excluded in bioreactors that have controlled flow rates and therefore do not require manual adjustment of saturation levels. Denitrifying bacteria are generally facultative and ubiquitously found in soil and water. Therefore, no inoculation is required as the bacteria rapidly colonize the woodchips once water begins passing though. Denitrifying bioreactors are also currently used to treat aquaculture waters and various other wastewaters (Lepine et al., 2016; Pluer et al., 2018; von Ahnen et al., 2016).

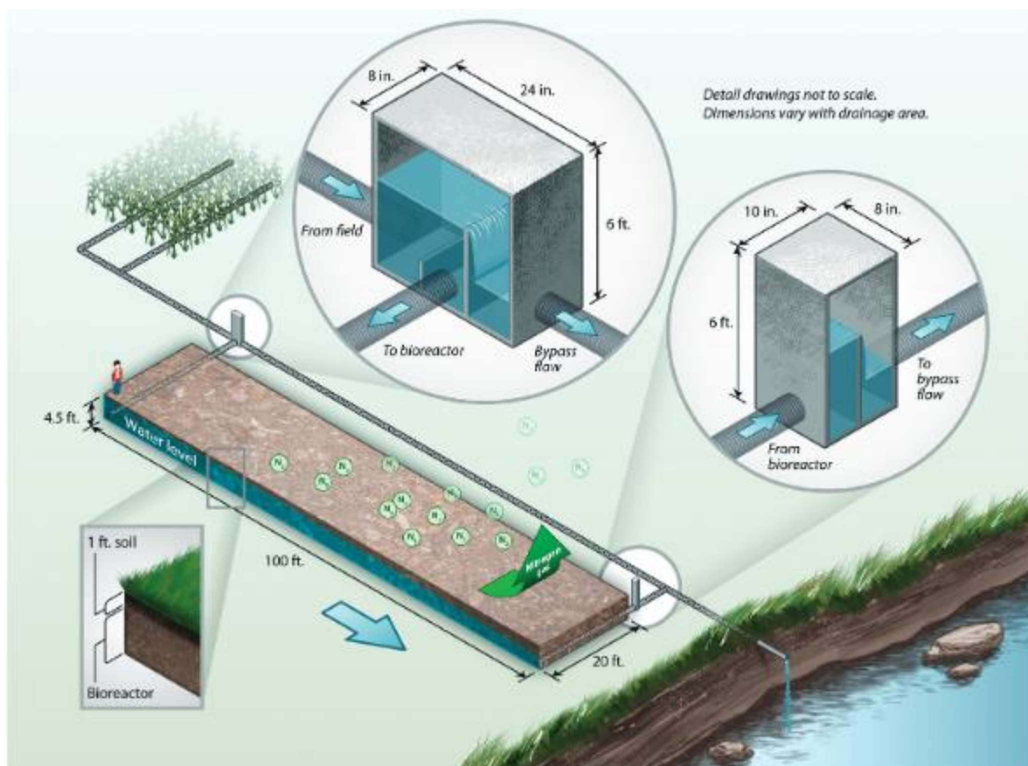


Figure 2.1. Design illustration of a denitrifying bioreactor showing the control structures and bypass line. (From Christianson & Helmers, 2011)

Denitrifying bioreactors are generally designed to last at least 10 years but could last as long as 20 years. These numbers will soon be able to be validated as several field-scale research bioreactors are nearing 10, 15, 20 years of age (Long et al., 2011; Moorman et al., 2010; Robertson et al., 2008). The typical bioreactor is designed for tile-drained fields (12-32 ha) and with site-specific factors such as drainage history, topography considered (Christianson & Helmers, 2011).

In the Midwestern United States, denitrifying bioreactors have been quickly adopted as a proven mitigation strategy for reducing NO_3^- pollution and several states have voluntarily included them as recognized created nutrient reduction strategies (IDALS, 2014; IEPA & IDOA, 2015; MPCA, 2014). Denitrifying bioreactors are also being installed on a voluntary basis throughout New Zealand and in Queensland, Australia.

2.1.2 Factors influencing N removal

Besides achieving anoxic conditions, other factors that determine the amount of N removed are the type of carbon source, the concentration of oxygen, NO_3^-, and temperature.

2.1.2.1 Carbon Source

Several carbon sources, or bioreactor media, have been researched including corn cobs, corn stalks, cardboard, wheat straw, newspaper, and chipped municipal storm debris (Cameron & Schipper, 2010b; Feyereisen et al., 2016; Healy et al., 2012). Woodchips are generally the recommended carbon fill media because they do degrade as quickly as some other alternatives, therefore, maintaining porosity. Woodchips are generally more uniform in size than other sources making porosity easier to quantify in design assumptions (Christianson & Helmers, 2011; USDA NRCS, 2017).

When selecting the type of woodchip to use, there are generally no restraints regarding tree species, however, the use of oak, cedar, and other high-tannin woods is not recommended as tannins cause discoloration of the water and potentially affect pH (USDA NRCS, 2017). Regardless of wood type, woodchips should be relatively free of fines or debris, so that water can pass through easily (Christianson & Helmers, 2011). Cameron & Schipper (2010b) determined that particle size did not affect NO_3^- removal rates likely due to the dual-porosity characteristics of woodchips where denitrification occurs within the woodchips and not just the surface area. It is also recommended that treated wood is not used is as carbon availability is restricted due to the treatment process as well as excluding any excess “green” debris due to quick degradation and introducing additional high N content (Christianson & Helmers, 2011).

If using a media other than woodchips for the carbon source, it has been recommended to avoid materials that support very high NO_3^- removal and degrades too quickly which can lead to a decreased lifetime of the bioreactor (Cameron & Schipper, 2010b; Feyereisen et al., 2016). Carbon availability may also be limited within a bioreactor due to natural degradation and may need to be replenished, both of which can affect NO_3^- removal ability (Addy et al., 2016; Feyereisen et al., 2016; Robertson, 2010). Available carbon can also be affected by wetting and rewetting cycles that can accelerate decomposition and enhance NO_3^- removal (Maxwell et al., 2019).

2.1.2.2 Oxygen

Anoxic conditions are critical for the denitrification process and so DO concentrations are important. This is because denitrifying bacteria are able to switch their metabolism to utilize O_2 as a terminal electron acceptor replacing NO_3^- and halting the denitrification process. For denitrification to occur, DO should be below 0.5 mg L^{-1} (Tchobanoglous et al., 2003) and this is usually achieved within the first few meters from the inlet (Christianson et al., 2011a; Christianson et al., 2011c; Warneke et al., 2011b). As a result, initially high levels of DO in the inlet water can lead to reduced or incomplete denitrification should DO concentrations not be reduced (Elgood et al., 2010).

2.1.2.3 NO_3^-

Besides DO concentrations, initial inlet N concentrations have been found to significantly influence the amount of NO_3^- removed (Addy et al., 2016; Schipper et al., 2010). A meta-analysis by Addy et al., (2016) found that high levels of inlet N concentrations (>30 mg NO_3-N L^{-1}) typically correlated to higher rates of NO_3^- removal. If the bioreactor has too low of inlet N levels initially or NO_3^- falls below ~0.5 mg NO_3^- L^{-1} before exiting the bioreactor, the system may become NO_3^- limited ceasing denitrification and triggering other unwanted redox reactions (Robertson & Merkley, 2009; Shih et al., 2011). Unwanted redox reactions are discussed further in design considerations (Section 2.3.3).

2.1.2.4 Temperature

Finally, it has been well established that as temperature increases so do biological processes and this has been observed frequently throughout bioreactor literature (Addy et al., 2016; Feyereisen et al., 2016; Hoover et al., 2016; Soupir et al., 2018). Christianson et al., (2012b) observed across current bioreactor literature the Q_{10} (the ratio of removal rates for every 10°C increase in temperature) averaged around 2. One New Zealand study has attempted to heat bioreactor beds to increase N removal, however, they saw no significant increase in N removal with an average increased bed temperature of 3.4°C (Cameron & Schipper, 2010a). This is an area of continued research with both column and field studies further investigating the relationship (Hoover et al., 2017; Soupir et al., 2018).

2.1.3 Bioreactor design

In addition to the factors discussed above, bioreactor design also plays an important role in NO_3^- removal. Many factors can influence denitrification bioreactor design, such as drainage area, seasonal rainfall, flow rate, hydraulic retention time, and other site-specific conditions (Christianson et al., 2011b; Cooke & Bell, 2014). A lengthier list of more specific factors (Table 2.1) to consider when installing a bioreactor was compiled by Christianson et al., (2012b). As a result of these site-specific factors, there is currently no single design that works for all scenarios. Cooke & Bell (2014) and Christianson et al. (2011b) offer two protocols on bioreactor design. These models heavily influenced the United States Department of Agriculture Natural Resource Conservation Service (USDA NRCS) design standard 605 which applies to all denitrifying bioreactors installed on agricultural subsurface drainage systems (USDA NRCS, 2017). More recently, Ghane et al., (2014) proposed a design method intended to better predict high flow rate conditions compared to recent models. Currently, New Zealand does not have guidelines on bioreactor design but any of the above protocols would likely be good reference points. One caveat to these design methodologies is that they have all been developed in the U.S. Midwest for subsurface agricultural systems and may require additional considerations in the younger New Zealand landscape, land uses, and environment. Further research is needed to know if additional considerations are needed for bioreactors treating wastewater.

Table 2.1. Further denitrifying bioreactor design considerations. Adapted from Christianson et al., 2012b.

Pre-Design			
Drainage Characteristics	Site Conditions	Materials Availability	Construction
Drainage area	Available space for the bioreactor	Inflow and outflow control structures	Uniform and consistent filling of media
Tile locations	Soil type	Suitable fill media	Use of a liner and woodchip covering fabric
Tile size	Proximity to sensitive or public water bodies	Plastic lining and geo-fabric to cover woodchips	Mounding soil cover
Tile slope	Equipment traffic-ability	Non-perforated pipe near the structures	Reseeding with appropriate seed mixture
Drainage co-efficient	Accessibility for installation, management, and sampling	Construction labor and equipment	Transport and use of surplus soil
Number of surface intakes (aim to minimize bioreactor influent sediment)	Identification of individual(s) to manage and monitor bioreactor	Labor for annual maintenance	Construction safety equipment and procedures
	Installation timing: availability of contractors and restrictions due to crops in the fields or nearby nesting birds		

Regardless of design method used, hydraulic retention time (HRT) and flow rate (Q), are always accounted for in bioreactor designs as they are highly influential on denitrification performance within bioreactors (Fenton et al., 2016; Feyereisen et al., 2016; Hoover et al., 2017). Hydraulic retention time is measured as the length of time for water to move from the inlet to the outlet of a bioreactor (Equation 2.1).

$$T = \frac{\rho V}{Q}$$

Equation 2.1

Hydraulic retention time (T) is directly affected by the bioreactor saturated volume (V), the media used (porosity, ρ), and the average Q of the water. If retention time is too short, dissolved oxygen may not be removed so that anoxic conditions required for denitrification do not develop. Typically, the longer the retention time, the greater the N removal efficiency, however, this may reduce removal rates as the HRT increases (Hoover et al 2016; Lepine et al., 2016). Addy et al., (2016) noted lower retention times could limit the removal rate. Christianson et al., (2013a) observed that there is no clear relationship between removal rates and HRT regarding bioreactor design. The Christianson et al., (2012b) review found that of the 23 bioreactors studied, retention time varied significantly from 26 minutes to six days, although most retention times were less than 24 hours. Woli et al., (2010) found that a significant design challenge is balancing bioreactor size for retention times to achieve complete removal with variable, unknown drainage systems.

2.1.4 Redox

Many studies have also shown that excessive retention times can result in complete NO_3^- removal and a decline in redox potential as other microbial processes using alternative electron acceptors (e.g. MnO_2, FeOOH, SO_4^{-2}, and CO_2) become prevalent. As NO_3^- is being reduced to near zero, denitrifying bacteria being to face competition for carbon from other anaerobic microorganisms such as SO_4^{2-} reducers and methanogens. The reduction of SO_4^{2-} can result in undesirable consequences such as the production of such as hydrogen sulfide, methane, and methylmercury if redox potential drops low enough (Addy et al., 2016; Blowes et al., 1994; Lepine et al., 2016; Shih et al., 2011). When these competing electron acceptors are oxidized is dependent on the amount of energy released (redox potential) with denitrification releasing more energy for microbes than SO_4^{2-} reduction (Tchobanoglous et al., 2003).

Sulfate reduction typically occurs during periods of low flow and high temperatures (i.e. summer) and in the winter with low N loads entering the systems (Blowes et al., 1994; Robertson & Cherry, 1995; Robertson & Merkley, 2009; Shih et al., 2011). Landowners are recommended to prevent these adverse effects by pulling boards to increase water flow prior to periods of low flow or

when the rotten egg smell of hydrogen sulfide is detected (Christianson & Helmers 2011). Some studies assume that if there is any sulfide present in bioreactor effluent that is likely oxidized back into SO_4^{2-} once the water was aerated (Christianson et al., 2016; Robertson et al., 2005). Shih et al., (2011) suggested designing bioreactors in a way to maintain at least a 0.5 mg NO_3^--N L^{-1} in the effluent from the bioreactor to minimize SO_4^{2-} reduction from occurring. However, few studies quantify SO_4^{2-} reduction, especially the production of methylmercury; unless NO_3^- concentrations are below 0.5 mg NO_3^--N L^{-1} and this is a concern about bioreactors that may deserve further exploration as climates change.

2.1.5 Phosphate in Bioreactors

While denitrifying bioreactors are targeted at removing NO_3^-, drainage systems also allow for phosphorus (P) to exit the field at a greater rate than usual (David et al., 2010). Phosphorus in the form PO_4^{3-} is another main contributor to hypoxia from both agricultural and wastewater sources. Algoazany et al., (2007) found that at 10 to 20 µg that phosphorus levels promote eutrophication. There is concern that bioreactors can be a source of P as woodchips (the predominantly used carbon source) contain P that if mineralized can leach from the bioreactor. Several studies have found that when bioreactors were first operational there was leaching of P, but after some time there was potential for P removal depending upon species (Dougherty, 2018; Husk et al., 2018; Sharrer et al., 2016; von Ahnen et al., 2016). From these studies, there is evidence of a P-leaching phase followed by neutral or positive removal. Addy et al., (2016) concluded a meta-analysis focused exclusively on P should be conducted in due time.

2.2 Calculation of NO_3^- removal in bioreactors

There are three different ways NO_3^- removal can be calculated and each is useful for different purposes. Before performing calculations, it is critical to consider any untreated bypass water if a bypass line is part of the bioreactor design depicted in Figure 2.1.

2.2.1 NO_3^- removal as a percent

The first way to evaluate bioreactor effectiveness is to calculate the percent load reduction or N removal efficiency. Nitrogen removal efficiency (NRE, %) is the amount of NO_3^- removed from the drainage water that enters the bioreactor subtracting the amount of NO_3^- leaving the bioreactor untreated. This is calculated as at the difference in concentrations of NO_3^- at the inlet (N_{IN}) and outlet (N_{OUT}) dividing by the inlet concentration (Equation 2.2).

$$NRE = \frac{N_{IN} - N_{OUT}}{N_{IN}} \times 100$$

Equation 2.2

Nitrate removal efficiencies are mainly used for comparing bioreactors with other nutrient-reducing agricultural practices such as saturated buffers and perennial/energy crops as this approach can account for untreated bypass flow. The N_{IN} value can be obtained from measurements taken in the inlet of a bioreactor which would not account for bypass flow or from the tile line prior which would represent the overall flow from the field. Nitrogen load efficiency can vary depending on the site and year ranging from 12-100% N load reduction Christianson et al., (2012b). Woli et al. (2010) reported NO_3^- load reductions of 23 and 50% of the tile drainage in a field bioreactors' first and second years, respectively. In the United States, bioreactors are estimated to remove 15-60% of the NO_3^- load from agricultural fields (Christianson & Helmers, 2011). Recent studies in the United States confirm this range with NO_3^- load reductions of 9-62% observed (Husk et al., 2017; Rosen & Christianson, 2017). New Zealand studies have shown that NO_3^- removal efficiencies ranged from <10 to >99% (Goeller et al., 2019; Long et al., 2011; Rambags et al., 2016; Schipper & Vojvodić-Vuković, 2001). Nitrogen removal efficiency rates in individual bioreactors are highly dependent on changes in flow rate through the bioreactor.

2.2.2 NO_3^- removal rates

Nitrate removal rate (NRR) is another index that measures NO_3^- removal and expressed as g N removed per m^3 of the bioreactor per day (g N m^{-3} d^{-1}) as shown in Equation 2.3.

$$NRR = \frac{gN_{IN} - gN_{OUT}}{m^3_{bioreactor} X\, t}$$

Equation 2.3

This calculation is based on physical dimensions and as such this calculation is more commonly used for bioreactor to bioreactor comparison and in the initial design of bioreactor sizing. A recent meta-analysis by Addy et al., (2016) found a mean NRR of 4.7 g N m^{-3} d^{-1} for denitrification beds (i.e. bioreactors). Schipper et al., (2010) reported denitrification beds had a range of removal rates 2-22 g N m^{-3} d^{-1}. New Zealand studies closely aligned to these numbers with removal rates that range between 3.0-19.8 g N m^{-3} d^{-1} (Cameron & Schipper, 2010b; Rambags et al., 2016; Tanner et al., 2012; Warneke et al., 2011a; Warneke et al., 2011b). Many of these studies were conducted on newly installed denitrifying bioreactors and as such removal rates may be temporarily high. Addy et al., (2016) confirmed that in the first year, high NRR were likely to be observed, but in year two and beyond NRR were typically lower.

Other instances of high NRR are when bioreactors treat drainage water from a source other than agricultural fields that contain high dissolved organic carbon (DOC). Having a high DOC in incoming water provides an additional carbon source for the denitrifying bacteria and may lead to increased NRR. Sources of DOC may include human waste effluent, dairy or livestock effluent, and even aquaculture (Goeller et al., 2019; Rambags et al., 2016; von Ahnen et al., 2018).

One important caveat of the Addy et al., (2016) meta-analysis is that it contained all types of studies (laboratory, pilot, field) which could mean that the average NRR could be higher than realistic particularly because of the inclusion of laboratory studies. Laboratory studies are generally of shorter duration and this may overestimate long term sustained NO_3^- removal. To address this, a survey of field scale bioreactor literature published since the Addy et al., (2016) was collated (Table 2.2). Selection criteria for inclusion were that (i) studies must have been peer-reviewed journal articles, (ii) be published after 2016, (iii) a field-scale study, and (iv) required NRR reported in as g N m^{-3} d^{-1}. The table consists of 13 studies that represented roughly 30 site years. Most of the data were from newly

installed up to two-year-old bioreactors and so long term NRR may be overestimated. From this table, NRR ranged from 0.21 to 50.9 g N m^{-3} d^{-1} with an average NRR for field studies of 5.4 g N m^{-3} d^{-1}. Within this table, there were eight bioreactors with influent that contained high DOC the average NRR for these studies alone was 12.6 g N m^{-3} d^{-1}. In contrast, bioreactors with low influent DOC had an average NRR of 4.7 N m^{-3} d^{-1} (Table 2.2). A Mann-Whitney Rank Sum Test (due to normality being violated) was conducted between the NRR from bioreactors treating average agricultural drainage and the NRR of bioreactors treating influent with high DOC determined the difference in NRR of the higher DOC was statistically significant (P-value = 0.036).

Table 2.2. Compilation of recent field studies reported after Addy et al. 2016 meta-analysis.

Source	Location	Type	Installed	V (m^3)	ha	HRT	Influent N (mg L^{-1})	NRE (%)	NRR (g N m^{-3} d^{-1})	Data Reported	Notes
Bock et al., 2018	Middlesex Co, VA	bed	Aug. 2014	25.3	6.5	3-20h	3.7	9.5†	0.56	Sept. 2015-Sept. 2016	90% WC10% biochar (v:v)
Christianson et al., 2017	Caroline Co, MD	stream bed	Nov. 2015	248.9	35	N/A	3.98	75.*, 25.†	0.97	295 d	Treated ditch water from rotating crops
Christianson et al., 2017	Somerset Co, MD	stream bed	Dec. 2015	20.8-29.3	6.6	~24h	2.53	65*	N/A	4 months of 1st year	In-Ditch bioreactor – built in 3 segments
Christianson et al., 2017	Somerset Co, MD	stream bed	Feb. 2015	19.8	0.08	N/A	5.5 & 4.9	>90*	1.9 & 2.9	~1 year	Sawdust wall
Goeller et al., 2019	Canterbury Plains, NZ	bed	Oct. 2015	25.0	160	0.9h	N/A	10*	50.9	Dec. 2015-Oct. 2017	Closed pit – tile drainage from dairy pasture‡
Goeller et al., 2019	Canterbury Plains, NZ	walls	Oct. 2015	20.0, 24.0	160	2.7h, 5.5h	N/A	57,* 99*	N/A	Dec. 2015-Oct. 2017	2 sawdust walls, riparian seep from dairy pasture
Gottschall et al., 2016	Eastern Ontario, Canada	bed	Oct. 2011	3.3, 3.3, 3.3	0.14, 0.14, 0.14	N/A	N/A	39* 62* 63*	6.58, 7.80, 6.36	Sept. 2013-May 2014	6 bioreactors - 2 replicates WC, WC and 10% WTR, WC and 20% WTR
Hassanpour et al., 2017	Tompkins Co, NY	beds	Oct. 2012	9.5	4	2.2d, 2.1d	9.3	42,* 55*	3.8, 4.7	2013-2015	1 WC and 1 WC+10% Biochar - closed pit
Hassanpour et al., 2017	Chemung Co, NY	beds	June 2013	9.5, 17.1	5	0.5d, 0.3d	6.2	68,* 66*	13.5, 15.1	2013-2015	1 WC and 1 WC+10% biochar – closed pit
Hassanpour et al., 2017	Steuben Co, NY	beds	July 2013	12.1, 17.1	6, 9	2.8d, 2.3d	18.4, 16.6	58,* 62*	4.7, 6.7	2013-2015	1 WC and 1 WC+2% biochar – closed pit
Hoffmann et al., 2019	Jutland, Denmark	beds	Fall 2012	100.0	78	N/A	5-14	55†, 56†, 43†, 46†, 50†, 55†	1.67-2.22	2013-2014	6 bioreactors with mixed media (seashells/willow WC) at 50:50 and 25:75 ratios. Closed pit with vegetation
Husk et al., 2017	South Central Quebec, Canada	beds	July 2012	33.0, 10.1, 22.7, 33.0	0.61, 0.69, 0.93, 1.28	14.1h, 5.3h 27h, 14.h	5.3	99†	6.84 (median)	2012-2015	4 bioreactors monitored 3 years for 12 site years of data cumulative. All closed pit
Pfannerstill, et al., 2016	North German lowlands	stream bed	2010	N/A	N/A	N/A	8.9	28* 15†	N/A	Aug. 2010-April 2012	drainage reactive ditch with tile from a farm - 12 m^3 WC
Pluer et al., 2018	Sarasota, FL		Nov. 2013	4.32	16.4	CF	N/A	N/A	7.9	Nov. 2013-Nov. 2014	open pit, Submerged bios in retention ponds‡
Pluer et al., 2018	Sarasota, FL		Oct. 2013	4.32	22.9	CF	N/A	N/A	3.4	Nov. 2013-Nov. 2014	open pit, Submerged bios in retention ponds‡

Source	Location	Type	Installed	V (m^3)	ha	HRT	Influent N (mg L^{-1})	NRE (%)	NRR (g N m^{-3} d^{-1})	Data Reported	Notes
Rambags et al., 2016	Newstead, NZ	bed	May 2013	114.0	CF	7-10d	avg 31.2	>99*	~14	Aug. 2013-June 2015	closed pit trapezoidal, treats wastewater‡
Rosen & Christianson, 2017	Caroline, Co, MD	bed	Nov. 2013	169.3	34.7	>10d	4.7, 7.7	9†, 16†	0.4, 0.21	Aug. 2014-Aug. 2015, Aug. 2015-May 2016	closed pit treats dairy farm
Rosen & Christianson 2017	Queen Anne's Co, MD	bed	Dec. 2013	90.9	25.2	> 1 week	9.2, 8.6	62†, 47†	5.36, 5.12	Aug. 2014-Aug. 2015, Aug. 2015-April 2016	closed pit treats organic grain farm
Rosen & Christianson 2017	Caroline, Co, MD	bed	Nov. 2014	252.6	40.1	42 ± 56 h	13.5	10†	1.53	Dec. 2014 – July 2015	closed pit treats conventional row crops
von Ahnen et al.,, 2016	North Jutland Region, Denmark	bed	Oct. 2015	12.5	CF	N/A	5.6	N/A	7.06	first 147 d	treats aquaculture - start-up - rainbow trout farm‡
von Ahnen et al., 2018	Denmark	bed	March 2017	300	CF	N/A	5.3	80*	4.8	52 weeks from install	Horizontal flow – rainbow trout aquaculture‡
von Ahnen et al., 2018	Denmark	bed	July 2017	660	CF	N/A	10.5	29*	4.5	28 weeks starting 6 weeks after install	vertical/down flow - rainbow trout aquaculture‡
von Ahnen, et al., 2018	Denmark	bed	Jan. 2017	1440	CF	N/A	9.5	48*	7.8	52 weeks starting 7 weeks after install	vertical/down flow - rainbow trout aquaculture‡

Where V is the volume of bioreactors, ha is number of drainage hectares treated, HRT is reported hydraulic retention times observed, NRE is N removal efficiency (noted as concentration or load), NRR is N removal rate, WC is woodchips, and N/A is not available.

Footnotes:
* = Concentration reduction, † = Load reduction, WTR = waste treatment plant residuals, CF = Constant pumped flow,‡ = these bioreactors have high dissolved organic carbon (DOC) in the influent likely resulting in "higher" NRE and NRR

2.2.3 NO_3^- removal cost

Finally, bioreactors can be evaluated through an efficiency/benefit analysis, or the cost per kg N removed ($/kg N removed). However, few papers have estimated the total cost of NO_3^- removal unless analyzing the overall installation cost of a bioreactor and usually only considers the first few years of activity. Schipper et al., (2010) performed a cost/benefit analysis using data from Robertson et al., (2009) and determined the experimental bioreactor removed 2.39 to 15.17 US$ kg-N^{-1}. Christianson et al., (2013b) calculated the mean annual cost (US$ kg N^{-1} yr^{-1}) for bioreactors in the United States of $1.30. This is an area that requires more research to show how the "real world" application of bioreactors can be cost-effective. Cost efficiency analyses such as those summarized in Table 2.3; provide tangible numbers for landowners to review when deciding what nutrient remediation strategies to use. These cost efficiencies are also useful to regulators in deciding which strategies to promote. Providing cost analysis such as that above can aid in decision making of whether a bioreactor provides returns on its initial installation cost in comparison to other approaches.

Table 2.3. Example cost for different N reduction strategies in USD and kilograms (kg). Adapted from the Illinois Nutrient Loss Reduction Strategy (IEPA & IDOA, 2015). Cost converted to NZ$ using Morningstar for Currency and Coinbase for Cryptocurrency on 31 October 2019.

Practice/scenario	Cost (US$/lb removed)	Cost (NZ$/kg removed)
Bioreactors on 50% of tile-drained land	2.21	1.57
Cover crops on all corn/soybean tile-drained acres	3.21	2.27
Wetlands on 35% of tile-drained land	4.05	2.88
Buffers on all applicable crop land (reduction only for water that interacts with active area)	1.63	1.16
Perennial/energy crops equal to pasture/hay acreage from 1987	9.34	6.64
Perennial/energy crops on 10% of tile-drained land	3.18	2.25

Regardless of how NO_3^- removal is calculated there is still a wide range of values for removal rates and efficiencies and better data is needed. Currently, grab samples are the primary way through which NO_3^- data and its removal are calculated. Grab sampling is problematic as these samples only represent points in time and are usually gathered weekly and as such do not necessarily capture fluctuating N concentrations which would provide more accurate removal data. Further discussion of grab sampling and an alternative, DGT are presented below.

2.3 Diffusive Gradients in Thin-films (DGT)

Water quality data is primarily collected via active sampling methods such as hand or autosampler grab sampling and then analyzed in the laboratory using a variety of analytical approaches (Christianson et al., 2012b; Hartz et al., 2017; Rozemeijer et al., 2010; Vrana et al., 2005). Samples collected in situ are also possible but are generally restricted to basic water quality parameters (e.g. temperature, pH, dissolved oxygen). However, in situ sampling is typically costly. Infrequent sampling can result in unrepresentative data due to a variety of factors. The first factor being that grab sampling only represents the concentration at the time the sample was collected and can, therefore, result in a highly variable data set (Audet et al., 2014; Vrana et al., 2005). Other reasons for high variability could be from low sampling frequency, seasonal N-cycling, land use, inputs from point and non-point sources, and the weather (Huang et al., 2016a). Christianson & Schipper (2016) concluded that additional advanced monitoring techniques were needed to more closely evaluate bioreactor performance as well as additional long-term monitoring to have a wider base of N removal knowledge.

An example of the high variability of NO_3^- concentrations that can occur within a bioreactor is given in Figure 2.2. This figure illustrates that even with hourly grab sampling, concentrations can fluctuate significantly with time with the inlet concentration having an hourly change of 40 mg N L^{-1}. Fluctuating NO_3^- concentrations make it difficult to accurately calculate NO_3^- removal, especially when there are large fluctuations as shown in the inlet concentrations which is the initial value used to determine NRR and NRE (Equations 2.2 and 2.3). A potential way to revolutionize the way nitrogen concentration data is collected is through the use of passive sampling techniques such as Diffusive Gradients in Thin-Films (DGT) (Davison, 2016; Menegario et al., 2017; Zhang & Davison, 1995).

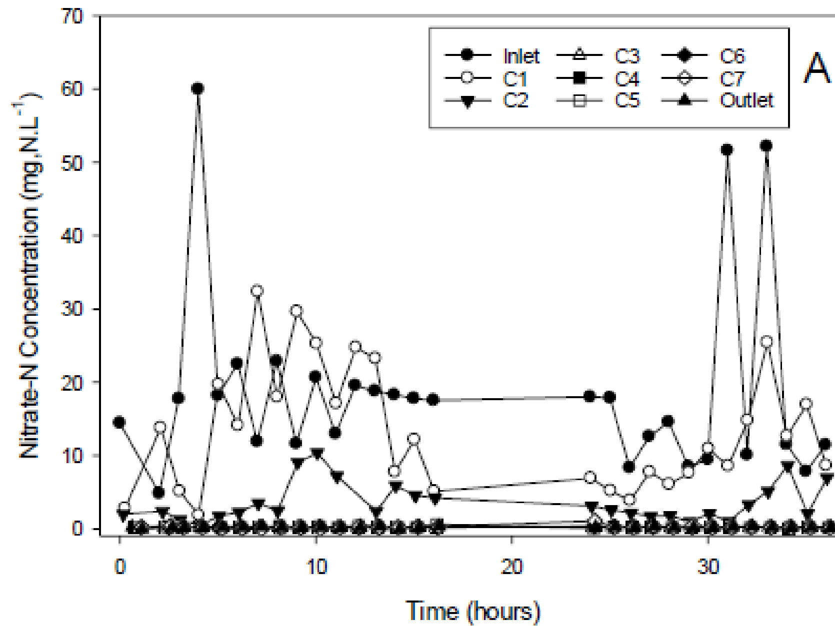


Figure 2.2. Nitrate concentrations collected by hourly grab sampling plotted against time collected from the previous study conducted at the LIC bioreactor in March and April 2019. Each line symbol in the legend represents the sampling well from where the data was collected. The inlet, C1-C7, and outlet represent different sampling points along the length of the bioreactor. (From Corbett et al., in press).

Diffusive Gradients in Thin-Film utilize Fick's first law of diffusion, the phenomena where molecules move from areas of high concentration to equilibrate concentrations, where Flux (J) is equal to the change in concentration (∂C), multiplied by the diffusion coefficient (D) over the change of position (∂x), as shown in Equation 2.4 (Schaschke, 2014).

$$J = \frac{-D\partial C}{\partial x}$$

Equation 2.4

Diffusion is temperature-dependent; therefore, the diffusion coefficient needs to be adjusted for temperature using the Stokes-Einstein equations (Huang et al., 2017; Huang, et al., 2016a; Zhang & Davison, 1999).

Diffusive Gradients in Thin-Films allow for the calculation of the average concentration in the water over a determined time and can be built to capture selective species (e.g. metals, NO_3^-, SO_4^{2-}, etc.) (Menegario et al., 2017). They are composed of five basic parts; a plastic cap (with an exposure window), a filter membrane, a diffusive layer, a binding layer, and a plastic base as depicted in Figure 2.3A. (Zhang & Davison, 1995).

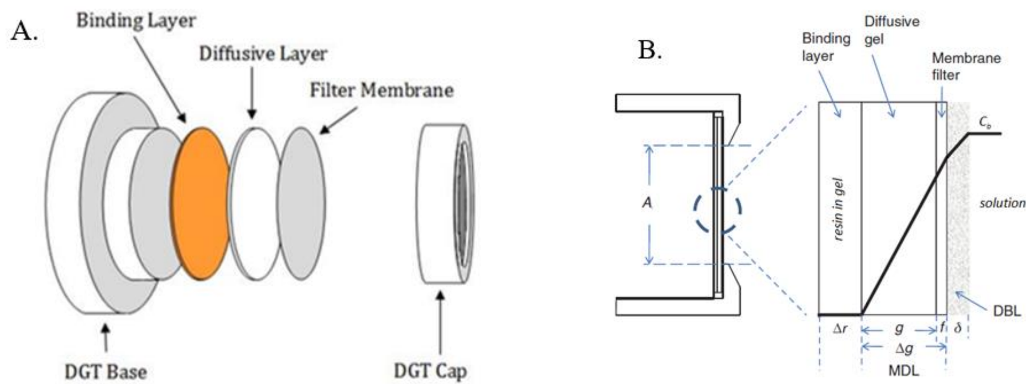


Figure 2.3. A) Expanded diagram of a DGT depicting each layer. See text for a full description of each DGT part. B) A side view depiction of how diffusion works within the DGT. Note that the diffusive boundary layer (DBL) is the area where the solution and DGT window interact while the material diffusive layer (MDL) is the width of the diffusive gel and filter membrane that the analyte has to cross to become bound to the resin gel. From T. Corbett at the University of Waikato, NZ.

When assembling the DGT, the layer that lays against the cap is the filter membrane which protects the underlying layers from microbial decomposition and prevents sediment clogging (Davison, 2016). The diffusive layer and the filter membrane forms the material diffusive layer (MDL) of a known thickness (Figure 2.3B) which the selected analyte species can pass through to the highly selective binding layer to be immobilized (Davison, 2016; Zhang & Davison, 1994; Zhang & Davison, 1995). The action of the analyte binding to the binding layer reduces the solute down to zero, creating a concentration gradient earning the name "diffusive gradient" (Zhang & Davison, 1995).

As DGT are a passive sampling technique, field deployments only require noting times deployed and removed and temperature throughout the study. DGT should have weights attached to ensure they stay submerged during the length of deployment. Total deployment time, calculated prior to deployment in the lab, is dependent upon the maximum saturation level of the binding layer for the selected species.

After retrieval from the bioreactor, the first two layers are removed. The accumulated analyte is eluted from the binding layer and measured using standard analytical approaches to allow calculation of the accumulated mass (C_{DGT}, mg cm^{-3}). The time-weighted average concentration of the analyte is calculated by using the thickness of the DBL (δ, cm, see below), the exposure window area ($A_{eff,}$, cm^2), the deployment time (t, s), the mass of the analyte eluted from the DGT (M, mg), and the appropriate diffusion coefficient of the diffusive layer (Davison, 2016; Zhang & Davison, 1994; Zhang & Davison, 1995).

For nitrogen remediation strategies, the DGT-measured NO_3^- concentrations are calculated following Equation 2.5. This was preferential to using the standard DGT equation (Equation 2.6), as it appropriately accounts for DBL and lateral diffusion through the diffusion layer (Davison, 2016).

$$C_{DGT} = \frac{M}{A_{eff}t}\left(\frac{\Delta g_{gel}}{D_{gel}} + \frac{\Delta_f}{D_f} + \frac{\delta}{D_w}\right)$$

Equation 2.5

In this expanded equation from Davison (2016), there are three diffusion coefficients; diffusive gel (D_{gel}), filter membrane (D_f), and DBL (D_w) all measured in cm^{-2} s^{-1} while the thickness of the diffusive gel (Δg_{gel}), filter membrane (Δ_f), and DBL (δ) are measured in cm (Figure 2.3B).

$$C_{DGT} = \frac{M\Delta g}{DAt}$$

Equation 2.6

Where M is the mass of the analyte eluted from the DGT, Δg is the known DBL thickness (cm), D if the analyte species diffusion coefficient (cm^2 s^{-1}), and A is the area of the exposure window (cm^2).

Both equations use a diffusion coefficient of 1.46 x 10-5 cm^2 s^{-1} at 25°C for NO_3^- through the diffusive gel (Huang et al., 2016a) which can also be used as the diffusion coefficient for the filter membrane (Davison, 2016).

As mentioned previously, diffusion is temperature-dependent and all diffusion coefficients (D_{gel}, D_f, D_w) need to be corrected for temperature using the following equation (Davison, 2016):

$$logD_T = \frac{1.37023(T-25) + 0.000836(T-25)^2}{109+T} + log\frac{D_{25}(273+T)}{298}$$

Equation 2.7

Where D_T = diffusion coefficient at temperature T (cm^2 s^{-1}), T = temperature (°C), and D_{25} = diffusion coefficient (cm^2 s^{-1}) at 25°C.

The DBL is the area where the solution and DGT window interact. DBL values can fluctuate depending on the flow rate (Davison, 2016). To calculate DBL Equation 2.8 was used; where D_{mdl} = mdl diffusion coefficient; y= y-intercept; s = slope (Davison, 2016), of the inverse of the accumulated mass (1/M), plotted against diffusive layer thicknesses versus (δ) (Levy et al., 2011; Zhang et al., 1998).

$$\delta = \frac{yD_w}{sD_{mdl}}$$

Equation 2.8

2.3.1 Previous uses of DGT

Originally developed for determining concentrations of freshwater metal and semi-metal sampling, this technique is still relatively new but has expanded rapidly and been used for many applications (Zhang & Davison, 1994). Davison & Zhang (2012) recorded the many ways DGT technology has expanded beyond water quality sampling to applications in research on sediment geochemistry, chemical speciation in solutions, bioavailability and dynamic processes in water and soils. Diffusive gradients in thin-films have been developed to measure oxyanions, trace metals, sulfide, inorganic nutrients and binding layers for PO_4^{3-}, NH_4^+, and NO_3^-. Though DGT have primarily been used for a single selected analyte, multiple or mixed binding layer methods have begun to be developed for multi-species analysis (Huang et al., 2017).

In terms of agricultural water quality monitoring, only recently have studies have shown that Purolite A520E-DGT anion can be used as the binding agent for NO_3^- while PrCH resin and zeolite have successfully been used as binding agents for ammonium (NH_4^+), (Corbett et al., in press; Huang et al., 2017; Huang et al., 2016a; Huang et al., 2016b). Huang et al., (2016b) first experimented with using Purolite's A520E as an N binding layer for DGT. Through two field deployments for 24 h periods over four days, they showed that the A520E resins would work in waters with pH between 3.5-8.5, worked best at binding NO_3^- than NO_2^-, and could be useful in the future for monitoring loads in waters. One drawback was that they were unsure if long term deployment would be feasible for high N waters due to saturation of the resin in the binding layer.

Huang et al., (2016a) also investigated the usability of commercially available AMI-7001 anion exchange membrane binding layers for NO_3^- instead of Purolite A520E as they are easier to prepare and handle than the Purolite A520E DGT resins. These layers were determined to be equally as effective while also reducing preparation time and were potentially less chemically hazardous. The AMI-DGT could be deployed for 72 h and were thought to have the potential for "stacking" for use with other membranes and binding layers thus next steps were to test them in a field setting. Huang et al., (2016c) deployed DGT constructed using A520E at seven sites (freshwater and wetlands) for 24 and 72 h and demonstrated the DGT data was equivalent to results obtained by grab sampling. They argued that the observed difference between DGT measured concentrations and grab samples was likely due to grab samples not adequately capturing a representative average result making DGT superior to low frequency grab sampling.

Huang et al., (2017) then demonstrated that A520E had the potential to be combined with other binding layers to monitor NH_4^+ and PO_4^{3-} as well. Research is still needed as it was observed that NH_4^+ capacity was reduced compared to existing single analyte DGT. Like the other previous studies, (Huang et al., 2016a; Huang et al., 2016b; Huang et al., 2017) this combination layer DGT reduced costs and preparation time. This study showed that one mixed binding layer sampler could be used in the place of three separate analyte-specific increasing cost-effectiveness and presenting a more practical larger scale monitoring tool.

Building on upon these previous studies, Corbett et al., (in press) improved upon the NO_3^- specific methodology described by Huang et al., (2016b) by grinding and sieving the Purolite, increasing the quantity used in the resin (from 2 g to 4 g), and mixing it with acrylamide instead of bisacrylamide. They then deployed these fuller Purolite resin DGT in denitrifying bioreactors to determine NO_3^- removal rates. In 16, 24, and 36 h deployments Corbett et al., (in press) determined that DGT were an attractive alternative to low frequency grab sampling and very closely matched high frequency grab sampling. The study refined the A520E resin methodology and showed real-life practicality in both time and cost-effectiveness as well as more representative overall equivalent to high frequency grab sampling and concluded that DGT were a worthy alternative to traditional techniques.

When compared to grab sampling, DGT represent a fraction of the cost in time, equipment, preservation, and overcome point in time sampling to obtain a time-weighted average. As such, DGT more closely represents high frequency grab sampling and can provide more representative N concentrations that occur over deployments compared to weekly grab samples. In terms of DGT for NO_3^-, DGT do not have to be analyzed immediately like grab samples as the NO_3^- is bound to Purolite resin and inaccessible to microorganisms and potential ongoing transformations. These passive sampling devices are an attractive option to replace traditional active sampling methods especially for basic monitoring of nitrogen remediation strategies such as denitrifying bioreactors.

2.4 Conclusion

Denitrifying bioreactors are a proven nitrogen mitigation technology. To keep improving the performance of bioreactors and other mitigation strategies (e.g. saturated buffers and wetlands) it is critical to accurately determine NRR to inform not only performance but improve designs and evaluate if a practice is working. The use of DGT have recently been proven as a successful alternative to grab sampling when monitoring NO_3^- within denitrifying bioreactors and other freshwaters. Moving forward in nitrogen mitigation research such as denitrifying bioreactors, DGT are an option to consider using instead of traditional grab sampling and expensive in situ methods. Future studies need to continue testing DGT as an alternative sampling approach under the different conditions present in denitrifying bioreactors and other strategies such as low flow. Additional studies will likely explore the use of multiple binding layers DGT to monitor additional water nutrients such as PO_4^{3-}, SO_4^{2-}, and NH_4^+.

Chapter 3

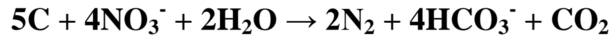
Evaluation of a denitrifying bioreactor to improve water quality of tertiary treated water using DGT

3.1 Introduction

Nitrogen is a building block of life and integral to the growth of all life and therefore is a key component for maintaining production in agricultural systems. In urban systems, nitrogen is present in septic systems and other wastewaters. Specifically, NO_3^- is of great concern to many due to its presence in nitrogen fertilizers and municipal wastewater treatment facilities, and as NO_3^- is easily lost from soils (Galloway et al., 2003). This is of even greater concern when subsurface drainage systems are present, as these drainage systems support rapid NO_3^- transportation to sensitive waterways.

When excess NO_3^- is present in ecosystems, both environmental and human health are at risk. The use of septic tanks allows untreated NO_3^- water to percolate to groundwater which can then contaminate drinking waters. With the increase in anthropogenic N from agricultural sources paired with the rise of subsurface drainage to improve lands for agricultural use, it is imperative to develop effective nitrogen mitigation strategies. In agricultural lands, current nitrogen remediation strategies include cover crops, drainage water management, saturated buffers, wetlands, and denitrifying bioreactors (Cooke & Verma, 2012, Groh et al., 2018, Kadlec & Wallace, 2009 Tonitto et al., 2005;). Denitrifying bioreactors are also used to treat wastewaters from urban systems (i.e. municipal waste) (Robertson & Cherry, 1995; Rambags et al., 2016).

A proven technology for nitrogen remediation, denitrifying bioreactors enhance naturally occurring denitrification by providing ideal conditions for microorganisms. For denitrification to occur four conditions need to be met (i) a source of NO_3^- or another N oxide, (ii) a carbon source, (iii) denitrifying bacteria, and (iv) anoxic conditions (Korom, 1992). Once met, denitrifying bacteria consume the carbon (C) converting NO_3^- into nitrogen gas (N_2), bicarbonate (HCO_3^-), and carbon dioxide (CO_2):



$$5C + 4NO_3^- + 2H_2O \rightarrow 2N_2 + 4HCO_3^- + CO_2$$

Equation 3.1

Denitrifying bioreactors are able to facilitate these conditions by providing a place (usually a trench or ditch) for a carbon-rich environment through which subsurface drainage water may pass. Water levels in the bioreactor are controlled with inlet and outlet structures to maintain anoxic conditions and flow rate (Blowes et al., 1994; Robertson & Cherry, 1995; Schipper & Vojvodić-Vuković, 1998).

When denitrifying bioreactors have influent NO_3^- or NO_3^- concentrations within the bioreactor and along the flow path of less than 0.5 mg NO_3^--N L^{-1}, microbes can begin to use carbon to reduce other electron acceptors such as SO_4^{2-}. Sulfate-reducing conditions allow for sulfide (i.e. rotten egg smell) to be released as well as conditions for methylmercury production. After all other electron acceptors have been reduced then methanogenic bacteria produce methane. All of which are unwanted reactions as (i) sulfate production corrodes and is toxic and (ii) methylmercury is toxic. As these conditions usually occur during periods of low flow, complete NO_3^- reduction, or both, these microbial reactions can be prevented by allowing water to flow freely (Blowes et al., 1994; Robertson & Cherry, 1995; Shih et al., 2011).

In addition to the above compounds, denitrifying bioreactors are also monitored for P. More specifically, they are monitored for PO_4^{3-} or dissolved phosphorus as excess phosphorus levels can also promote eutrophication. There is concern that bioreactors can be a source of P as woodchips (the predominantly used carbon source) contain P that if mineralized can leach from the bioreactor. There has not been extensive research on P within bioreactors, however, some studies have found initial leaching of P followed by the potential of P removal, however, more studies are needed for a better consensus (Dougherty, 2018; Husk et al., 2018; Sharrer et al., 2016; von Ahnen et al., 2016).

Throughout literature, bioreactors have been able to reduce annual NO_3^- loads from anywhere between 9-62% (Christianson et al., 2012a; Husk et al., 2017; Rosen & Christianson, 2017). In the past, it has been reported that denitrifying bioreactors had NO_3^- removal rate (NRR) range of 2 to 22 g N m^{-3} d^{-1} (Schipper et al., 2010). A more recent meta-analysis of an array of bioreactor studies including laboratory, field, and pilot, reported a mean NRR of 4.7 g N m^{-3} d^{-1} (Addy et al., 2016). However, the value Addy et al., (2016) found was likely somewhat high due to the inclusion of laboratory and pilot studies which are generally controlled short term studies rather than to long term field bioreactor studies. Additionally, the NRR of 4.7 g N m^{-3} d^{-1} may due elevated due to the inclusion of bioreactors studies using data from bioreactors less than two years old which are known to have temporarily high NRR and do not illustrate longer removal rates.

Denitrifying bioreactors that treat waters other than from agricultural groundwater (e.g. wastewaters) may see higher NRR removal due to increased available dissolved organic carbon (DOC) influent in the bioreactor. Waters that typically have increased DOC concentrations include; wastewaters (including human waste), livestock effluent, and aquaculture systems (Goeller et al., 2019; Rambags et al., 2016; von Ahnen et al., 2016; von Ahnen et al., 2018). For example, denitrifying bioreactors treating aquaculture effluent have been found to have NRR ranging from 4.5 to 7.8 g N m^{-3} d^{-1} (von Ahnen et al., 2018) which is slightly higher than the average presented by Addy et al., (2016).

All of the data used to determine these NRR were collected by grab sampling or automated grab sampling. While grab sampling is currently the most widely used method for ascertaining nutrient removal such as NO_3^-, there are some problems with this approach. Grab samples only represent a single point in time and can, therefore, miss rapid temporal changes in NO_3^- concentration, which can lead to a mischaracterization of average NO_3^- concentrations. This is especially important during growing seasons and around livestock operations as additional NO_3^- is introduced from seasonal fertilizers and manure as well as during times of rainfall.

One potential way to overcome the problem of NO_3^- quantification is to measure the time-weighted mean concentration. To do this, diffusive gradients in thin-films (DGT) can be deployed. Diffusive Gradients in Thin-films are based on Fick's First Law of Diffusion. An analyte, such as NO_3^-, diffuses from areas of high concentrations to areas of lower concentration; this concentration gradient is maintained by the binding of NO_3^- to a binding layer (Zhang & Davison, 1995). A requirement of the DGT methodology is an accurate measurement of time (length of deployment) and temperature (as diffusion is temperature dependent). Ideally, the temperature is logged throughout a DGT deployment, however, bioreactors are isothermal, therefore temperature at DGT deployment and removal often may suffice (Corbett et al., in press) DGT have been shown as effective for monitoring NO_3^- within denitrifying bioreactors (Corbett et al., in press).

The focus of Corbett et al., (in press) was to test whether DGT could be used to accurately determine NO_3^- concentration in a bioreactor with less emphasis on determining NRR. In this study, A520E-DGT were deployed in a controlled flow wastewater denitrifying bioreactor during the winter season to also determine long-term NO_3^- concentrations in place of traditional grab sampling methods. The resulting measurements were used to calculate bioreactor performance (NRR and NRE) and were then compared to the previous study of Corbett et al., (in press) that made measurements during higher summer temperatures. Nitrate removal rates were also summarized from recently reported field studies of bioreactors with since the meta-analysis by Addy et al., (2016). A secondary objective here was to determine whether SO_4^{2-} and PO_4^{3-} was removed and lastly if methylmercury was formed.

3.2 Materials and Methods

3.2.1 Study site

A controlled-flow full-scale denitrifying bioreactor used to treat human wastewater from a laboratory and ablution block was selected for this study. The effluent that enters the denitrifying bioreactor was pre-treated by a septic tank and recirculating filter systems (AdvanTex AX100, Orenco Systems Inc.) prior to entering the bioreactor through a slotted plastic arch vault as part of Livestock Improvement Corporation (LIC) buildings water septic system, located at the LIC

facility in Newstead, New Zealand. This bioreactor was constructed in May 2013 (Figure 3.1) with a trapezoidal trench design with a 20 m top length, 7 m top width, 1 m depth and side slopes of ~1:1 (width: height) filled with woodchips (*Pinus radiata*) (Rambags et al., 2016). The woodchips were layered with geotextile mesh to prevent root growth into the woodchips below, and then a soil cap was placed and planted with vegetation (Rambags et al., 2016). The original sampling wells described in Rambags et al., (2016) had a smaller diameter and were replaced with larger slotted PVC wells (50 mm diameter) in March 2019 to accommodate the DGT units (see below). The flow rate was monitored independently by an electromagnetic flowmeter before the bioreactor inlet providing total daily flow only. The amount of water that passed through each day varied depending on the people on site. This variation in the number of people resulted in fluctuating NO_3^- concentrations entering the bioreactor particularly during the weekend when the wastewater loads were low.

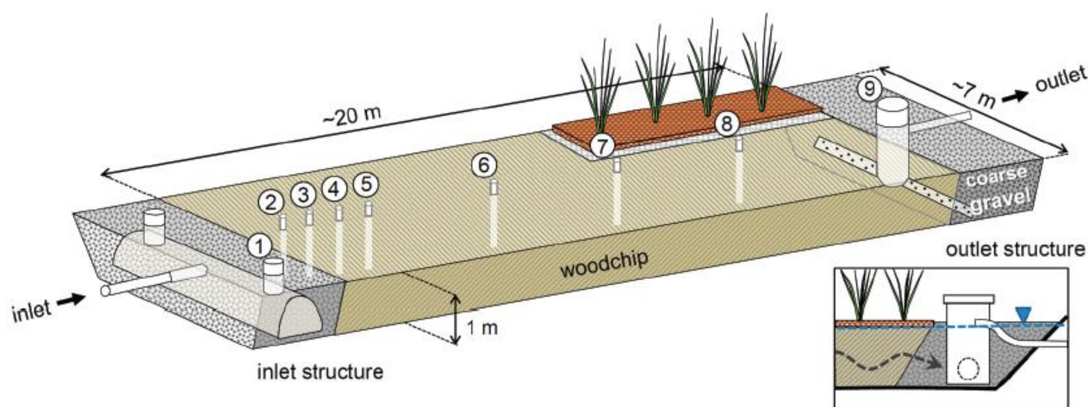


Figure 3.1. The design of the LIC bioreactor. Water enters through the inlet (left) and traveling the length to exit at the outlet (right). The numbers indicate the sampling wells (including the inlet and outlet). (From Rambags et al. 2016).

3.2.2 DGT procedures

3.2.2.1 General laboratory procedures & DGT preparation

Solution preparation, reagents, and washing all used deionized water (MΩ = 17.6-18.2). All containers used for experiments, glass plates for preparing gels, and DGT components were washed in 10 % (v/v) HCl for a minimum of 24 hours (h), before being thoroughly rinsed with deionized water (Huang et al., 2016a).

Agarose acrylamide gel solution was prepared by mixing gel cross-linker 2% (DGT research Ltd, Lancaster, UK), deionized water, and acrylamide solution (40%) following the methods of Zhang & Davison, (1994). Agarose cross-linked polyacrylamide (APA) hydrogels were prepared by mixing gel solution (10 mL), ammonia peroxydisulfate (70 µL of 10% solution), and N,N,N',N'-tetramethylethane-1,2-diamine (TEMED, 25 µL) then cast between glass plates separated by spacers (0.4 & 0.5 mm) and polymerized at 45°C for 90 minutes (Huang et al., 2016a). Once set, gels were rinsed with DI water and washed six times on a shaker table for 24 h. The APA diffusive gels were stored in 0.01 mol L^{-1} NaCl in (~750 mL) (Huang et al., 2016a).

Nitrate specific binding gels were based on the A520E developed and described by Huang et al. (2016b) and modified by Corbett et al., (in press). The A520E Purolite resin gels were made of air-dried, ground, and sieved resin (4 g) thoroughly mixed with 10 mL gel solution, 70 µL of 10% solution ammonia peroxydisulfate, and 25 µL TEMED (Corbett et al., in press). The mixture was then cast between glass plates with a 0.5 mm spacer and polymerized at 45°C for 100 minutes. The resins were washed four times over 24 h but stored in ~750 mL DI water at 4°C. Blanks from each set of Purolite resin gels were analyzed for background NO_3^- concentrations of the binding gels, to adjust for any background NO_3^-.

Filter membranes were cut from sheets of polyethersulfone (Sterlitech, Washington, US) and stored in deionized water until assembly. DGT with three different material thickness layers were assembled by layering cut Purolite resin gels (3.8cm^2 disk), APA diffusive gels, then the filter membrane (3.8cm^2) before being sealed with the DGT cap. DGT were deployed in triplicate with the various diffusive gel layer thicknesses so that the diffusive boundary layer (DBL; see below) could be calculated (Corbett et al., in press). All procedures were done in clean laboratory conditions to minimize contamination.

3.2.2.2 Field deployment

A study conducted earlier in the year also at the LIC bioreactor involved a lot of exploratory work on how to best use of DGT to determine NO_3^- concentrations (Corbett et al., in press). This previous study provided much of the groundwork for DGT in bioreactors and influenced the study outlined in this paper's hypothesis and field deployments. A description of making and extracting DGT is given below.

DGT were strung together on a fishing line in triplicate with different diffusion thicknesses (D_{mdl} =0.095, 0.079, and 0.015 cm, see below) and then weighted by a fishing weight or rock to ensure DGT remained submerged during deployment. To measure NO_3^- concentrations within the LIC bioreactor 4 DGT deployments (24 h each) were made during the winter season, with triplicate DGT deployed from the inlet to well 4 as well as the outlet. Corbett et al, (in press) and Rambags et al., (2016) previously found that the bioreactor had achieved complete NO_3^- removal by well 4.

Temperature was recorded by iButtons (iButtonLink, Wisconsin USA) attached to each set of DGT. Dissolved oxygen (DO) was measured at the inlet, all wells, and outlet using an Einstein Tablet (Fourier Systems Ltd., Israel) to determine where anoxic conditions were occurring. Additional grab samples were taken from each well after deploying the DGT from the length of the bioreactor to analyze for NO_3^-, SO_4^{2-}, and PO_4^{3-}. These samples were stored on ice until returned to the lab and then stored at -20°C, then thawed at 4°C until analyzed via ion chromatography. Before running the grab samples, iron was precipitated out by adding a known amount of NaOH, placed on a shaker table for ~30 minutes, and centrifuged for 10 minutes before 4 mL was decanted and analyzed.

A sub-set of grab samples were analyzed for methylmercury (MeHg) and inorganic mercury (Hg) using Agilent 1260 BioInert HPLC operated in tandem with an Agilent 8900 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS; Agilent Technologies, Santa Clara, California, USA) controlled by MassHunter Workstation (version 4.5).

3.2.2.3 Processing and analysis of DGT

Upon retrieval, DGT were rinsed clean with deionized water and stored at 4°C until disassembled and eluted. To analyze NO_3^- concentrations, the A520E Purolite resin disks were kept eluted in a 4 mL solution of 2 mol L^{-1} for 24 h on a shaker table then diluted with 6 mL of deionized water. (Corbett et al., in press, Huang et al., 2016b)

Nitrate concentrations from the eluted DGT were measured using a Dionex ICS-200 Ion Chromatograph (Dionex, California, United States) alongside commercial anion standards (Dionex 220 Seven Anion Standard) using a. As a chloride solution was used to elute the DGT, a gradient concentration method (41-minute isocratic method at 1 mL per minute) was used to ensure that chloride was eluted prior to NO_3^- providing optimal peak resolution. The measured NO_3^- concentrations were converted to mass and an elution factor (0.93) was applied (Corbett et al., in press), the NO_3^- mass was then used to calculate the DBL and subsequently used (Davison, 2016):

$$C_{DGT} = \frac{M}{A_{eff}t}\left(\frac{\Delta g_{gel}}{D_{gel}} + \frac{\Delta_f}{D_f} + \frac{\delta}{D_w}\right)$$

Equation 3.2

Where C_{DGT} is DGT measured concentration in units mg L^{-1}; M (mg) is the mass of the analyte eluted from the DGT; $A_{eff,}$ (cm^2) is the exposure window area; t is time; Δg_{gel} (cm) is the thickness of the diffusive gel; Δ_f (cm) is the filter membrane; δ (cm) is the DBL. With diffusive gel (D_{gel}), filter membrane (D_f), and DBL (D_w) as the diffusion coefficients all measured in cm^2 s^{-1}.

(Huang et al., 2016c) found 1.46×10^{-5} at 25°C to be the diffusion coefficient of NO_3^- through APA. For the filter membrane, the above diffusion coefficient was also used as these layers have been determined to be indiscernible from each other (Davison, 2016). The D_{MDL} was determined by combing the filter and gel components which were then also the mdl and diffusion layer thickness (Δg).

However, the diffusion coefficients (D_{gel}, D_f, D_w) all needed to be temperature corrected since diffusion is temperature dependent:

$$logD_T = \frac{1.37023(T-25) + 0.000836(T-25)^2}{109+T} + log\frac{D_{25}(273+T)}{298}$$

Equation 3.3

Where T = temperature (^{o}C), D_T = diffusion coefficient at temperature T ($cm^2\ s^{-1}$), and D_{25} = diffusion coefficient ($cm^2\ s^{-1}$) at 25°C (Davison, 2016).

3.2.2.4 Determining DBL

The diffusive boundary layer (DBL) is the volume where the solution and DGT window interact that needs to be accounted for when calculating the total diffusion distance. Prior work from the autumn study showed DBL values can fluctuate between wells depending on flow rate and mixing throughout the bioreactor (Corbett et al., in press; Davison, 2016). To calculate DBL, the following equation was used:

$$\delta = \frac{yD_w}{sD_{mdl}}$$

Equation 3.4

Where D_{mdl} = mdl diffusion coefficient; y= y-intercept; s = slope (Davison, 2016), of the inverse of the accumulated mass (1/M) plotted against diffusive layer thicknesses versus (δ) (Levy et al., 2011; Zhang et al., 1998).

3.2.3 Nutrient removal

The N removal efficiency was calculated as the difference between inlet and outlet concentration divided by the inlet concentration (Equation 3.5). As there was no bypass line in this system's design "bioreactor removal efficiency" and "overall removal efficiency" were interchangeable.

$$NRE = \frac{N_{IN} - N_{OUT}}{N_{IN}} \times 100$$

Equation 3.5

Typically, NRR is calculated using Equation 3.5 is for bioreactors which do not have monitoring wells. As complete NO_3^- removal was achieved within the first four meters of the bioreactor, NO_3^- removal was calculated using the modified Equation 3.6 to reflect where NO_3^- became limited:

.

$$\boldsymbol{NRR = \frac{gN_{IN} - gN_{OUT}}{m^3_{bioreactor}\, X\, t}}$$

Equation 3.6

$$\boldsymbol{NRR = \frac{(\Delta N) \times Q_{avg}}{V_{limited}}}$$

Equation 3.7

Therefore, NO_3^- removal was calculated as the slope of a linear fit of NO_3^- concentration from inlet to well 4 (ΔN), multiplied by the average flow rate (Q_{avg}) for the DGT deployment time divided by bioreactor volume up the well where N was limited. Removal rates for SO_4^{2-} were calculated using the same method.

3.3 Results and Discussion

3.3.1 NO_3^- removal

During the four study deployments, the LIC bioreactor had an average influent N concentration of ~13 mg N L^{-1} (Table 3.1). The influent N from the grab samples was lower than in well 1 likely as these samples were collected in the early morning before most staff arrived on site. Nitrate concentration collected may have been from wastes collected the day before. Alternatively, these low concentrations may have been a result of O_2 being present in the inlet allowing for NH_4^+ to be converted to NO_3^- between the inlet and well 1. Or the lower concentrations may have resulted from a combination of both of the above theories. The NRR and NRE for the grab sampling data were calculated using the values from wells C1 to C4 to obtain the highest rates of NO_3^- removal and more accurately reflect denitrification rates (as seen in Deployment 3). The influent N concentrations and NRR from Table 3.1 were in agreement with conclusions drawn by Addy et al., (2016) that initial elevated levels of N concentrations typically result in higher removal rates.

Table 3.1. Bioreactor influent nitrate concentrations, nitrate removal rates (NRR), nitrate removal efficiency (NRE), and temperature for DGT and grab sampling (GS). Grab sample values are averages of samples taken at the time of deployment and removal of the DGT. Deployment averages (AVG) and standard deviations (STD) are included. Data from Rambags et al., (2016) and Corbett et al., (in press) included for comparison.

Deployment	**Influent N ($mg\ N\ L^{-1}$)**		**NRR ($g\ N\ m^{-3}\ d^{-1}$)**		**NRE (%)**		**Temperature (°C)**
	DGT	GS	DGT	GS	DGT	GS	AVG
1	13.5	15.2	9.4	16.6	>99.9	96.6	15.0
2	15.9	4.9	8.3	6.3	>99.9	88.3	14.9
3	9.7	17.5	5.7	19.6	>99.9	97.0	14.2
4	14.5	13.8	8.2	14.5	>99.9	96.3	14.3
AVG	14.7	12.8	7.9	14.3	99.9	94.5	14.6
STD	2.7	5.5	0.3	0.6	---	0.4	0.4
Rambags et al., 2016 AVG	---	~31.2	---	14	---	99.9	13-23
Corbett et al., in press AVG	16.4	14.5	26.0	18.7	>99.9	99.0	23.4
STD	3.8	4.5	7.6	3.4	---	0.2	0.6

The previous studies conducted at the LIC bioreactor measured a NRR of ~ 14 g N m^{-3} d^{-1} (over two years via grab samples; Rambags et al., 2016) and 14 to 31 g N m^{-3} d^{-1} (via both grab samples and DGT; Corbett et al, in press). Addy et al., (2016) in a meta-analysis reported a mean NRR of 4.7 g N m^{-3} d^{-1}, however, this analysis included a diverse array of laboratory and field studies. From the more recent field studies collated in the literature review section (Table 2.2; Figure 3.2), an average NRR of 5.4 g N m^{-3} d^{-1} was calculated. These studies included bioreactors that had higher DOC in the influent like the LIC bioreactor. By constraining analysis to the subset of studies with higher DOC loading (Figure 3.2) the NRR increased to 12.6 g N m^{-3} d^{-1}. A non-parametric test (due to non-normal data) was conducted between the NRR from the low DOC and high DOC bioreactors (Figure 3.2) determined the difference in NRR of the higher DOC was statistically significant (P-value = 0.036). In this study, NRR calculated from the grab samples averaged 14.3 g N m^{-3} d^{-1} while NRR from the DGT averaged 7.9 g N m^{-3} d^{-1} (Table 3.1). The DGT time-weighted averages had consistently lower removal rates than the grab sampling which previous studies suggest, due to the variability of the grab samples (Table 3.1).

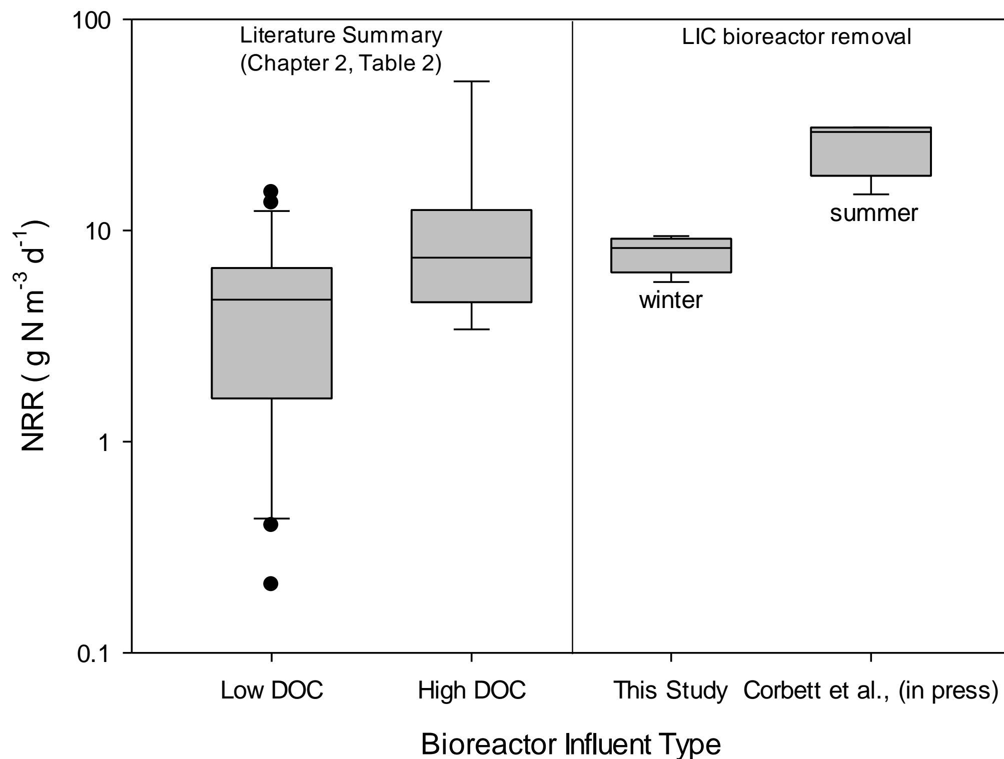


Figure 3.2. Boxplots of NRR within the field scale studies used for Table 2.2 presented on the left-hand side. The 'Low DOC' plot shows the rates of the low dissolved organic carbon (DOC) bioreactors treating field agriculture subsurface drainage. The 'High DOC' plot summarizes rates of bioreactors that treat effluent with high DOC. The 'This study' and the 'Corbett et al., (in press)' plots represent the removal rates calculated from the DGT deployed in those studies.

A study conducted earlier in the year during (summer and autumn, ~23°C) measured higher NRR with an average 26.1 g N m^{-3} d^{-1} for DGT and an average of 18.7 g N m^{-3} d^{-1} for grab samples (Table 3.1; Corbett et al., in press). Compared to the values from this study in Table 3.1, the measurements from the previous study were significantly higher than the current study where the average temperature was ~15°C. The difference in temperature (~9°C) was likely responsible for the lower removal rates measured during this study's winter deployment.

The factor at which the removal rate increased with every 10°C increase is referred to as a Q_{10}. A Q_{10} of 3.9 was calculated for the NRR between this study and Corbett et al., (in press) indicating that temperature played a significant part in NRR. Cameron & Schipper (2010b) had similar values of those use to calculate Q_{10} in this study (14°C and 23.5°C) and also observed a significant difference in removal rates due to this temperature difference. Other bioreactor studies have observed that denitrifying bioreactors have an average Q_{10} of 2 (Elgood et al., 2010; Warneke et al., 2011a).

From these Q_{10} values it is clear that temperature played an important role in the lower NRR values from this study compared to prior measurements from Rambags et al., (2016) and Corbett et al., (in press). Although the lower temperatures did not hinder the bioreactor's ability to achieve complete N reduction, it does demonstrate how seasonal variation can affect N removal at different times of the year even in a controlled flow system. This importance of temperature has clear consequences for design criteria to ensure bioreactors can remove sufficient nitrate at colder times of the year. Even over the relatively short 24 h deployments, DGT concentrations provided more representative NO_3^- concentrations given they accounted for the temporal change in NO_3^-, and therefore more representative of NRR.

3.3.2 DBL Calculation

Previous studies testing the usability of DGT for freshwater nutrient measurement demonstrated that determining the DBL was integral for calculating the correct concentration of NO_3^- (Huang et al., 2016b; Zhang et al., 1998). The width of the DBL is dependent on the flow rate past the DGT window with greater DBL observed at low flow rates. So, DGT were deployed in triplicate in each pre-determined location (Inlet, C1-C4, Outlet) to determine DBL for each location (Davison, 2016). The experimental design and placement of DGT into bioreactors meant that the DBL had to be calculated for each well to ensure correct calculation of NO_3^- concentration (Table 3.2). From Table 3.2, it can be seen that from day-to-day and even sampling sites within the same deployment that DBL was not constant. In general, the inlet had smaller DBL's (0.186 ± 0.07 cm) than the wells within the bioreactor, reflecting better mixing in the inlet within the flow path of the bioreactor. As DBL can only be calculated when NO_3^- concentrations greater than zero, no DBL could be calculated after meter 4 (well C4) to the outlet as NO_3^- had been reduced to zero.

Within Table 3.2 it can also be seen that flow rate can affect DBL. In Deployment 2, a larger DBL was calculated for each well along the length of the bioreactor likely due to the lower flow that took place during this deployment (10620 L d^{-1}). Both Corbett et al., (in press) and Huang et al., (2016b) also calculated smaller DBL at lower flow rates in both bioreactors and freshwaters. From the DBL's calculated in C3 it could be interpreted that better mixing occurred at this well due to the lower DBL's compared to the other wells (Table 3.2). If correct this variance in DBL might indicate preferential flow paths within the bioreactor.

Calculating the size of the DBL for each well was important to accurately determine NO_3^- concentration. For example, at the inlet during Deployment 2 the DBL was determined to be 0.281 cm which resulted in a NO_3^- concentration of 15.9 mg N L^{-1}. If the DBL had been only 0.1 cm thicker, then the NO_3^- concentration would be calculated as 20.4 mg N L^{-1}. This represents a 78% overestimation of NO_3^- concentration and demonstrated the need to calculate site-specific DBL when flow rates are low. As flow rates increase the DBL decreases and have much less of an impact on calculated NO_3^- concentrations.

Table 3.2. Calculated Diffusive Boundary Layer (DBL) (cm ± STD) for each deployment at each location illustrating how the layer can fluctuate therefore affecting N concentration calculations. Deployment 1 has no value for well C2 DGT due to no DBL able to be calculated.

Deployment	Inlet	C1	C2	C3	C4	Deployment AVG ± STD	Flow rate
				cm			L d^{-1}
1	0.131	0.339	---	0.259	0.222	0.238 ± 0.09	12827
2	0.281	0.214	0.214	0.122	0.222	0.211 ± 0.06	10620
3	0.193	0.189	0.567	0.103	0.567	0.324 ± 0.22	12425
4	0.138	0.193	0.334	0.043	0.222	0.186 ± 0.11	11562

3.3.3 DGT and grab sample correlation

Nitrate concentrations gathered from the four 24 h deployments in July 2019 via both traditional grab samples and DGT and these both supported previous findings (Figure 3.3) where N was shown to be removed to near zero by well C4. Note that the influent N concentrations from the grab samples were lower than well C1 in comparison to the values from the DGT (Figure 3.3), further illustrating how grab samples, especially low frequency samples, may be misleading. While NO_3^- concentrations in grab sampling and DGT showed the same patterns of removal along the length of the bioreactor, there were weak correlations between grab samples within the bioreactor compared to the time-weighted averages calculated from the DGT samples (Figure 3.3).

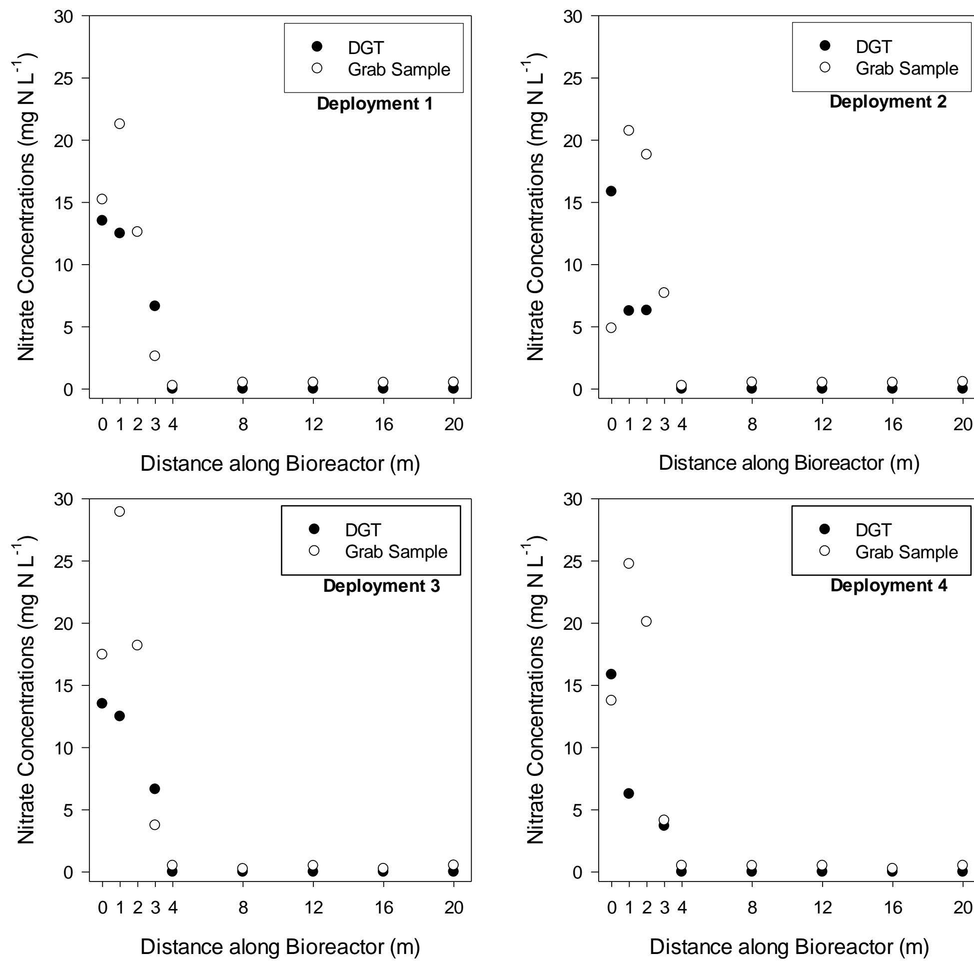


Figure 3.3. Nitrate concentration graphs comparing the grab sample measured concentrations versus (white circles) the DGT measured concentrations (black circles) on each deployment day.

For example, in Deployment 1 and Deployment 3 (Figure 3.4), the concentration of NO_3^- calculated from grab samples and DGT were similar. In contrast, Deployment 2 and Deployment 4 were not as well correlated. In Figure 3.3, it can be seen that the NO_3^- concentrations calculated from the DGT were similar in trend as the low frequency grab sampling. This matched similar results Corbett et al., (in press) saw in the summer experiment. Like that experiment, it is assumed that the difference between the NO_3^- concentrations collected by grab sample and DGT increased as the inlet grab sample concentrations were more variable.

While general patterns of NO_3^- removal from grab samples and DGT were similar, absolute correlations between DGT and grab samples for each well were poorer (Figure 3.4). In contrast, when comparing high frequency sampling to DGT (hourly samples for 16, 24, and 36 h per well) grab sampling concentrations and DGT concentrations exhibited similar linear trends (Corbett et al., in press). While low frequency (2 samples per well) sampling under or overestimated NO_3^- removal (Corbett et al., in press).

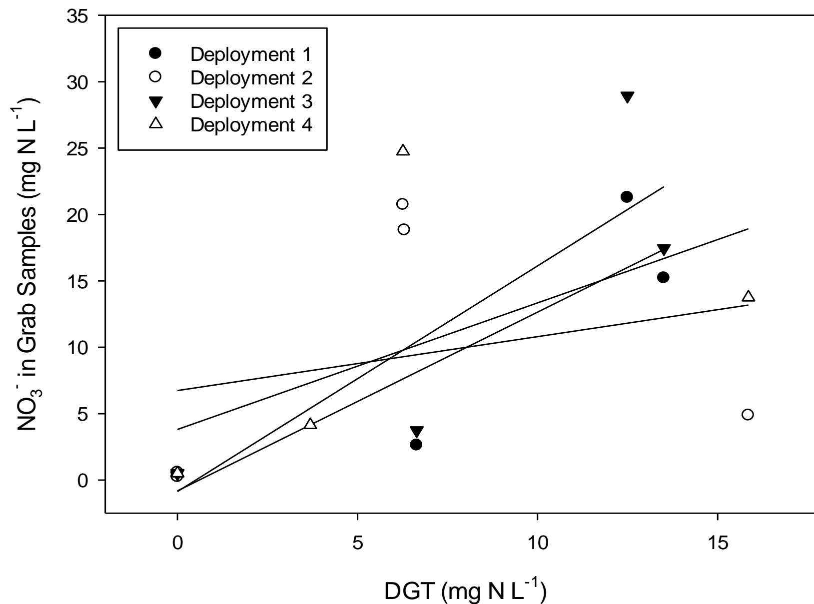


Figure 3.4. Correlation between the NO_3^--N concentrations calculated using the average of all DGT and the N concentrations calculated using the average of all grab sampling.

In terms of field deployment, the DGT were much easier to set up compared to grab sampling. While it took around 5 minutes at each well to place each set of DGT, collecting grab samples was more time consuming when collecting using a syringe and the need to 'purge' the wells to ensure well-mixed samples. Furthermore, if there is a need to collect high frequency samples such as hourly to daily this sampling time adds up to a large labor cost. If sample collection is automated this results in a large start-up and equipment cost and maintenance costs. DGT are low cost in comparison in terms of onsite time and expense to an extent this is balanced by the increase in laboratory time, however, with experience this is minimal.

3.3.4 Dynamics of O_2 and SO_4^{2-}

From the grab samples still needed for monitoring other freshwater nutrient pollutants such as O_2, SO_4^{2-}, the sequential depletion of microbial electron acceptors was evident in the LIC bioreactor.

Supplemental DO measurements taken at three of the deployments to determine when anoxic conditions (DO of <0.5 mg L^{-1}) were reached (Tchobanoglous et al., 2003). Anoxic conditions were reached in the inlet, further explaining the ability for the LIC bioreactor to completely remove NO_3^- (Figure 3.5B). As the LIC

bioreactor is part of a closed secondary wastewater treatment system there was additional dissolved organic carbon available for denitrifiers, denitrification can begin immediately in the inlet. This system was more efficient compared to field bioreactors which typically have lower DO in influent concentrations, but still achieve anoxic conditions in the first few meters (Christianson et al., 2011a; Christianson et al., 2011c; Warneke et al., 2011b).

As NO_3^- was reduced to zero or near zero consistently by meter four, it was assumed that other redox processes occur (Figure 3.6). When replacing the wells for this study visual observations were made that the chips in the first five meters were fairly decomposed and covered in black 'slime'. Further down the bioreactor chips looked practically new and generated a sulfide smell (i.e. rotten egg). From the grab sampling, it can be seen that from meter two (well C2) to meter eight (well C5) SO_4^{2-} was reduced, with a slight increase at the outlet (Figure 3.5C). Increase of SO_4^{2-} concentrations at the outlet may have been due to the sulfide oxidation as the outlet had oxygen present in the overlying headspace of the large diameter of the outlet structure (~50 cm) outlet. Sulfate removal rates ranged from 1.7 to 5.1 g S m^{-3} d^{-1} with an average of 3.6 g S m^{-3} d^{-1} over the four deployments with SO_4^{2-} removal efficiencies ranging from 37 to 99%. Sulfate reduction rates for the same area averaged 6.5 g S m^{-3} d^{-1} (equating to 59 to 74% removal) measured via high frequency grab sampling (Corbett et al., in press). Again, the difference in temperatures between these two studies sampling periods likely accounted for the differences in rates and yielded a Q_{10} of 3.9. Rambags et al., (2016) measured higher reduction rates of SO_4^{2-} with a 94% removal efficiency, however, that study used infrequent grab sampling over two years which may have allowed for a higher removal efficiency to be caculated as opposed to these ~24 h studies.

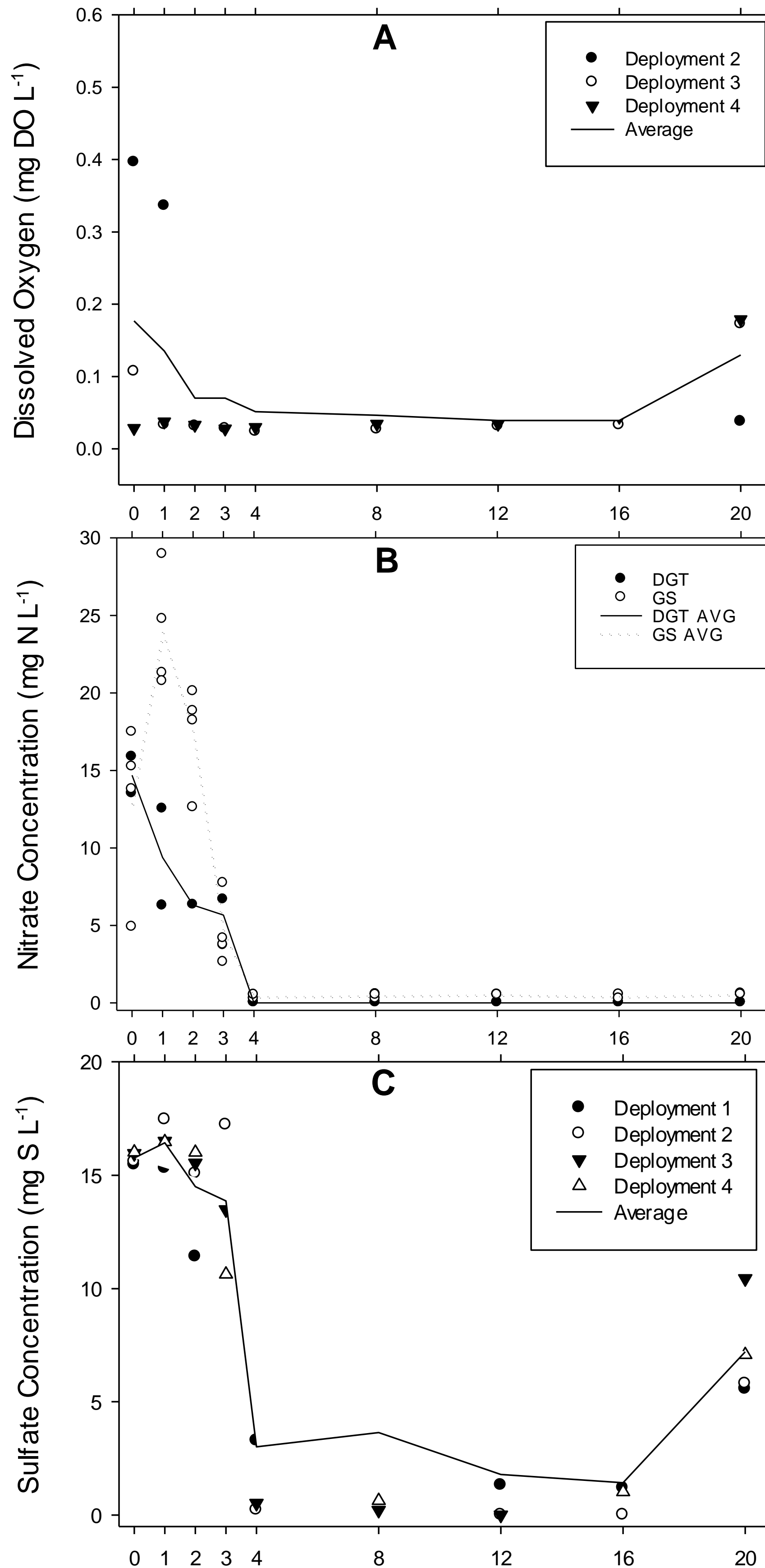


Figure 3.5. Average data from the four deployments along the length of the bioreactor A) dissolved oxygen concentrations graph of the DGT and grab sample average B) nitrate concentrations C) sulfate concentrations.

When replacing the wells in March 2019, a black coating on the woodchips was observed nearer to the outlet which was suspected to be metal sulfides. In addition to H_2S observed with the SO_4^{2-} removal, SO_4^{2-} reduction can also result in the production of methylmercury (Blowes et al., 1994). Methylmercury is of concern as it can bioaccumulate in shellfish and fish and then can be toxic to central and peripheral nervous systems and impair neurological development when consumed by humans (WHO, 2017).

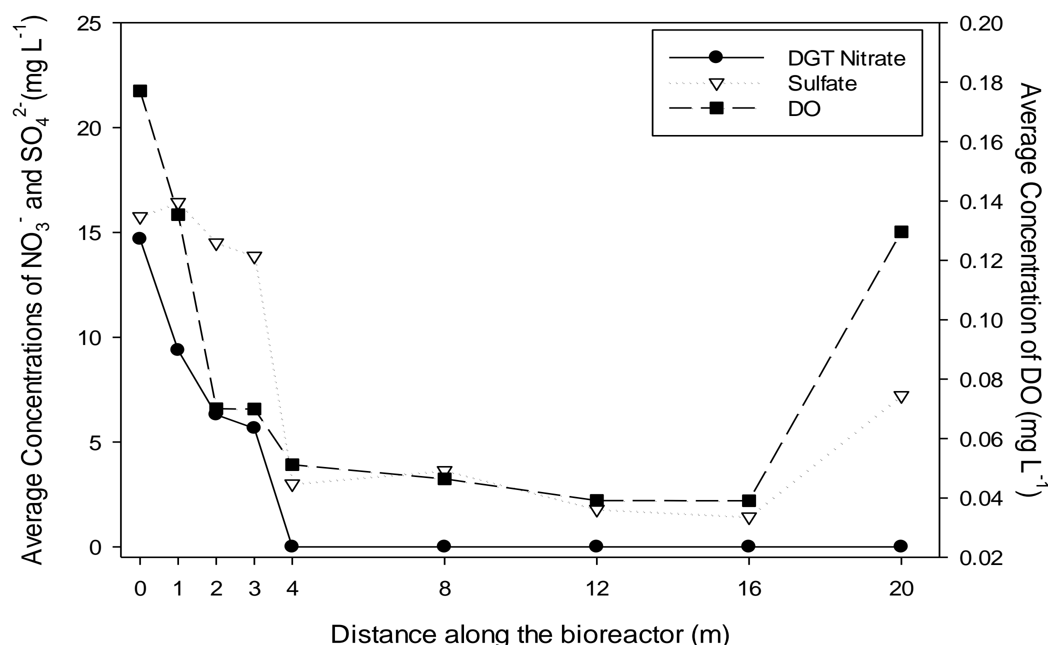


Figure 3.6. Comparative graph of the nitrate, dissolved oxygen, and sulfate concentration averages from Figure 3.5 demonstrating the sequential removal of electron acceptors.

To date, only one study has looked in-depth at the dynamics of mercury and methylmercury production in bioreactors (Shih et al., 2011). As it is known that SO_4^{2-} reducing conditions occur at the LIC bioreactor, a subset of samples were analyzed for both methylmercury (MeHg) and total mercury (Hg) (Figure 3.7). Patterns of both Hg and MeHg along the length of the bioreactor were not consistent. In Deployment 2, there was an increase in MeHg at meter 8 (from 0.05 mg L^{-1} to 0.16 mg L^{-1}) which decreased by the outlet (0.05) in the first sampling (Deployment 2). The Deployment 4 subset started with an elevated MeHg of 0.20 mg L^{-1}, which decreased to 0.03 mg L^{-1} in the outlet. Generally, Hg concentration was stable (Figure 3.7). This bioreactor discharges to groundwater and so any MeHg will be diluted and the full impact is not well known.

Shih et al., (2011) previously measured 1.0×10^{-8} to 7.6×10^{-7} 0.01 mg MeHg L^{-1} and 1.3×10^{-6} to 3.4×10^{-6} mg Hg L^{-1} in a streambed bioreactor which was significantly lower than the values seen during this study. This difference is very likely due to the difference in the influents treated by the bioreactors as the LIC bioreactor treats human waste and greywater these are likely additional sources initially coming into the bioreactor which would not enter an agricultural bioreactor. Typically, bioreactors are designed to maintain concentrations of 0.5 mg NO_3^--N L^{-1} to ensure that H_2S and MeHg do not develop (Shih et al., 2011; Robertson & Merkely, 2009).

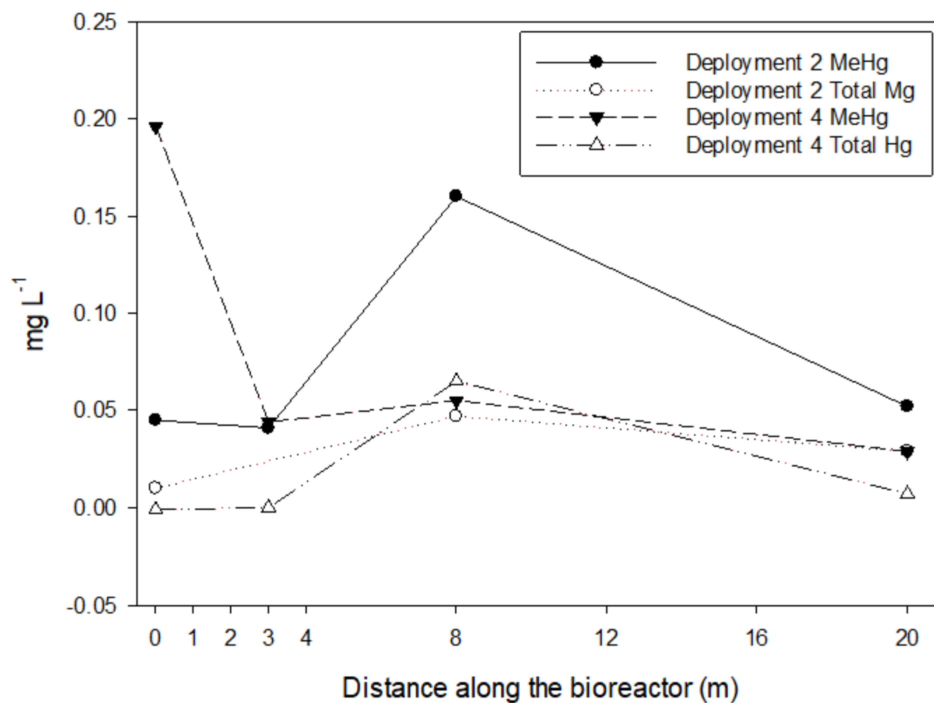


Figure 3.7. Measurement of methylmercury (MeHg) and total mercury (Hg) from grab samples.

3.3.5 PO_4^{3-}

The previous study by Corbett et al., (in press) measured an average increase of 0.63 mg P L^{-1} during their deployments while Rambags et al., (2016) observed a 7% increase in P over their two-year study at the outlet of the LIC bioreactor. In this study, however, no PO_4^{3-} removal was observed, instead, there was a general increase from meter eight toward the outlet (Figure 3.8). This increase of 9.65 mg P L^{-1} from meter eight (well C5) to the outlet could potentially be due to variability associated with low frequency sampling. As the LIC bioreactor was ~6

years old, this increase is of interest due to there being very little literature on PO_4^{3-} dynamics, especially in older bioreactors. Though concentrations remained under 10 to 20 µg P L^{-1} which is the level at which eutrophication is promoted (Algoazany et al., 2007). Further research is still needed regarding PO_4^{3-} dynamics at this site and other field scale bioreactors.

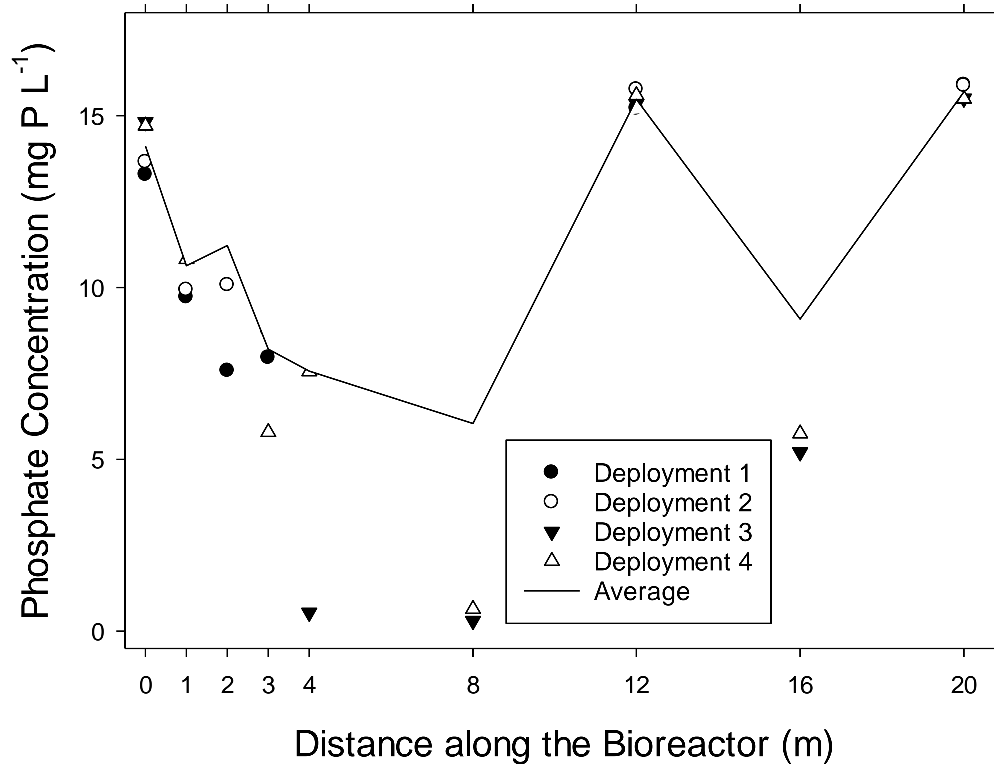


Figure 3.8. Phosphate concentrations determined by grab sampling over the four deployments

3.4 Conclusions and future work

Diffusive Gradients in Thin-films generated a NRR of 7.9 g N m^{-3} d^{-1} (ranging between 5.67 to 9.37 g N m^{-3} d^{-1}) which provided better NRR than the grab samples NRR of 14.3 g N m^{-3} d-1 (6.31 to 19.59 g N m^{-3} d^{-1}) which appeared to overestimate NRR. These removal values fall above the range to be found in many denitrifying bioreactors but in line with NRR for bioreactors with high DOC in the influent.

Using DGT to monitor a denitrifying bioreactor provided lower NO_3^- values to grab sampling and NRR, however, they likely provided more representative NRR given they accounted for changing NO_3^- as opposed to point-in-time grab sampling. Additionally, as DGT binds NO_3^- to the binding layer, samples did not have to be immediately analyzed as the grab samples had to reduce the risk of biological transformation during transport and storage. The DGT were easier to use and less time-consuming in the field than the traditional grab sampling method (syringe collection) but required more laboratory preparation. Overall, this study demonstrated that DGT were useful tools for measuring NO_3^- in denitrifying bioreactors and could easily replace active sampling methods. Given the chemical and physical complexity of the bioreactor systems, DGT could be as effective for determining the performance of other nitrogen remediation strategies such as wetlands and saturated buffers.

The next step for DGT research would be the implementation of DGT in a long-term monitoring project to be compared to current continuous sampling methods such as auto sampling. A potential concern could be the effectiveness of DGT at extreme low flows for long term bioreactor monitoring as DBL may be too large for the analyte to become bound to the binding resin. If proven successful even during low flow and other periods DGT could replace grab sampling methods easily in terms of financial and labor costs. In terms of extension and for private landowners, the establishment of a lab to make DGT and subsequent analysis would help in getting wide-spread adoption of DGT. Additional future research goals for DGT could be the development and trial of mixed-binding layers to use within water quality strategies such as denitrifying bioreactors and other nitrogen remediation strategies.

Chapter 4

Summary and Conclusions

4.1 Conclusions

As the implementation of strategies for nutrient remediation is expected to increase around the globe, quantifying their removal capabilities will help both landowners and regulators decide which practices are best implemented and promoted. The current way that these practices are evaluated using traditional grab sampling methods that are machine analyzed to determine nutrient concentrations. Corbett et al., (in press) and this study has shown that DGT could easily replace these traditional grab sampling methods in denitrifying bioreactors and other nutrient remediation studies by providing time-weighted concentrations means which can overcome temporal variability and provide more accurate data.

Building upon the previous DGT study conducted at the LIC bioreactor, this study sought to further evaluate the effectiveness of the A520E DGT method as an alternative to traditional grab sampling methods and as a tool to monitor nitrate (NO_3^-) removal within nitrogen remediation strategies.

This thesis has made several key findings:

(i) A520E-DGT methods can be used to monitor NO_3^- in denitrifying bioreactors and are more effective than grab sampling.

This study showed DGT are easier, more cost-effective, and likely more accurate than traditional grab sampling than low frequency grab sampling at two times. During field deployments, the DGT were easier to place as opposed to collecting water samples with a syringe and required less overall labor time. This may have been offset by the additional laboratory time needed to make and assemble the units; however, previous experience with the DGT methodology indicated this time was not substantial. Another benefit of the DGT was that they did not have to be immediately analyzed as the NO_3^- was bound to the AE520 layer, unlike the water samples which needed to be refrigerated and swift analysis to maintain the

integrity of the samples. Based on the NO_3^- data collected from both the DGT, ~7.9 g N m^{-3} d^{-1}, and grab samples, 14.3 g N m^{-3} d^{-1}, the NRR calculated from the grab samples appeared to be an overestimation when compared to the DGT NRR. These overestimations were presumably due to the nature of point in time grab sampling which DGT overcome by providing a time-weighted average. This further corroborates with results reported by Corbett et al., (in press) that DGT are a reasonable alternative to traditional grab sampling methods.

(ii) The NO_3^- removal data observed was consistent with previous findings at the site and in literature.

The previous study conducted during the late summer/early autumn found DGT NRR average of 26.1 g N m^{-3} d^{-1} and the grab sample NRR average of 18.7 g N m^{-3} d^{-1} (Corbett et al., in press). These authors also observed that with low frequency grab sampling, the grab sample NRR from the autumn deployments tended to underestimate the concentration compared to the DGT calculated NO_3^- concentration values. As mentioned above, compared to this previous study grab sampling tended to overestimate the DGT calculated concentrations showing how variable temporal NO_3^- concentrations can be throughout 24h. Corbett et al., (in press) measured NRR values significantly higher than the NRR calculated in the study presented here, likely due to higher water temperatures. In bioreactor literature, the ratio of removal rates for every 10°C increase in temperature (Q_{10}) averaged around 2 (Christianson et al., 2012b). Using the NRR from Corbett et al., (in press) and this study a Q_{10} of 3.9 was calculated indicating that a 10°C decrease in temperature will decrease rates by 3.9 at this site. This indicated that temperature was a key driver behind removal rates and an important design criterion.

(iii) Demonstrated that NRR were significantly higher in bioreactors with high DOC inputs than those without.

Recent removal rates collected from across all of bioreactor literature in Addy et al., (2016) showed an average NRR of 4.7 g N m^{-3} d^{-1}. An updated estimate of more recent field studies (Table 2.2) averaged NRR of 5.4 g N m^{-3} d^{-1} for all bioreactors. Bioreactors that treated effluent high with DOC averaged NRR of

12.6 g N m^{-3} d^{-1} and significantly higher than bioreactors receiving low DOC influent. A simple t-test comparing the low DOC influent bioreactors to the high DOC bioreactors found that the high DOC influent was statistically different from the low DOC. Rambags et al., (2016) observed an average of 14 g N m^{-3} d^{-1} over two years which aligned with the high NRR trend observed in the forming Table 2.2. The DGT NRR from Corbett et al., (in press) and this study (26.1 g N m^{-3} d^{-1} and 7.9 g N m^{-3} d^{-1}, respectively) were used in Figure 3.2 and matched the average NRR calculated for high DOC influent denitrifying bioreactors.

(iv) Sequential removal of electron acceptors was observed where O_2 was removed in the influent, NO_3^- by well 4 (~4 meters), and then SO_4^{2-} by well 5 (~8 meters).

This current study had an average SO_4^{2-} removal rate of 3.6 g S m^{-3} d^{-1} (1.67 to 5.10 g S m^{-3} d^{-1}) calculated from low frequency data. While Corbett et al., (in press) measured an average SO_4^{2-} reduction of 6.5 g S m^{-3} d^{-1} (4.8 to 8.2 g S m^{-3}) d^{-1} and sulfate removal efficiencies of 59-74% removal linked to high frequency grab sampling. Rates of SO_4^{2-} removal were likely less in the current study due to lower temperature than when Corbett et al., (in press) made their measurements.

Methylmercury and total mercury were also measured, however, no discernible pattern was observed and outlet concentrations were determined to exceed health guidelines. Finally, no phosphate removal was observed as it had been in both previous studies at this site (Corbett et al., in press; Rambags et al., 2016).

4.2 Future research

4.2.1 DGT

Thus far, DGT have mainly been used to collect data on trace metals and few studies have focused on freshwater applications and common nutrients of concern. Moving forward, DGT research needs more replicate studies in varying climates, water systems, and locations to provide a better understanding of the strengths and limitations of DGT. This would include determining and implementing long-term monitoring studies in place of grab sampling for denitrifying bioreactors and other nutrient remediation strategies. For instance, there is a need for testing DGT at

extreme low flow, such as during summer periods where water can back up and stagnate under practices integrated with subsurface drainage systems (e.g. denitrifying bioreactors, saturated buffers, and drainage water management). This need is to understand how DBL within DGT will react at such low flows and if analytes are able to be bound to the binding layer. Additionally, while the above strategies focus mainly upon the removal of NO_3^-, to eliminate the need for grab sampling mixed-binding layer DGT, such as those currently being trialed by Huang et al., (2017) need further explored so that other water quality nutrients such a sulfate and phosphate can be monitored using DGT.

Additional DGT limitations include needing knowledge of how to make and assemble them. Not knowing this would increase laboratory time. For private use, landowners would need to know water temperature or have a close estimate to accurately calculate NO_3^- concentration. The other critical factor is ensuring that time deployed is recorded to be used.

As DGT are a relatively new practice, one hurdle that DGT needs to overcome is a place for standard lab analysis. To gain wide-scale use, it would be helpful to have commercial production and analysis labs established so that the technology is more accessible to non-academics. As nutrient monitoring and water quality concerns are more prevalent than ever, having low-cost solutions such as DGT to help determine where problem areas are in fields and catchments would assist landowners in identifying and mitigating problem areas.

4.2.2 Denitrifying Bioreactors

While denitrifying bioreactors have been proven a useful nitrogen remediation practice, in surveying the literature for this thesis I see there is still somewhat of a lack of consistency in reporting removal rates. Christianson & Schipper (2016) called for more complete reporting of inlet and outlet nitrate concentrations, flow rates, hydraulic retention times, bed temperature, and whether NRR was calculated using active or saturated volumes. Many published papers failed to report a majority of these measurements which made it difficult to summarize data in Table 2.2 and will make future comparison studies difficult. Additionally, the reporting of seasonal Q_{10} is a parameter worth reporting more to more fully

understand temperature dynamics as climate changes. While compiling data (Table 2.2), there appeared to still be significantly more laboratory and pilot studies than field studies being published. Therefore, more long-term monitoring studies (e.g. beyond 2-3 years) at field scale are needed to fill the media longevity gap in bioreactor knowledge.

Finally, the overall naming of 'bioreactors' within papers is still confusing with 'bio-filters' and 'permeable reactive barriers' continuing to be used. This resulted in overlooking these studies when performing keyword searched of 'denitrifying bioreactor' or 'bioreactor' and resulted in these studies being excluded.

In the case of the LIC bioreactor, which is over 5 years old, it would be difficult to compare its results with those of younger bioreactors which are known to have high rates of removal in the first two years (Addy et al., 2016). Additionally, a more thorough characterization of the influent into bioreactors is needed as the LIC bioreactor has shown to have high NRR likely correlated to increased dissolved organic matter (DOC) coming in through the inlet. From the field studies gathered in Table 2.2, it can be seen that bioreactors with known elevated DOC typically had higher NRR. Some future studies on bioreactor longevity with higher DOC and NRR would be an insightful contribution to bioreactor literature. A meta-analysis could be useful as well as bioreactors are used to treat wastewaters as opposed to agricultural drainage waters.

Future studies at the LIC bioreactor, in particular, should also measure DOC to see how a 5+-year-old bioreactor can maintain high N removal rates. Such a study would give great insight into redox dynamics within a bioreactor and also explain the visual decomposition and lack of decomposition made when replacing the wells within the bioreactor earlier this year.

In summary, the LIC denitrifying bioreactor is still efficient at removing NO_3^-, though long-term research into the internal dynamics of the bioreactor would be useful. Using DGT to monitor the NO_3^- within bioreactors could provide more precise annual removal rates and longevity.

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Appendix

Table A.1 Nitrate concentrations (mg N L^{-1}) calculated using DGT data. The dashed lines (---) indicate that a concentration was unable to be calculated.

Sample Site	Deployment 1	Deployment 2	Deployment 3	Deployment 4	Average
			(mg N L^{-1})		
Inlet	13.51	15.85	13.51	15.85	14.68
C1	12.49	6.26	12.49	6.26	9.38
C2	---	6.30	---	---	6.30
C3	6.64	---	6.64	3.69	5.66
C4	0.00	0.00	0.00	0.00	0.00
C5	0.00	0.00	0.00	0.00	0.00
C6	0.00	0.00	0.00	0.00	0.00
C7	0.00	0.00	0.00	0.00	0.00
Outlet	0.00	0.00	0.00	0.00	0.00

Table A.2. Nitrate concentrations (mg N L^{-1}) calculated using grab sample data.

Sample Site	Deployment 1	Deployment 2	Deployment 3	Deployment 4	Average
			(mg N L^{-1})		
Inlet	15.22	4.88	17.46	13.76	12.83
C1	21.26	20.74	28.94	24.75	23.92
C2	12.60	18.82	18.19	20.09	17.43
C3	2.62	7.70	3.74	4.14	4.55
C4	0.26	0.25	0.50	0.50	0.38
C5	0.52	0.52	0.25	0.50	0.45
C6	0.51	0.50	0.50	0.50	0.50
C7	0.51	0.51	0.25	0.25	0.38
Outlet	0.52	0.57	0.53	0.51	0.53

Table A.3. Dissolved oxygen (DO; mg DO L^{-1}) concentrations measured across deployments 2-4. Deployment one did not have DO measurements taken.

Sample Site	Deployment 2	Deployment 3	Deployment 4	Average
		(mg DO L^{-1})		
Inlet	0.40	0.11	0.03	0.18
C1	0.34	0.03	0.04	0.14
C2	0.15	0.03	0.03	0.07
C3	0.15	0.03	0.03	0.07
C4	0.10	0.02	0.03	0.05
C5	0.08	0.03	0.03	0.05
C6	0.05	0.03	0.03	0.04
C7	0.04	0.03	0.04	0.04
Outlet	0.04	0.17	0.18	0.13

Table A.4. Sulfate concentrations (mg S L^{-1}) calculated using grab sample data.

Sample Site	Deployment 1	Deployment 2	Deployment 3	Deployment 4	Average
			(mg S L^{-1})		
Inlet	15.46	15.59	15.96	16.02	15.75
C1	15.30	17.45	16.50	16.47	16.43
C2	11.40	15.06	15.54	16.01	14.50
C3	14.14	17.22	13.49	10.63	13.87
C4	3.28	0.21	0.52	8.03	3.01
C5	4.15	9.57	0.22	0.63	3.64
C6	1.31	0.00	0.00	5.86	1.79
C7	1.18	0.00	3.54	1.02	1.43
Outlet	5.56	5.79	10.45	7.07	7.22

Table A.5. Phosphate concentrations (mg P L^{-1}) calculated using grab sample data.

Sample Site	Deployment 1	Deployment 2	Deployment 3	Deployment 4	Average
			(mg P L^{-1})		
Inlet	13.29	13.65	14.81	14.70	14.11
C1	9.72	9.93	12.05	10.82	10.63
C2	7.58	10.07	13.48	13.76	11.22
C3	7.96	10.48	8.60	5.78	8.20
C4	10.78	11.38	0.55	7.55	7.57
C5	11.74	11.49	0.31	0.64	6.04
C6	15.21	15.76	15.34	15.59	15.47
C7	12.66	12.72	5.21	5.74	9.08
Outlet	15.89	15.88	15.50	15.48	15.69

Table A.6. Temperature from the iButtons (°C) during each deployment

Sample Site	Deployment 1	Deployment 2	Deployment 3	Deployment 4	Average
			°C		
Inlet	17.0	16.7	16.4	16.4	16.6
C1	14.0	13.9	13.6	14.1	13.9
C2	15.1	15.0	13.6	13.5	14.3
C3	14.5	14.5	13.5	13.3	13.9
C4	14.5	14.5	13.5	13.3	13.9
C5	14.5	14.5	13.5	13.3	13.9
C6	14.5	14.5	13.5	13.3	13.9
C7	14.5	14.5	13.5	13.3	13.9
Outlet	14.0	14.0	13.3	13.1	13.6

Table A.7. Methylmercury (mg MeHg L^{-1}) and total mercury (mg Hg L^{-1}) concentrations from the subset of grab samples. The BD indicates that no concentration was able to be measure due to being below detection limit.

Sample Site	Deployment 2	Deployment 2	Deployment 4	Deployment 4
	mg MeHg L^{-1}	mg Hg L^{-1}	mg MeHg L^{-1}	mg Hg L^{-1}
Inlet	0.045	0.010	0.196	-1.00E-03
C3	0.041	BD	0.044	BD
C5	0.160	0.047	0.055	0.065
Outlet	0.052	0.029	0.029	7.00E-03