Carbon Dosing of Denitrifying Bioreactors to Remove Nitrate from Agricultural Drainage

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Abstract

Several studies have demonstrated that denitrifying woodchips bioreactors effectively remove nitrate from a broad spectrum of wastewater. Even though bioreactors have a comparatively small spatial footprint and are straightforward to construct, the low long-term nitrate removal efficiency they provide compared to other mitigation options, such as constructed wetlands, may limit their utilization by catchment managers and landowners. This thesis investigated the use of external carbon dosing in bioreactors to improve nitrate removal and quantified some potential adverse effects of carbon dosing on bioreactors and downstream aquatic environments. Through observations ranging from field to mesocosm experiments, this thesis sought to (1) determine whether and to what extent external carbon dosing could improve nitrate removal in bioreactors and (2) assess the potential adverse effects of carbon dosing, such as reduced hydraulic performance and pollution swapping.

The first component of the study evaluated the carbon dosing of a pilot-scale (58 m^3) bioreactor treating drainage waters from a 0.65 ha paddock on a dairy farm. The effects of carbon dosing on the bioreactor's nitrate removal rates were assessed following a constant dosage of 8% (v/v) methanol solution at 10 mL min^{-1} to the bioreactor's inlet distributor. Sulfate reduction and losses of added methanol from the bioreactor's outlet were quantified as the potential adverse effects of external carbon dosing. Methanol dosing increased seasonal nitrate removal rates to 8 g N m^{-3} day^{-1} in 2020 and 5 g N m^{-3} day^{-1} in 2021 (with a halved dosing rate), compared to 1 g N m^{-3} day^{-1} in the undosed bioreactor in 2018. Even under nitrate-limiting conditions, the added methanol was effectively removed in the bioreactor, with a mean removal rate of 106 g CH_3OH-C m^{-3} day^{-1}. Methanol concentrations decreased by order of

magnitude along the bioreactor (lengthwise) and never exceeded 50 mg CH_3OH-C L^{-1} at the outflow. The added carbon caused sulfate reduction with a mean removal rate of 8.5 g $SO4^{2-}$-S m^{-3} day^{-1} in 2020 (higher dosing rate) and 0.5 g $SO4^{2-}$-S m^{-3} day^{-1} in 2021 (halved dosing rate). Carbon dosage at a continuous rate was shown to be a viable method for increasing nitrate removal while minimizing capital and operational costs. The simplicity of this approach is a significant advantage, and it could be used by a variety of industries, including farmers.

While field observations showed that carbon dosage could improve nitrate removal, the very variable flows and nitrate concentrations made determining nitrate, sulfate, and methanol removal rates difficult. Therefore, the second component of the thesis was to assess nitrate, sulfate and added carbon removal rates more accurately using mesocosms. An empirical model was also developed to calculate the amount of methanol required to remove specific loads of nitrate. Mesocosm bioreactors were designed to share some characteristics with the field bioreactor (for example, added carbon component and dosage ratio) but diverged in that they were hydrologically regulated to eliminate the impact of transient operating circumstances on bioreactor performance. Carbon dosing increased nitrate removal rates from 7 g N m^{-3} day^{-1} in the control treatment bioreactors (without methanol addition) to 27 g N m^{-3} day^{-1} in the methanol-dosed treatment bioreactors. Added methanol was removed in either the absence or presence of nitrate, with mean methanol removal rates of 23 g CH_3OH-C m^{-3} day^{-1} in nitrate prevailing conditions and 17 g CH_3OH-C m^{-3} day^{-1} in nitrate limiting conditions. The empirical model suggested that a methanol to nitrate ratio of 0.7 resulted in complete nitrate removal.

In the third component of this thesis research, observations from both mesocosm and full-scale bioreactors were used to assess the hydraulic characteristics of bioreactors

under various carbon dosage regimes. Field measurements revealed a decrease in hydraulic conductivity from 4601 m day^{-1} in 2018 (season without carbon dosing) to 1600 m day^{-1} in 2021 (second year of dosing). Based on tracer tests of the mesocosm bioreactors, I investigated the effects of added carbon on the internal hydraulic performance of bioreactors. The results of the tracer tests revealed that carbon dosing had no significant effect on the hydraulic parameters of the mesocosm bioreactors (p-value > 0.05).

The findings of this thesis have implications for improving the performance of existing bioreactors or for developing new, externally dosed bioreactors with smaller spatial footprints. The constant dosage strategy, demonstrated in the full-scale bioreactor, was effective in increasing nitrate removal rates while also being safe regarding added carbon losses to the receiving environments. The ease of implementation of the constant dosage strategy could increase take-up from landowners and managers. The thesis also provides an understanding of whether and to what extent carbon dosing affects the hydrology of bioreactors, which can be used to develop new bioreactor designs with less backflow and bypass.

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RR	Removal rates
RE	Removal efficiency
CI	Confidence interval
CD	Controlled drainage
DOC	Dissolved organic carbon
SD	Standard deviation
MMRT	Macromolecular rate theory
DO	Dissolved oxygen
NA	Not available
HRT	Hydraulic residence/retention time
EPS	Extracellular polymeric substance
RTD	Residence time distribution
NHRT	Nominal hydraulic residence/retention time
AHRT	Actual hydraulic residence/retention time
TIS	Tanks in series
LCA	Environmental life cycle analysis
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
EPDM	Ethylene propylene diene monomer
PVC	Polyvinyl chloride
TOC	Total organic carbon
EC	Electrical conductivity
ORP	Oxidation-reduction potential

DIC	Dissolved inorganic carbon
NDIR	Nondispersive infrared sensor
FID	Flame ionization detection
GC	Gas chromatography
ANOVA	Analysis of variance
BNM	Methanol+nitrate+sulfate dosed bioreactors
BN	Nitrate+sulfate dosed bioreactors
BM	Methanol+sulfate dosed bioreactors
MLR	Multi linear regression
RSE	Residual standard error

Chapter 1

Introduction, Thesis Aim, and Outline

1.1 Introduction

Nitrate pollution of aquatic environments can result in a number of environmental problems, including eutrophication and fish death (Anas et al., 2020; Basu et al., 2022; Hoffmann et al., 2020). Attempts have been made to develop simple and effective mitigation techniques for removing nitrate from a wide range of point and non-point sources, including agricultural tile drainage waters (Basu et al., 2022; Christianson et al., 2013; Hoffmann et al., 2020). These mitigation strategies include saturated buffers, constructed wetlands, and denitrifying bioreactors.

Denitrifying bioreactors are simple and passive ecotechnologies that have been shown to remove nitrate from a wide range of wastewaters effectively, including agricultural tile drainage and municipal wastewater (Addy et al., 2016; Christianson and Schipper, 2016; Schipper et al., 2010a; Schipper et al., 2010b). Denitrifying bioreactors are lined excavated pits filled with a solid organic carbon medium (typically woodchip) through which nitrate-laden water flows (Christianson et al., 2021; Schipper et al., 2010b). The conversion of nitrate to harmless dinitrogen gas is mainly accomplished by denitrification, which is dependent on the labile carbon produced during the decomposition of woodchips in denitrifying bioreactors (Addy et al., 2016; Christianson and Schipper, 2016; Schipper et al., 2010a; Schipper et al., 2010b).

One of the primary reasons for bioreactors' current growing popularity among catchment managers and landowners such as farmers is their simplicity and low maintenance requirements compared to other nitrate mitigation strategies. However, denitrifying bioreactors can have a lower nitrate removal effectiveness when compared

to other mitigation measures. For example, Christianson et al. (2020) measured an average nitrate removal effectiveness of 20% (including untreated bypass flows) in 37 field-scale denitrifying bioreactors. The average removal efficiency of bioreactors is lower than that of alternative mitigation strategies, such as constructed wetlands, which have been shown to reduce nitrate loads by 30 to 50% (Tanner et al., 2012).

The relatively low long-term nitrate removal efficiency of denitrifying bioreactors has been attributed to the depletion of carbon supply from the solid substrate with time (Addy et al., 2016; Christianson et al., 2021; Robertson, 2010; Schipper et al., 2010b; Schmidt and Clark, 2013). The supply of available carbon can limit nitrate removal in denitrifying bioreactors and has spurred a number of alternative approaches to maintain sufficient nitrate removal. External carbon dosing, biochar addition, and periodic drainage are some potential approaches that can either stimulate carbon release from the solid medium or compensate for the solid medium's carbon shortage, supporting improved long-term nitrate removal efficacy (Christianson et al., 2011; Maxwell et al., 2019a; Maxwell et al., 2019b; Roser et al., 2018).

There is some evidence that carbon dosing (e.g., ethanol, methanol, acetate) in denitrifying bioreactors can increase nitrate removal rates (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Palomo et al., 2013; Roser et al., 2018). Roser et al. (2018) evaluated the dosing of acetate in denitrifying bioreactors and reported nitrate removal rates of 120.6 $g\ N\ m^{-3}\ day^{-1}$ compared to 7.1 $g\ N\ m^{-3}\ day^{-1}$ in control bioreactors. Several carbon dosing studies are small-scale column experiments, necessitating field-scale trials. Furthermore, there are significant limitations in the literature on the potential adverse effects of carbon dosing, and improved nitrate removal rates vary across studies. Carbon dosing may cause pollution swapping, such as increased carbon losses from bioreactor outlets as well as a faster shift in bioreactor hydrology. Furthermore, no research has been conducted on carbon dosing in

woodchip bioreactors operating under transient operating conditions, which are typical of agricultural catchments.

1.2 Thesis aim

The first component of this thesis investigated the effects of carbon dosing on nitrate removal rates in a field-size bioreactor located in an agricultural catchment in Waikato, New Zealand. This study also assessed sulfate reduction and potential losses of added carbon as adverse effects of carbon dosing. The second component of this thesis had some similar aims as the field component but differed in that the experiments were conducted in hydrologically controlled mesocosm bioreactors. The third component incorporated measurements from both the field bioreactor and the mesocosm bioreactors to determine the effects of carbon dosing on the hydraulic performance of denitrifying bioreactors.

1.2.1 Carbon dosing of a full-scale bioreactor

When compared to denitrifying bioreactors operating under controlled flow conditions, full-scale denitrifying bioreactors in agricultural catchments typically offer lower nitrogen removal rates and efficiency (Addy et al., 2016; Christianson et al., 2021; Rivas et al., 2020). This low nitrate removal efficiency in comparison to flow-controlled denitrifying bioreactors could be attributed to transient flow conditions that regularly place the bioreactor in either nitrate or carbon limiting conditions (Addy et al., 2016; Christianson et al., 2021; Maxwell et al., 2020; Rivas et al., 2020). Carbon dosing of denitrifying bioreactors operating in agricultural catchments can increase seasonal (drainage season) removal rates by targeting large pulses of nitrate that may occur in drainage waters (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018). Carbon dosing can either compensate for the carbon shortage in existing denitrifying bioreactors or be used to design denitrifying bioreactors with smaller spatial footprints

due to the increased nitrate removal rates. Given the varying nitrate input loads to bioreactors in agricultural catchments, the added carbon should ideally be matched to the input nitrate loads. However, this strategy necessitates costly nitrate and flow monitoring in the field and an electronic controller capable of regulating the added carbon dosage rate based on real-time nitrate and flow measurements. A more feasible approach would be adding carbon at a constant rate regardless of the bioreactor's nitrate input, which removes large nitrate pulses. However, added carbon may cause nitrate-limiting conditions in the bioreactor, potentially increasing the risk of unwanted carbon losses from the bioreactor to the receiving waters. Additionally, other microbial processes, such as sulfate reduction, may be activated when carbon is added in excess of nitrate input. Sulfate reduction, in particular, emits noxious hydrogen sulphide into the environment.

1.2.2 Carbon dosing of mesocosm denitrifying bioreactors

Mesocosm experiments seek to replicate the natural environment under controlled settings (Spivak et al., 2011; Stewart et al., 2013). Denitrifying bioreactors in agricultural catchments, particularly in pastoral fields, are subjected to extremely transient flow conditions, and the length of drainage seasons may be insufficient (Christianson et al., 2021; Maxwell et al., 2020; Rivas et al., 2020). Controlled mesocosm carbon dosing experiments allow for more accurate determination of the enhancement of nitrate removal rates in bioreactors with added carbon sources. The removal rates of nutrients, such as nitrate and sulfate, as well as added carbon, could provide design parameters for either improving the performance of existing denitrifying bioreactors or designing new denitrifying bioreactors with external carbon dosing.

1.2.3 Hydraulic determination of denitrifying bioreactors under different carbon dosing treatments

The clogging of the porous medium of bioreactors is a potential problem for their deployment in catchments (Ashoori et al., 2019; Christianson et al., 2016; Lepine et al., 2020). Reduced hydraulic performance may either reduce the overall nitrate removal efficiency through increased by-pass flow or increase the risk of backflow to the upstream field (Cameron and Schipper, 2010; Christianson et al., 2010; Ghane et al., 2014). External carbon dosing may contribute to the change in bioreactor hydraulic reduction since a few studies, such as Christianson et al. (2016), have demonstrated a positive correlation between influent carbon concentrations and decreases in bioreactor hydrology. However, none of the carbon dosing studies have explored the effects of external carbon dosing on the various hydraulic parameters of denitrifying bioreactors, such as hydraulic conductivity (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018). A combination of field and mesocosm observations can determine the effects of carbon dosage on bioreactor hydraulic performance.

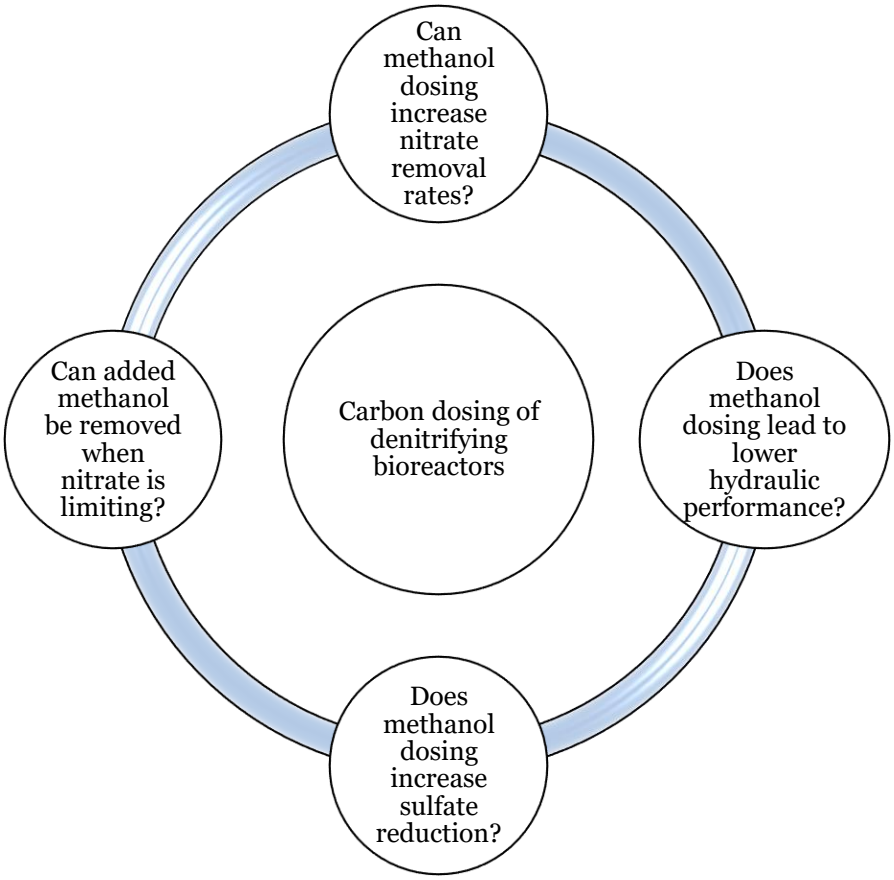

Figure 1-1 The main research questions addressed in this thesis

1.3 Thesis outline

The next chapter provides a review of the literature on the problem of excess nitrogen in the environment, as well as an overview of proposed mitigation approaches for removing nitrogen from diffuse pollution sources (e.g., agricultural tile drainage). The literature review then expands on denitrifying bioreactors and their efficacy in removing nitrate by focusing on research to date, concentrating on external carbon dosing to improve bioreactor performance, and highlighting knowledge gaps. The literature review is followed by three chapters outlining the research conducted. The third chapter investigated the carbon dosing of a pilot-scale bioreactor in an agricultural catchment over two consecutive drainage seasons. Carbon dosing in

mesocosm denitrifying bioreactors is explored in Chapter 4. Chapter 5 delves into the hydraulic modeling of denitrifying bioreactors under various carbon dosing regimens. Chapter 6 presents an overall assessment of the thesis and potential implications and recommendations for future bioreactor research. Chapters 3-5 detail the research conducted during this Ph.D. and are written in standard research paper format (abstract, introduction, methods, results, discussion, and references).

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Chapter 2

Literature review

2.1 Addressing the environmental nitrogen issue

2.1.1 An introduction to Earth's nitrogen cycle

The nitrogen cycle on Earth is a set of physical, chemical, and biological cycles that include both biotic (such as bacteria and animals) and abiotic (environmental) factors that transform nitrogen between a variety of chemical forms across different ecosystems (Basu et al., 2022; Fowler et al., 2013; Haqq-Misra et al., 2022; Mosier et al., 2013; Stevens, 2019). As the majority of the nitrogen on Earth is molecular nitrogen (N_2), which is relatively inert, nitrogen availability has been recognized as the major constraint to primary production in a variety of aquatic and terrestrial environments (Basu et al., 2022; Fowler et al., 2013; Stevens, 2019). Energy-intensive chemical approaches, such as the Haber-Bosch process, have been developed to convert inert dinitrogen gas to bioavailable forms such as ammonia and nitrate (Billen et al., 2013; Fowler et al., 2013). These industrial processes, however, rely on chemical energy derived from fossil fuels to produce reactive nitrogen at high pressure and temperature, which can then be used as fertilizers (Basu et al., 2022; Fowler et al., 2013; Modak, 2002).

Nonindustrial nitrogen fixation combines physical mechanisms (such as lightning) as well as biological sources (e.g., crops such as legumes). The annual biological and abiotic nitrogen fixation rates are approximately 193×10^6 and 9×10^6 tonnes of nitrogen, respectively. The amount of nitrogen fixed industrially per year is around 85×10^6 tonnes of nitrogen, compared to a total nitrogen fixation of 287×10^6 tonnes per

year (Basu et al., 2022; Brady et al., 2008; Fowler et al., 2013). Industrial nitrogen fixation accounts for 29% of the total nitrogen fixation and is a substantial anthropogenic nitrogen input into the biosphere.

Increased nitrogen inputs have allowed for more outstanding food production through intensive agriculture, but these inputs have also resulted in significant environmental issues, particularly eutrophication of nutrient-sensitive aquatic ecosystems (Billen et al., 2013; Davidson et al., 2011; Fowler et al., 2013; Haqq-Misra et al., 2022; Stein and Klotz, 2016; Stevens, 2019). The efficiency of nitrogen uptake by agricultural plants varies between 30 and 53% of the nitrogen applied by farmers (Basu et al., 2022; Carstensen et al., 2020; Haqq-Misra et al., 2022; Kronvang et al., 2020). Significant nitrogen losses occur in agricultural soils primarily because of poor farming practices and the over-application of fertilizers (Basu et al., 2022; Haqq-Misra et al., 2022).

Nutrient losses to receiving ecosystems occur when nitrate and other nutrients flow away from agricultural soils into receiving aquatic habitats (Anas et al., 2020; Cui et al., 2020; Di and Cameron, 2002; Wang et al., 2019). Due to its toxicological characteristics, nitrate may also be harmful to human health if it is present in drinking water in large amounts (Haqq-Misra et al., 2022; Stevens, 2019).

2.1.2 A closer look at denitrification

Denitrification is an important part of the Earth's nitrogen cycle, as it regulates the biogeochemical balance of nitrogen in the environment (Anas et al., 2020; Kuypers et al., 2018). This microbial process is carried out by a wide variety of bacteria and archaea that can use nitrate (NO_3^-) as a terminal electron acceptor in the absence of oxygen. Denitrification consists of four enzymatic steps that convert nitrate to nitrite

(NO_2^-), nitric oxide (NO), and nitrogen dioxide (NO_2) before being liberated as nitrogen gas (N_2) (Basu et al., 2022; Fowler et al., 2013; Stevens, 2019).

The reduction of nitrate to nitrite by the enzyme nitrate reductase is the first step in denitrification. Denitrifying bacteria such as *Paracoccus* sp. *to Pseudomonas* sp. carry out this reaction (Carlson and Ingraham, 1983; Davies et al., 1989). The second step in denitrification involves the action of nitrite reductase, also known as nitrite oxidoreductase, to convert nitrite to nitric oxide (Kraft et al., 2011; Maia and Moura, 2015; Rajeev et al., 2015). The reduction of nitric oxide to nitrogen dioxide by the action of nitric oxide reductase is the third step in denitrification. The final step involves the action of nitrous oxide reductase to convert nitrogen dioxide to nitrogen gas (Kraft et al., 2011; Maia and Moura, 2015; Rajeev et al., 2015).

Several environmental factors regulate the rate of denitrification in terrestrial and aquatic environments, including the availability of nitrate, organic carbon, pH, temperature, and the presence of oxygen as a terminal electron acceptor. In general, denitrification thrives in environments with low oxygen concentrations, high nitrate concentrations, and the presence of organic carbon as an energy source (Basu et al., 2022; Fowler et al., 2013; Stevens, 2019)

2.1.3 Mitigation measures

Even though farming practices such as optimum nitrogen application can increase nitrogen uptake to 70% of total applied nitrogen, edge-of-field mitigation strategies may still be necessary to limit nitrogen losses to receiving water bodies (Anas et al., 2020; Bloem et al., 2020; Carstensen et al., 2020). The edge-of-field nitrate mitigation

practices are becoming more widespread in developed countries to reduce nitrogen pollution from agriculture (Carstensen et al., 2020; Carstensen et al., 2021).

These nitrate-mitigation strategies employ natural ecological processes to remove nitrate from agricultural systems (Bowles et al., 2018; Carstensen et al., 2020; Christianson et al., 2013b; Hoffmann et al., 2020). Constructed wetlands, saturated buffers, control drainage, and denitrifying woodchip bioreactors have been proposed as potential solutions to lower nitrate levels in agricultural runoff (Carstensen et al., 2020; Christianson et al., 2013b; Christianson et al., 2013c). The most efficient nitrate treatment mitigation techniques are primarily dependent on the hydrology of the watershed and the climate of the agricultural region (Carstensen et al., 2020; Chandrasoma et al., 2022; Christianson et al., 2013b; Christianson et al., 2013c; Christianson et al., 2021).

2.1.3.1 Nitrate removal performance

The effectiveness of the nitrate removal offered by the nitrate mitigation measures has led to the establishment of different metrics of performance, including nitrate removal efficiency (%), for evaluating other treatment systems' performance (Carstensen et al., 2020; Christianson et al., 2013b). Nitrate removal efficiency is commonly used to compare mitigating strategies performance (Christianson et al., 2013b; Christianson et al., 2013c). Volumetric nitrate removal rates (the amount of nitrate removed per volume per time) are also used to quantify the performance of the mitigation measures. Nitrate removal rates might be used to build a treatment system that targets specific nitrate removal efficacy (Schipper et al., 2010b; Warneke et al., 2011a).

The next section of the literature review will provide a brief overview of various nitrate mitigation options before delving into bioreactors and the factors that regulate their removal efficacy.

2.1.4 Constructed wetlands

In constructed wetlands, drainage water runs through a vegetated basin, where nitrate removal occurs progressively as the water flows through (Figure 2-1) (Bauwe et al., 2022; Carstensen et al., 2021; Tanner et al., 2012). Denitrification is responsible for most nitrate removal in constructed wetlands (Bauwe et al., 2022; Persson et al., 1999; Tanner et al., 2012). However, anammox (anaerobic ammonium oxidation) contributes to nitrate removal when ammonium is generated through the anaerobic decomposition of organic nitrogen (nitrogen mineralization) (Tanner et al., 2012). In addition to denitrification, plant nitrogen uptake has also been demonstrated to contribute to nitrate removal in constructed wetlands (Bauwe et al., 2022; Carstensen et al., 2021; Tanner et al., 2012). In their typical design and operating conditions, constructed wetlands have been shown to lower nitrate loads by between 30 and 50% (Tanner et al., 2012).

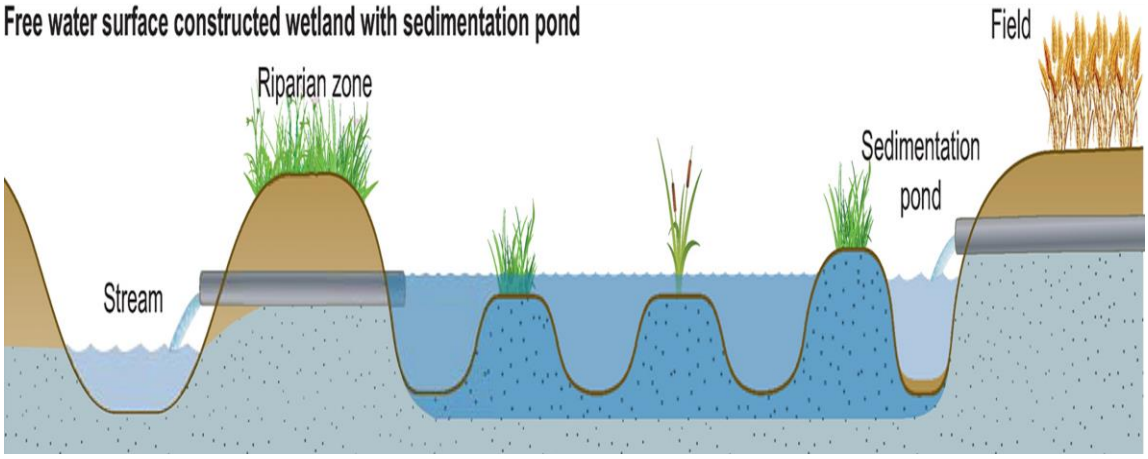

Figure 2-1 Conceptual design of constructed wetlands adapted from Carstensen et al. (2020)

2.1.5 Saturated buffers

In saturated buffers (SBs), tile drainage is diverted to a perforated lateral pipe parallel to the downstream river, where drainage water is distributed into riparian soil rather than flowing directly into the river (Figure 2-2) (Jaynes and Isenhart, 2014; 2019). Soil carbon in SBs and infiltrating water creates anoxic conditions, allowing denitrification to occur, mimicking the hydrology of naturally occurring riparian buffers (Jaynes and Isenhart, 2014). As a mitigation strategy, SBs were first reported by Jaynes and Isenhart (2014) and have been mainly employed in the United States (Carstensen et al., 2020). In a meta-analysis of removal efficiencies of SBs, Carstensen et al. (2020) reported an average nitrate removal efficiency of 37% (CI: 17–57%), including the flow that bypassed the SBs.

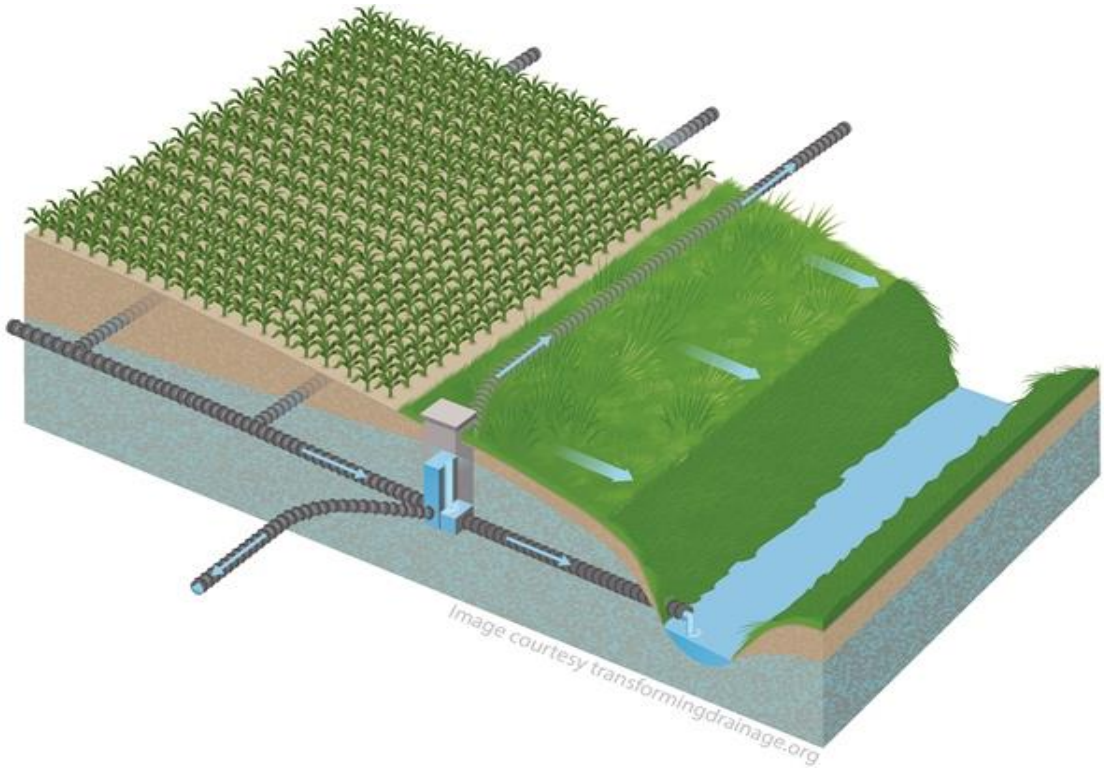

Figure 2-2 Conceptual design of saturated buffers adapted from Dan Jaynes (2018)

2.1.6 Controlled drainage

Controlled drainages (CDs) are structural mitigation strategies that raise and lower the water table in the field to regulate the volume of drainage and, as a result, the nutrients into downstream water bodies (Figure 2-3) (Carstensen et al., 2020; Christianson et al., 2013b; Christianson et al., 2013c). The drainage from the field is controlled by adjusting the water depth at an outlet structure, typically done with stoplogs (Figure 2-3) (Carstensen et al., 2020). Controlled drainages as conservation drainage practices were initially introduced by Gilliam et al. (1979) in the 1970s in the United States and have since gained popularity in other countries such as Germany. Controlled drainages reduce nutrient loss and could combat summer droughts by managing the water table in the field (Carstensen et al., 2020). In a meta-analysis of 17 CD field experiments, Carstensen et al. (2020) found that CDs reduce yearly drainage loads by approximately 46% and annual nitrate losses by around 48%.

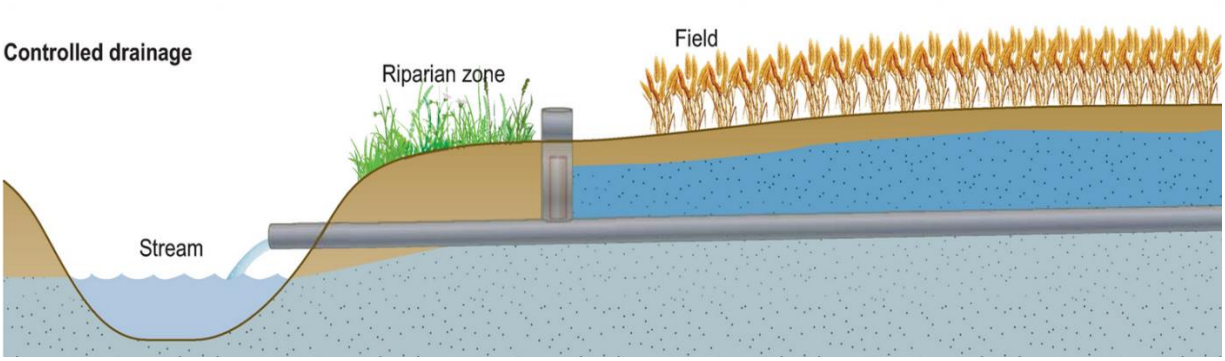

Figure 2-3 Conceptual design of controlled drainage adapted from Carstensen et al. (2020)

2.1.7 Denitrifying bioreactors

Bioreactors are lined trenches filled with carbon-rich solid materials such as woodchips, maize cobs, and sawdust in which nitrate is transformed into gaseous nitrogen by denitrification (Figure 2-4) (Addy et al., 2016; Christianson and Schipper, 2016; Schipper et al., 2010a). Denitrification is fuelled by the slow and gradual release of dissolved organic carbon into the water matrix (Figure 2-4) (Addy et al., 2016; Cameron and Schipper, 2010). Schipper et al. (2010b) classified denitrifying bioreactors into three types based on their hydrological settings: denitrification walls that intercept shallow groundwater flow (wall's cross-section perpendicular to the flow), denitrifying beds that intercept subsurface discharge (flow path along the length of the bed), and denitrifying layers that are horizontal covers of solid carbon substrate that reduce nitrate loads from leachate into groundwater. Extensive studies have focused on denitrifying beds and their use in treating various wastewaters such as agricultural tile drainage, aquaculture wastewater, stormwater, and nitrified wastewater (Addy et al., 2016; Ashoori et al., 2019; Christianson et al., 2021; Christianson et al., 2016; Christianson and Schipper, 2016; Goeller et al., 2019; Lepine et al., 2016; Rambags et al., 2016; Schipper et al., 2010b; Tanner et al., 2012). Denitrifying beds should ideally have a bypass structure that can bypass flow

exceeding the hydraulic capacity of the bioreactor (Addy et al., 2016; Christianson and Schipper, 2016; Schipper et al., 2010a). Section 2.20 explores the factors that regulate nitrate removal in bioreactors, with a particular focus on those installed in agricultural catchments.

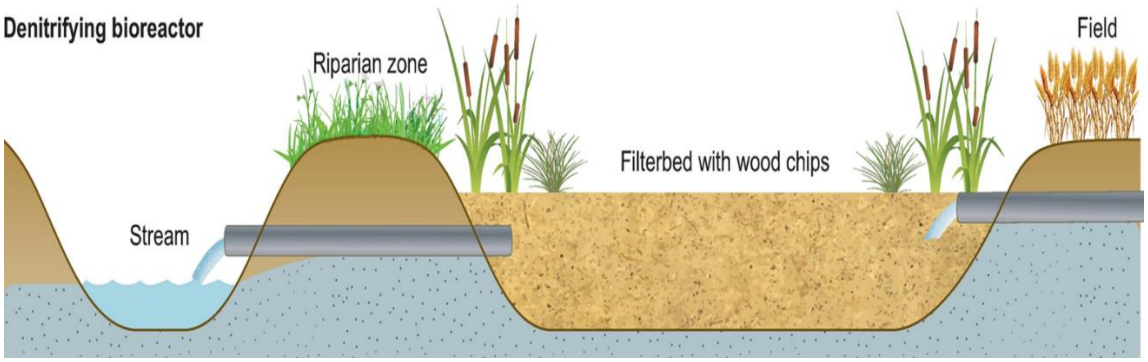

Figure 2-4 Conceptual design of bioreactors adapted from Carstensen et al. (2020)

2.1.8 Synopsis: mitigation strategies

In their usual design and operational settings, nitrate mitigation techniques generally have less than 50% nitrate removal efficiency. Most of these mitigation strategies are based on ecological systems that rely on physiochemical mechanisms to provide the carbon needed for nitrate removal through denitrification. As a result, there are not many possibilities for increasing system efficiency; thus, high removal efficiency needs larger systems to renovate a greater volume of water and nitrate. However, larger systems would result in more land being removed from production, lowering the acceptability of these techniques by watershed managers and landowners. However, bioreactors are rapid to establish, needing minimal efforts to stabilize the systems before start-up. Therefore, developing and testing simple and economical approaches that could improve bioreactor's nitrate removal performance would be of interest to promote these systems as the preferred nitrogen removal approach in agricultural catchments.

2.2 An introduction to bioreactors

Stewart et al. (1979) proposed using a solid organic substrate to improve denitrification for nitrate removal. So far, multiple studies have employed this technique to remove nitrate from a variety of pollution sources, including agricultural tile drainage (Audet et al., 2021; Christianson et al., 2021; Christianson and Schipper, 2016; Maxwell et al., 2020; Rivas et al., 2020b; Schipper et al., 2010b), aquaculture wastewater (Aalto et al., 2022; Christianson et al., 2016; Lepine et al., 2020), stormwater (Ashoori et al., 2019; Lynn et al., 2016), municipal wastewater (Rambags et al., 2019a; Rambags et al., 2016; 2019b), and mine drainage (Nordström and Herbert, 2017; 2018; 2019). Most of the bioreactor research has focused on either demonstrating and enhancing bioreactor performance in terms of nitrate removal and hydraulic efficiency (such as Cameron and Schipper (2011)) or minimizing pollution swapping, such as sulfate reduction and DOC losses (Christianson et al. (2017) and Rivas et al. (2020b)). A few studies have examined the removal of other sources of pollutants, such as ammonium and pathogens, suggesting that bioreactors could provide further benefits in removing a wide variety of redox-sensitive pollutants (Rambags et al., 2019a; Rambags et al., 2016; 2019b).

Bioreactors have been thoroughly studied in a few in-depth reviews, including those by Schipper et al. (2010b), Addy et al. (2016), and Christianson et al. (2021). As a result, a full investigation of bioreactors is not the focus of this chapter; instead, a broad summary of factors regulating nitrate removal rates, with a particular emphasis on carbon availability, is provided.

2.2.1 Factors affecting bioreactor's performance

The performance of bioreactors, like that of other biological treatment systems, is regulated by environmental and operational parameters such as temperature and substrate content (Addy et al., 2016; Schipper et al., 2005; Schipper et al., 2010b; Warneke et al., 2011b). However, because bioreactors are passive (i.e., there are no substantial controls on bioreactor inputs and operating settings), environmental circumstances would predominantly regulate or limit nitrate removal efficiency (Cameron and Schipper, 2010; Manca et al., 2020; Schipper et al., 2010b; Warneke et al., 2011b; Warneke et al., 2011c). The nitrate removal rates of bioreactors have been demonstrated to differ significantly between studies, presumably due to differences in bioreactor inputs, bioreactor design, and methodologies for measuring nitrate concentrations and removal rate estimations. For example, Addy et al. (2016) conducted a meta-analysis of peer-reviewed bioreactor studies that removed nitrate from diverse water inputs (including tile drainage and municipal wastewater) and reported mean nitrate removal rates of 10.5 g N m^{-3} d^{-1}, 95% CI [8.5 g N m^{-3} d^{-1}, 12.3 g N m^{-3} d^{-1}] with nitrate inputs greater than 30 mg N L^{-1}. Christianson et al. (2021) reported nitrogen removal rates of 7.2 ± 9.6 g N m^{-3} d^{-1} (mean ± SD) in 27 field woodchip bioreactors with a variety of effluent inputs, including partially nitrified wastewater. However, Lepine et al. (2016) and Goeller et al. (2019) demonstrated substantial nitrate removal rates of 44 g N m^{-3} d^{-1} and 50 g N m^{-3} d^{-1} in bioreactors removing nitrate from aquaculture wastewater and an agricultural stream. In general, controlled mesocosm experiments in the lab have been shown to provide higher nitrate removal rates than full-scale bioreactors operating in the field (Addy et al., 2016; Cameron and Schipper, 2010; Schipper et al., 2010b).

The methods used to quantify nitrate concentrations in bioreactors have also been shown to affect calculations of removal rates. For instance, Corbett et al. (2020) reported nitrate removal rates of 26 g N m^{-3} d^{-1} in a full-scale bioreactor treating partially nitrified municipal wastewater via diffusive gradients in thin films (DGTs) while accounting for the temporal dynamics of nitrate in the bioreactor. Rambags et al. (2016), on the other hand, reported removal rates of 16 g N m^{-3} d^{-1} in the same bioreactor measuring nitrate concentrations using grab samples.

2.2.1.1 Temperature

The temperature is a significant environmental factor in regulating nitrate removal rates in bioreactors (Addy et al., 2016; Nordström and Herbert, 2019; Schipper et al., 2010b; Schmidt and Clark, 2013). The correlation between temperature and nitrate removal rate has been commonly modelled using the Arrhenius equation with Q_{10} as the temperature dependence metric. Q_{10} has been reported to range between 0.16 and 5 across different studies (Cameron and Schipper, 2010; Robertson, 2010; Schmidt and Clark, 2013; Warneke et al., 2011b; Warneke et al., 2011c). However, Nordström and Herbert (2019) used the macromolecular rate theory (MMRT) (Schipper et al., 2014) as a novel approach for modelling the temperature dependence of denitrification in a full-scale bioreactor, calculating a mean temperature optimum of 24.2 °C in the first year of bioreactor operation, which then decreased to 16.0 °C. Nordström and Herbert (2019) attributed this decline in temperature optimum to the denitrifier population's homogenization with time in the bioreactor.

2.2.1.2 Dissolved oxygen

Dissolved oxygen (DO) has been shown to inhibit denitrification in bioreactors (Addy et al., 2016; Schipper et al., 2010b). Because it is more energy-efficient, aerobic decomposition may hamper denitrification by competing for labile carbon generated

by woodchips (Addy et al., 2016; Schipper et al., 2010b; Warneke et al., 2011c). The rates of aerobic decomposition have been reported to range from 1.0 to 20 mg O_2 L^{-1} h^{-1} (David et al., 2016; Halaburka et al., 2017; Schmidt and Clark, 2013), with limiting DO concentrations ranging from 0.05 to 0.2 mg O_2 L^{-1} (Doussan et al., 1997; Higashino et al., 2008). Given the comparatively high removal DO rates in bioreactors compared to denitrification rates of 0.05–2.0 mg N L^{-1} h^{-1} (David et al., 2016; Halaburka et al., 2017; Schmidt and Clark, 2013), DO removal would occur in the early sections of the bioreactor, leaving the rest of the bioreactor suitable for denitrification to occur.

2.2.1.3 Substrate content

Nitrate and labile carbon as the substrate fuel denitrification in bioreactors (Addy et al., 2016; Bremner and Shaw, 1958; Schipper et al., 2010b) and are addressed in the following sections:

a. Nitrate

The nitrate dependence of denitrification rates in bioreactors has been examined using two methods (Ghane et al., 2015; Nordström and Herbert, 2019). Michaelis-Menten kinetics and the nth-order rate law have been used to model the nitrate removal kinetics based on the experimental results (Halaburka et al., 2017; Nordström and Herbert, 2019). The nth-order rate law's equation is as follows:

$$\nu = kC_i^n \qquad \text{Equation 2-1}$$

Where ν is the nitrate removal rate, C_i is the nitrate concentration, n is the rate order, and k is the temperature coefficient constant. The rate order (n) is reported between zero and one (Halaburka et al., 2017; Nordström and Herbert, 2019; Robertson, 2010).

The Monod equation is another common technique for modelling the nitrate dependency of denitrification rates in bioreactors (Ghane et al., 2015; Halaburka et al., 2017; Kouanda and Hua, 2021):

$$v = \frac{V_{max} C_n}{k_m + C_n} \quad \text{Equation 2-2}$$

Where V_{max} stands for maximal nitrate removal rates, k_m represents Michaelis constant, and C_n is nitrate concentrations. Nitrate removal rates when nitrate concentrations are less than k_m are considered increasingly nitrate limited so that the removal rate then becomes a linear function of concentration (Kouanda and Hua, 2021); however, removal rates in concentrations larger than k_m become independent of nitrate concentrations approaching V_{max}. There has been a broad range of k_m values (indicative of nitrate limited removal rates) reported for heterotrophic denitrification. However, k_m values have ranged between 2 and 10 mg N L^{-1} (Ghane et al., 2015; Kouanda and Hua, 2021).

b. Carbon

The DOC release from woodchips provides the labile carbon necessary for denitrification in the nitrate removal (Abusallout and Hua, 2017; Halaburka et al., 2017; Rivas et al., 2020b; Warneke et al., 2011b). However, microbiological degradation of woodchips (hydrolysis and fermentation) is slow and often limits denitrification (Abusallout and Hua, 2017; Halaburka et al., 2017; Nordström and Herbert, 2019; Rivas et al., 2020b; Warneke et al., 2011b). The hydrolysis of woody material (cellulosic and lignocellulosic substrates) into large, less recalcitrant carbonaceous molecules is the first step in producing DOC from woodchips (Nordström and Herbert, 2019). Under anaerobic conditions, these large molecules are then fermented into more labile carbon sources such as sugars and volatile fatty acids (VFAs), which are then available for denitrifiers to remove nitrate (Nordström

and Herbert, 2019). The DOC supply from woodchips may initially be abundant but progressively decline, potentially limiting denitrification (Abusallout and Hua, 2017; Rivas et al., 2020b).

Various solid carbon substrates have been examined to provide bioreactors with sufficient DOC supply for long-term nitrate removal. Examples of carbon substrates include woodchips, wheat straws, maize husks, and cardboard chips (Cameron and Schipper, 2010; Warneke et al., 2011c). While specific carbon sources, such as woodchips, may have lower nitrate removal rates but can be more durable, others, like wheat straw and maize cobs, supply more readily available carbon in the short term, but nitrate removal rates then decline more quickly (Addy et al., 2016; Cameron and Schipper, 2010). Due to their accessibility, woodchips are bioreactors' most often used carbon source (Addy et al., 2016; Rivas et al., 2020a; Schipper et al., 2010b). Employing woodchips also has a fundamental drawback: the generation of labile carbon decreases with time. This temporal decline in DOC productivity of woodchips has been quantified in various studies, including Robertson (2010) and Abusallout and Hua (2017). Robertson (2010), for example, showed that the DOC productivity of woodchips falls by 50% over the first year of bioreactor operation. Several studies have also identified a significant decrease in nitrate removal rates in bioreactors due to a reduction in DOC generation from woodchips (Maxwell et al., 2019a; Maxwell et al., 2019b; McGuire et al., 2021; Rivas et al., 2020b; Robertson, 2010; Warneke et al., 2011b). This DOC deficiency in bioreactors has generated initiatives to improve bioreactors for better long-term sustainable nitrate removal.

2.2.2 Synopsis: Factors regulating nitrate removal in bioreactors

Bioreactor's simplicity and somewhat small spatial footprint compared to other mitigation measures are critical reasons for explaining their current appeal. However, nitrate removal in bioreactors appears to decrease with time as carbon availability limits denitrification. After installation, bioreactors are often not managed, and external environmental factors, such as the influent's temperature and chemistry, regulate nitrate removal. However, if simple and inexpensive strategies were developed to address the carbon shortage of bioreactors, these systems would be more often adopted as a nitrate mitigation option. Some researchers have used physical and chemical strategies to either accelerate the carbon release from woodchips or incorporate external carbon sources into bioreactors to compensate for this carbon deficiency. Periodic drainage, biochar amendment, and external carbon dosage are the principal ways to compensate for nitrate removal's carbon restriction. The benefits and drawbacks of each of these strategies are discussed in the following section.

2.3 Overcoming the carbon constraint of the nitrate removal

2.3.1 Periodic drainage

The concept of exposing the bioreactors to drying–rewetting cycles via periodic drainage was inspired by soil science literature and was first investigated by Maxwell et al. (2019a). Soils redox drying–rewetting cycles are shown to increase carbon leaching and shift microbial and fungal populations (Beare et al., 2009; Borken and Matzner, 2009). Maxwell et al. (2019a) showed that exposing bioreactors to the aerobic phase for 8 h once a week improved nitrate removal efficiency by 80%. In a follow-up experiment, Maxwell et al. (2019b) found that increasing the duration of drying–rewetting cycles boosted nitrate removal by measuring nitrate removal rates

subjected to either 2 h, 8 h, or 24 h. They reported that the longest dry (aerobic) period of 24 h resulted in a 172% increase in nitrate removal efficiency over control bioreactors.

2.3.2 Biochar amendment

Biochar is a solid substrate produced by the thermochemical conversion of organic feedstock in the absence of oxygen (Christianson et al., 2011; Maleki Shahraki and Mao, 2022). There has been conflicting evidence of biochar increasing and decreasing nitrate removal rates in bioreactors. Depending on the operating conditions and biochar age, biochar has been demonstrated to function as an electron acceptor and donor (Ashoori et al., 2019; Berger et al., 2019; Bock et al., 2015; Maleki Shahraki and Mao, 2022). More precisely, the age of the biochar has been shown to determine its influence on nitrate removal, with fresh biochar likely to enhance nitrate removal and old or oxidized biochar likely not to alter nitrate removal when added with woodchips. For instance, Hassanpour et al. (2020) demonstrated that adding fresh biochar to bioreactors boosted nitrate removal by 23%. However, some studies revealed that adding biochar did not significantly improve nitrate removal (Ashoori et al., 2019; Christianson et al., 2011; Hassanpour et al., 2020).

2.3.3 Carbon dosing

Carbon dosing has been tested in a few studies to enhance nitrate removal rates in bioreactors (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018). Herbert Jr et al. (2014) first evaluated this approach by adding acetate to a full-scale bioreactor for treating mine drainage wastewater. They reported nitrate removal rates ranging from 5 to 10 g N m^{-3} day^{-1}, compared to 1.3-4.8 g N m^{-3} day^{-1} for the control treatment without carbon dosage. This strategy was expanded upon by Hartz

et al. (2017), who dosed mesocosm and full-scale bioreactors treating agricultural subsurface drainage water with methanol and glycerine. According to Hartz et al. (2017), carbon dosage increased nitrate removal rates by 29–40 g N m^{-3} day^{-1} compared to the control removal rates of 2.5 g N m^{-3} day^{-1}. Section 2.4 will cover carbon dosing in bioreactors in greater detail.

2.3.4 Synopsis: various approaches to improving bioreactors

Various procedures to improve bioreactor performance resulted in a small to substantial improvement in nitrate removal rates and efficiency. Periodic drainage has been proven to have nearly double nitrate removal effectiveness, whereas biochar addition has resulted in a negligible to a slight improvement in nitrate removal rates. On the other hand, external carbon dosing has resulted in a significant rise in nitrate removal rates. However, increases in nitrogen removal rates have been found to differ widely throughout different investigations, with limited attempts to assess the adverse impacts of external carbon dosing on downstream water bodies. The following section aims to thoroughly investigate the nitrate removal rates offered by various carbon sources and the main adverse effects that exogenous carbon dosage may have on receiving water bodies.

2.4 Carbon dosing of bioreactors

Low molecular weight carbon compounds (e.g., methanol, succinate, ethanol, or acetate), disaccharides (e.g., glucose, fructose), as well as industrial and agricultural by-products, such as molasses, are the most common liquid carbon sources used in denitrification systems such as bioreactors (Jansen et al., 2019; Jiang et al., 2022; Ortmeyer et al., 2021; Roser et al., 2018). Carbon dosing has generally resulted in

substantial nitrate removal enhancement in bioreactors, with the highest removal rates found in Roser et al. (2018), who reported removal rates of 121 g N m^{-3} day^{-1}.

Jansen et al. (2019) employed ethanol as an external carbon source in a passive dosing mechanism (no energy supply for the operation) in a bioreactor with no solid substrate to promote microbial growth. Nitrate removal rates of 50 g N m^{-3} d^{-1} and 1 g N m^{-3} d^{-1} were observed in the ethanol reactor and woodchip bioreactor, respectively. Since the system Jansen et al. (2019) tested was a sealed vessel rather than a wood-based bioreactor, the removal rates of nitrate from the ethanol reactor are not strictly comparable to those provided by woodchip bioreactors. A thorough breakdown of the various carbon compounds offering improved nitrate removal rates compared to the control removal rates is given in Table 2-1.

Depending on the operating scale, the influent's chemistry, and the operating conditions, nitrate removal rates enhancement due to carbon dosing widely varies across investigations (Table 2-1). These differences in nitrate removal rates between experiments suggest that assessing the effect of carbon dosing on the performance of different bioreactors may require a case-by-case study. A more practical approach would be to compare the performance of a particular bioreactor with and without carbon addition.

Table 2-1 Comparison of different compounds used in bioreactors running under different operating conditions

Carbon compound	Hydraulic retention time (hours)	Temperature (°C)	Saturated volume (m^3)	Control removal rates	Enhanced removal rate	Reference
				g N m^{-3} d^{-1}		
Ethanol	2.4	NA	0.7	1	50	Jansen et al. (2019)
Sodium acetate	1.5	14.6 5 14.9	9.07×10^{-3}	6.5 2.2 7.1	43 29.9 120.6	Roser et al. (2018)
	8	14.6 5 14.9	9.07×10^{-3}	5.8 0.9 7.8	26.7 29.8 26.5	
	12	14.6 5 14.9	9.07×10^{-3}	6.4 1.5 7.8	19.6 21.3 18.4	
	24	14.6 5 14.9	9.07×10^{-3}	7.3 1.6 7.1	10.2 11.8 10.3	
Sodium acetate	24	Without dosing (18 ± 2.5 °C) With dosing (16 °C)	27	1.3–4.8	5–10	Herbert Jr et al. (2014)
Glycerine	48	NA	14×10^{-3}	2.25	29-40	Hartz et al. (2017)
Methanol	48	NA	14×10^{-3}	2.25	31-40	Hartz et al. (2017)
Methanol	48	(17 ± 13 °C)	12.7	9	36	Hartz et al. (2017)

2.4.1 Potential side effects of carbon dosing

Adding liquid organic carbon can certainly enhance nitrate removal rates by order of magnitude (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018). However, some unwanted consequences of carbon dosing have yet to be investigated (Jansen et al., 2019; Roser et al., 2018). Carbon dosage should ideally be applied at a rate where nitrate is the dominant terminal electron acceptor so that denitrification consumes all the carbon supplied. This optimal redox state could be

supported provided the extra carbon is carefully matched to the bioreactor's nitrate input and the dosed bioreactor operated under adequate hydraulic retention time (HRT). This matching technique necessitates continuous monitoring of bioreactor input, which the controller then uses to match the added carbon with input nitrate and flow. However, given the matching technique's increased capital and operational costs, this strategy may not be feasible for upscaling carbon dosing to increase nitrate removal.

A more cost-effective alternative to the carbon matching technique would be to dose added carbon at a constant rate independent of input nitrate. However, regular carbon dosage may result in more extreme redox state fluctuations, which can have several adverse consequences, such as added carbon losses from the bioreactor outlet (Jansen et al., 2019; Roser et al., 2018). The following sections will outline the potential adverse effects of the constant carbon dosing method.

2.4.1.1 Added carbon losses to the receiving environment

Dissolved organic carbon (DOC) losses from bioreactors, particularly during the start-up stage, are one of the potential detrimental impacts of installing bioreactors in catchments (Abusallout and Hua, 2017; Addy et al., 2016; Christianson et al., 2013b; Christianson and Schipper, 2016; Rivas et al., 2020b). Excessive DOC in water bodies can reduce the amount of dissolved oxygen through aerobic decomposition, stressing aquatic habitats (Fukushima et al., 1996; Withers et al., 2014; Wurtsbaugh et al., 2019).

Adding soluble carbon at a constant rate may exacerbate DOC losses from bioreactors, resulting in excessive DOC (including added carbon) seeping into the receiving aquatic habitats. The prospect of increased DOC losses due to carbon dosing is especially important in bioreactors with varying nitrate concentrations in the input water (such

as those in agricultural catchments (Christianson et al., 2013b; Christianson et al., 2021; Maxwell et al., 2020; Rivas et al., 2020b)). Furthermore, some soluble carbon sources, including methanol, can potentially be hazardous to aquatic life (Kaviraj et al., 2004).

The variable nitrate input may result in low nitrate concentrations in bioreactors, limiting denitrification. However, less effective microbial DOC removal processes, including sulfate reduction, may consume the extra soluble carbon before it is discharged.

2.4.1.2 Sulfate reduction

Sulfate reduction is one of the anaerobic biogeochemical pathways frequently observed in bioreactors (Aalto et al., 2022; Christianson et al., 2017; Corbett et al., 2020; Easton et al., 2015; Nordström and Herbert, 2018; Rambags et al., 2016). Reduced conditions in bioreactors brought on by carbon dosing may speed up the sulfate reduction process and consume some added carbon, especially under nitrate-limited conditions. Sulfate reduction produces poisonous hydrogen sulphide (H_2S), which can emit an unpleasant smell into the surrounding atmosphere (Easton et al., 2015; Wiener et al., 2013). In the presence of trace quantities of mercury in bioreactors input, sulfate reduction can result in the formation of methyl mercury, which is toxic to aquatic organisms (Easton et al., 2015; Hartfiel et al., 2022; Shih et al., 2011).

2.4.1.3 Greenhouse gas production

One of the potential adverse effects of bioreactors is the generation of greenhouse gases, including nitrous oxide (N_2O) and methane (CH_4), which have global warming potentials that are 265- and 28-times that of carbon dioxide (CO_2), respectively (Addy et al., 2016; Hassanpour et al., 2020; Manca et al., 2020; Rivas et al., 2020b; Warneke

et al., 2011b). The release of greenhouse gases might occur either as emissions from the top of the bioreactors or as dissolved gases in the bioreactor outflow (Hassanpour et al., 2020; Manca et al., 2020; Rivas et al., 2020b; Warneke et al., 2011c). However, a denitrification wall in Queensland, Australia, for example, was shown to be a net N_2O sink reported (i.e., the outflow load of N_2O was less than the inflow N_2O) (Manca et al., 2020). Carbon dosing may minimize N_2O generation in bioreactors, as N_2O production has been correlated with a shortage of accessible carbon (Nordström and Herbert, 2018). Hartz et al. (2017) reported zero production of dissolved N_2O in methanol-dosed bioreactors. This was comparable to 12% (percent of total denitrified nitrate) in mesocosm-size bioreactors without methanol dosing. However, research is needed to evaluate the generation and consumption of N_2O and CH_4 in bioreactors with various carbon dosing loads, particularly under conditions of fluctuating nitrate concentration, such as those seen in agricultural catchments.

2.4.1.4 Bioreactors hydraulic capacity

One concern with long-term bioreactors deployment in catchments is the clogging of the porous medium in bioreactors, which limits the effective volume of bioreactors over time (Ashoori et al., 2019; Christianson et al., 2016; Ma et al., 2021; Seki et al., 1998). Clogging in bioreactors can develop due to a range of physical and biological causes (Christianson et al., 2016; Ma et al., 2021). Woodchip settling, also known as woodchip subsidence, is a significant source of physical hydraulic deterioration in bioreactors, particularly those with dynamic fluxes (Christianson et al., 2013a). The most prevalent biological reasons for bioreactor clogging include the formation of microbial biomass in bioreactors (such as bacteria and fungus) and their extracellular metabolites, such as extracellular polymeric substance (EPS) (Christianson et al., 2013a; Christianson et al., 2016; Ma et al., 2021; Ortmeyer et al., 2021; Seki et al.,

1998). Due to void volume reduction in the porous woodchip medium, the combination of these factors would induce a progressive change in the bioreactor intrinsic permeability and a decline in the effective bioreactor volume (Ashoori et al., 2019; Christianson et al., 2016; Lepine et al., 2020).

External carbon dosage may make the biological clogging worse, as a lower redox state may increase the activity of chemoheterotrophic bacteria (Christianson et al., 2016). However, the potential impact of external carbon dosage on bioreactor hydrology has not been explored in any of the earlier studies on carbon dosing (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018).

The bioreactors decreased effective hydraulic conductivity might cause a rise in water level at their inlet structure (Christianson et al., 2013a; Ghane et al., 2014; Ghane et al., 2016). This would increase the bypass flow or flooding while lowering the overall efficiency of nitrate removal (Christianson et al., 2013a). The problem with this spike in water level at the inlet structure might be worse, especially in bioreactors without bypass structure and which could cause flooding and possibly trigger backflow into the field (Ashoori et al., 2019; Christianson et al., 2013a; Christianson et al., 2016; Ma et al., 2021).

2.4.2 Synopsis: external carbon dosing and potential adverse effects

External carbon sources, such as methanol, ethanol, and acetate, notably increase nitrate removal rates in bioreactors. However, the degree of nitrate removal improvement appears to vary significantly between investigations, presumably due to differences in carbon compounds, bioreactor input, and design. Designing new bioreactors with external carbon sources is tricky because of the variations in nitrate removal. The dosed bioreactor volume needs to be precisely determined using a stable

figure for long-term enhanced nitrate removal rates. As a result, long-term research on carbon dosing on a mesocosm to full-scale bioreactors is needed to measure the enhanced nitrate removal rates for design purposes. The adverse effects of carbon dosage should also be assessed to set the proper dosing load. Overloading the bioreactors with external carbon may adversely impact the receiving waters. Hence there needs to be a limit on the amount of carbon dosing. A thorough investigation that quantifies the increased nitrate removal rates and the potential adverse effects of dosing is required to establish carbon dosing as a safe and effective way to improve the performance of bioreactors.

2.5 Hydraulic flow in bioreactors

As discussed in section 2.4.1.4, changes in the hydrology of bioreactors are likely with time and, in theory, could be exacerbated by external carbon dosage. The next phase of the literature review explores the approaches for assessing the hydrology of bioreactors that might be used to investigate the impact of the added carbon on changes in hydrology.

2.5.1 Linear hydraulics

Darcy's equation is commonly used to model porous media hydrology, notably bioreactors. Darcy's equation, in its most basic version, is as follows (Ghane et al., 2014; Ghane et al., 2016; Whitaker, 1986):

$$q = K\frac{\Delta H}{L} \qquad \text{Equation 2-3}$$

Where q (m day^{-1}) is the specific discharge through the bioreactor, K is the saturated hydraulic conductivity (m day^{-1}), ΔH is the head differences (m), and L is the length of the bioreactor (m).

The original Darcy's equation requires that the cross-section of the medium remains constant, which is not often the case with bioreactors due to their trapezoidal form (Ghane et al., 2014; Ghane et al., 2016). Darcy's equation for trapezoidal beds has been adapted by Ghane et al. (2014):

$$Q = K_D \times \left(\frac{b(h_i^2 - h_o^2)}{2L_B} + \frac{z(h_i^3 - h_o^3)}{3L_B} \right) \quad \text{Equation 2-4}$$

Where Q is the flow rate through the bioreactor ($m^3\ day^{-1}$), h_i and h_o are the matched heights of water at the inlet and outlet structures (m), respectively, L is the length of the bed (m), b is the bioreactor's bottom width (m), K is hydraulic conductivity ($m\ day^{-1}$), and z is the bioreactor's side slope.

2.5.2 Non-linear hydraulics

Darcy's law applies exclusively to viscous (laminar, i.e., Reynolds numbers up to 10) flow. For porous medium flow, the Reynolds number (a dimensionless metric) is often employed as an index of the flow regime (laminar or turbulence) through the porous material and is expressed as (Sobieski and Trykozko, 2014):

$$Re = \frac{\rho q d}{\mu} \quad \text{Equation 2-5}$$

Where ρ is the water density ($kg\ m^{-3}$), d is the size of the woodchips (m), and μ is the water's dynamic viscosity coefficient ($kg\ m^{-1}\ s^{-1}$).

The Reynolds number is commonly employed as a flow regime indicator, with Reynolds numbers less than ten indicating laminar flow and Reynolds numbers more than ten indicating turbulent flow (Ghane et al., 2014; Ghane et al., 2016; Sobieski and Trykozko, 2014; Whitaker, 1986). There are a few ways to expand Darcy's equation for turbulent flow regimes, such as Forchheimer's equation (Ghane et al., 2014; Ghane et

al., 2016; Sobieski and Trykozko, 2014). Forchheimer's equation would account for inertial forces in the porous medium, making it appropriate for turbulent flow (Ghane et al., 2014; Ghane et al., 2015; Ghane et al., 2016; Sobieski and Trykozko, 2014).

$$i = \frac{1}{K_f} q + \alpha q^2 \qquad \text{Equation 2-6}$$

Where i stands for hydraulic gradient (m m^{-1}), q stands for specific discharge (m day^{-1}), K_f stands for post-linear hydraulic conductivity (also known as Forchheimer hydraulic conductivity, m day^{-1}), and α is Forchheimer's coefficient. Forchheimer's equation approaches Darcy's equation as α approaches zero and is often employed as a sign of flow divergence from linear behavior.

2.5.3 Conservative Tracer Test

Conservative tracer experiments model the flow processes through porous media (Cameron and Schipper, 2011; Christianson et al., 2013a; Ghane et al., 2015; Lynn et al., 2016). To accurately determine the hydrology of porous media, the substance used in the tracer test should preferably be physiochemically inert. Tracer tests determine the heterogeneity of porous media by monitoring the speed at which the tracer is recovered at the porous medium downstream (Christianson et al., 2013a; Schipper et al., 2005).

The tracer (typically salts like sodium chloride) is supplied upstream of a porous medium (in the case of bioreactors, the inlet structure, or the head of the input water) (Cameron and Schipper, 2011; Christianson et al., 2016). The concentrations of the tracer recovered downstream (bioreactor's outlet structure) are monitored over time to generate the residence time distribution (RTD) plot (Cameron and Schipper, 2011; Christianson et al., 2016).

The RTD obtained from the tracer experiment can then be used to analyze hydraulic performance in bioreactors, such as flow regime (i.e., plug flow, mixed flow, or a combination of the two), and several hydraulic indicators, such as actual hydraulic residence/retention time (AHRT).

2.5.3.1 Actual hydraulic residence time

AHRT is the mean retention time of the conservative tracer in the bioreactor and is calculated as follows (Christianson et al., 2016):

$$AHRT = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i} \qquad \text{Equation 2-7}$$

t_i and C_i are the time and tracer concentration of the i^{th} measurement, respectively, while t_i is the time interval between measurements.

2.5.3.2 Effective volume ratio

The effective volume of the bioreactor determines the effective utilization of the saturated woodchips volume (Christianson et al., 2013a; Guo et al., 2017; Persson and Wittgren, 2003). When there is a short circuit, the inflow takes the quickest way out of the bioreactor rather than flowing uniformly through the medium (Christianson et al., 2013a; Guo et al., 2017). A short circuit reduces the effective bioreactor volume and thus nitrate removal efficiency (Cameron and Schipper, 2011; Christianson et al., 2013a; Christianson et al., 2016).

The effective volume ratio can be calculated using the AHRT calculated from Equation 2-7 with the nominal hydraulic retention time (derived from porosity, flowrates, and saturation volumes) given by:

$$e = \frac{AHRT}{THRT} \qquad \text{Equation 2-8}$$

Where e = 1 demonstrates plug flow, e < 1 suggests short-circuiting in the bioreactors.

2.5.3.3 Tanks-in-series model

The number of tanks in the tanks-in-series (TIS) model can be used to estimate the extent of ideal (plug) flow regimes (Holland et al., 2004; Persson, 2000; Persson et al., 1999; Persson and Wittgren, 2003):

$$N = \frac{(AHRT)^2}{\sigma^2} \qquad \text{Equation 2-9}$$

Where N is the number of tanks in the TIS model, and σ^2 denotes the variance of the RTD curve (h^2). The variable σ^2 represents how far the RTD curve deviates from the AHRT (centroid of RTD curve) and is calculated as follows (Guo et al., 2017; Persson, 2000; Persson and Wittgren, 2003):

$$\sigma^2 = \sum_{i=1}^{n-1} (t_i - AHRT)^2 \times \frac{A_i}{A} \qquad \text{Equation 2-10}$$

2.5.3.4 Hydraulic efficiency

The hydraulic efficiency of bioreactors may be calculated using the effective volume ratio e and the peak time in the RTD curve t_p. The hydraulic efficiency based on effective volume (λ_e) might be computed as follows (Guo et al., 2017; Holland et al., 2004; Persson et al., 1999; Persson and Wittgren, 2003):

$$\lambda_e = e(1 - \frac{1}{N}) \qquad \text{Equation 2-11}$$

The hydraulic efficiency based on peak time (λ_P) may be calculated as follows (Guo et al., 2017):

$$\lambda_p = \frac{t_p}{t_n} \qquad \text{Equation 2-12}$$

2.5.4 Summary and conclusion

The hydraulic efficiency of bioreactors gradually degrades because of a combination of physical and biological causes. Due to increased microbial activity, the external carbon dosing might cause the hydraulic performance of bioreactors to degrade more quickly. Previous research on carbon dosing has not measured whether or how much the hydrology of bioreactors would change over time because of carbon dosing. The lifespan of a bioreactor may be decreased even more by an accelerated loss of hydraulic function, necessitating more frequent solid medium replacement. As a result, research is required to precisely measure changes in the bioreactor hydrology across different scales of operation to enhance the design of new bioreactors with external carbon sources.

2.6 Why methanol?

Various carbon substrates, such as methanol and acetate, have been investigated in several denitrification nitrate removal processes, including bioreactors (Hartz et al., 2017; Horova et al., 2020; Jansen et al., 2019; Jiang et al., 2022; Ortmeyer et al., 2021; Roser et al., 2018; Timmermans and Vanhaute, 1983; Wunderlich et al., 2012). The cost of procurement and operation, health and safety, and the higher nitrate removal rates of additional carbon would influence the choice of an appropriate carbon compound. This thesis chose methanol as the carbon compound to be evaluated.

Methanol has been widely employed as an external carbon source for denitrification in industrial wastewater treatment plants (Badia et al., 2021; Hartz et al., 2017; Mahmoud et al., 2022; Timmermans and Vanhaute, 1983). Methanol's application in both activated sludge reactors and denitrification filter processes is widely established (Badia et al., 2021; Hartz et al., 2017; Mahmoud et al., 2022; Timmermans and

Vanhaute, 1983; Torresi et al., 2017). When compared to alternative carbon sources, methanol has distinct advantages, such as a reasonably low C:N ratio (the quantity of methanol-carbon required to remove a particular amount of nitrate-nitrogen) compared to other carbon compounds such as acetate (Badia et al., 2021; Fu et al., 2022). This relatively low C:N ratio would result in a lower dosing ratio, lower biomass production, and reduced operational and purchasing expenses (Badia et al., 2021; Fu et al., 2022).

The environmental life cycle analysis (LCA) of using methanol as a carbon source in denitrification has also shown that methanol is a more environmentally friendly choice than other available carbon source options such as ethanol and acetic acid (De Faria et al., 2015; Fernández-Nava et al., 2014; Houillon and Jolliet, 2005). The environmental life cycle analysis comprehensively determines the consequences of chemical usage on ecological concerns such as global warming and eutrophication (Badia et al., 2021; De Faria et al., 2015; Fernández-Nava et al., 2014; Houillon and Jolliet, 2005). As an illustration, methanol has been shown to generate less CO_2 per amount of nitrate removed than ethanol and acetate in denitrification. Compared to 2.7 and 2.1 of acetate and ethanol, respectively, methanol produces 1.4 kg CO2 per kilogram of nitrate removed (Badia et al., 2021).

2.6.1 Biogeochemical pathways of methanol removal

In addition to heterotrophic denitrification, added carbon might be removed via a variety of aerobic, anoxic, and anaerobic processes, including sulfate reduction (Table 2-2). Given that added carbon can be metabolized by these various biogeochemical pathways, the likelihood of excess carbon removal increases, particularly when nitrate is limiting denitrification (Badia et al., 2021; Fu et al., 2022). However, some by-

products of these biogeochemical pathways can be harmful. For example, sulfate reduction may result in poisonous hydrogen sulfide (H_2S) emitting a stench into the surrounding environment (Aalto et al., 2022; Easton et al., 2015; Mohanakrishnan et al., 2011; Wiener et al., 2013). Methanogenesis, which might be activated in reducing conditions caused by carbon dosing, produces methane, a potent greenhouse gas (Weijma and Stams, 2001; Yanagawa et al., 2016).

The most energetic anoxic microbial mechanism for removing methanol is denitrification (Table 2-2) (Fischer et al., 2021; Yanagawa et al., 2016). Following denitrification, the most to least energy-efficient microbial pathways that can metabolize methanol are manganese reduction, iron reduction, sulfate reduction, acetogenesis, and methanogenesis (Table 2-2).

Table 2-2 Pathways of methanol breakdown in anoxic environments

Reaction	pathway	Gibbs free energy changes	Reference
$NO_3^- + \frac{5}{6}CH_3OH + \frac{1}{6}H_2CO_3 \rightarrow \frac{1}{2}N_2 + HCO_3^- + \frac{4}{3}H_2O$	Denitrification	-1135 kJ/reaction	Calculated Based on (Thauer et al., 1977)
$12Fe^{3+} + 2CH_3OH + 5HCO_3^- \rightarrow 12Fe^{2+} + 7CO_2 + 7H^+ + 3H_2O$	Iron reduction	-1037 kJ/reaction	Calculated Based on (Thauer et al., 1977)
$3MnO_2 + 1CH_3OH + 2HCO_3^- + 1H^+ \rightarrow 3MnCO_3 + 5H_2O$	Manganese reduction	-957 kJ/reaction	Calculated Based on (Thauer et al., 1977)
$3SO_4^{2-} + CH_3OH \rightarrow 3HS^- + 4HCO_3^- + 4H_2O + H^+$	Sulfate reduction	-364 kJ/reaction	Fischer et al. (2021)
$CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	Methanogenesis	-331 kJ/reaction	Fischer et al. (2021)
$4CH_3OH + 2HCO_3^- \rightarrow +3C_2H_3O_2^- + 4H_2O + H^+$	Acetogenesis	-221 kJ/reaction	Fischer et al. (2021)

2.6.2 Synopsis: denitrification with methanol

Continual carbon dosing of bioreactors could contribute to carbon losses and/or the formation of undesired by-products, depending on environmental variables and bioreactor design. Estimating the bioreactor input each year is challenging, especially in bioreactors with fluctuating inputs, such as those in agricultural catchments. The variability in bioreactor input makes determining proper dosing load difficult. A long-term study on the export dynamics of nitrogen and other electron acceptors from artificially drained farms is necessary to properly calibrate the supplied carbon rate to target a specific amount of nitrate removed and also minimize possible carbon losses and pollution swapping. Another difficulty in estimating the dosing load is local regulatory restrictions that may limit carbon losses from bioreactors. It is necessary to

investigate carbon removal and losses in bioreactors under a gradient of nitrate prevalent and limiting conditions to more confidently determine the proper dosing rate.

2.7 Summary and conclusion

Extensive research has found that nitrate removal declines with time in bioreactors, with a long-term nitrate removal efficiency of 20% (Christianson et al., 2021). Despite clear advantages such as compact footprints and modest maintenance requirements, compared to alternative mitigation options, bioreactors' relatively poor nitrate removal effectiveness may limit bioreactor adoption. There is solid evidence that adding a soluble carbon source increases nitrate removal rates in bioreactors. However, nitrate removal rates differ between investigations, and no research has been conducted to assess carbon dosage in full-scale bioreactors operating in agriculture catchments. Furthermore, no study has been conducted on the potential adverse effects of carbon dosing on bioreactors or downstream aquatic environments.

External carbon should ideally be adjusted to the nitrate input and inflow rates to minimize the adverse effects of carbon dosing, such as sulfate reduction and accelerated hydraulic change. However, this approach may be costly and limit widespread adoption among stakeholders, including farmers. Regular carbon dosage is an alternative strategy that would cost less upfront and in the long run than the matched technique. This strategy, however, may impose some potential adverse effects on downstream water bodies or bioreactors due to extreme redox fluctuations caused by changes in the input to the bioreactors.

The main goals of this thesis were to: (1) determine whether and to what extent external carbon dosage, in particular methanol, can increase nitrogen removal rates;

and (2) quantify some of the potential adverse effects of external carbon dosage, i.e., added carbon losses, sulfate reduction, and accelerated change in bioreactors hydrology. A better understanding of enhanced nitrate removal and the potential adverse effects of external carbon dosage may result in the development of more efficient and compact bioreactors, resulting in the faster adoption of these novel approaches to addressing the environmental nitrogen problem.

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Chapter 3

A Simple and Effective Carbon Dosing Method for Improving Nitrate Removal in a Full-Scale Denitrifying Bioreactor

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Published in Ecological Engineering:

Moghaddam, R., Barkle, G., Rivas, A., Torres-Rojas, D., & Schipper, L. (2023). Constant carbon dosing of a pilot-scale denitrifying bioreactor to improve nitrate removal from agricultural tile drainage. Ecological Engineering, 187, 106851.

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Highlights

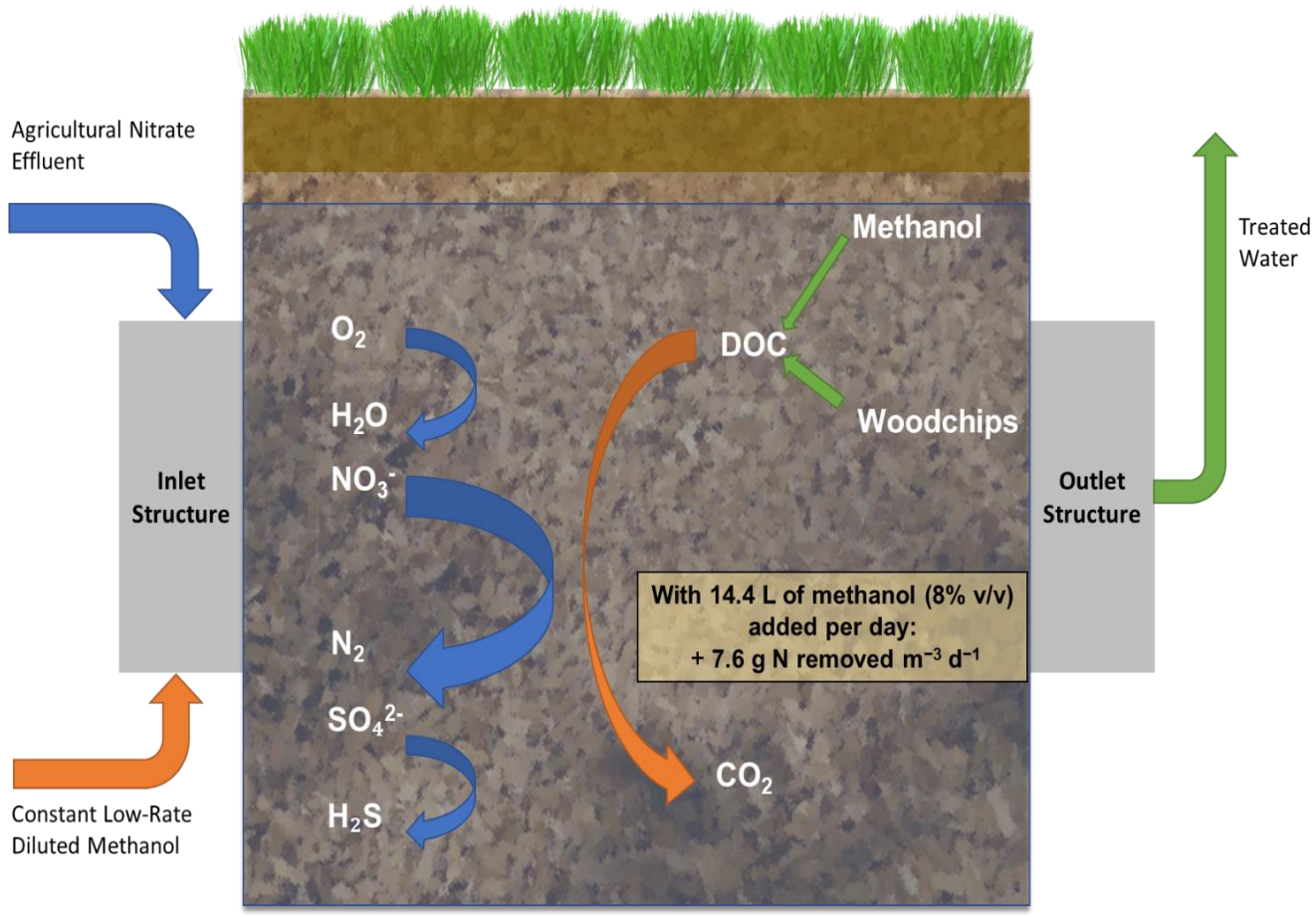
- Nitrogen removal becomes carbon-limited in denitrifying bioreactors with time.
- Methanol dosing substantially enhanced nitrogen removal in the bioreactor.
- Constant methanol dosage did not result in considerable methanol losses.
- Methanol dosing markedly increased sulfate reduction in the bioreactor.

Abstract

Denitrifying bioreactors are effective tools for removing nitrate from agricultural drainage water. However, as woodchips age with time, a shortage of labile carbon

supply limits nitrate removal by microbial denitrification when high nitrate pulses occur in drainage waters. In this study, we investigated the potential of methanol dosing at a constant rate to increase nitrate removal rates in a 58 m^3 pilot-scale bioreactor (25 m^3 saturated volume) installed on a dairy farm for two consecutive drainage seasons. The drainage water in the bioreactor had a mean hydraulic retention time (HRT) of 12.7 days and 13.5 days in the 2020 and 2021 drainage seasons. With 14.4 L of methanol solution (8% v/v) added per day, the seasonal nitrate removal rates were 8.6 g N m^{-3} d^{-1} in 2020 and 5.1 g N m^{-3} d^{-1} in 2021 when the methanol dosing rate was halved. Both rates (2020 and 2021) were enhanced as compared to seasonal rates of 0.67–1.60 g N m^{-3} d^{-1} in previous years (2017 and 2018) when the bioreactor was not dosed. When there were very large pulses of nitrate into the bioreactor, which exhausted added methanol, nitrate was observed at concentrations above limiting ranges (> 3 mg N L^{-1}) at the outlet on several occasions in 2021. Cumulative nitrate load reductions of 1859 g N and 1620 g N occurred in 2020 and 2021, respectively, resulting in overall nitrate removal efficiencies of 85 and 73%, respectively. Methanol concentrations decreased by order of magnitude along the bioreactor length, from mean inlet concentrations of 327 mg CH_3OH-C L^{-1} in 2020 (higher dosing rate) to concentrations of less than 50 mg CH_3OH-C L^{-1} at the outlet. The mean methanol removal rates of 106 g CH_3OH-C m^{-3} d^{-1} in 2020 and 109 g CH_3OH-C m^{-3} d^{-1} in 2021. A 90 % (2020) and 100 % (2021) overall methanol removal efficiency was calculated. With methanol dosing, sulfate removal occurred in the bioreactor, with an average sulfate removal rate of 8.5 g SO_4^{2-}-S m^{-3} d^{-1} in 2020 and 0.5 g SO_4^{2-}-S m^{-3} d^{-1} in 2021. This work demonstrated that methanol additions to bioreactors could enhance denitrification rates even when nitrate was limiting without considerable losses of methanol being released to the receiving waters.

Graphical abstract



3.1 Introduction

Farmers worldwide have been using nitrogen (N) fertilizers and N-fixing crops to boost food output to feed a growing global population (Fowler et al., 2013). The dramatic rise of N inputs to agriculture over recent decades has resulted in water contamination from drainage and the leaching of nitrate from agricultural soils into the receiving water bodies. By effectively short-circuiting the soil profile, tile drainage

systems can drastically contribute to elevated nitrate levels, resulting in various negative environmental consequences, including harmful algal growth (Stevens, 2019).

Several mitigation strategies, such as controlled drainage, denitrifying bioreactors, and constructed wetlands, have been proposed for reducing nitrate losses from drained lands (Christianson et al., 2013a; Christianson et al., 2013b; Stark and Richards, 2008). Denitrifying bioreactors have received particular attention as they can be simple and cost-effective passive systems implemented on the edge of farms to treat high N concentration drainage waters (Addy et al., 2016; Christianson et al., 2021; Christianson and Schipper, 2016; Rambags et al., 2019). Bioreactors are generally lined pits filled with a solid carbon source (commonly woodchips) that provide an anaerobic environment suitable for the growth of heterotrophic denitrifying microorganisms that remove nitrate (Schipper et al., 2010).

A range of different carbon sources, such as corn cobs, wheat straw, and woodchips, can support nitrate removal under a range of environmental conditions (Addy et al., 2016; Cameron and Schipper, 2010). Solid carbon sources are generally recalcitrant organic molecules (such as cellulose), which require microbial processing (i.e., hydrolysis and fermentation) before they can be utilized to support denitrification (Nordström and Herbert, 2019). Extracellular enzymes produced by a diverse spectrum of bacteria and fungi break down the solid sources of carbon into more labile carbon sources, which denitrifiers can subsequently utilize to remove nitrate (Malherbe and Cloete, 2002; Nordström and Herbert, 2019). Carbon availability from the woodchips to the denitrifier population decreases with time, potentially limiting denitrification (Lopez-Ponnada et al., 2017; Nordström and Herbert, 2019). Some carbon sources, such as maize cobs, have more available carbon, allowing faster nitrate removal in the short term. However, because of the higher C:N ratio in the bioreactor,

the more available carbon provided by solid substrate increases the likelihood of a few adverse effects such as DNRA and subsequent ammonium production. Other solid substrates, such as woodchips, have lower short-term nitrate removal rates but are more persistent over time, with a lower likelihood of adverse effects due to excess carbon availability in bioreactors (Addy et al., 2016; Cameron and Schipper, 2010). Woodchips are the most commonly used carbon source in bioreactors due to their ease of access and low cost in many countries (Christianson et al., 2021; Schipper et al., 2010). The main disadvantage of using woodchips is that labile carbon production declines with time. Several studies (Abusallout and Hua, 2017; Robertson, 2010) have reported that dissolved organic carbon (DOC) supply can decrease by half during the first year of operation. Woodchip's temporal decline of DOC production has often accompanied a large reduction in nitrate removal rates in bioreactors. Addy et al. (2016) reported nitrate removal rates between 4.7-16 g N m^{-3} (of bioreactor volume) d^{-1} during the first thirteen months of operation, which decreased to 1.4 - 5.4 g N m^{-3} d^{-1} during the second year. The substantial reduction in nitrate removal rates under similar nitrate inputs and environmental conditions was attributed to available carbon constraints in the bioreactors. Christianson et al. (2021) reported nitrogen removal rates of 7.2 ± 9.6 g N m^{-3} d^{-1} (mean ±SD) in 27 field woodchip bioreactors. These removal rates include diverse effluent inputs including partially nitrified wastewater which may have elevated biochemical oxygen demand (BOD) levels that could enhance denitrification rates. Some of the studied bioreactors reported operating with low nitrogen inputs, suggesting nitrate-limiting conditions (Christianson et al., 2021).

In a few studies (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018), the addition of soluble carbon to bioreactors improved nitrate removal rates, particularly in bioreactors with aged woodchips where initial removal rates had decreased.

Denitrification rates were enhanced by liquid carbon sources such as ethanol, glycerin, acetate, and methanol, which provided denitrifiers a more readily available carbon energy source (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018). Hartz et al. (2017) demonstrated that constant methanol dosing increased denitrification rates, resulting in complete nitrate removal in a mesocosm experiment and a field-scale bioreactor with a 2-day hydraulic retention time (HRT) and high nitrate inflow concentrations (150–193 mg N L^{-1}). With methanol dosing, the equivalent nitrate removal rate of ~36 g N m^{-3} d^{-1} was much higher than ~9 g N m^{-3} d^{-1} without methanol dosing in control bioreactors. Roser et al. (2018) reported nitrate removal rates of 10.2, 11.8, and 10.3 g N m^{-3} d^{-1} at 14.6 °C, 5.5 °C, and 14.6 °C in acetate-dosed mesocosms under 24 hours HRT. These removals were higher than control bioreactors that had nitrate removal rates of 7.3, 1.6 and 7.1 g N m^{-3} d^{-1}, respectively under the same hydrological and temperature conditions. Jansen et al. (2019) measured nitrate removal rates in two types of field-scale denitrifying bioreactors to determine the possible benefit of ethanol dosage on nitrate removal rates. The ethanol treatment used a passive dosing mechanism (without energy supply for the operation) in a bioreactor with no solid substrate to support microbial growth compared to a larger control woodchip bioreactor without dosing. They reported nitrate removal rates of 50 g N m^{-3} d^{-1} and 1 g N m^{-3} d^{-1} for the ethanol reactor and the woodchip bioreactor, respectively.

There is reasonable evidence that adding soluble carbon to denitrifying bioreactors could increase nitrate removal rates. However, few reports have quantified nitrate removal rates to support the construction of field-scale bioreactors with an added soluble carbon source. Furthermore, adding soluble carbon could result in excessive DOC (including added carbon) leaching from bioreactors, resulting in adverse

environmental effects in receiving environments (Abusallout and Hua, 2017). Increased DOC outputs from bioreactors may raise the biological oxygen demand in receiving water bodies, affecting local aquatic life (Fukushima et al., 1996). Variable nitrate concentrations and inflow rates may result in low nitrate loads to the bioreactor, causing added carbon to be available in excess of nitrate, rendering the denitrification process nitrate-limited. In this case, the added carbon may not be removed by denitrification, increasing the risk of added carbon losses from the bioreactor. If denitrification is nitrate-limited, less efficient-microbial removal processes (such as sulfate reduction and methanogenesis) may consume the added carbon before it is discharged with outflow. However, undesirable products (e.g., hydrogen sulfide and methane) may be released due to these side microbial pathways, necessitating the establishment of an appropriate dosing rate to minimize these adverse effects while increasing nitrate removal rates.

Additionally, some soluble carbon sources, such as methanol, have potentially toxic effects on the aquatic inhabitants (Kaviraj et al., 2004). Any carbon loss is especially important for bioreactors installed in agricultural catchments, which might experience severe nitrate load changes, causing the system to become nitrate-limited regularly (Rivas et al., 2020b). Another concern is that the added soluble carbon can cause an increase in microbial biomass growth, potentially resulting in hydraulic clogging in the bioreactors. For example, Christianson et al. (2016) reported flow reduction after 105 operating days in bioreactors treating wastewater with a relatively high chemical oxygen demand (COD).

One possible technique for reducing carbon loss from the bioreactors and mitigating potential clogging issues is adjusting the carbon dosing rate based on incoming nitrate inputs. Such a matching technique might be feasible in principle for highly managed

bioreactors (e.g., wastewater treatment plants) but not affordable or pragmatical for farmers who are looking for passive treatment systems with modest maintenance requirements and costs. The alternative and preferable technique is to continuously dose bioreactors at rates targeted to treat the larger nitrate input pulses. When nitrate is limiting, other microbial pathways (such as sulfate reduction) may effectively remove excess added carbon at the expense of undesirable products (e.g., hydrogen sulfide, methane). However, added carbon removal has not been examined in any studies on carbon dosing (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018).

Our primary goals in this work were to (I) examine if and by how much methanol dosing of a field-scale denitrifying bioreactor increased nitrate removal rates and (II) determine if methanol was removed when nitrate was limiting and no longer available as a terminal electron acceptor.

To achieve these goals, an existing woodchip field-scale bioreactor, which had been in operation for over three drainage seasons (2017 to 2019) treating the tile drainage from a dairy farm, was dosed with methanol and monitored over two drainage seasons (2020 and 2021). Without dosing, seasonal average nitrate removal rates ranged between 0.67 to 1.60 g N m^{-3} d^{-1} (Rivas et al., 2020b).

3.2 Methods

3.3 Study site and system design

This study was conducted during two annual drainage seasons that extended over 44 days in 2020 and 65 days in 2021 at a field-scale bioreactor (Figure 3-1) installed on a dairy farm in Tatuanui, Waikato, New Zealand. Subsurface drainage is often used in this area to keep shallow groundwater from rising into the root zone of dairy pastures during the winter. Tile drainage from approximately 0.65 ha of farmland was

intercepted with a trapezoidal denitrifying bioreactor (9 m long x 5 m wide at the bottom x 1.2 m deep) filled with woodchips (54 m3 untreated Monterey pine, Pinus radiata). An impermeable ethylene propylene diene monomer (EPDM, 1.1 mm Firestone GeoGard) liner separated the bioreactor's bottom and side surfaces from the surrounding soil. The top of the woodchips was covered with a geotextile liner (Bidim A14, 155 g/m2) and capped with 0.7 m of excavated soil. The incoming tile drainage water was distributed across the top of the bioreactor using a perforated drainage pipe and exited through a collector installed at the bottom of the bioreactor (Figure 3-1). A V-notch weir installed at the bioreactor outlet was used to measure flow rates. Three fully perforated sampling wells were installed along the bioreactor centreline, creating approximately four-quarter compartments of the bioreactor, for sampling. Rivas et al. (2020b) provide a complete description of the bioreactor being studied.

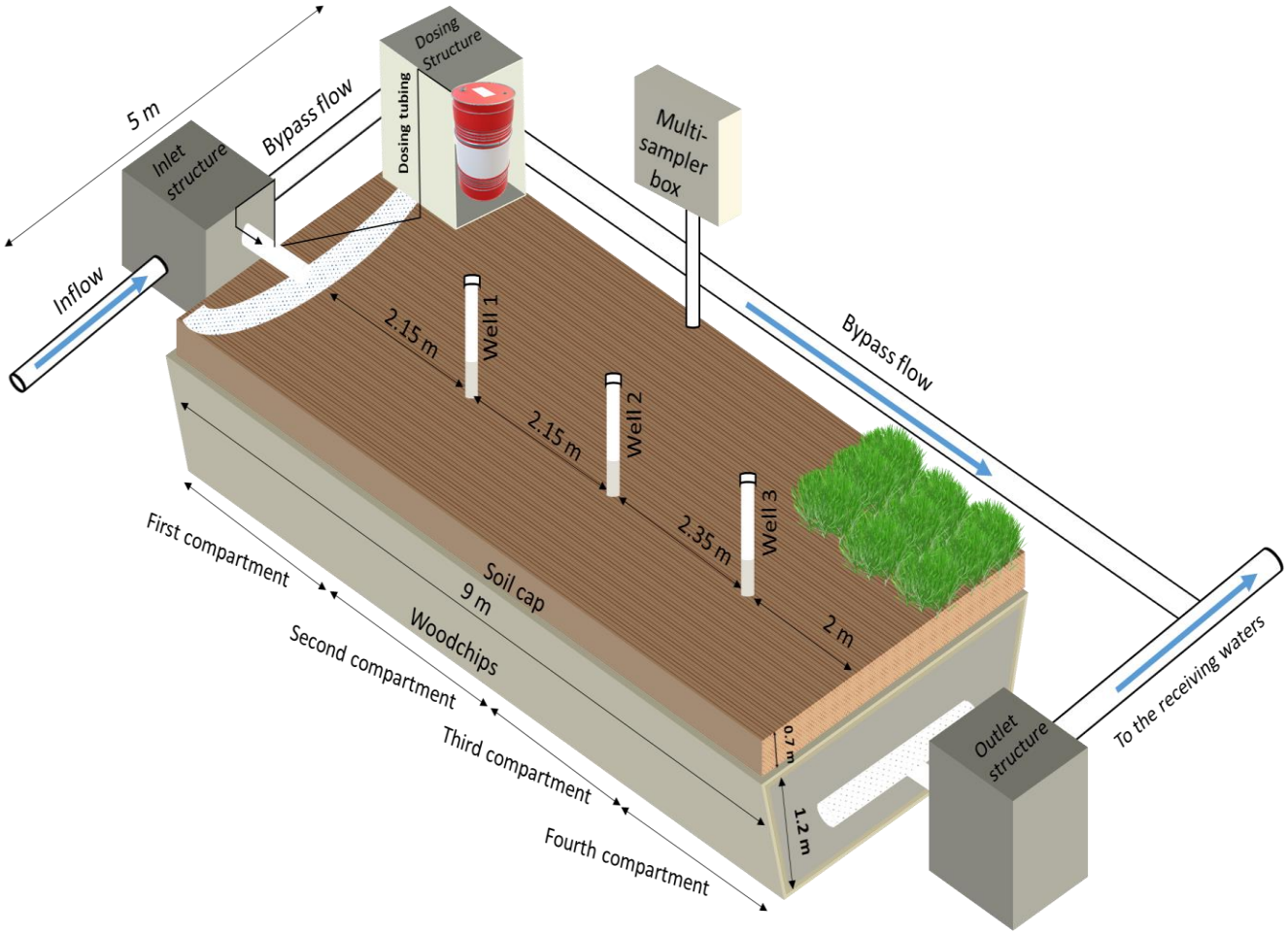


Figure 3-1 Tatuanui bioreactor schematic. Inlet and outlet structures, longitudinal middle wells (for sampling), multi-sampler system (taking samples from the inlet, outlet, and sampling wells every two hours), and dosing structure (methanol-filled drum delivering 8% (v/v) methanol at the inlet at a constant rate of 10 mL min^{-1} for 2020 and 5 mL min^{-1} for 2021). The sampling wells separated the bioreactor into four compartments.

3.3.1 Methanol dosing strategy

During the drainage season of 2020, the methanol dosing rate was based on the stoichiometric ratio determined by Hartz et al. (2017) of 1.48 MeOH-C: NO3--N to increase the nitrate removal rates to at least 5.7 g N m-3 d-1 from the previously reported seasonal removal rates of 0.67-1.6 g N m-3 d-1 (Rivas et al., 2020b). The C:N ratio of 1.48 used in this study diverged with the theoretical value of 0.6 CH3OH-C:NO3−-N for denitrification using methanol (Timmermans and Vanhaute, 1983):

$$NO_3^- + 1.1\ CH_3OH + H^+ \rightarrow 0.5\ N_2 + 0.71\ CO_2 + 2.4\ H_2O + 0.078\ C_5H_7O_2N \quad \text{Equation 3-1}$$

The target removal rate was calculated using the previously untreated nitrate load from the bioreactor in the 2019 drainage season (Rivas pers comm, in preparation). The bioreactor inflow rates in the drainage seasons of 2020 and 2021 varied from 0 to 177 m3 d-1 and 0 to 77 m3 d-1, respectively.

Dosing with a constant rate of methanol into a variable drainage flow produced theoretical concentrations of methanol into the bioreactor of between 30 and 565 mg CH3OH-C L–1 in 2020 and 4 and 90 mg CH3OH-C L–1 in 2021, assuming complete mixing at the inlet structure.

After analyzing the first drainage season data (2020), the methanol dosing rate was reduced by half. This was done to determine if a lower dosing rate would still generate sufficient nitrate removal rates while further reducing the risks associated with excess carbon in the bioreactor (e.g., sulfate reduction and carbon loss to the receiving water). It also decreased the amount of methanol used for dosing.

A PVC drum (200 L) enclosed in a wooden casing was installed near the inlet structure (Figure 1) to store (8% v/v) methanol onsite for dosing the bioreactor. A peristaltic pump delivered methanol to the inlet distributor at a constant rate of 10 mL min–1 in 2020 and 5 mL min–1 in 2021; the dosing rates were checked during each field campaign in both seasons. Methanol dosing was triggered when drainage occurred into the bioreactor (details regarding flow rate measurements are included in section 2.4) and halted when outflow stopped. While methanol dosing occurred during the entire 2020 season, due to the COVID-19 pandemic lockdown in New Zealand, the field site could not be accessed from August 17th to September 19th, 2021, to replenish the methanol supply. It was determined from the dosing flow rate and volume of

methanol onsite that methanol dosing stopped on August 17th. It was possible to reaccess the site on September 20th; the methanol reservoir was replenished, and dosing occurred again until October 1st.

3.3.2 Flow rate measurement

The water height at the outlet was measured using a V-notch weir and Solinist level recorder (Model 3001 Levelogger Junior Edge F15/M5) every five minutes. The flow rate was determined based on the following equation (Maxwell et al., 2020):

$$Q = 0.5681 \times (H + 0.001621)^{2.5} \qquad \text{Equation 3-2}$$

Q is the flow rate in $m^3\ s^{-1}$, and H is water height (m) above the 45° V-notch weir. Since the bioreactor was lined with an impermeable membrane, we assumed water mass conservation in the system, and therefore, inflow and outflow rates were considered equal.

3.3.3 Sampling and analytical methods

In situ nitrate concentration measurements were made every two hours continuously from the inlet, outlet, and sampling wells by a TriOS Opus multispectral nitrate sensor (TriOS, Oldenburg, Germany) installed on a high-frequency, multipoint sampling system (Rivas et al., 2020a). The sensor's nitrate measurements were calibrated separately for each drainage season using a linear regression calibration based on nitrate concentrations determined by Ion Chromatography (see next section). After a complete sampling circuit, the probe was automatically cleaned with a deionized water flush. The optical sensor was manually cleaned weekly, using ethanol and acetone solutions to reduce biofouling. During methanol dosing, grab samples were collected at various times from the inlet, outlet, and pore water samples from the centreline wells (roughly every 1-2 weeks) for analysis. Samples were filtered in situ through 0.45

μm filters (Sartorius AG, Germany), stored at 4 °C within two hours of collection, and analyzed for methanol, nitrate, sulfate, and total organic carbon (TOC) concentrations (see next section). Electrical conductivity (EC), pH, dissolved oxygen (DO), temperature, and oxidation-reduction potential (ORP) were measured in situ with a YSI ProQuatro Multiparameter probe (YSI Inc, Ohio, United States) lowered into the inlet, outlet, and centreline wells.

3.3.4 Sample analysis

TOC concentrations in collected water samples were determined using an OI Analytical Aurora 1030 TOC analyzer (Xylem, New York, USA) and potassium hydrogen phthalate as carbon standards. To separate dissolved inorganic carbon (DIC) from DOC, phosphoric acid was first added to the samples, and then the samples were purged with dinitrogen (N_2) as an inert gas. The generated carbon dioxide (CO_2) was determined using non-dispersive infrared (NDIR) spectroscopy after high-temperature TOC oxidation with sodium persulphate as the oxidizing agent (Hartland et al., 2012).

Methanol concentrations were determined by a gas chromatograph equipped with a DB-WAX (30 m × 0.25 mm × 0.5 μm, Agilent) column and a flame ionization detector (FID). Liquid samples (1 μL) were directly injected into the gas chromatograph by autosampler with a 20:1 split ratio. The initial oven temperature for each injection was 60°C, which was held for 5 minutes, and then elevated to 240 °C at a rate of 10°C min^{-1}.

Nitrate, nitrite, and sulfate analyses of pore water samples along with anion standards (Dionex five anion standard (Thermo Fisher Scientific, Massachusetts, United States)) were conducted using an IonPac AS11-HC (4 × 250 mm) column equipped on a Dionex ICS-200 Ion Chromatograph (Dionex, California, United States).

3.3.5 Bioreactor Performance

3.3.5.1 Nitrate removal

Many bioreactor studies calculate nitrate removal rates based on the assumption that samples of nitrate concentrations at the inlet and outlet and flow rate at the time of sampling are representative of the same water at the inlet and outlet. This assumption is perhaps reasonable for bioreactors with relatively constant flow rates and when a large number of grab samples are taken, allowing for a representative removal rate to be calculated by average or cumulative nitrate mass of inflow and outflow. However, due to the highly variable characteristics of the drainage flows through the studied bioreactor, accurate nitrate removal rate estimations could not be determined using this analysis method.

In our study, nitrate concentrations were continuously measured (every 2 hours at the inlet, outflow, and sampling wells), allowing us to track individual water parcels through the bioreactor with known nitrate concentrations and discharge volume. The method calculated the two-hour average of the outflow rate to determine the discharge volume referred to as a "parcel of water" (Rivas et al., 2020a). These outlet parcels were matched to the relevant parcels and nitrate concentrations at the inlet and sampling wells, assuming piston flow and the estimated drainable pore volume between sampling points. Once parcels of water and nitrate concentrations were matched, instantaneous (parcel-based) and cumulative nitrate loads for each section of the bioreactor, along with volumetric nitrate removal rates, were calculated. The hydraulic retention time (HRT) of each parcel passing through each compartment (each ~25% of the bioreactor) was estimated based on the backward calculation of cumulative flow at the outlet and the bioreactor's saturated volume in each compartment and measured drainable pore volume. Nitrate concentrations and HRT

of each parcel, passing through different wells and compartments, were estimated once enough new drainage water was fed to the bioreactor indicated by cumulative volumes exceeding drainable pore volumes between the wells, namely, 6.25 m3 for well 3; 12.5 m3 for well 2; 18.75 m3 for well 1; and 25 m3 for the inlet. This provided the nitrate concentrations at the inlet, three center wells, and outflow for each parcel of water, as well as HRT for each compartment. This data was utilized to determine volumetric nitrogen removal rates (g N m−3 d−1) for each given parcel:

$$N_{rr} = \frac{(N_{i+1} - N_i) \times \phi}{HRT} \qquad \text{Equation 3-3}$$

Where N_i ($i \in$ (inlet, well1, well2, well3, outlet)) is the nitrate concentration of each parcel (mg N L^{-1}) passing through the bioreactor, HRT is the corresponding hydraulic retention time (days), and ϕ equals 0.462, which was the average drainable porosity of the bioreactor (Rivas et al. 2020b).

For calculating the seasonal average nitrate removal rates, we filtered out nitrate-limited removal rates for both seasons based on nitrate concentrations that were below the half-saturation concentration, K_m value of 3 mg N L^{-1} (Kouanda and Hua, 2021).

The cumulative nitrate load reduction over the season (R_{cn}%) was calculated as the difference between a load of nitrate (g NO_3^--N) coming in and out of the bioreactor during the entire drainage season when dosing was occurring:

$$R_{cn}\% = \frac{NC_{in} - NC_{out}}{NC_{in}} \times 100 \qquad \text{Equation 3-4}$$

Where NC_{in} (g NO_3^--N) was inlet cumulative load and NC_{out} (g NO_3^--N) was outlet nitrate cumulative load.

3.3.5.2 Methanol removal rates

Methanol volumetric removal rates (M_{rr}) were calculated based on methanol concentrations of water grab samples, the daily average outflow rates at each sampling day, and the bioreactor's drainable volume of the section where the methanol concentrations were consistent (i.e., no notable change observed in the following downstream wells):

$$M_{rr} = \frac{\left(M_{inlet} - M_{limiting}\right) \times Q_{average}}{V_{limited}} \qquad \text{Equation 3-5}$$

Where M_{inlet} and $M_{limiting}$ are the methanol (CH_3OH-C) concentrations at the inlet and the limiting well (the first well where methanol concentrations were consistently unchanged in the rest of the bioreactor) (mg CH_3OH-C L^{-1}), respectively, $Q_{average}$ is the average flow rate (m^3 d^{-1}) and $V_{limited}$ is the saturated volume of the bioreactor's section where methanol removal is calculated (m^3).

The seasonal methanol removal efficiency (R_m %) was calculated as the percentage of methanol removed from the inlet to the outlet of the bioreactor:

$$R_m\% = \frac{M_{inlet} - M_{outlet}}{M_{inlet}} \times 100 \qquad \text{Equation 3-6}$$

Where M_{outlet} (mg CH_3OH-C L^{-1}) is the methanol concentration at the outlet and M_{inlet} (mg CH_3OH-C L^{-1}) is the methanol concentration at the inlet.

3.3.5.3 Sulfate removal

Sulfate removal rates were determined using the same method described for methanol removal rates:

$$S_{rr} = \frac{(S_{inlet} - S_{limiting}) \times Q_{average}}{V_{limited}}$$ Equation 3-7

Sulfate removal efficiency:

$$R_S\% = \frac{S_{inlet} - S_{outlet}}{S_{inlet}} \times 100$$ Equation 3-8

3.3.6 Statistical analysis

The student's t-test was used to compare the mean inflow and outflow methanol concentrations and mean sulfate removal rates in the bioreactor between two seasons using R version 4.0.3 (R Core Team, 2021).

3.4 Results

3.4.1 Nitrate concentrations

The inlet nitrate concentrations ranged between 20-35 mg N L^{-1} and 20-40 mg N L^{-1} during the 2020 and 2021 seasons, respectively (Figure 3-2). During the 2020 season (higher methanol dosing), the largest reduction in nitrate concentrations occurred in the first compartment. In the remaining bioreactor compartments, nitrate declined at a lower rate. Similarly, during the 2021 season (half methanol dosing rate), a significant decline in concentration occurred between the inlet and the first well, while nitrate levels remained relatively similar in the bioreactor's downstream wells (varying between 0 and 25 mg N L^{-1} across the season). At the start of the 2020 season, the outlet nitrate concentrations were roughly one-third of the inlet nitrate concentrations (around 12 mg N L^{-1}). However, as methanol dosing continued, the outlet nitrate

concentrations decreased to < 1 mg N L^{-1} until the end of the drainage season (9/9/2020) (Figure 3-2a). However, when methanol was added to the bioreactor at the start of the 2021 drainage season, there was no delay in enhanced nitrate removal (Figure 3-2b).

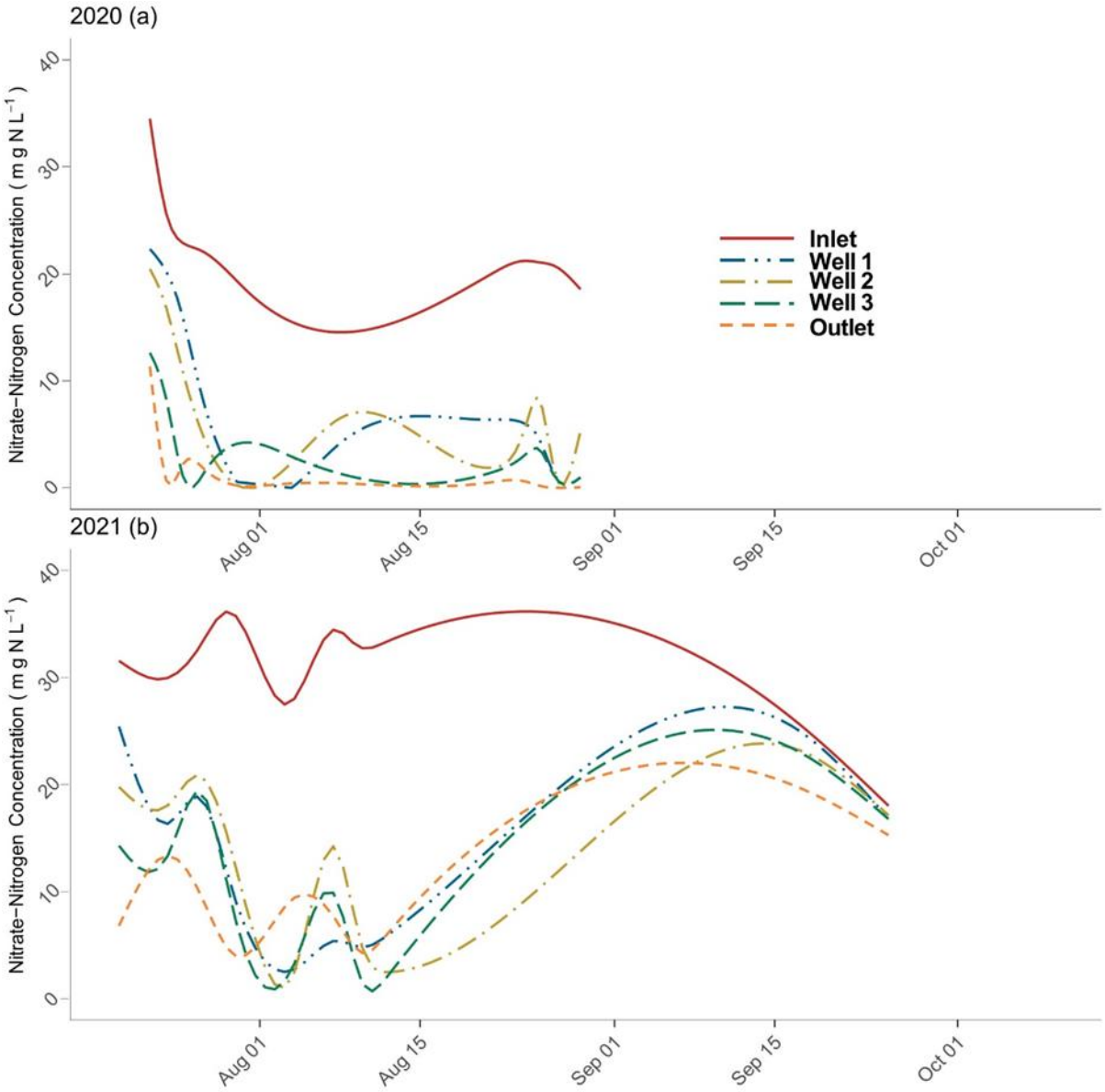


Figure 3-2 Nitrate concentrations at the inlet, outlet, and sampling wells over the drainage seasons of (a) 2020 and (b) 2021

3.4.2 Nitrate removal

During methanol dosing of the bioreactor in 2020 and 2021, nitrate removal rates increased considerably compared to the average seasonal removal rate in 2018 (Figure

3-3) when the bioreactor was not dosed (Rivas et al. 2020b).

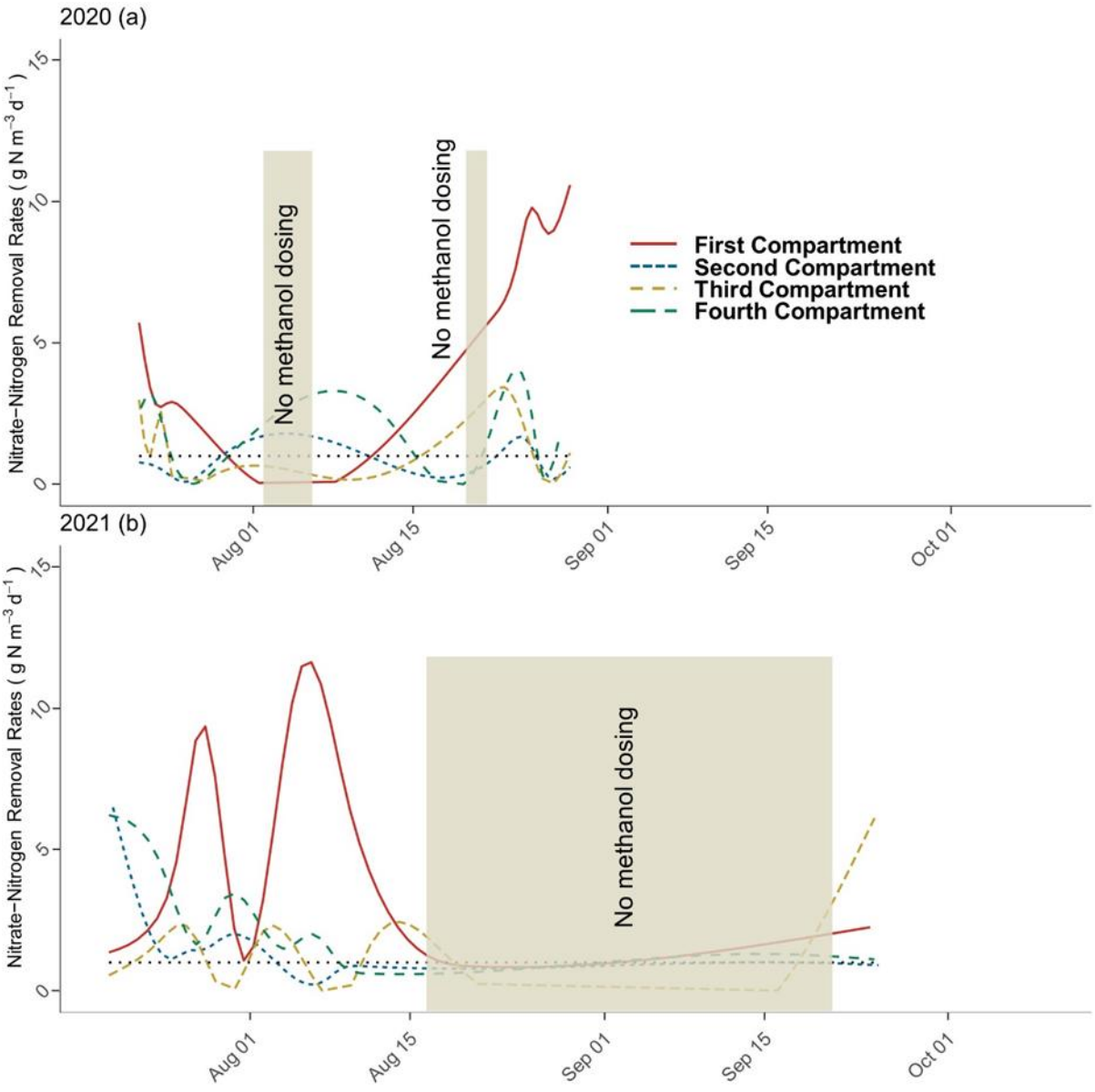


Figure 3-3 Performance of the bioreactor in removing nitrate from different compartments in (a) 2020 and (b) 2021, the two seasons when the bioreactor was dosed with methanol. The black dash-dotted line depicts mean nitrate removal rates without methanol dosing throughout the 2018 drainage seasons (Rivas et al., 2020b).

The first compartment generally had the highest removal rates, while the following three downstream compartments had rates broadly comparable to those reported without dosing in 2018.

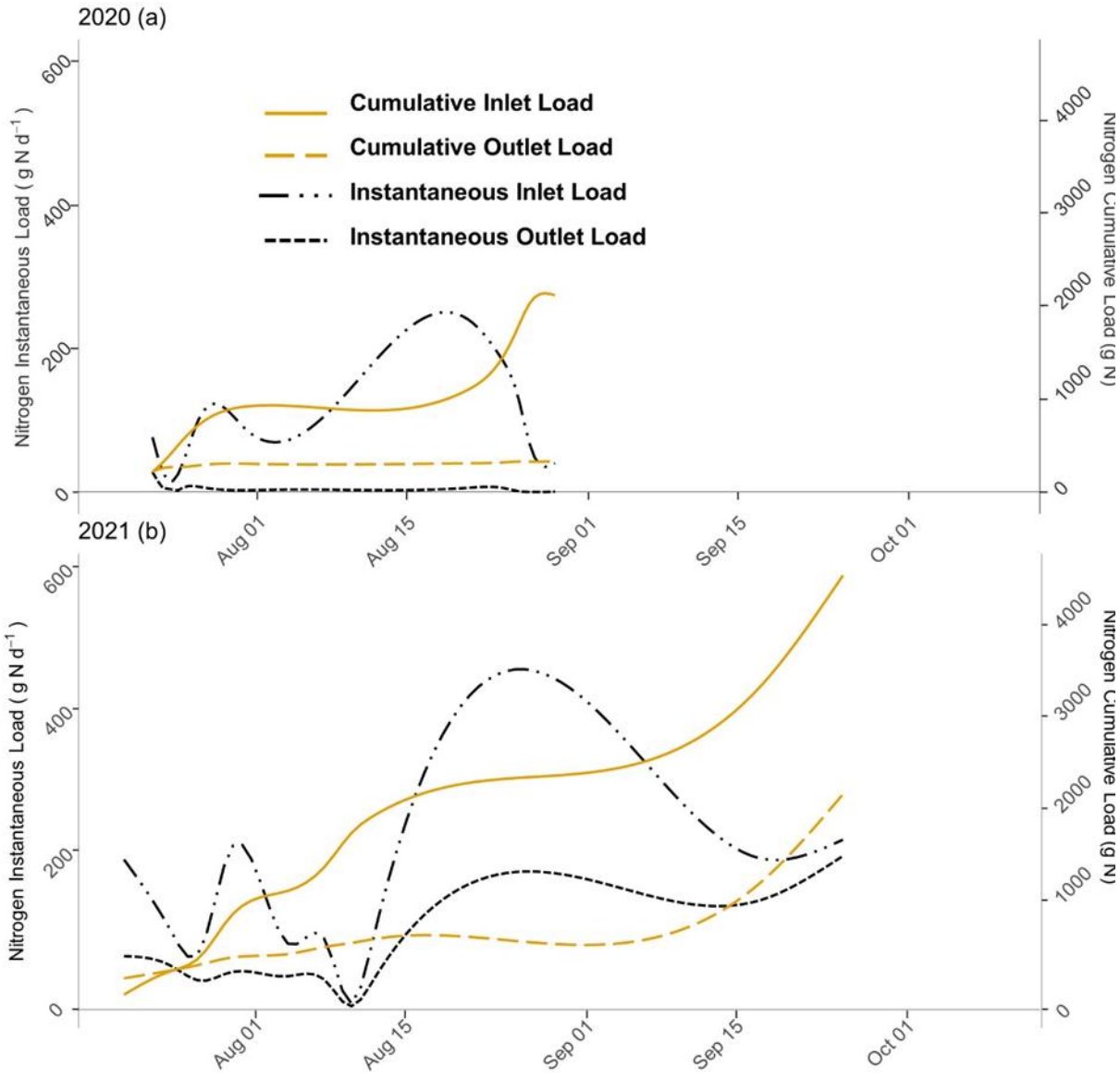


Figure 3-4 Instantaneous and cumulative nitrate loads entering and exiting the bioreactor in the seasons of (a) 2020 and (b) 2021 when the bioreactor was dosed with methanol.

In 2020, the first nitrate pulse (visual peaks in inlet nitrate instantaneous load) (approximately 100 g N d^{-1}) occurred when dosing started on July 22nd (Figure 3-4a). At this time, the first compartment's nitrate removal rate was roughly 6 g N m^{-3} d^{-1} (Figure 3-3a). Once the pulse in the first compartment started to decline, the nitrate removal rates in the second, third, and fourth compartments increased (reaching about 2 to 5 g N m^{-3} d^{-1}). Two additional instantaneous nitrate pulses with different

orders of magnitude were observed at the inlet on August 1st (~80 g N d^{-1}) and August 20th (~230 g N d^{-1}). The outlet loads corresponding to these input loads were near-zero in both cases, demonstrating near-complete nitrate removal (Figure 3-4a). These two pulses corresponded to maximum nitrogen removal rates in the first compartment of 3 and 12 g N m^{-3} d^{-1} (Figure 3-3a).

In the 2021 drainage season, with a half methanol dosing rate, the first three first pulses of nitrate input occurred on July 26th, August 1st, and August 10th with maximum daily nitrogen loads at the inlet of 200, 210, and 70 g N d^{-1}, respectively. The bioreactor entirely removed nitrate from these three pulses (Figure 3-4b), with maximal removal rates in the first compartment of 12 g N m^{-3} d^{-1} (Figure 3-3b). When methanol was not added, from August 15th to September 1st, the daily nitrate load increased at the inlet, reaching a peak value of 420 g N d^{-1} on September 15th. The instantaneous outlet load peaked at 200 g N d^{-1} (Figure 3-4b). During that time, the nitrogen removal rate in all compartments remained at approximately 1 g N m^{-3} d^{-1}. In the absence of methanol dosing, nitrogen removal rates were low, similar to those reported by Rivas et al. (2020b), confirming that methanol dosing had ceased. Nitrate removal rates for this period were not included when calculating the overall removal rates for 2021.

3.4.3 Cumulative nitrate reduction

By the end of the 2020 drainage season, the cumulative inflow nitrogen load was 2188 g N. The cumulative outflow nitrogen load was 400 g N, resulting in an overall removal of 85% of the cumulative nitrogen load (Figure 3-4a). By the end of the 2021 season (excluding the period of no methanol dosing), the cumulative inlet and outflow nitrogen loads were 2200 g N and 580 g N, respectively, resulting in a 73% reduction

in cumulative nitrogen loads (Figure 3-4b). In 2019, the bioreactor reduced cumulative nitrogen loads by 13% without methanol dosing, from 15828 g N of total input loads to 1376 g N of total output loads.

3.4.4 Overall nitrate removal performance

In 2020, the first compartment showed better performance than the remainder of the bioreactor, with a median nitrate removal rate of 4 g N m^{-3} d^{-1} (25^{th} and 75^{th} percentiles of 2.6 and 7.4, respectively; Supplementary figure 3-1) compared to 2021 when the median nitrate removal rate was 2.8 g N m^{-3} d^{-1} (25^{th} and 75^{th} percentiles of 2.1 and 7.3, respectively; Supplementary figure 3-1). The removal rates of the remaining compartment's medians were within the range of removal rates without methanol dosing, ranging from 0.67 to 1.60 g N m^{-3} d^{-1} (Rivas et al. 2020b). The overall seasonal average nitrate removal rates were 8.6 g N m^{-3} d^{-1}, 95% CI [8.55 g N m^{-3} d^{-1}, 8.65 g N m^{-3} d^{-1}] and 5.1 g N m^{-3} d^{-1}, 95% CI [5.07 g N m^{-3} d^{-1}, 5. 13 g N m^{-3} d^{-1}] for 2020 and 2021 drainage seasons when dosing was occurring respectively.

3.4.5 Methanol removal

Methanol concentrations decreased in the first compartment of the bioreactor, decreasing from maximum concentrations of 617 mg CH_3OH-C L^{-1} at the inlet to 30 mg CH_3OH-C L^{-1} at the first well, observed on July 31^{st}, 2020 (Figure 3-5a). The methanol concentrations remained relatively stable between 1-30 mg CH_3OH-C L^{-1} in the rest of the bioreactor (Figure 3-5a). In 2020, the seasonal average methanol removal rate was 109 g CH_3OH-C m^{-3} d^{-1} (95% CI: 216 g CH_3OH-C m^{-3} d^{-1} to 0 g CH_3OH-C m^{-3} d^{-1}) with a removal efficiency of 99%. In 2020, the seasonal methanol removal rate over the seasonal nitrate removal rate was 12.1 g CH_3OH-C g^{-1} N compared to the theoretical C:N ratio of 0.6.

In 2021, the first compartment (Figure 3-6a) had a similarly large reduction in methanol as in 2020, with all outflow methanol concentrations falling below the detection limit (1 mg CH_3OH L^{-1}). In 2021, the average methanol removal rate was 106 g CH_3OH-C m^{-3} d^{-1} (95% CI: 218 g CH_3OH-C m^{-3} d^{-1} to 0 g CH_3OH-C m^{-3} d^{-1}), with a removal efficiency of 100%. In 2021, the seasonal methanol removal rate over the seasonal nitrate removal rate was 15.9 g CH_3OH-C g^{-1} N.

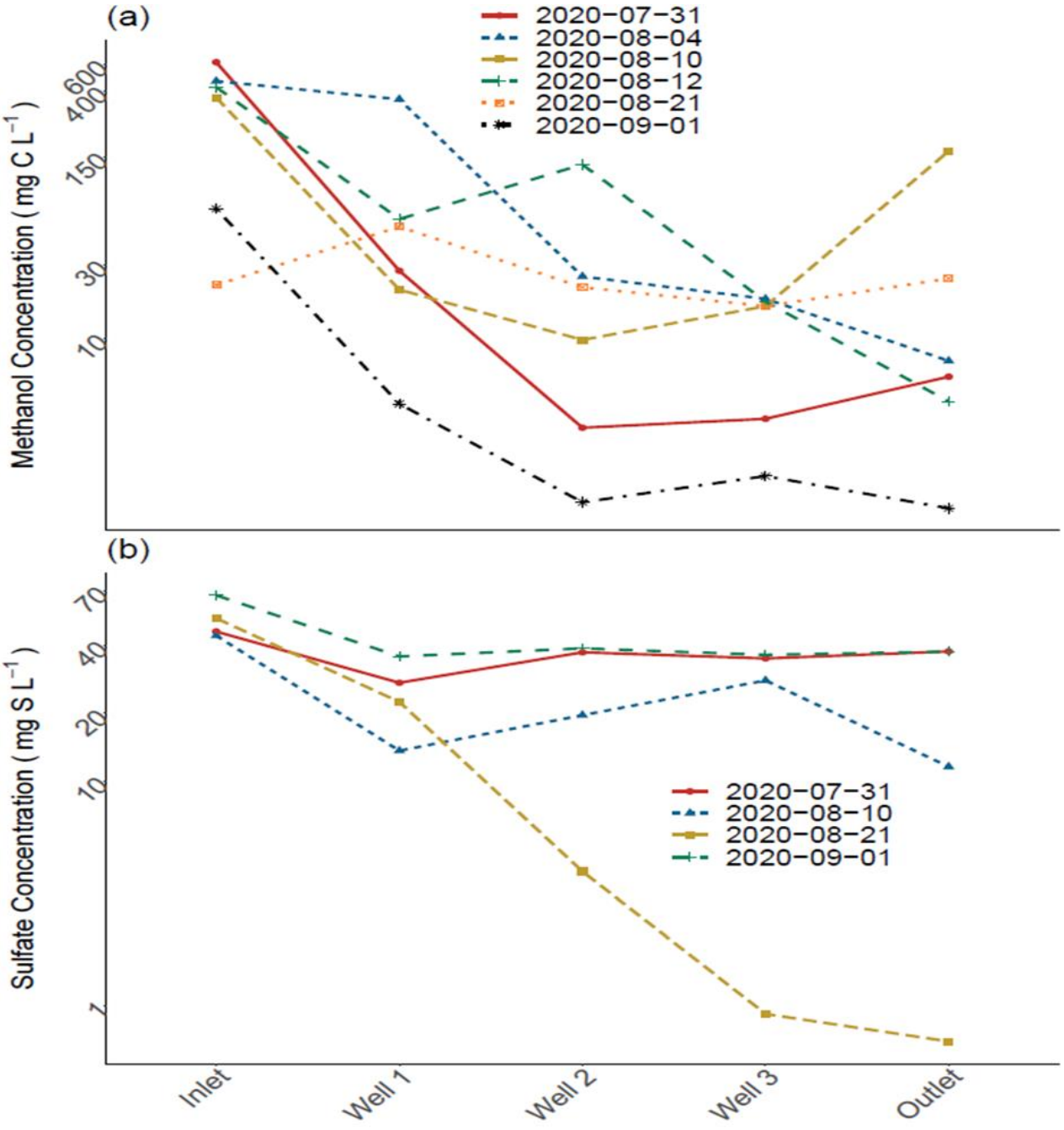


Figure 3-5 Methanol (a) and sulfate (b) concentrations along the bioreactor during the different sampling events in 2020. Variable drainage flows that diluted added methanol caused variable varying methanol concentrations at the inlet. Note that the y-axis for both figures is on a square root scale logarithmic scale.

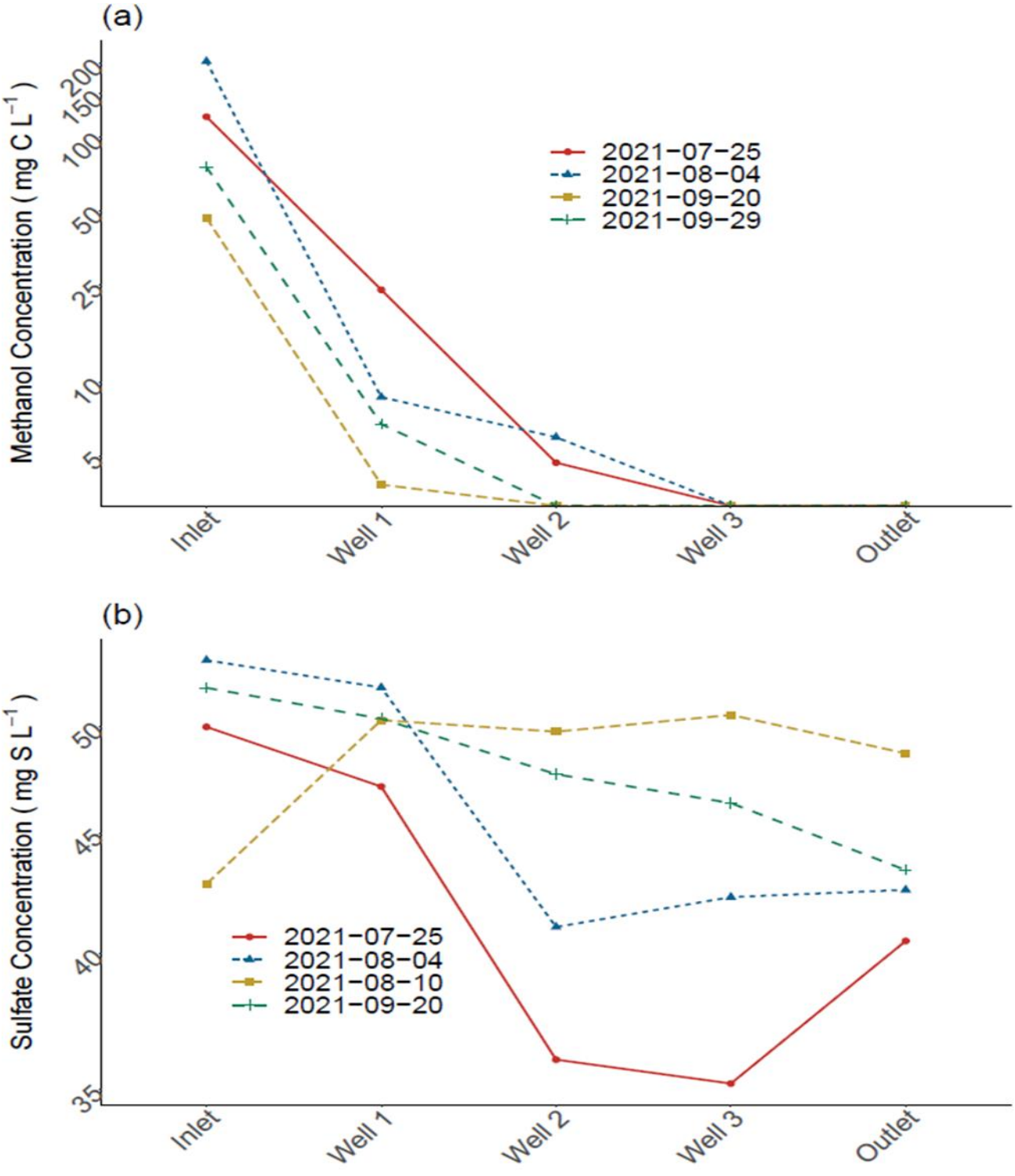


Figure 3-6 Methanol (a) and sulfate (b) concentrations along the length of the bioreactor during the different sampling events in 2021. Transient water inputs caused variable methanol concentrations at the inlet, which diluted added methanol. Note that the y-axis for figure a is on the logarithmic scale.

3.4.6 Sulfate and nitrite

Sulfate removal mainly occurred in the first compartment in both seasons, even with nitrate present (Figure 3-5b and Figure 3-6b). At the inlet, sulfate concentrations in 2020 ranged from 50 to 66 mg SO_4^{2-}-S L^{-1} during the season and decreased to 16.5 to 33 mg SO_4^{2-}-S L^{-1} at the first well. Sulfate concentrations generally remained unchanged beyond the first well on July 31st, August 10th, and September 1st but decreased to nearly zero on August 21st (Figure 3-5b). The average sulfate removal rate in 2020 was 8.5 g SO_4^{2-}-S m^{-3} d^{-1} with a 95% confidence interval [19.3 g SO_4^{2-}-S m^{-3} d^{-1}, 0 g SO_4^{2-}-S m^{-3} d^{-1}]. Sulfate concentrations in the first compartment remained similar in 2021 (with a halved methanol dosage rate) compared to 2020 when a comparatively considerable sulfate reduction occurred in the first compartment (Figure 3-6b). A mean sulfate removal rate of 0.50 g SO_4^{2-}-S m^{-3} d^{-1} was observed with a 95% confidence interval [1.02 g SO_4^{2-}-S m^{-3} d^{-1}, 0 g SO_4^{2-}-S m^{-3} d^{-1}] in the bioreactor's second compartment in 2021. During the drainage season in 2020 (higher dosing rate), nitrite concentrations were below the detection limit (0.2 mg $NO2^{-}$-N L^{-1}) along the length of the bioreactor.

3.4.7 Environmental parameters

Dissolved oxygen concentration decreased sharply in the first compartment of the bioreactor (ranging from 2.5 to 9.5 mg L^{-1} at the inlet to 1 to 2.5 mg L^{-1} in the first well; Supplementary figure 3-3a). During four sampling events, the dissolved oxygen increased slightly (approximately 1 mg L^{-1}) between the first and second well. Dissolved oxygen in the remaining compartments showed no consistent behaviour.

In most cases, the temperature rose from approximately 12 °C at the inlet to 14-18 °C over the length of the bioreactor (Supplementary figure 3-3b). The pH remained

relatively constant and somewhat acidic along the bioreactor, ranging from 5.2 to 6.3 (Supplementary figure 3-3c). The redox potential decreased along the bioreactor, ranging from 0 to 100 mV at the inlet to approximately -100 to -220 mV at the outlet (Supplementary figure 3-3d).

3.5 Discussion

3.5.1 Methanol: a denitrification booster

Overall, we demonstrated that methanol dosing increased seasonal average nitrate removal rates to 8.6 g N m^{-3} d^{-1} in 2020 and 5.1 g N m^{-3} d^{-1} in 2021 compared to the 1 g N m^{-3} d^{-1} in the undosed bioreactor (Rivas et al., 2020b). However, removal rates obtained in our study were generally lower than the nitrate removal rates observed in prior carbon dosing studies (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018). Differences in nitrate removal rates between our study and others could be attributed to differences in inflow nitrate loads, hydraulic regimes, carbon sources, and operating scales.

The nitrate removal rates reported by Roser et al. (2018), who used acetate as an external carbon source, were based on a relatively small column study (15.2 cm ID, 49.5 cm length) conducted under hydrologically controlled conditions with no nitrate spikes occurring in the inflow. This study assessed nitrate removal rates at four distinct HRTs of 1.5 hours, 8 hours, 12 hours, and 24 hours. The shortest HRT (1.5 hours) yielded average removal rates of 64 g N m^{-3} d^{-1} in acetate-dosed bioreactors, which is considerably higher than the removal rates in our study. However, the HRT of 24 hours in Roser et al. (2018) resulted in average removal rates of 10.5 g N m^{-3} d^{-1}, which is 2 g N m^{-3} d^{-1} greater than the removal rates in our trial. The average HRT in our study bioreactor was 12.7 days in 2020, which could probably explain why the nitrate

removal rates in our study were close to Roser et al. (2018) under longer HRTs. Longer HRTs would give the microbial population more time to remove nitrate in the bioreactors, leading to lower outflow nitrate concentrations, mainly when the bioreactor operates in nitrate-limited conditions (Rivas et al., 2020b). However, long HRTs would lead to lower nitrate removal rates in bioreactors as longer HRTs require either larger woodchip volumes or lower inflow rates. Long HRTs in bioreactors, on the other hand, may result in secondary effects such as sulfate reduction and subsequent hydrogen sulfide (H_2S) production, which is toxic and emits an unpleasant odor into the surrounding environment (Easton et al., 2015; Rivas et al., 2020b; Shih et al., 2011).

The inlet and output nitrate concentrations in Jansen et al. (2019) were 5 and 0 mg N L^{-1}, respectively, indicating nitrate-limited conditions, which may suggest that removal rates would have been even greater if higher quantities of nitrate were delivered to the reactor.

Hartz et al. (2017) performed methanol dosing in a field bioreactor under steady-state inflow conditions (2-day HRT), pumping tile drain from collection sumps. The added methanol in this study was matched to the nitrate inputs at the bioreactor's inlet every 15 minutes using a C:N ratio of 1.4:1. Nitrate removal rates were not reported in Hartz et al. (2017), but we estimated the removal rates based on the difference in inlet and outlet nitrate concentrations (155 mg N L^{-1}) and HRT (2 days) in their study with an assumption of drainable porosity of 0.47 (average drainable porosity in Cameron and Schipper (2010)). An equivalent removal rate of 36 g N m^{-3} d^{-1} was achieved, which was 4.5 folds greater than average nitrate removals (2020) in our study. The nitrate inputs in Hartz et al. (2017) ranged from 150 to 193 mg N $L^{-1,}$ which was approximately 8 times higher than the nitrate inputs in our study. These extremely elevated nitrate

inputs in Hartz et al. (2017) may explain the higher removal rates compared to our study.

When both nitrate and carbon are abundant, improved nitrate removal rates may be limited by other environmental factors, such as dissolved oxygen in the pore water (Nordström and Herbert, 2019). Additionally, bioclogging of bioreactors' porous media is likely to arise, which could limit the carbon dosing in field applications with certain thresholds (Christianson et al., 2016; Ma et al., 2021). Furthermore, the Hartz et al. (2017) study was carried out in rather steady hydrological conditions, whereas ours was conducted under extremely flashy drainage waters characteristics. The transient flow conditions may hinder the establishment of a stable microbial population in bioreactors, resulting in a varying microbial flush from hydrologically dynamic bioreactors. This change in the microbial community in the bioreactors may limit nitrate removal rates (Narihiro et al., 2018). Moreover, Hartz et al. (2017) had the advantage of matching the added methanol based on the in situ nitrate inputs. In contrast, our dosing study was based on a constant dosage of methanol, irrespective of nitrate inputs into the bioreactor. However, the additional construction and maintenance costs associated with this carbon dosing matching would restrict its widespread application.

Overall, the differences in nitrate removal rates observed across different carbon dosing studies, each with its site-specific characteristics, suggest that evaluating the effects of carbon dosing on the performance of other bioreactors should be done on a case-by-case basis. Comparing the performance of a specific bioreactor with and without carbon dosing might be a more realistic approach, as our results showed a 5 to 8 times increase in bioreactors nitrate removal performance compared to the same undosed bioreactor (Rivas et al., 2020b).

3.5.2 Bioreactor's response to the new substrate

In our study, the nitrate load at the outlet remained close to zero throughout the 2020 season (Figure 3-4a), with the exception of a brief delay at the start of dosing (7 days), when nitrate exited from the outlet. Subsequently, the slow and progressive decrease of the output nitrate load, which coincided hydraulically with the start of methanol dosing, reflected a nitrate removal delay in the dosed bioreactor. This delay was likely due to an acclimatization period by the microbial population to the presence of methanol as the new substrate (Paradis et al., 2022). We did not observe a similar delay in 2021, suggesting that the denitrifier population capable of using methanol remained present when the second drainage season began. The maintenance of the acclimatized denitrifier population between drainage seasons increases the chance of removing the first nitrate pulse at the start of each season. It also eliminates the need to inoculate bioreactors with a denitrifier population acclimatized to methanol as the dominant substrate before adding methanol as a carbon source.

In 2021, the nitrate removal rates in the bioreactor appeared to be carbon limited when nitrate concentrations at the outlet and most downstream wells were below the K_m value of 3 mg N L^{-1} (Kouanda and Hua, 2021). Carbon limitation, in 2021, was supported by lower methanol concentrations in downstream compartments, as the bioreactor's first half had removed the added methanol (Figure 3-6a). This carbon-limited condition in 2021, imposed by a lower methanol dosing rate, would strongly suggest a methanol dosing rate threshold in the bioreactor, identified based on the average seasonal load of nitrate transported from the field into the bioreactor.

3.5.3 The fate of methanol in the bioreactor

Although recent research (e.g., Hartz et al., 2017; Roser et al., 2018) has shown the benefits of carbon dosing for increasing nitrate removal rates, the fate of additional carbon removal in bioreactors with changing nitrate levels has not been well examined. Excess carbon discharges may have various detrimental environmental consequences in downstream water bodies, including toxicity to aquatic species (Kaviraj et al., 2004). The aerobic and anaerobic degradation of methanol via different biogeochemical routes resulted in a sustained and significant decrease (p-value < 0.01) in methanol concentrations in the bioreactor. Methanol has been found to be degraded through a variety of biogeochemical pathways, i.e. aerobic decomposition, heterotrophic denitrification, iron reduction, sulfate reduction, acetogenesis, methanogenesis, and fermentation (Kolb, 2009; Sousa et al., 2018; Yanagawa et al., 2016). Given the methanol removal at varying nitrate inputs in our study bioreactor, we suggest that, under nitrate-limiting conditions, methanol was degraded by biogeochemical routes other than heterotrophic denitrification, such as dissimilatory sulfate reduction. As a result of these different methanol removal mechanisms, methanol concentrations at the outflow fell below 34 mg CH_3OH-C L^{-1} in 2020 and reached zero throughout the 2021 drainage season. The considerably higher observed C:N ratios of 12.1 g CH_3OH-C g^{-1} N and 15.9 in 2020 and 2021, respectively, compared to the theoretical C:N ratio of 0.6, suggest that other electron acceptors were abundant and may have consumed added methanol in addition to denitrification and nitrate removal.

As considerable removal of methanol was found in the bioreactor under nitrate-limited conditions, the extent to which such pathways would be active in the bioreactor would need to be investigated further.

Furthermore, sulfate removal rates in 2021 were significantly lower (p-value < 0.01) than in 2020, potentially due to the reduction of the methanol dosing rate and increased nitrate inputs to the bioreactor (cumulative nitrogen input of 2188 g N in 2020 and 2200 g N in 2021). Due to the rapid methanol removal in the first compartment, more sampling wells between well 1 and the inlet structure would be required to more precisely determine the contribution of the first compartment's involvement in various methanol removal processes.

Overall we demonstrated that continuous dosing of the bioreactor, regardless of nitrate inputs, did not result in dramatic methanol losses in the outflow, provided the dosing rate is set at the right rate and load. On the other hand, maximum methanol removal limits in the bioreactors are dependent on environmental inputs such as flow, nitrate, and other electron acceptors (including sulfate), necessitating the establishment of a proper dosing rate specific to the target bioreactor and its operating conditions.

3.5.4 Dosing rate and setup implementation

Our results suggest that carbon dosing optimization is necessary to ensure maximum nitrate and methanol removal efficiency. Our findings showed that increasing the methanol dosage rate resulted in greater nitrate removal effectiveness but increased the chance of methanol loss from the system. The seasonal and variable composition of electron acceptors in agricultural drainage waters (Kroger et al., 2007) makes determining optimal methanol dosing rates somewhat difficult in bioreactors deployed in agricultural catchments. There is a need for long-term studies on the export dynamics of nitrogen and other electron acceptors from artificially drained farms to establish methanol dosing rates with greater confidence.

In our study, a peristaltic pump (along with electrical equipment such as solar panels and batteries) continuously delivered methanol into the bioreactor. A better option would be designing a simple dosing system that requires less capital investment for construction and maintenance and can deliver methanol safely without a pump, ideally with minimal efforts by the landowner.

3.6 Conclusion

We showed that continual methanol dosing at a constant rate greatly improved nitrate removal in a field-scale bioreactor treating tile drainage water. The addition of methanol at two different regular rates for two consecutive drainage seasons resulted in substantially higher nitrate removal rates (+ 7.6 g N m^{-3} d^{-1}) when compared to seasons without dosing. Halving the dosing rate from (10 to 5 mL min^{-1}) resulted in a lower seasonal nitrate removal rate, but these were still enhanced as compared to the removal rates without dosing. Increased nitrate inputs to the bioreactor resulted in some nitrate exiting from the bioreactor due to carbon-limiting conditions. Methanol losses to the receiving surface waters were minimized as it was removed even with variable nitrate inputs. Overall, in old bioreactors with aged woodchips, methanol dosing can be an alternative carbon source to enhance nitrate removal rates rather than the need to change the bioreactor's substrate, presuming the hydraulic conductivity had not been reduced. Our findings could also be utilized to build new bioreactors that use methanol as an additional carbon source, reducing the bioreactor's footprint (i.e., the amount of land required for bioreactor construction) and lowering the capital cost of construction. Carbon dosing, however, necessitates landowner upkeep. The ideal methanol dosing rate could be determined by the amount of nitrate and other electron acceptors in the influent and could be investigated in a more controlled and matched dosing system. Additionally, supplying a portion of the

added methanol (e.g., half) to the bioreactor's downstream compartments (e.g., between first and second wells) to examine the bioreactor's response to spatially varied dosing would be recommended.

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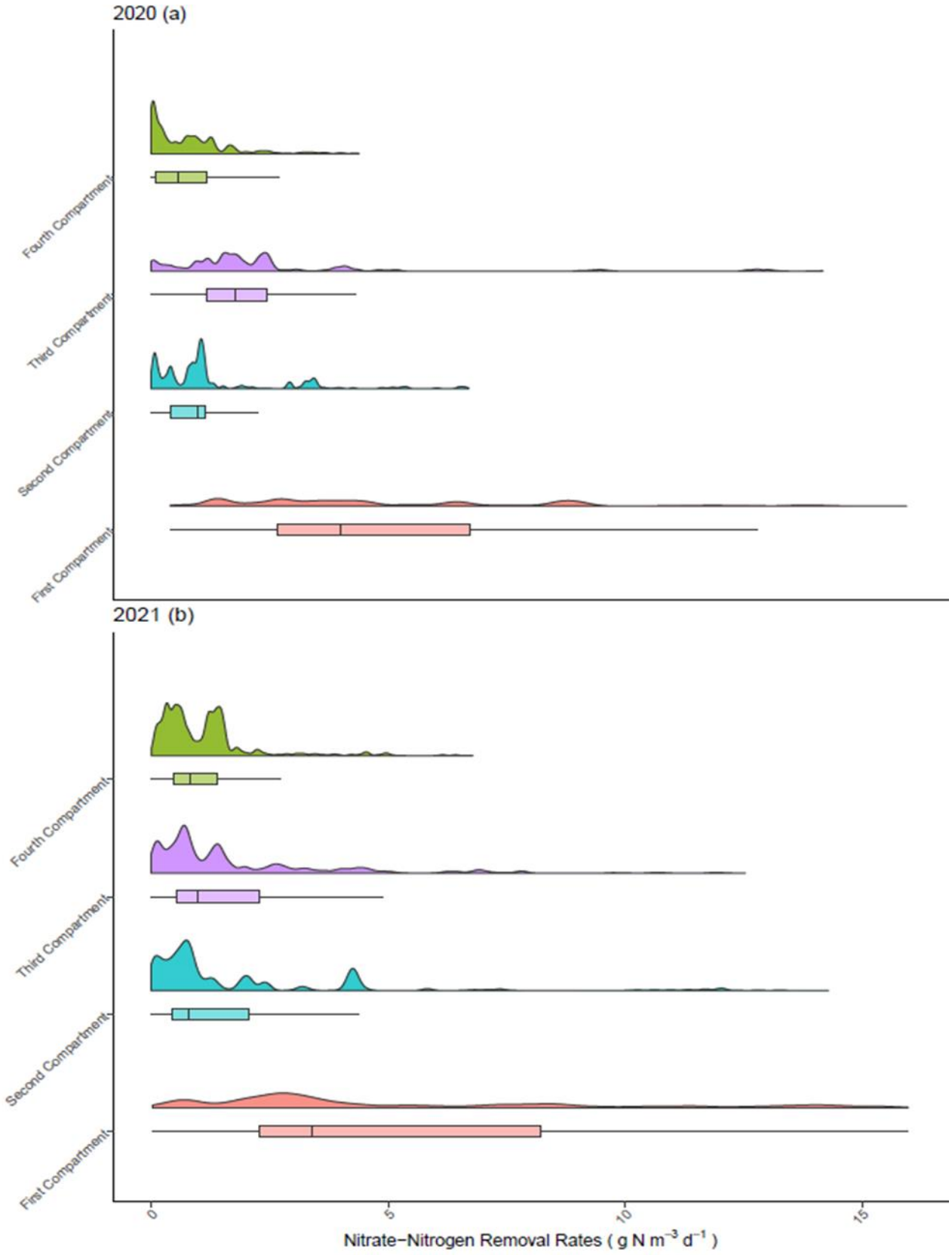
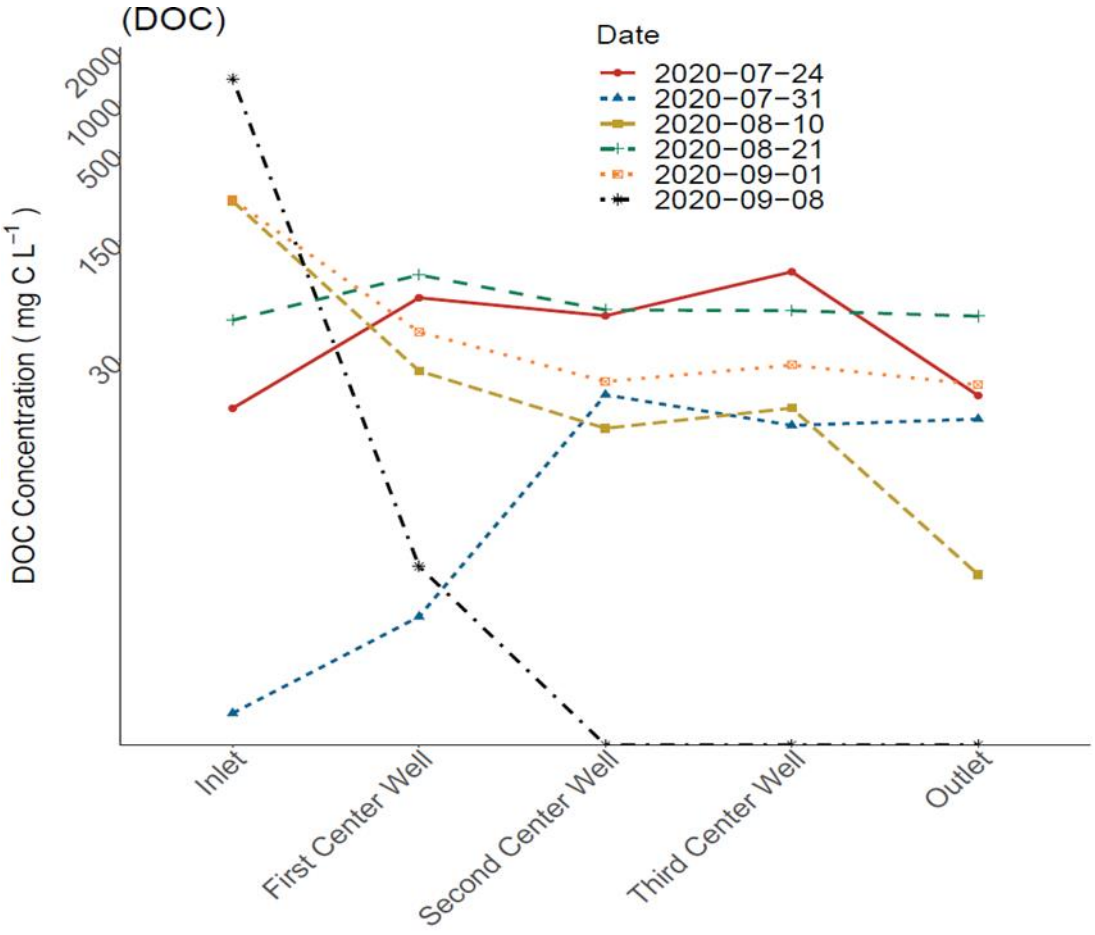
Supplementary figures

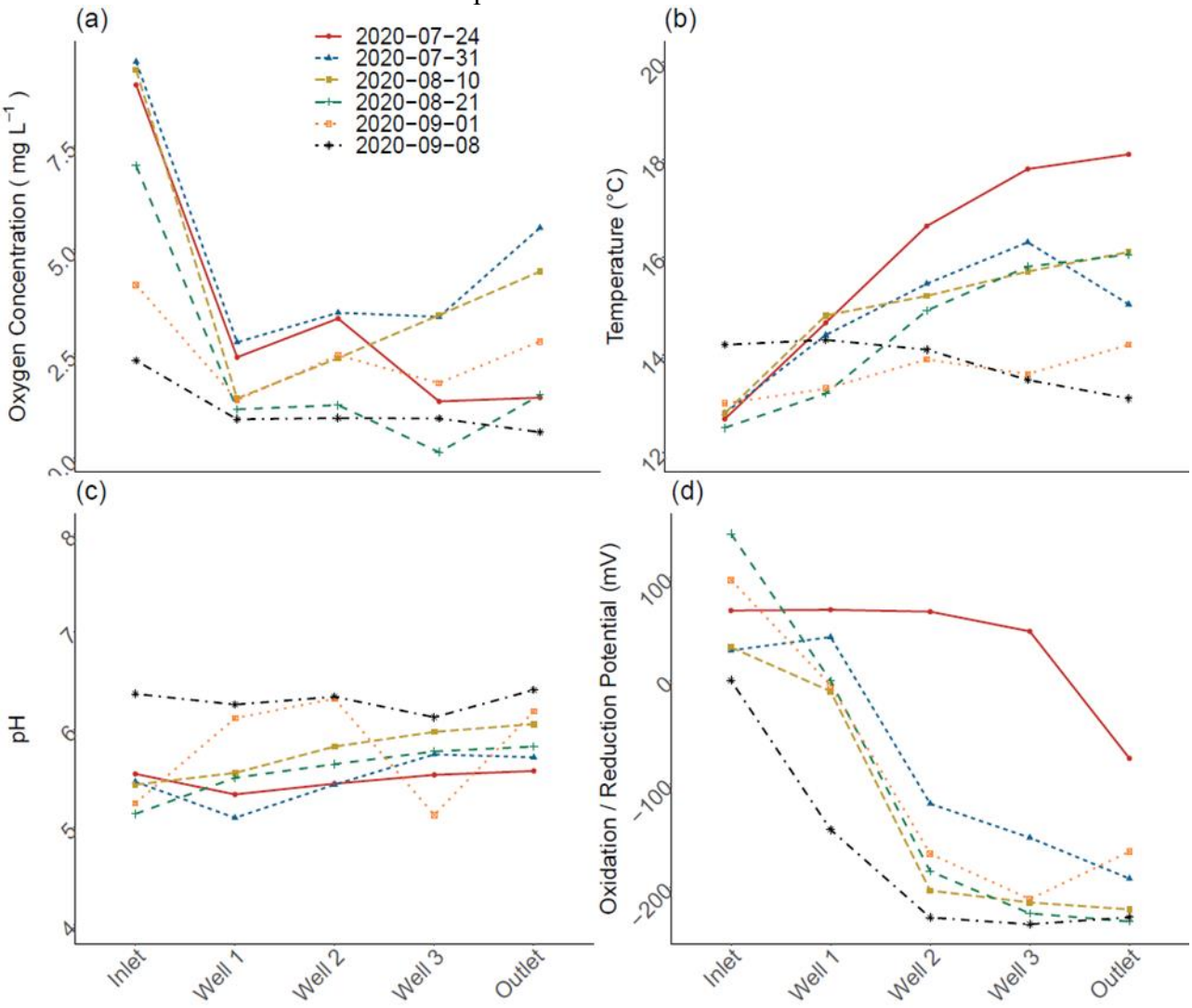


Supplementary figure 3-1 Boxplots and split-half violins depicting the nitrate removal rates in two seasons with methanol dosing through different compartments of the bioreactor. The split-half violins represent the kernel density of nitrate removal rates.



Supplementary figure 3-2 DOC concentrations in drainage water grab samples along the length of the bioreactor during the 2020 drainage seasons. Note that the y-axis is on the

square root scale.



Supplementary figure 3-3 Dissolved oxygen (a), temperature (b), pH (c), and redox potential (d) along the bioreactor over the 2020 drainage season.

Chapter 4

Enhanced Nitrate Removal and Side Effects of Methanol Dosing in Denitrifying Bioreactors

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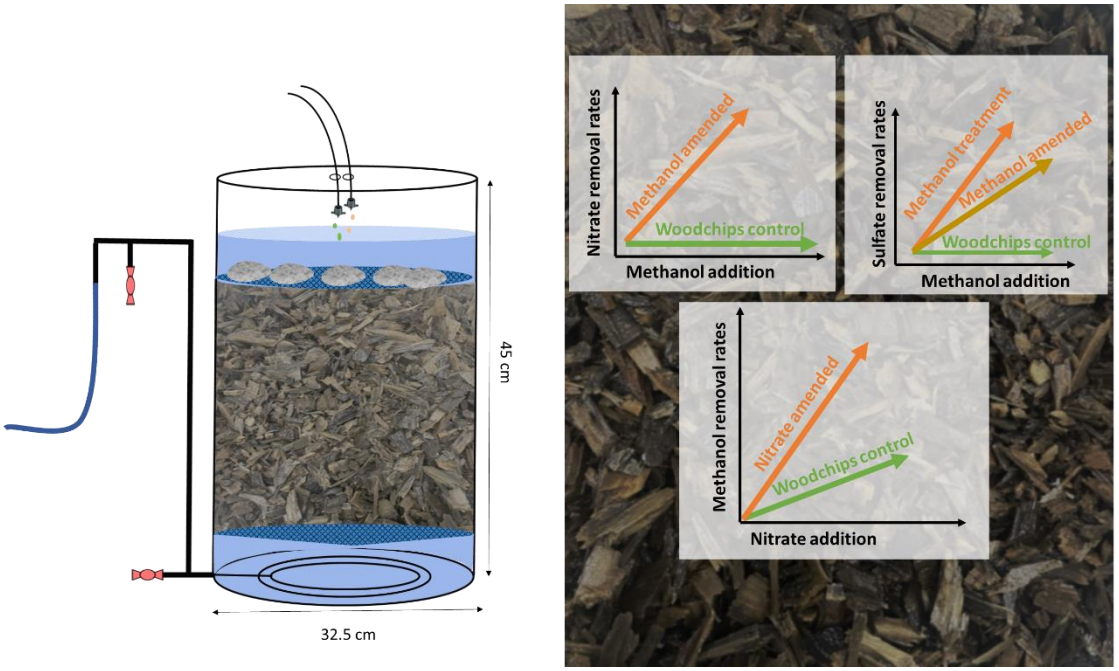
Abstract

The nitrate removal efficiency of denitrifying bioreactors decreases when the carbon supply from woodchips is insufficient, particularly during large nitrate pulses. This study aimed to assess the effects of methanol dosing, as an external carbon source, on nitrate removal rates in mesocosm-scale bioreactors while monitoring the secondary effects of dosing that may occur. A secondary goal was to quantify sulfate reduction rates and methanol consumption in the presence and absence of nitrate to aid in determining the dosing load to minimize undesirable effects such as methanol entering receiving waters and minimizing sulfate reduction. We continuously dosed the bioreactors with nitrate (~20 mg NO_3^--N L^{-1}), methanol (~35 mg CH_3OH-C L^{-1}), and sulfate (~9 mg SO_4^{2-}-S L^{-1}), which was already present in the tap water. In a long-term controlled mesocosm experiment, we established three bioreactor treatments to investigate nitrate, methanol, and sulfate removal rates with and without the presence

of nitrate and methanol. Compared to the woodchip control treatment, methanol dosing resulted in an approximately fourfold increase in nitrate removal rates from 7 to 27 g N m^{-3} day^{-1}. Methanol dosing increased sulfate removal rates, from average sulfate removal rates of 1.5 g SO_4^{2-}-S m^{-3} day^{-1} under nitrate-prevailing conditions to 5.5 g SO_4^{2-}-S m^{-3} day^{-1} removal rates under nitrate-limiting conditions compared to woodchip control removal of 0.3 g SO_4^{2-}-S m^{-3} day^{-1}. Mean methanol removal rates were 23 g CH_3OH-C m^{-3} day^{-1} under nitrate-prevailing conditions compared to 18 g CH_3OH-C m^{-3} day^{-1} in the woodchip control experiment. Improved nitrate removal rates, as well as methanol consumption and sulfate removal rates, might be leveraged to develop innovative low-footprint bioreactors.

Graphical Abstract



Highlights

- Methanol dosage increased nitrate removal fourfold in denitrifying bioreactors.

- Sulfate removal increased in bioreactors with methanol dosing.
- Methanol was consumed in bioreactors with and without nitrate.
- Methanol dosing offers a low-cost approach to improving bioreactor performance.

4.1 Introduction

Farmers have gradually increased the use of organic and inorganic sources of nitrogen and phosphorus in agriculture to increase crop yields and fulfil the world's food needs (Basu et al., 2022; Godfray, 2013; Mosier et al., 2013; Wurtsbaugh et al., 2019). Crops cannot always utilize all of the nitrogen and phosphorus supplied and are subsequently lost to the receiving ecosystems (Basu et al., 2022; Godfray, 2013; Mosier et al., 2013). Half of the nitrogen applied to agricultural soils is lost to the environment during the drainage of surplus water from farm fields (Basu et al., 2022; Davidson et al., 2011), raising concerns about surface and groundwater resources. Diffuse (nonpoint) agricultural water pollution has prompted initiatives to develop simple and novel management strategies to mitigate the impact of intensive agriculture on downstream aquatic ecosystems (Basu et al., 2022; Shortle et al., 2021).

Several edge-of-field mitigation measures reduce diffuse nitrate pollution from the drained agricultural lands to the downstream water bodies (Carstensen et al., 2020; Christianson et al., 2013b). Constructed wetlands, saturated buffers, and denitrifying woodchip bioreactors are the primary practices utilized to lower nitrate levels in agricultural runoff (Carstensen et al., 2020; Christianson et al., 2013a; Christianson et al., 2013b). The most appropriate nitrate treatment mitigation measure depends on the hydrology and climate of the watershed (Carstensen et al., 2020).

Denitrifying bioreactors have gained popularity due to their cost-effectiveness, relatively small footprints, and somewhat rapid establishment compared to other mitigation measures such as constructed wetlands (Addy et al., 2016; Christianson et al., 2021; Schipper et al., 2010). Bioreactors consist primarily of a trench filled with a solid carbon source (such as woodchips and sawdust) that supports heterotrophic denitrification, transforming nitrate into gaseous nitrogen (Cameron and Schipper, 2010; Schipper et al., 2010; Warneke et al., 2011). Bioreactors are generally classified as passive nitrate removal systems since they do not require pumping systems to remove nitrogen-polluted water, resulting in considerable energy savings and fewer maintenance requirements (Christianson et al., 2021; Schipper et al., 2010). While alternative biological nitrogen removal pathways, such as anammox, have been found to contribute to nitrogen removal (Herbert Jr et al., 2014; Rambags et al., 2019), heterotrophic denitrification is still widely regarded as the primary nitrogen removal mechanism in bioreactors (Aalto et al., 2022; Nordström and Herbert, 2018; Schipper et al., 2010).

Carbon availability, environmental characteristics, and other controlling factors such as temperature and influent nitrate content can regulate the nitrate removal performance of bioreactors (Addy et al., 2016; Rivas et al., 2020). Woodchips in bioreactors release dissolved organic carbon (DOC) that is required for denitrification. However, the amount of DOC produced by woodchips declines with time (Abusallout and Hua, 2017) and may eventually limit nitrate removal rates, especially in bioreactors with older woodchips (Addy et al., 2016; Rivas et al., 2020; Robertson, 2010). Christianson et al. (2021) reported an average nitrate removal efficiency of 20% (including the untreated bypass flow) in 37 field-scale bioreactors. The relatively low nitrate removal efficiency compared to other mitigation measures was due to

woodchip's lack of carbon input. This low removal efficiency suggested that bioreactors would not perform as well as other mitigation options, such as constructed wetlands, which have been shown to consistently reduce nitrate loads by 30 to 50 percent (Tanner et al., 2012).

In an attempt to enhance bioreactor performance, a few studies have examined the effects of cycling bioreactors through aerobic phases (through temporary drainage) to stimulate carbon release from woodchips, increasing nitrate removal rates in bioreactors (Maxwell et al., 2019a; Maxwell et al., 2019b; McGuire et al., 2021). Maxwell et al. (2019b) demonstrated that exposing bioreactors to the aerobic phase once a week effectively increased nitrate removal efficiency. A 24-hour aerobic cycle resulted in a nitrate removal efficiency of 172% over control bioreactors. Maxwell et al. (2019b) attributed the increase in nitrate removal efficiency to higher carbon release by cycling the bioreactor between aerobic and anaerobic phases, suggesting that nitrate removal in the bioreactors was carbon-limited. While drying–rewetting cycles stimulate carbon release and increase nitrate removal, this management practice might not always be practical or fully compensate for the carbon shortage in bioreactors treating large nitrate pulses in agricultural drainage waters.

A few studies have shown that adding a soluble organic carbon source (e.g., ethanol, methanol, acetate, and succinate) to woodchip bioreactors improves nitrate removal rates (Hartz et al., 2017; Jansen et al., 2019; Palomo et al., 2013; Roser et al., 2018). Palomo et al. (2013) found that adding succinate ($C_4H_6O_4$) as an external carbon source enhanced nitrate removal efficiency in a field denitrification wall treating groundwater flow from 15% to 73%. Roser et al. (2018) examined the effect of acetate dosage on nitrate removal rates in hydrologically controlled mesocosm-scale bioreactors. When the bioreactors were dosed with acetate, Roser et al. (2018)

measured nitrate removal rates of 120.6 ± 12.9 g N m^{-3} day^{-1} (mean ± SD). These improved nitrate removal rates were compared to control rates of 7.1 ± 3.8 g N m^{-3} day^{-1} (mean ± SD) without carbon dosing under the same operating circumstances (i.e., same temperature and hydraulic conditions). Hartz et al. (2017) studied the effects of carbon dosing on laboratory-scale bioreactors (14 L) with two different loads of methanol and glycerin. The study reported removal efficiency improvements of 78% and 100% in methanol-dosed bioreactors and 75% and 100% in glycerine-dosed bioreactors, respectively, compared to 5% in woodchip bioreactors. Methanol and glycerine dosage resulted in equivalent nitrate removal rates of 31-40 g N m^{-3} day^{-1} and 29-40 g N m^{-3} day^{-1}, respectively, compared to 2.25 g N m^{-3} day^{-1} in the woodchip control experiment. In a field trial of methanol dosing in a full-scale bioreactor, Moghaddam et al. (2023) showed that methanol dosing was effective, reporting nitrate removal rates of 10.8 g N m^{-3} day^{-1} compared to 0.67–1.60 g N m^{-3} day^{-1} without methanol dosing in the same bioreactor (Rivas et al. 2020). While Moghaddam et al. (2023) demonstrated a substantial increase in nitrate removal rates with methanol dosing, the nitrate removal rates were considerably different from other carbon dosing studies (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018). The variations in nitrate removal rates between the prior dosing studies and those of Moghaddam et al. (2023) were most likely attributable to differences in scale of operation, environmental conditions, and bioreactor inputs. Overall, adding an external carbon supply to bioreactors improves nitrate removal rates, although the rate increase is poorly constrained.

Even while adding external carbon sources to bioreactors has been shown to improve nitrate removal, there is still a lack of understanding of the potential adverse effects carbon dosage may have on either the bioreactors or downstream aquatic habitats. For

instance, carbon dosing could result in exacerbated carbon losses from bioreactors and higher sulfate reduction rates. Dissolved organic carbon leaching from bioreactors has potentially harmful environmental implications for catchments, especially during the start-up phases, when a significant flush of DOC occurs (Abusallout and Hua, 2017; Rivas et al., 2020; Schipper et al., 2010). Significant losses of DOC from bioreactors may result in dissolved oxygen (DO) depletion in receiving water bodies, subjecting downstream aquatic biota to further stresses (Chambers et al., 2000). Dissolved organic carbon losses from bioreactors could worsen if added carbon is not entirely removed through denitrification and other biogeochemical pathways. Moghaddam et al. (2023) demonstrated that added methanol was effectively removed during transient nitrate inputs with greater than 90% removal efficiency. The dosed bioreactor in Moghaddam et al. (2023) intercepted tile drainage with highly variable nitrate inputs. Methanol's contribution to nitrate and sulfate removal is poorly constrained, necessitating a long-term hydrologically controlled experiment to determine nitrate, sulfate, and methanol removal rates more precisely. The variable flow through the bioreactor also made it difficult to determine methanol and sulfate removal rates when nitrate was in excess and when it was limiting. If added methanol considerably increased the rate of sulfate reduction in bioreactors in the presence and absence of nitrate, the end products of sulfate reduction may be another detrimental consequence of methanol dosing.

Sulfate reduction in bioreactors in the presence of trace levels of mercury can result in the formation of methyl mercury, which is harmful to aquatic organisms (Shih et al., 2011; Ullrich et al., 2001). In addition, sulfate reduction leads to hydrogen sulphide (H_2S) production, which is toxic and results in the emission of an unpleasant odour into the adjacent environment while also causing acid corrosion of bioreactor

components and pipework (Easton et al., 2015; Wiener et al., 2013). Sulfate reduction in methanol-amended bioreactors must be minimized if methanol dosing in bioreactors is to be used.

The purpose of the present work was to (I) conduct a long-term study to examine the effect of methanol dosing on the nitrate removal performance of mesocosm-scale bioreactors; (II) to measure sulfate and methanol removal rates in the presence and absence of nitrate under steady-state conditions for design purposes; and (III) conduct mass balance modelling to establish a relationship between methanol, sulfate, and nitrate removed in methanol-dosed bioreactors, enabling the estimate of methanol required to remove a certain amount of nitrate.

4.2 Materials and Methods

A mesocosm study was designed, built, and operated at the University of Waikato to investigate the effects of methanol dosing on nitrate removal rates on bioreactors while determining some of the secondary effects (i.e., sulfate reduction and methanol loss). Methanol and nitrate were dosed into the bioreactors, while sulfate was present in the tap water used in the experiment.

4.2.1 Experiment design

Three treatments were established to determine the removal rates of nitrate, methanol, and sulfate in the bioreactors. The three treatments were “BN treatment” for nitrate + sulfate fed bioreactors; “BM treatment” for methanol + sulfate fed bioreactors; and “BNM treatment” for nitrate, sulfate, and methanol-fed bioreactors. BN and BM treatment bioreactors were supplied with tap water at the same flow rate as BNM treatment bioreactors (with two flows of methanol and nitrate) to maintain the same hydraulic retention time (HRT) across all treatments.

Each treatment received steady-state nutrients and methanol flows and was replicated four times (Supplementary figure 4-1), resulting in twelve 30L-barrels (hereafter referred to as bioreactors). Methanol and nitrate solutions were gravity fed to bioreactors to maintain steady-state flow conditions (full details below, Supplementary figure 4-1). Concentrated methanol and nitrate flows were pumped on a regular basis from 200L dosing drums to 60L dosing tanks 3m above ground to feed the mixing tanks for dilution before filling receiving tanks and bioreactors at lower elevations on stainless steel scaffolding shelves.

A CR1000X control datalogger (Campbell Scientific, Logan, UT, USA) was used to power, regulate, and automate process devices, which was subsequently reinforced with a relay controller module (Campbell Scientific, Logan, UT, USA) to enhance the logger's capacity to control more of the experiment's parameters. Methanol (1.65 g CH_3OH-C L^{-1}) and nitrate (1.1 g NO_3^--N L^{-1}) solutions made up in the dosing drums were diluted with municipal tap water in mixing tanks regulated by eight centrifugal pumps (RS Components, London, United Kingdom) and solenoid valves (HR Products, Sydney, Australia) to provide the required nitrate and methanol inputs into the bioreactors (Supplementary figure 4-1). Mixing tanks were replenished three times per day to ensure steady-state flow supplies based on the bioreactor's feeding rates to administer methanol and nitrate into the treatment bioreactors using pressure compensating drippers (Aquadrip, Växjö, Sweden). Unless otherwise specified, all materials for the experiment (including drums, barrels, hoses, tubes, and tapes) were obtained locally. The experiment was disrupted three times due to COVID-19 lockdowns in New Zealand and electrical technical issues. There were three significant interruptions to the experiment due to COVID-19 lockdowns in New Zealand and technical issues. The interruptions occurred during the experimental days 60 to 74,

145 to 175, and 253 to 298. Bioreactors remained saturated during this period with no input of nutrients and methanol.

4.2.2 Bioreactor design

With a few adjustments, the design of the bioreactors followed Cameron and Schipper (2010) (Supplementary figure 4-2). Bioreactors were flushed with tap water for 2 weeks before dosing with methanol and nitrate to remove the initial DOC release commonly associated with fresh woodchips. The influents were added to the bioreactors at the top, gravity-fed through the woodchips, and collected via a perforated ring at the bottom, to encourage plug flow through the medium. Stainless steel wire mesh was placed on top of the woodchips to establish a uniform surface of woodchips at the top of each bioreactor and stabilized with stones to counteract buoyant force (Supplementary figure 4-2). A perforated tube was inserted at the bottom of the barrels, separated from the above woodchips by a steel mesh, to collect the treated water. A polyvinyl chloride (PVC) riser pipe was installed on the barrels' side to keep a constant water level above the woodchips, supporting a uniform flow through the medium (Supplementary figure 4-2).

4.2.3 Dosing ratio

The influent nitrate and methanol concentrations delivered to the bioreactors were generally kept between 20-30 mg NO_3^--N L^{-1} and 30-40 mg CH_3OH-C L^{-1}, respectively. The influent concentrations for this study represented field bioreactor nitrate inputs Moghaddam et al. (2023). A 1.48 CH_3OH-C:NO_3^--N ratio (Hartz et al., 2017) was maintained. We increased the methanol and nitrate concentrations entering the bioreactors near the end of the trial to assess the bioreactors' performance under elevated inputs.

4.2.4 Sampling and hydrochemical analysis

Water samples were collected from the inflow and outflow for analysis approximately every two weeks, but this varied due to three major interruptions in the experiment (see section 4.2.1). After collection, the samples were filtered through 0.45 μm (Sartorius AG, Germany) membranes and stored at 4 °C until analysis. The samples were analysed for nitrate, sulfate, and methanol concentrations. Dionex ICS-200 Ion Chromatograph (Dionex, California, United States) with an IonPac AS11-HC guard (4 μm, 2 × 50 mm) column (Dionex, California, United States) was used to measure nitrate and sulfate concentrations in the grab samples calibrated with the anion standards (five anion standard, Dionex, California, United States).

Methanol concentration was determined using a gas chromatograph equipped with a polar column DB-WAX (30 m × 0.25 mm × 0.5 μm, Agilent Technologies, Santa Clara, California, United States) and a flame ionization detector (FID). With a 20:1 split ratio, liquid samples (1 μL) were directly injected into the gas chromatograph via an autosampler. For each injection, the initial oven temperature was 60°C and was kept for 5 minutes before increasing it to 240 °C at 10°C min^{-1}.

Inlet and outlet dissolved oxygen (O_2), temperature, and pH were measured using a YSI ProQuatro Multiparameter probe (YSI Inc, Ohio, United States) when influent and effluent samples were taken at each bioreactor.

4.2.5 Removal rates and efficiency

We calculated nitrate, sulfate, and methanol volumetric removal rates as follows:

$$RR = \frac{(C_{inlet} - C_{Outlet}) \times Q}{V_{saturated}} \qquad \text{Equation 4-1}$$

Where C_{inlet} and C_{Outlet} were either NO_3^--N, SO_4^{2-}-S, or CH_3OH-C concentrations (mg L^{-3}) at the inlet and the outlet, respectively, Q was the flow rate (m^3 d^{-1}) and $V_{saturated}$ was the saturated volume of woodchips in the bioreactors (m^3).

The removal efficiencies (RE %) of the bioreactors were determined as the percentage of either nitrate, sulfate, or methanol removed from the bioreactors between the inlet and the outlet:

$$RE\% = \frac{C_{inlet} - C_{outlet}}{C_{inlet}} \times 100 \qquad \text{Equation 4-2}$$

Where, $C_{inlet} - C_{Outlet}$ are the concentrations of NO_3^--N, SO_4^{2-}-S, and CH_3OH-C at the inlet and the outlet (mg L^{-3}), respectively.

4.2.6 Statistical analysis

All statistical analyses, data modelling, and visualizations were performed using R programming language (R Core Team, 2021) in conjunction with other R external packages, Tidyverse (Wickham et al., 2019), Rstatix (Kassambara, 2020), and mosaic (Pruim et al., 2017). A two-way repeated-measures analysis of variance (ANOVA) was used to assess the effects of treatments and time on dependent variables (removal rates of nitrate, sulfate, and methanol). The Shapiro–Wilk test was used to determine whether the dependent variables in each treatment (BN, BM, and BNM) were normally distributed. To visually assess the normality assumption of data points, a Q–Q (quantile-quantile) plot was utilized for dependent variables (removal rates of nitrate, sulfate, and methanol) under different treatments (plots not shown). The paired t-test was performed for post hoc pairwise comparisons to examine the effects of treatment at each time point and the influence of time on each treatment when the interaction effect between treatment and time was significant.

4.2.7 Mathematical Modelling

We fitted a multiple linear regression model (MLR) based on experimental findings from each sampling time to estimate methanol removal in BNM treatment bioreactors based on the quantity of sulfate and nitrate removed.

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 \qquad \text{Equation 4-3}$$

Where Y represents the mass of methanol removed in the bioreactors, β_i represents the coefficients of independent variables (mass of nitrate and sulfate removal) as well as their interaction, and X_i represents the independent variables.

4.3 Results

4.3.1 Nitrate removal

BNM bioreactors removed significantly more nitrate than BN bioreactors (Figure 4-1 and Table 4-1). The outlet nitrate concentrations of the BN bioreactors were initially around 7 mg NO_3^--N L^{-1} compared to about 20 mg NO_3^--N L^{-1} at the inlet. Nitrate removal rates decreased during the second month of operation as the outlet nitrate concentration in the BN treatment bioreactors approached the inlet nitrate concentrations (Figure 4-1a). As a result, the nitrate removal rate of the BN treatment bioreactors was 10.4 g N m^{-3} (of saturated woodchips) day^{-1}, 95% CI [8.5 g N m^{-3} d^{-1}, 12.3 g N m^{-3} d^{-1}] during the first two months. Subsequently, nitrate removal rates decreased to 5.9 g N m^{-3} d^{-1}, 95% CI [4.2 g N m^{-3} d^{-1}, 7.7 g N m^{-3} d^{-1}], and remained within this range until the end of the trial (Figure 4-2b and Table 4-1).

The outlet nitrate concentration of the BNM bioreactors was generally in the limiting ranges (concentrations below the Michaelis-Menten K_m of 3 mg NO_3^--N L^{-1} for heterotrophic denitrification (Kouanda and Hua, 2021)) with nitrate removal rates of 27 g N m^{-3} d^{-1} 95% CI [23 g N m^{-3} d^{-1}, 30 g N m^{-3} d^{-1}] (Figure 4-1 and Table 4-1).

When nitrate and methanol inputs to the bioreactors were doubled, nitrate removal rates increased to 54 g N m^{-3} d^{-1} 95% CI [52 g N m^{-3} d^{-1}, 56 g N m^{-3} d^{-1}], and the output nitrate concentrations increased to around 13 mg NO_3^--N L^{-1} (Figure 4-1).

Table 4-1 Nitrate, sulfate, and methanol removal rates and efficiency for the different treatments

Compound	Treatment	Mean RRs*	RRs 95% CI*	Mean RE*	RE 95% CI*
Nitrate	BN	7.4	[6, 8.8]	28.3	[22.4, 34]
	BNM	29	[25, 34]	87.4	[84, 90]
Sulfate	BN	0.3	[0.2, 0.5]	4.3	[2.4, 6.3]
	BNM	1.5	[1.11, 1.9]	19.9	[14.9, 24.8]
	BM	5.1	[4.5, 5.7]	67.4	[60.7, 74.1]
Methanol	BM	17.6	[15.8, 19.5]	48.4	[43.5, 53.4]
	BNM	23.8	[21.7, 25.9]	65.2	[60, 70.4]

*RRs: removal rates (g m^{-3} day^{-1}), CI: confidence interval, and RE: removal efficiency (%)

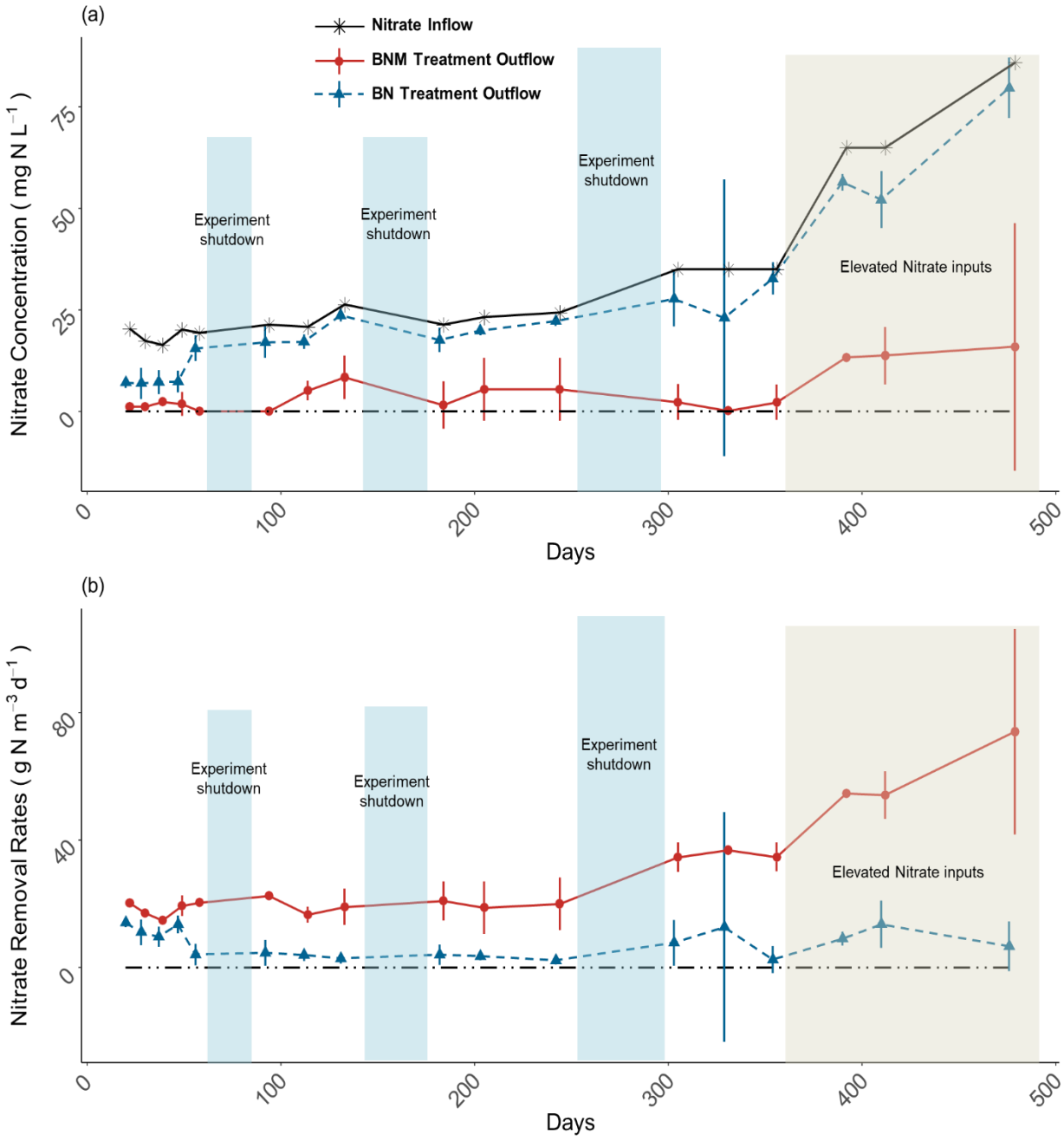


Figure 4-1 Inflow and outflow nitrate concentrations for the control and methanol amended bioreactor (a) and equivalent nitrate removal rates (b). The error bars are a 95% confidence interval for the mean.

Two-way repeated-measures ANOVA revealed a statistically significant effect of treatment (BN and BNM treatments, P-value < 0.001) and time (P-value < 0.001) as well as the interaction on nitrate removal rates (Table 4-2). The effect of each treatment

at each observation time was evaluated, and so was the influence of time on each treatment. At all observation times, the effect of each treatment (BN and BNM) was significant (P-value < 0.001, Supplementary Table 4-1, and Supplementary Table 4-2). The effect of time was also significant for both treatments (P-value < 0.001, Supplementary Table 4-1, and Supplementary Table 4-2).

Table 4-2 Results of the repeated measures ANOVA determining the effect of treatment, time, and their interaction on nitrate, sulfate, and methanol removal rates

Compound	Effect	DFn*	DFd*	F-value	P-value	ges*
Nitrate	Time	15	78	39.642	<0.001	0.884
	Treatment	1	78	1010.283	<0.001	0.928
	Treatment: Time	15	78	26.919	<0.001	0.838
Sulfate	Time	15	126	14	<0.001	0.63
	Treatment	2	126	416	<0.001	0.87
	Treatment: Time	30	126	4.9	<0.001	0.54
Methanol	Time	12	73	8.1	<0.001	0.58
	Treatment	1	73	28.8	<0.001	0.29
	Treatment: Time	12	73	3.2	<0.001	0.35

*DFn: degrees of freedom in the numerator, DFd: degrees of freedom in the denominators, and ges: generalized Eta-Squared (η^2) measure of effect size

4.3.2 Sulfate removal

The sulfate concentrations in the inlet water were usually between 7 and 9 mg SO_4^{2-}-S L^{-1}. There was no significant sulfate removal in the BN treatment bioreactors. The output sulfate concentrations remained near the inputs levels, and removal rates were less than 0.3 g SO_4^{2-}-S m^{-3} day^{-1} (Figure 4-2 and Table 4-1). Sulfate concentrations declined marginally in the BNM treatment bioreactors, with a difference of 1 to 4 g SO_4^{2-}-S m^{-3} d^{-1} between input and outflow and mean removal rates of 1.5 g SO_4^{2-}-S m^{-3} day^{-1} (Figure 4-2 and Table 4-1). In the BM treatment bioreactors (nitrate-

limiting), however, sulfate concentrations decreased considerably, with outflow concentrations ranging from 0 to 4 mg SO_4^{2-}-S L^{-1} and mean removal rates of 5.1 g SO_4^{2-}-S m^{-3} day^{-1} (Figure 4-2 and Table 4-1). The odour of hydrogen sulphide (H_2S) was detected throughout the study, notably in BM bioreactor outflow samples.

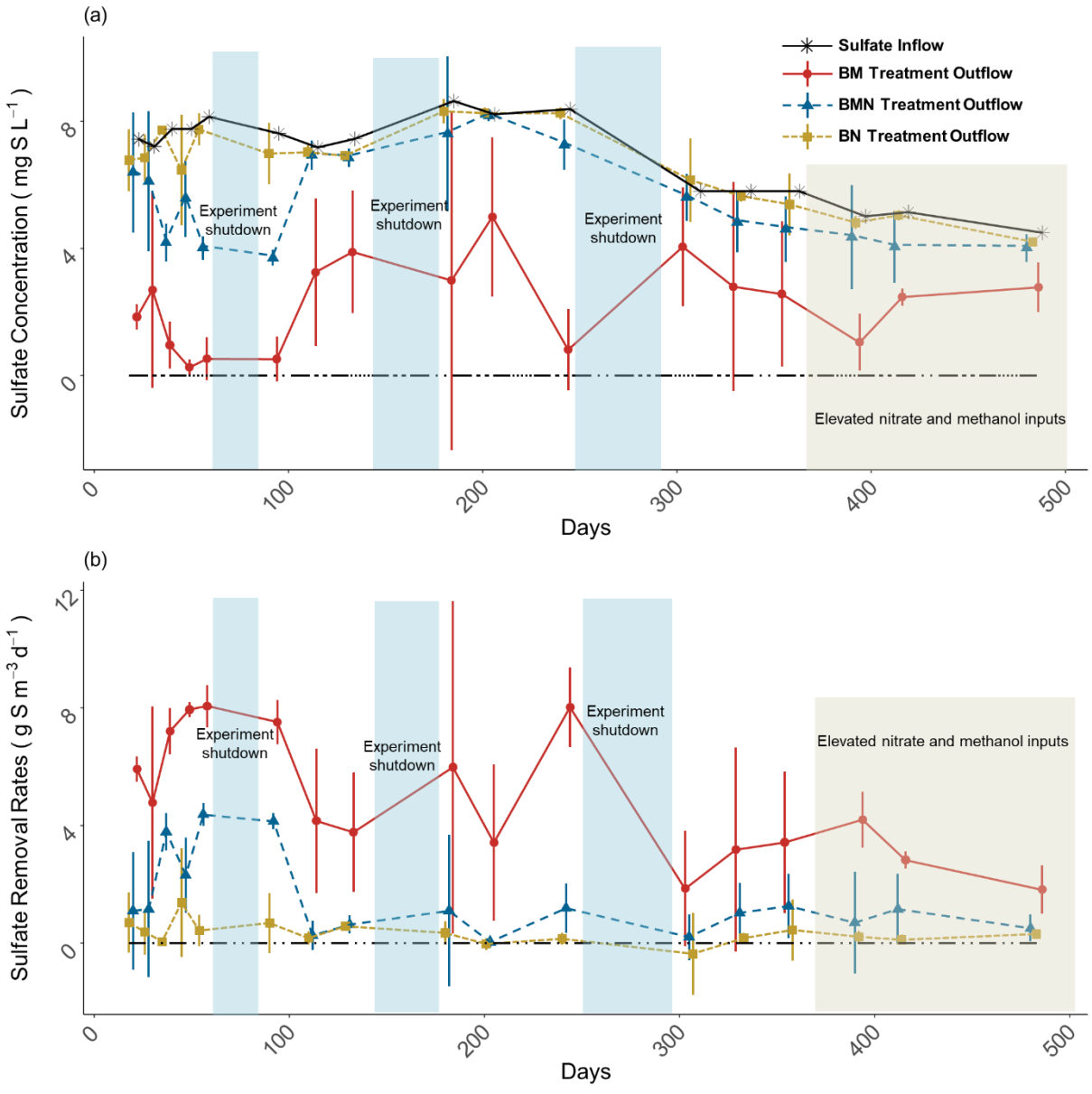


Figure 4-2 Inflow and outflow sulfate concentrations in BN, BNM, and BM bioreactors (a) and corresponding removal rates (b). The error bars are a 95% confidence interval for the mean.

Two-way repeated measurements ANOVA revealed that treatment and time had a statistically significant effect on sulfate removal rates and their interaction (Table 4-2). As a result, the effects of each treatment during each observation period were evaluated, and so was the effect of time on each treatment. BM treatment had significantly higher sulfate removal rates than BN and BNM treatment (P-value <0.001;

Supplementary Table 4-3). However, the effect of treatment on sulfate removal rates was not significant in three observations in the BN and BNM (P-value > 0.001,

Supplementary Table 4-3). Furthermore, for all treatments, the effect of time on sulfate removal rates was significant (P-value < 0.001, Supplementary Table 4-4).

4.3.3 Methanol removal

The methanol concentrations in the inlet water generally varied from 30 to 50 mg CH_3OH-C L^{-1}. The outlet methanol concentrations in the BNM treatment bioreactors generally ranged from 7 to 20 mg CH_3OH-C L^{-1} with mean methanol removal rates of 23.8 g CH_3OH-C m^{-3} day^{-1} (Figure 4-3 and Table 4-1). Methanol removal rates in the BM treatment bioreactors ranged from 10 to 25 mg CH_3OH-C L^{-1} with mean removal rates of 17.6 g CH_3OH-C m^{-3} day^{-1} (Figure 4-3 and Table 4-1).

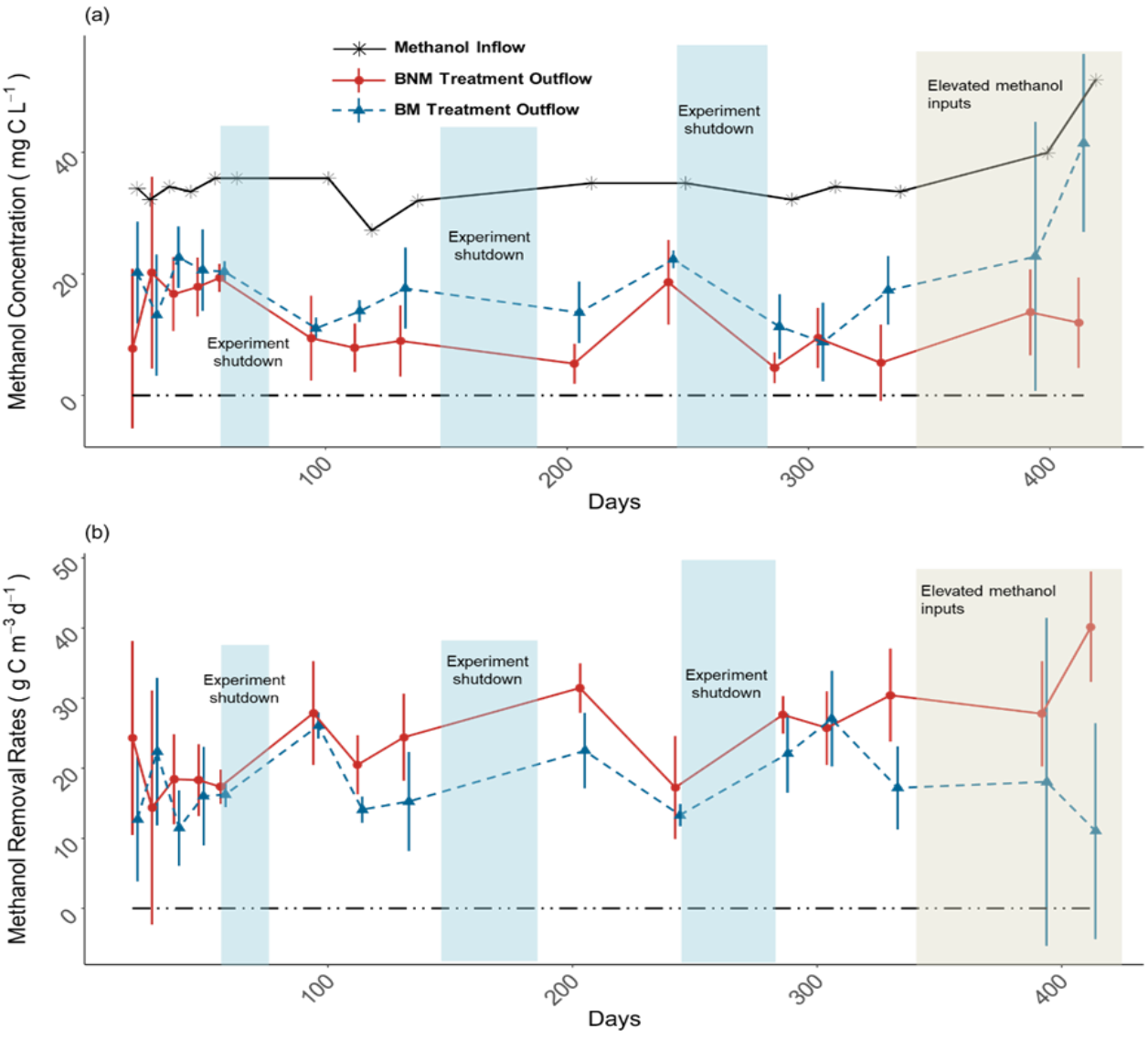


Figure 4-3 Inflow and outflow methanol concentrations in the control and nitrate amended bioreactor (a) and corresponding removal rates (b). The error bars are a 95% confidence interval for the mean.

The effects of treatment and time on methanol removal rates were significant, as was their interaction (Table 4-2). The treatments showed no significant effect on methanol removal rates in the bioreactors on a few observation dates (P-value > 0.05,

Supplementary Table 4-5). Furthermore, time had a significant effect on each treatment (P-value < 0.001, Supplementary Table 4-6).

4.3.4 Oxygen removal

The BNM, BN, and BM treatment bioreactors had mean oxygen removal rates of 3.2, 3, and 3.7 g O_2 m^{-3} day^{-1}, respectively (Supplementary figure 4-3).

4.3.5 Modelling

Figure 4-4 depicts a contour plot of sulfate and nitrate removal for varying amounts of methanol removed (contour lines). This graph, which combines Figures 1, 2, and 3, provides an MLR model of the quantity of sulfate (mg SO_4^{2-}-S) and nitrate (mg NO_3^--N) removed from bioreactors per amount of methanol (mg CH_3OH-C) consumed based on experimental observations in BNM treatment bioreactors (Figure 4-4, Table 4-3).

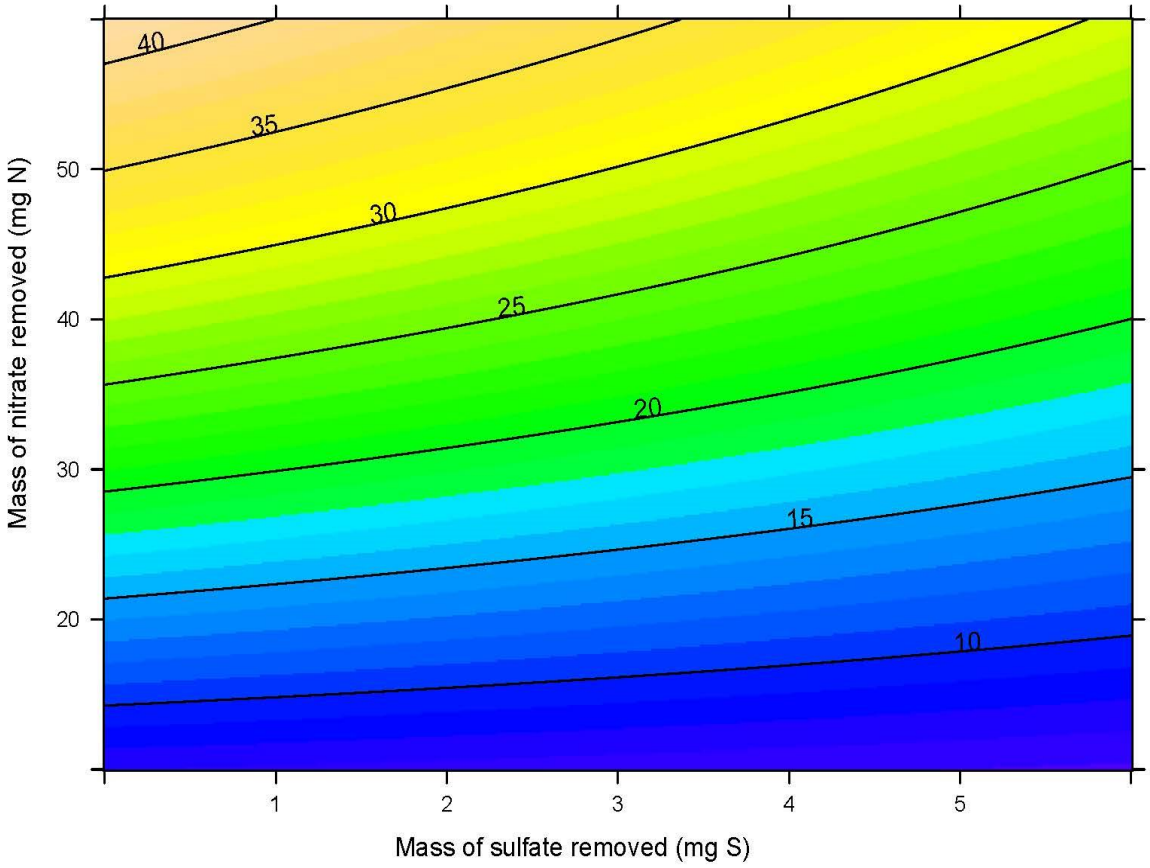


Figure 4-4 Contour plot of the regression model based on the experimental observations illustrating the amount of nitrate and sulfate removed in the bioreactors for a particular amount of methanol removed. The lines depict the quantity of methanol removed from the bioreactors.

Table 4-3 MLR model regression results

Parameter	Estimation	Standard error	T-value	P-value
β_1(Sulfate removal)	0.17	0.002	78	<0.001
β_2(Nitrate removal)	0.7	0.04	14.8	<0.001
β_3 (Interaction effect)	-0.03	0.001	-33	<0.001

The residual standard error (RSE): 0.53 on 429 degrees of freedom

This model estimates the minimum amount of methanol required to remove a specific amount of nitrate. In the presence of sulfate, nitrate removal (after reaching a minimum value, the y-intercept of each contour line) occurs concurrently with sulfate reduction. The sulfate and nitrate removal combination will further remove the additional methanol (Figure 4-4) .

4.4 Discussion

Increasing nitrate removal in bioreactors has been attempted using a variety of approaches, such as organic carbon dosing (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018), periodic drainage (oxic–anoxic cycling) (Maxwell et al., 2019a; Maxwell et al., 2019b; McGuire et al., 2021), passive solar heating (Cameron and Schipper, 2011), bioaugmentation (Aldossari and Ishii, 2022), mixing woodchips with activated sludge (Nordström and Herbert, 2017; 2018), and biochar addition (Bock et al., 2015).

Carbon dosing, in particular, has proven to increase bioreactor nitrate removal efficiency. However, the improvement of nitrate removal rate has been shown to vary among studies. There have been fewer attempts to quantify the adverse side effects due to carbon dosing. In our research, methanol dosing in bioreactors increased nitrate removal rates nearly fourfold, with sulfate reduction and methanol losses assessed as secondary impacts of methanol dosing.

4.4.1 Enhanced nitrate removal

The average nitrate removal rate in control bioreactors (BN treatment) was 7.4 g N m^{-3} day^{-1}, which was somewhat higher but consistent with Addy et al. (2016). They reported nitrate removal rates from 2.9 to 7.9 g N m^{-3} day^{-1} in a meta-analysis of twenty-seven column studies with nitrate inputs ranging from 10 to 30 mg NO_3^--N L^-

[1]. At concentrations well above the K_m value of 3 mg NO_3^--N L^{-1} for denitrification, significant nitrate losses from the control bioreactors demonstrated that denitrification was limited by factors other than nitrate (Kouanda and Hua, 2021). The nearly fourfold increase in average nitrate removal rates of methanol-amended bioreactors (BMN treatment) operating under identical environmental conditions as BN treatment bioreactors demonstrated the significance of labile carbon in bioreactor nitrate removal performance. The increase in nitrate removal rates in bioreactors due to carbon dosage in our investigation was consistent with earlier bioreactor dosing studies. Roser et al. (2018) reported average nitrate removal rates of 27 g N m^{-3} day^{-1} in acetate-dosed mesocosm bioreactors under 8-hours HRT flow conditions, similar to our findings in methanol-amended bioreactors. The similarity in nitrate removals between our work and Roser et al. (2018) might be attributed to comparable flow conditions (HRT of 8 hours), same nitrate inputs (inlet nitrate concentrations of 20 mg NO_3^--N L^{-1}), and the steady-state operating settings used in both studies. However, the outflow nitrate concentrations in Roser et al. (2018) and our observations (except for elevated nitrate inputs in our study, discussed later) were within nitrate-limiting concentrations (i.e., below the K_m value of 3 mg NO_3^--N L^{-1}), suggesting that nitrate removal rates would have been greater if the bioreactors had received higher nitrate inputs. The increase in nitrate removal rates relative to input nitrate concentrations under carbon-prevailing conditions was more pronounced in Hartz et al. (2017). They reported total denitrification (output nitrate concentrations below 1 mg NO_3^--N L^{-1}) in methanol and glycerine-dosed bioreactors with equivalent nitrate removal rates of 40 g N m^{-3} day^{-1} under 2-day HRTs. Toward the end of our study, when nitrate inputs were approximately doubled, nitrate removal rates increased to around 55 g N m^{-3} day^{-1}, demonstrating that the bioreactors had the capacity to remove even more nitrate when carbon was not limiting denitrification. However, when nitrate inputs were

increased, outlet nitrate concentrations rose to roughly 12 mg NO_3^--N L^{-1}, demonstrating that the bioreactor's performance was no longer nitrate-limited and that other factors other than nitrate began to restrict the denitrification rate. Several environmental variables (e.g., temperature, carbon availability, biomass concentrations in bioreactors) have been found to limit denitrification under varied operational conditions (Aalto et al., 2022; Addy et al., 2016; Schipper et al., 2010). Given the significant methanol losses in methanol-amended bioreactors, we contend that factors other than carbon were limiting denitrification at high nitrate inputs. This would suggest that the near upper limit of nitrate removal in bioreactors is 55 g N m^{-3} day^{-1}.

4.4.2 Methanol as the dominant electron donor

Given that nitrate removal rates in methanol-amended bioreactors were higher than in control bioreactors early in the experiment, we argue that denitrifier populations established swiftly in methanol-amended bioreactors. Using ethanol as the carbon source for nitrate removal in groundwater, Paradis et al. (2022) studied the microbial community of denitrifier populations. They reported denitrifiers in the ethanol-exposed treatment shifted as soon as ethanol was administered and persisted when ethanol was not supplied to the treatment wells. The acclimation and maintenance of denitrifier populations during the feast and famine period in Paradis et al. (2022) were similar to our findings, evidenced by nitrate removal rates in methanol-amended bioreactors remaining high (relative to control bioreactors) despite three significant disruptions (no addition of nutrients and methanol) throughout the experiment operation (Figure 4-1). Our full-scale bioreactor findings (Moghaddam et al., 2023) further supported the denitrifier population maintenance under dynamic substrate inputs. The current study showed that nitrate removal rates stayed high after zero

nitrate and methanol inputs during and between drainage seasons (lag of more than six months, Moghaddam et al. (2023)). The sustained presence of denitrifier populations in bioreactors under feast and famine periods would eliminate the need for inoculation of the bioreactor during or at the beginning of the operation of the methanol-dosed bioreactor.

4.4.3 Sulfate removal

Several bioreactor investigations have demonstrated dissimilatory sulfate reduction in bioreactors, most notably during the bioreactor start-up stage (high DOC availability) or under nitrate-limiting conditions (Christianson et al., 2017; Corbett et al., 2020; Nordström and Herbert, 2018; Rambags et al., 2016). However, none of the previous bioreactor dosing studies investigated sulfate reduction in bioreactors under reducing conditions as a result of carbon dosage in nitrate-limiting conditions, which may occur in many bioreactors with dynamic nitrate inputs, particularly those installed in agricultural catchments (Hartz et al., 2017; Jansen et al., 2019; Roser et al., 2018).

In our study, the mean sulfate removal rates in the BN treatment bioreactors (control treatment) were 0.3 g SO_4^{2-}-S m^{-3} day^{-1} with an average removal efficiency of 4%, compared to 1.5 g SO_4^{2-}-S m^{-3} day^{-1} with an average removal efficiency of 20% in the BNM treatment bioreactors (Figure 4-2 and Table 4-1). The significantly higher sulfate removal rates in BNM treatment bioreactors compared to BN treatment bioreactors can be likely attributed to methanol availability in excess of that used deriving denitrification in BNM bioreactors. This rise in sulfate removal rate was more pronounced in BM treatment bioreactors compared to both BNM and BN treatment bioreactors with mean sulfate removal rates of 5.2 g SO_4^{2-}-S m^{-3} d^{-1} and average removal efficiency of 67%. The increased average sulfate removal rate in BM treatment bioreactors compared to BNM treatment bioreactors was most likely due to nitrate-

limiting conditions in BM treatment bioreactors, which further increased sulfate removal.. The sulfate removal rates in the control treatment were lower than those reported by Corbett et al. (2020), who measured sulfate removal rates of 4.8–8.2 g SO_4^{2-}-S m^{-3} day^{-1} with removal efficiencies of 59–74% in a full-scale bioreactor treating partially nitrified wastewater with elevated biological oxygen demand (BOD) in the influent which presumably acted as an additional carbon source. Corbett et al. (2020) reported sulfate reduction in the bioreactor's section where nitrate was limiting. The sulfate removal rates in Corbett et al. (2020) matched sulfate removal rates in the BM treatment bioreactors (nitrate-limiting) in our study. The higher sulfate removal rates in BNM treatment bioreactors showed that bioreactors with high carbon availability and nitrate-limiting conditions would result in considerable sulfate removal.

Enhanced sulfate reduction in bioreactors with methanol dosing might be exploited to improve the efficacy of sulfate-reducing bioreactors (SRBRs) used to treat mining-influenced waters (MIW) with high levels of sulfate, metals, metalloids, and acidity (Al-Abed et al., 2017; Ayangbenro et al., 2018; Neculita et al., 2008). These pollutants are removed by sulfate-reducing bacteria (SRB) in SRBRs bioreactors following precipitations as metal sulphides, yet their metal removal performance has been carbon-limited (Al-Abed et al., 2017).

However, in denitrifying bioreactors, sulfate reduction and subsequent H_2S production are also associated with an increased risk of methyl mercury production (Compeau and Bartha, 1985; Ullrich et al., 2001) and disagreeable odour in the neighbouring environments (Easton et al., 2015; Shih et al., 2011). Some minerals, including ferric oxide, can effectively poise the redox potential in some soils and bioreactors to mitigate sulfate reduction (Easton et al., 2015; Lovley and Phillips,

1986). Easton et al. (2015) reported that the addition of amorphous ferric hydroxide ($Fe(OH)_3$) into bioreactors considerably mitigated sulfate reduction in the denitrifying bioreactors. Sulfate reduction was minimized by increasing the activity of iron reducer bacteria, which outcompeted SRB by poising the redox of the bioreactor (Easton et al., 2015). The redox poising method could be used in methanol-dosed bioreactors with dynamic nitrate inputs as a sulfate reduction mitigation strategy to limit H_2S production and the likelihood of methyl mercury formation.

In our study, SRB were established immediately in the methanol treatment bioreactors, as evidenced by a rapid decrease in sulfate concentrations in the BM treatment bioreactors outflow when the experiment began (Figure 4-2a). However, there was approximately a 20-day lag before the sulfate removal was observed in BNM treatment bioreactors. The nitrate present in the BNM treatment bioreactors may have inhibited SRB establishment. During the early part of the experiment, SRB were presumably outcompeted by denitrifiers, resulting in a relatively long lag before SRB proliferation (Mohanakrishnan et al., 2011; Rajeev et al., 2015).

4.4.4 Methanol removal

Liquid carbon dosing (e.g., methanol, acetate, and ethanol) has been proven to enhance nitrate removal rates in bioreactors (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018). However, enhanced nitrate removal by carbon dosing may come at a cost if higher DOC is discharged from bioreactors before it can be effectively removed. This is particularly concerning for bioreactors in agricultural catchments that experience fluctuating nitrate inputs, sometimes resulting in nitrate-limited conditions (Conant et al., 2011; Rivas et al., 2020). The significantly higher methanol removal rates in BNM treatment bioreactors, with an average of 24 g

CH_3OH-C m^{-3} day^{-1} compared to 18 g CH_3OH-C m^{-3} day^{-1} of BM treatment bioreactors, may be explained by the fact that nitrate, as a terminal electron acceptor, also contributed in the removal of added methanol as opposed to BM treatment bioreactors where nitrate was limiting and removal would be by other anaerobic microbial processes.

The mean methanol removal rate in a full-scale bioreactor was 106 g CH_3OH-C m^{-3} day^{-1} (Moghaddam et al., 2023), which was greater than removal rates in the current investigation in both BM and BNM treatment bioreactors. The substantial difference in methanol removal rates between the two investigations might be explained by greater sulfate availability (~60 mg SO_4^{2-}-S L^{-1}) in the field bioreactor inputs and other possible electron acceptors (Rivas et al., 2020) in comparison to our study.

Biological methanol decomposition has been found to occur in both aerobic and anaerobic biogeochemical pathways, particularly in anoxic environments (oxygen limiting).

In nitrate-limited conditions, anoxic biogeochemical processes, namely sulfate reduction, acetogenesis, and methanogenesis, have been demonstrated to effectively metabolize methanol (Kolb, 2009; Sousa et al., 2018; Yanagawa et al., 2016). The extent to which such biogeochemical pathways would be active in the methanol amended bioreactors is determined by the water inputs and the ambient conditions in which bioreactors operate and needs to be researched further.

4.4.5 Appropriate dosage load

In our study, increasing the methanol dosage in BNM treatment bioreactors considerably boosted nitrate removal rates towards the end of the trial (Figure 4-1). On the other hand, increasing the dosing rate increased the risk of methanol losses

from the bioreactors (Figure 4-3). For a given carbon dosing rate, the quantity of sulfate and nitrate removal rates in bioreactors would depend on several characteristics, including bioreactor design and input water chemistry (Aalto et al., 2022).

Higher nitrate removal efficiency with methanol dosage would inevitably result in sulfate reduction and/or methanol losses, assuming no other substantial methanol removal processes. Excess sulfate would remove the additional methanol in the bioreactors, resulting in enhanced methanol removal efficiency in some water inputs, such as agricultural tile drainage, where sulfate concentrations are high (Rivas et al., 2020).

The theoretical value of 0.6 CH_3OH-C:$NO3^-$-N for denitrification using methanol as the carbon and energy source might be derived using the following stoichiometric equation (Timmermans and Vanhaute, 1983):

$$NO_3^- + 1.1\ CH_3OH + H^+ \rightarrow 0.5\ N_2 + 0.71\ CO_2 + 2.4\ H_2O + 0.078\ C_5H_7O_2N \quad \text{Equation 4-4}$$

Using the nitrate variable coefficient of 0.7 in Table 4-3 as our study's C:N ratio, the theoretical 0.6 C:N value is lower than the C:N ratio in our investigation and the 1.48 C:N reported by Hartz et al. (2017). The larger C:N ratio in our study compared to the theoretical one might be due to other electron acceptors (apart from sulfate, which was incorporated in the model) that may have contributed to methanol consumption.

4.5 Conclusion

The effects of methanol dosing on nitrate removal, sulfate reduction, and methanol losses were examined in mesocosm-scale woodchip bioreactors. Throughout the study, methanol dosing significantly increased nitrate and sulfate removal rates. Added

methanol was partially removed when nitrate was either in excess or limited. Methanol-acclimatized denitrifier populations were established quickly in methanol-dosed-bioreactors shortly after the experiment began, leading to consistently high nitrate removal rates. Enhanced nitrate removal rates, as well as methanol consumption and sulfate removal rates, might be utilized as design criteria for low-footprint bioreactors with few side effects, such as methanol losses to receiving water bodies. More studies on carbon dosing in bioreactors could include the assessment of additional negative consequences of carbon dosing, such as increased greenhouse gas (GHG) generation and clogging and investigate alternate carbon sources.

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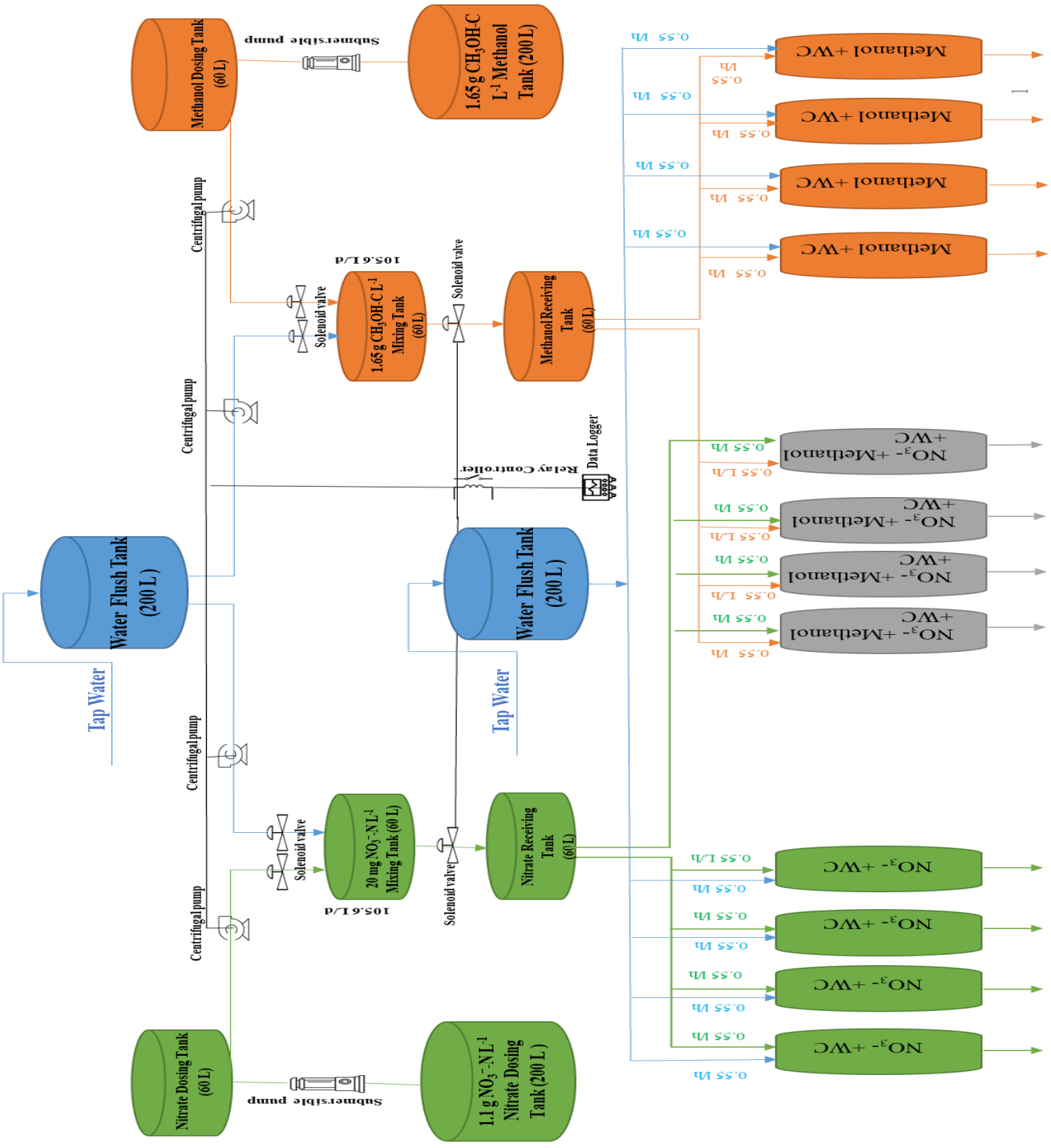
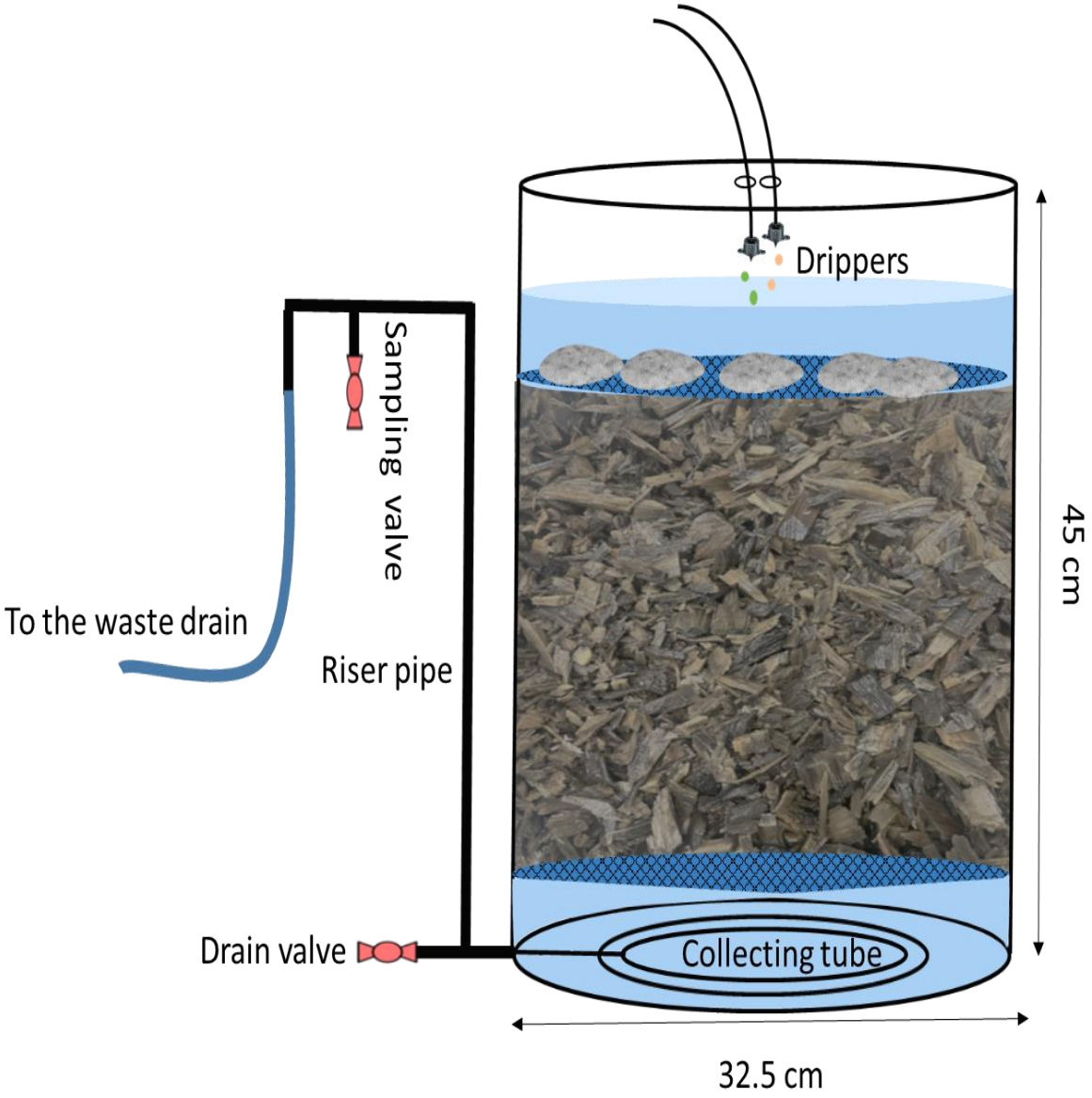
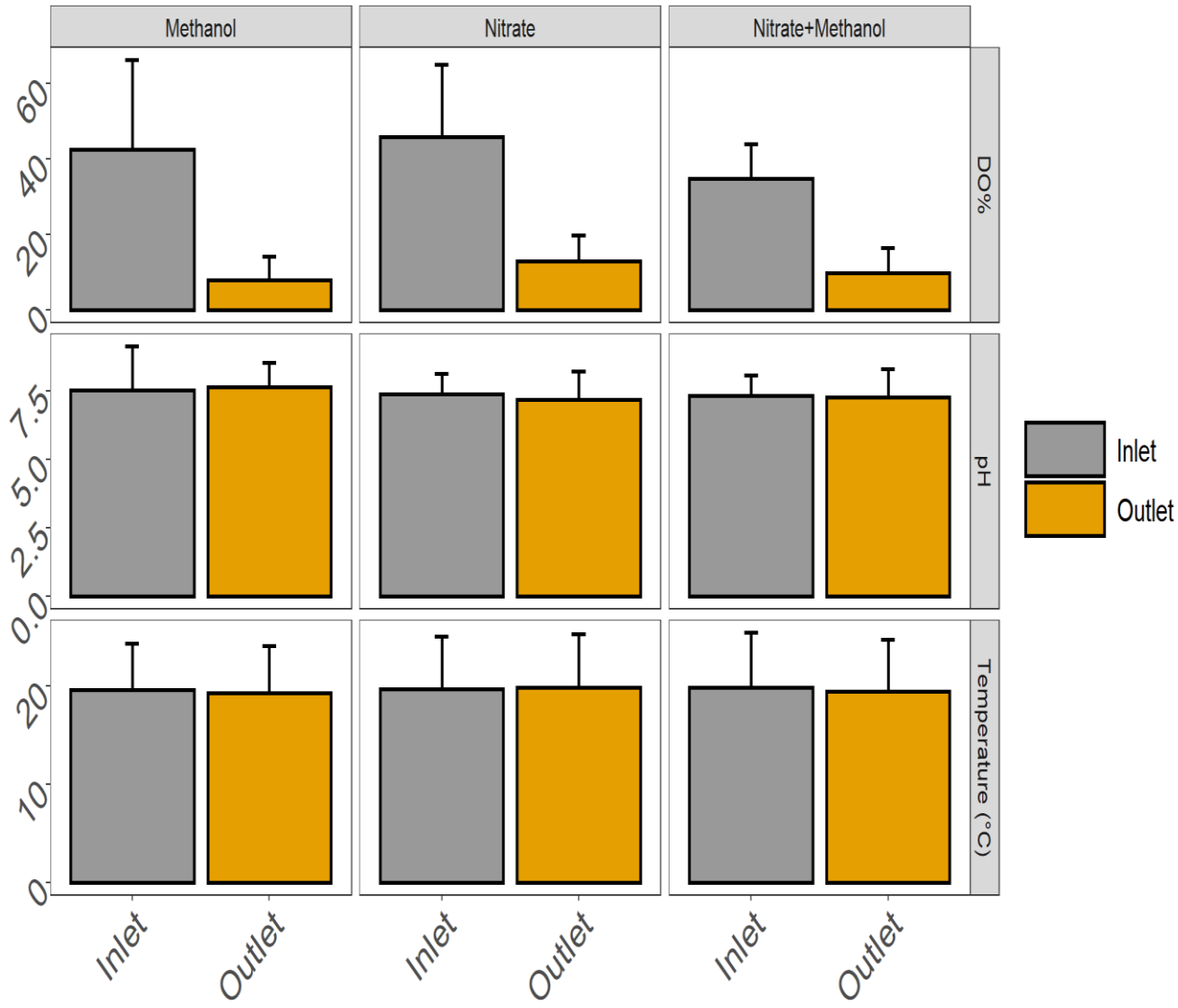
Supplementary material



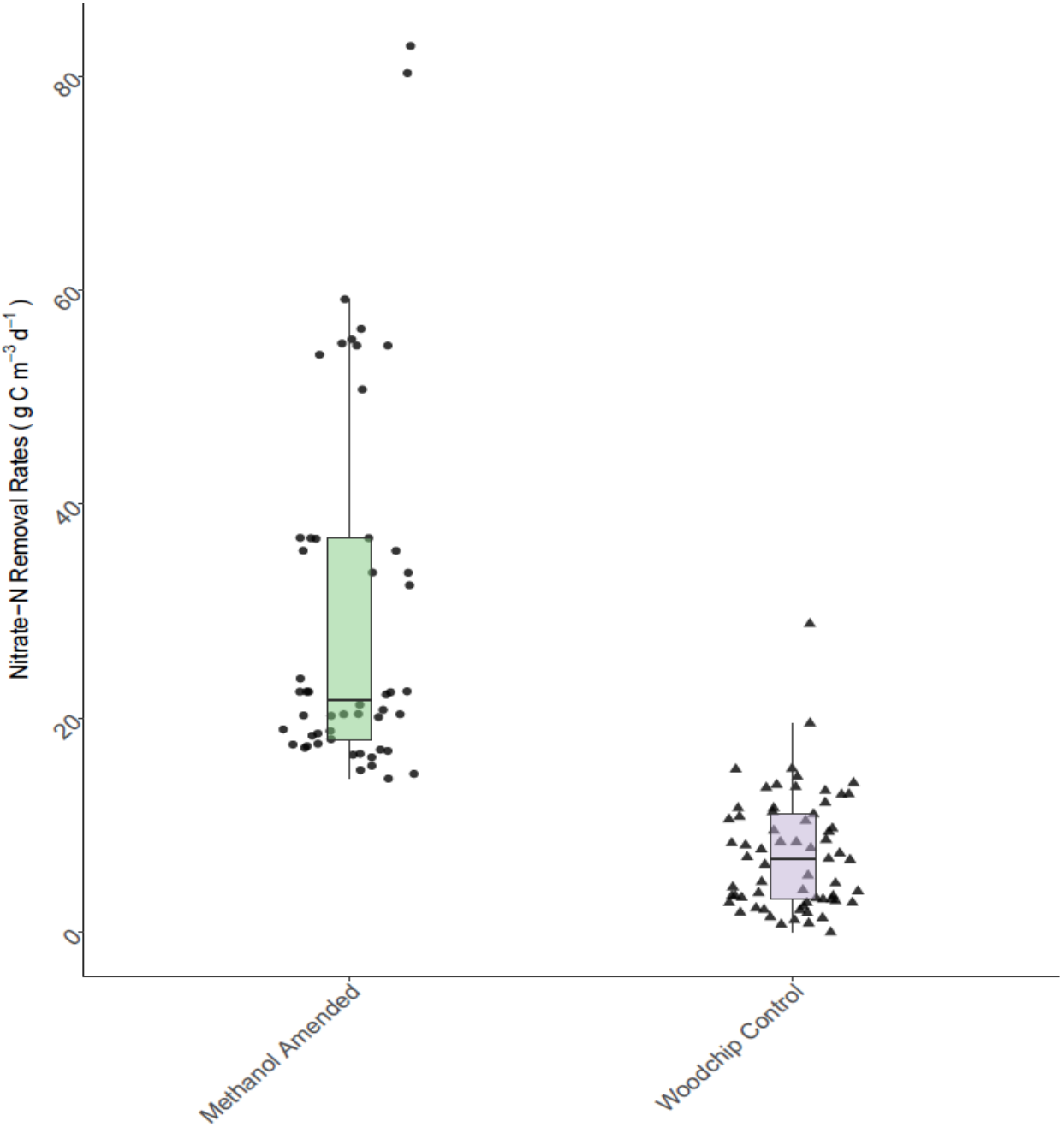
Supplementary figure 4-1 Process flow diagram displaying various process components



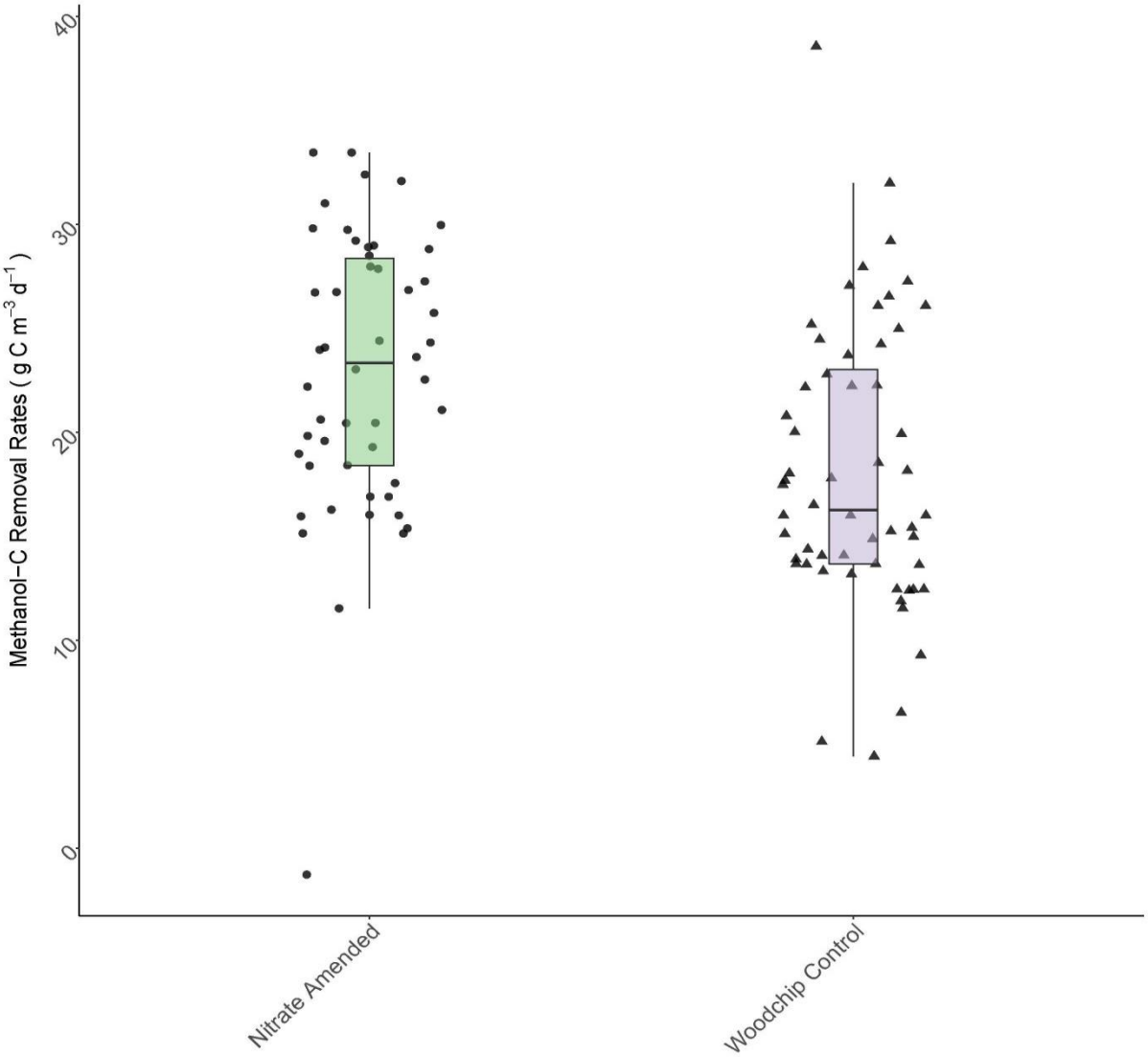
Supplementary figure 4-2: Bioreactor design



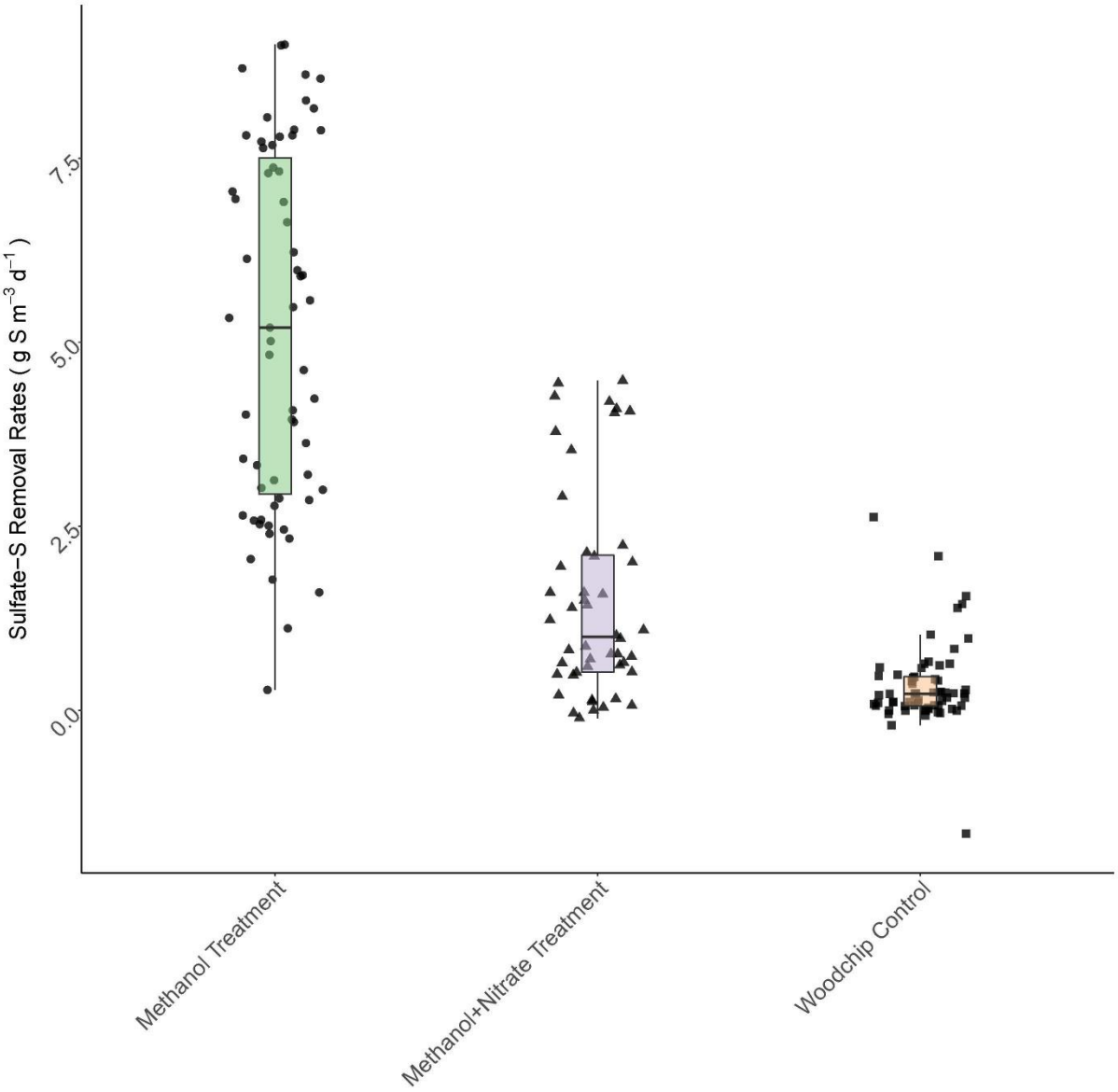
Supplementary figure 4-3: Mean (with 95 percent confidence interval) of dissolved O_2 (percent of saturation at the observation temperature), pH, and temperature of different treatment bioreactors during the experiment (n=6)



Supplementary figure 4-4: Scattered boxplots depicting nitrate removal rates under two different treatments (BNM and BN)



Supplementary figure 4-5: Scattered boxplots depicting methanol removal rates under two different treatments (BNM and BM)



Supplementary figure 4-6: Scattered boxplots depicting sulfate removal rates under three different treatments (BN, BNM, and BM)

Supplementary Table 4-1 ANOVA results comparing the effects of each treatment (BNM and BN) on nitrate removal rates

Date	Treatment 1	Treatment 2	n1	n2	adjusted P-value
9/02/2021	BNM	BN	3	4	<0.01
17/02/2021	BNM	BN	3	4	<0.01
26/02/2021	BNM	BN	3	4	<0.01
8/03/2021	BNM	BN	3	4	<0.01
17/03/2021	BNM	BN	3	4	<0.01
22/04/2021	BNM	BN	3	4	<0.01
12/05/2021	BNM	BN	3	3	<0.01
31/05/2021	BNM	BN	3	4	<0.01
21/07/2021	BNM	BN	3	4	<0.01
11/08/2021	BNM	BN	3	4	<0.01
19/09/2021	BNM	BN	3	4	<0.01
19/11/2021	BNM	BN	3	4	<0.01
15/12/2021	BNM	BN	3	3	<0.01
9/01/2022	BNM	BN	3	3	<0.01
12/02/2022	BNM	BN	4	4	<0.01
28/02/2022	BNM	BN	3	4	<0.01

Supplementary Table 4-2: ANOVA results assessing the effect of time on different treatments (BNM and BN) nitrate removal rates

Treatment	**DFn***	**DFd***	**F-value**	**P-value**	**ges***
BNM	15	33	22.177	<0.001	0.91
BN	15	45	22.022	<0.001	0.88

*DFn: degrees of freedom in the numerator, DFd: degrees of freedom in the denominators, and ges: generalized Eta-Squared (η^2) measure of effect size

Supplementary Table 4-3 ANOVA results comparing the effect of each treatment treatments (BM, BNM, and BN) on sulfate removal rates

Date	Treatment 1	Treatment 2	n1	n2	adjusted P-value
9/02/2021	BM	BNM	4	3	<0.01
9/02/2021	BM	BN	4	4	<0.01
9/02/2021	BNM	BN	3	4	<0.01
17/02/2021	BM	BNM	4	3	<0.01
17/02/2021	BM	BN	4	4	<0.01
17/02/2021	BNM	BN	3	4	<0.01
26/02/2021	BM	BNM	4	3	<0.01
26/02/2021	BM	BN	4	4	<0.01
26/02/2021	BNM	BN	3	4	<0.01
8/03/2021	BM	BNM	4	3	<0.01
8/03/2021	BM	BN	4	4	<0.01
8/03/2021	BNM	BN	3	4	<0.01
17/03/2021	BM	BNM	4	3	<0.01
17/03/2021	BM	BN	4	4	<0.01
17/03/2021	BNM	BN	3	4	<0.01
22/04/2021	BM	BNM	4	3	<0.01

Supplementary Table 4-4: ANOVA results assessing the effect of time on three different treatments (BM, BNM, and BN) on sulfate removal rates

Treatment	DFn*	DFd*	F-value	P-value	ges*
BM	15	47	4.427	<0.001	0.586
BNM	15	32	25.999	<0.001	0.924
BN	15	47	23.408	<0.001	0.882

*DFn: degrees of freedom in the numerator, DFd: degrees of freedom in the denominators, and ges: generalized Eta-Squared (η^2) measure of effect size

Supplementary Table 4-5 ANOVA results assessing the effects of two different treatments (BNM and BM) on methanol removal rates

Date	Treatment 1	Treatment 2	n1	n2	adjusted P-value
2021-02-09	BNM	BN	4	4	0.0663
2021-02-17	BNM	BN	4	4	0.245
2021-02-26	BNM	BN	4	4	0.0377
2021-03-08	BNM	BN	4	4	0.436
2021-03-17	BNM	BN	4	4	0.286
2021-04-24	BNM	BN	4	4	0.492
2021-05-12	BNM	BN	4	4	0.00469
2021-05-31	BNM	BN	4	4	0.0212
2021-08-11	BNM	BN	3	4	0.00835
2021-09-20	BNM	BN	3	4	0.0509
2021-11-01	BNM	BN	3	4	0.0509
2021-11-19	BNM	BN	3	4	0.652
2021-12-15	BNM	BN	3	4	0.00354
2022-02-12	BNM	BN	3	4	0.322
2022-02-28	BNM	BN	3	4	0.00448
2021-02-09	BNM	BN	4	4	0.0663

Supplementary Table 4-6: Summary ANOVA results assessing the effect of time on outflow methanol concentrations

Treatment	DFn*	DFd*	F-value	P-value	ges*
BNM	12	34	5.787	<0.001	0.671
BN	12	39	6.791	<0.001	0.676

*DFn: degrees of freedom in the numerator, DFd: degrees of freedom in the denominators, and ges: generalized Eta-Squared (η^2) measure of effect size

Chapter 5

Flow analysis and hydraulic performance of denitrifying bioreactors under different carbon dosing treatments

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Highlights

- Hydraulic impacts of C dosing were assessed at mesocosm and pilot-scale bioreactors
- Carbon dosing had no significant effect on the hydraulics of bioreactors
- Carbon dosing did not cause hydraulic failure in bioreactors

Abstract

Denitrifying bioreactors are an effective approach for removing nitrate from a variety of non-point wastewater sources, including agricultural tile drainage. However, compared to alternate mitigation approaches such as constructed wetlands, nitrate removal in bioreactors may decline with time and low temperature, resulting in poor

long-term nitrate removal rates. To address the low nitrate removal rates in bioreactors, the addition of an external carbon source has been found to be an effective method for enhancing and maintaining nitrate removal rates. While carbon dosing has led to a significant improvement in nitrate removal, some of the possible adverse effects of carbon dosing, such as clogging and reduction in hydraulic efficiency, remain unknown and need to be investigated. Using observations from both field and mesocosm trials, we compared the hydraulic performance of bioreactors with and without carbon dosing. The pilot-scale field bioreactor (58 m^3 total woodchip volume, 25 m^3 saturated volume, referred to as field bioreactor in this work) treated drainage water from a paddock of a dairy farm. The bioreactor received an exogenous carbon dose of 8% methanol (v/v) at 10 mL min^{-1} and 5 mL min^{-1} in the 2020 and 2021 drainage seasons, respectively. The field bioreactor had a statistically higher hydraulic conductivity in 2018 when not carbon-dosed of 4601 m day^{-1}, reducing to 1600 m day^{-1} in 2021 which was the second year of carbon dosing. Field observations could not establish whether the addition of liquid carbon could affect the bioreactor's internal hydraulics performance, such as actual hydraulic retention time (AHRT), despite a significant decline in hydraulic conductivity in the field bioreactor. Separate experiments on replicated bioreactor mesocosms were conducted to investigate the effects of carbon dosing on the internal hydraulic parameters of bioreactors. These mesocosm bioreactors had previously been used to study the long-term effects of methanol dosing on bioreactor performance, such as nitrate removal under steady-state conditions. The mesocosm and field bioreactors shared some characteristics, such as the use of methanol as an external carbon source, but the mesocosm experiments were hydrologically controlled contrary to the field bioreactor's transient operating conditions. We found that methanol dosing in either carbon or nitrate limiting conditions had no significant effects (p-value > 0.05) on internal hydraulic

parameters (e.g., effective utilization of media) when compared to control bioreactors. The present study offers insight into the long-term hydraulic performance of bioreactors and may help develop small-footprint bioreactors that incorporate external carbon dosing.

5.1 Introduction

Various mitigation approaches, including denitrifying bioreactors and constructed wetlands, have been developed to address diffuse nitrate pollution from intensive agricultural activities (Basu et al., 2022; Bowles et al., 2018; Carstensen et al., 2020; Christianson et al., 2013b). Denitrifying bioreactors are passive treatment methods that include a trench filled with a solid carbon substrate that offers a favorable environment for the growth of denitrifying bacteria that remove nitrate from wastewater (Addy et al., 2016; Schipper et al., 2010; Warneke et al., 2011a). Because of their availability and low cost, woodchips are often employed as solid carbon sources in bioreactors (Cameron and Schipper, 2010; Schipper et al., 2010; Warneke et al., 2011a).

Most of the nitrogen removal in bioreactors is accomplished through heterotrophic denitrification, which is dependent on the availability of dissolved organic carbon (DOC) from woodchips in the water matrix (Cameron and Schipper, 2010; Rivas et al., 2020; Schipper et al., 2010; Warneke et al., 2011a). However, denitrifier bacteria feed on labile carbon, which is limited in bioreactors to slow microbial hydrolysis and fermentation of woody materials (Nordström and Herbert, 2019). The availability of labile carbon in bioreactors may be high in the early months of operation but gradually declines, potentially limiting nitrate removal (Addy et al., 2016; David et al., 2016; Rambags et al., 2019; Rivas et al., 2020). Addy et al. (2016) conducted a meta-analysis of different bioreactors' nitrate removal rates based on peer-reviewed studies, finding

mean nitrate removal rates of 9.2 g N m^{-3} day^{-1} in the first 13 months of operation (n = 16) but dropping to 2.8 g N m^{-3} day^{-1} in the second year of operation (n = 13). Despite the fact that bioreactors are low-cost and easy-to-maintain nitrate mitigation methods, two major downsides of deploying bioreactors in catchments are relatively low long-term nitrate removal and media clogging (Addy et al., 2016; Cameron and Schipper, 2010; Christianson et al., 2016; Schipper et al., 2010). A few approaches, such as dosing the bioreactors with organic carbon compounds (Jansen et al., 2019; Roser et al., 2018), periodic emptying and resting (oxic-anoxic cycling) (Maxwell et al., 2019a; Maxwell et al., 2019b), and biochar addition (Ashoori et al., 2019; Hassanpour et al., 2020) have been trialed to compensate for the temporal reduction of DOC to maintain a higher consistent nitrate removal rate.

A few recent studies have shown that external carbon (e.g., methanol, ethanol, and acetate) dosing of bioreactors can significantly increase nitrate removal rates in bioreactors (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018). For instance, Roser et al. (2018) measured nitrate removal rates of 120.6 g N m^{-3} day^{-1} in acetate-dosed bioreactors. Under the same operating conditions, these enhanced nitrate removal rates contrasted with removal rates of only 7 g N m^{-3} day^{-1} without carbon dosing in 2018 (Roser et al., 2018).

While carbon dosing has been demonstrated to substantially increase nitrate removal rates, possible problems such as increased bioclogging, have received little consideration. Christianson et al. (2016) demonstrated a decrease in the flow capacity of bioreactors treating aquaculture effluent with relatively high biological oxygen demand (BOD) after 105 days. In Christianson et al. (2016), the elevated BOD in the influent was assumed to have acted as an external carbon source, enhancing the activity of heterotrophic microorganisms, but also causing progressive bioclogging.

Bioreactors are essentially porous media that can clog due to a variety of physical and biological causes (Christianson et al., 2020; Christianson et al., 2016; Ma et al., 2021; Seki et al., 1998). Woodchip consolidation (also known as woodchip subsidence) and sedimentation are regarded as the primary causes of physical blockage in bioreactors with dynamic inflow hydraulics such as tile drainage (Christianson et al., 2016; Ma et al., 2021). Microbial biomass and biofilm formation in bioreactors (such as bacteria and fungus) and their extracellular metabolites, such as tightly bound extracellular polymeric substances (TB-EPS), are the most common causes of biological clogging (Christianson et al., 2016; Lepine et al., 2020; Ma et al., 2021). A combination of these factors would result in a progressive change in bioreactors hydraulics with time. This decrease in bioreactor effective volume and intrinsic permeability may eventually result in an increase in water height at the inlet structure of the bioreactors, potentially increasing bypass flow and reducing the nitrate removal efficiency (Christianson et al., 2013a; Christianson et al., 2016). In the absence of a by-pass structure, this increase in water height at the inlet structure may result in flooding of the bioreactors, and consequently backflow onto the upstream adjacent field (Christianson et al., 2013a; Christianson et al., 2016).

Carbon dosing may exacerbate biological clogging in bioreactors due to increased microbial activity, as some studies have demonstrated a faster change in bioreactor hydrology with increasing BOD inputs, such as Christianson et al. (2016). Furthermore, the reduction in bioreactor's hydraulic efficiency might be more pronounced in bioreactors located in agricultural catchments with transient water inputs. For instance, Christianson et al. (2016) demonstrated that the pulsed inflow of the bioreactors was a factor contributing to the reduction of hydraulic conductivity, as the pulsed flow pushed the woodchips away from the bioreactor's inlet resulting in downgradient compaction. Given that agricultural catchment bioreactors function in

flashy drainage waters, transient drainage may also contribute to a flow decline through the bioreactor.

In a field study, Moghaddam et al. (2023) demonstrated that dosing with methanol, as an external carbon source, was effective in increasing nitrate removal rates in a pilot-scale bioreactor. Enhanced seasonal (2020) nitrate removal rates of 8 g N m^{-3} day^{-1} were reported by Moghaddam et al. (2023) compared to 0.67-1.60 g N m^{-3} day^{-1} in the same bioreactor without carbon dosing, as reported by Rivas et al (2020). Moghaddam et al. (2023) also demonstrated added methanol removal efficiency greater than 90%, even under nitrate-limited conditions. However, they did not investigate the change in the hydrology of the bioreactor due to the dosing. The effective in-situ hydraulic conductivity is critical as a measure of the bioreactor's hydraulic capacity to capture and treat more drainage. For bioreactors without bypass options, hydraulic conductivity is also an important factor in minimizing backflow to the upstream vicinity (Cameron and Schipper, 2010; Christianson et al., 2010; Ghane et al., 2016; Ghane et al., 2019; Schipper et al., 2005).

Although analysing the in-situ change of hydraulic conductivity in bioreactors provides insight into flow capacity, internal hydraulic performance metrics such as actual hydraulic retention time (AHRT) have yet to be investigated for design purposes. The divergence of AHRT from nominal hydraulic retention time (NHRT) is critical for the proper sizing of bioreactors with an external carbon source. Using AHRT to determine optimal bioreactor volume reduces the likelihood of potential pollution swapping due to oversizing and lower nitrate removal efficiency due to under sizing of bioreactors.

Conservative tracer testing is a well-established approach for investigating internal bioreactors' hydraulics (Christianson et al., 2013a; Ghane et al., 2015; Schipper et al.,

2005) and is used to determine the AHRT and other hydraulic efficiency parameters. The retention time distribution (RTD) curves based on multiple tracer tests could also be used to determine hydraulic parameters to measure the extent of non-ideal flow through the bioreactors. The RTD parameters could also be used to assess the effects of carbon dosing on bioreactor performance under different carbon dosing regimens. The majority of research examining bioreactor's hydraulic performance has used conservative tracers (such as bromide and chloride) results to calculate AHRT in bioreactors (Cameron and Schipper, 2011; Christianson et al., 2013a; Ghane et al., 2015). However, this technique alone may not be thorough enough to assess the hydraulic capacity of the bioreactors to cover the very dynamic water inflows, regardless of internal performance, particularly for bioreactors placed in agricultural catchments (Christianson et al., 2013a; Rivas et al., 2020). In other words, even with adequate mean AHRT, there is a possibility of bypass or overflow in bioreactors with insufficient hydraulic conductivity, which might reduce the efficacy of total nitrate removal. Overall, determining both hydraulic conductivity and internal hydraulic performance may be a more realistic approach to designing more efficient bioreactors. Accordingly, the main objectives of the current study were to (i) evaluate the temporal evolution of hydraulic properties in a pilot-scale bioreactor during seasons with and without methanol dosing, and (ii) Conduct tracer tests in replicated mesocosm bioreactors to determine the effects of added carbon on bioreactor internal hydraulic performance.

5.2 Methodology

5.2.1 Field observations

The field observations were made in a full-scale bioreactor at Tatuanui, Waikato, New Zealand. The bioreactor had been in operation without external carbon dosing from 2017 to 2019 and was dosed with a constant flowrate of methanol (8% (v/v), 23.7 g C L^{-1}) at 10 mL min^{-1} in 2020 and 5 mL min^{-1} in 2021 (Moghaddam et al., 2023). The added methanol load was based on the C:N ratio of 1.4 which was designed to treat the nitrate loads that went untreated during the 2019 drainage season. A peristaltic pump transported methanol solution from a PVC drum (200 L) covered in a wooden casing. The pump was triggered when drainage into the bioreactor occurred and halted when outflow ended. Due to flow fluctuations, the C:N ratio varied between 342 and 1.48 in 2020 and 171 and .34 in 2021 (halved dosing rate). Under time-varying nitrate loads at the inlet, the bioreactor performance improved considerably from the mean removal rate of 1 g N m^{-3} day^{-1} reported by Rivas et al. (2020) in 2018 to mean removal rates of 8 and 5 g N m^{-3} day^{-1} with carbon dosing, in 2020 and 2021, respectively. The field bioreactor was also found to attenuate excess methanol even under nitrate-limiting conditions, reaching mean methanol removal rates of 110 g C m^{-3} day^{-1}. The bioreactor featured trapezoidal cross sections (in both width and length) with top and bottom width dimensions of 6 and 5 meters, respectively, 9 meters length and 1.2 meters depth. The bioreactor was isolated from the surrounding subsoils by ethylene propylene diene monomer (EPDM, 1.1 mm Firestone GeoGard), and covered with excavated soil at the top (Figure 5-1). To keep the soil, backfill from mixing with the woodchips, a geotextile membrane (Bidim A14, 155 g m^{-2}) was laid on top of the woodchips. Untreated Monterey pine (*Pinus radiata*) woodchips with a median width of 8.6 mm and a thickness range of 0.8 to 13.1 mm were employed in the bioreactor

(Rivas et al., 2020). Based on limited field data on nitrate loads and a denitrification removal rate of 3.5 g N m^{-3} day^{-1}, the bioreactor was designed to remove about half of the seasonal nitrate input. The bioreactor's input flow rates and nitrate concentrations changed with time. Following each drainage season, the water in the bioreactor was pumped out to keep the woodchip media dry until the following drainage season. The field bioreactor is described in detail in Rivas et al. (2020).

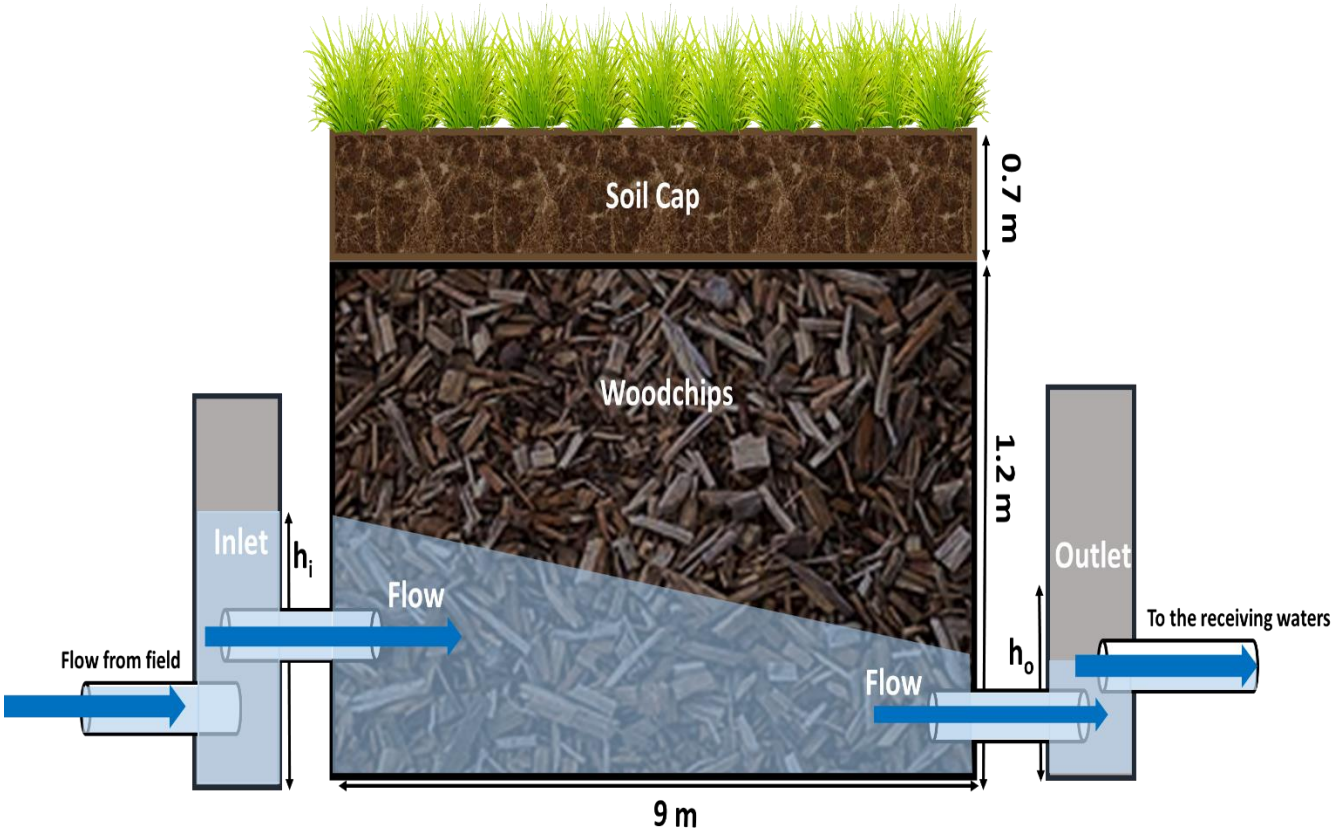

Figure 5-1 Schematic of the field bioreactor depicting the inlet and outlet structures, flow path, and bioreactor dimensions. The bottom of the bioreactor was 5 m wide.

5.2.2 Water depth and flowrates measurements

Two control structures were installed at the bioreactor's entrance and exit to route the flow direction and were used to measure the depth of the inflow and outflow water in order to calculate flow rates. During the drainage seasons, the bypass structure of the field bioreactor was shut down, and all drainage water was routed to the bioreactor's distributor. Water level dataloggers (Model 3001 Levelogger Junior Edge F15/M5)

were used every five minutes to measure the head of water above the V-notch at the inlet and outlet structures. The flow rate was calculated using the equation below (Maxwell et al., 2020):

$$Q = 0.5681 \times (H + 0.001621)^{2.5} \quad \text{Equation 5-1}$$

where Q was the flow rate in $m^3\ s^{-1}$ and H was water height (m) above the 45° V-notch weir.

2.1.2 Flow regime estimation

The Reynolds number was calculated as an indicator of the flow regime (laminar or turbulent) through the bioreactor porous medium (Sobieski and Trykozko, 2014):

$$Re = \frac{\rho q d}{\mu} \quad \text{Equation 5-2}$$

ρ was the water density (kg m^{-3}), q was the specific discharge (m day^{-1}), d was the size of the woodchips (m), and μ was the water's dynamic viscosity coefficient (kg $m^{-1}\ s^{-1}$). The Reynolds number was calculated using the average d_{50} of 10 mm from the particle size characterization of the woodchips utilized in the field bioreactor. Reynolds numbers under ten typically indicate laminar flow regimes, which suggests that Darcy's equation applies to the flow through the porous media (Sobieski and Trykozko, 2014).

5.2.3 Non-Darcy flow coefficients

We used Darcy's equation for the trapezoidal beds driven by Ghane et al. (2014) for hydraulic conductivity calculations based on the experimental data:

$$Q = K_D \times \left(\frac{b(h_i^2 - h_o^2)}{2L_B} + \frac{z(h_i^3 - h_o^3)}{3L_B} \right) \quad \text{Equation 5-3}$$

where Q was the flow rate through the bioreactor ($m^3\ day^{-1}$), h_i and h_o were the heights of drainage water at the inlet and outlet structures (m), respectively, L was the length

of the bed (m), b was the bioreactor's bottom width, K was the hydraulic conductivity (m day^{-1}), and z was the bioreactor's side slope.

The specific discharge from the bioreactor was determined using the flow through the bioreactor divided by the bioreactor's cross-sectional area:

$$q = \frac{Q}{A} \quad \text{Equation 5-4}$$

Where q was the specific discharge (m day^{-1}), Q was the flow rate through the bioreactor (m^3 day^{-1}), and A was the bioreactor's cross-sectional area (m^2). The hydraulic gradient was calculated by dividing the matching difference in water height at the inlet and outlet structures at the same time by the bioreactor's length:

$$i = \frac{\Delta h}{L} \quad \text{Equation 5-5}$$

Where i was the hydraulic gradient (m m^{-1}), Δy was the corresponding difference in water height at the inlet and outlet structures (m), and L was the length of the bioreactor (m).

5.2.4 Forchheimer's coefficients

Darcy's law was extended to nonlinear flows using the Forchheimer equation to determine whether non-laminar flow in the field bioreactor was significant during high flow events during the drainage seasons. The Forchheimer coefficients were calculated by fitting the hydraulic gradient as a function of the specific discharge for the seasons of 2018 (no carbon dosing), 2020 (higher carbon dosing rate), and 2021 (lower carbon dosing rate)

$$i = \frac{1}{K_f} q + \alpha q^2 \quad \text{Equation 5-6}$$

Where i denotes hydraulic gradient (m m^{-1}), q was specific discharge (m day^{-1}), K_f was post-linear hydraulic conductivity (also known as Forchheimer hydraulic

conductivity) (m day^{-1}), and α was Forchheimer's coefficient (day^2 m^{-2}). When α approaches zero, the quadratic flow equation reduces to the Darcy equation, suggesting laminar flow through the bioreactor.

5.2.5 Mesocosm experiment

5.2.5.1 Experimental design

The nine mesocosm bioreactors (26 L saturated woodchips; Monterey pine (*Pinus radiata*)) used in the tracer test were the same as those used by Moghaddam et al. (2022). Supplementary figure 1 depicts the process flow diagram for the mesocosm experiment. The bioreactors were gravity and downflow fed (Moghaddam et al., 2022). The mesocosm bioreactors had been in operation for 18 months. Continuous doses of methanol, nitrate, and sulfate were administered to the bioreactors to create three experimental treatments. The three different treatments were: the "BN treatment" for nitrate-fed bioreactors (carbon limiting), the "BM treatment" for methanol bioreactors (nitrate limiting), and the "BNM treatment" for methanol + nitrate-fed bioreactors. All treatments received sulfate, present in the tap water. The dosed concentrations were nitrate (80 mg NO_3^--N L^{-1}), methanol (60 mg CH_3OH-C L^{-1}), and sulfate (8 mg SO_4^{2-}-S L^{-1}). Methanol dosage was demonstrated to be effective in increasing nitrate removal rates from 7 g N m^{-3} day^{-1} in BN treatment bioreactors to 60 g N m^{-3} day^{-1} in BNM bioreactors. Outlet nitrate concentrations in BNM and BN treatment bioreactors were above non-limiting ranges (> 10 mg NO_3^--N L^{-1}, Kouanda and Hua (2021)) with significant losses of methanol at the outlet.

5.2.5.2 Conservative Tracer Test

The tracer test used an 8 mL instantaneous slug of NaCl (80 g L^{-1}) poured into the inlet head of each bioreactor. The bioreactor heads were then gently hand-mixed with a

stainless-steel laboratory spatula to provide a uniform tracer concentration at the inlet head. Electrical conductivity (EC) was recorded in real-time at the bioreactor's outflow with a YSI Procurator Multiparameter sensor (YSI Inc, Ohio, United States). The tracer test RTD curves were used to determine AHRT, hydraulic efficiency (λ), and the theoretical number of continuous stirred-tank reactors (CSTRs) in series (N).

5.2.5.3 Actual hydraulic retention time

The conservative tracer's mean retention time (actual hydraulic retention time: AHRT) in the mesocosm bioreactors was calculated as follows (Tchobanoglus et al., 2003):

$$AHRT = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i} \qquad \text{Equation 5-7}$$

t_i and C_i were the time and tracer concentration of the i^{th} measurement, respectively, and Δt_i was the time interval between measurements.

5.2.5.4 Effective volume ratio

The effective volume ratios in mesocosm bioreactors were calculated by combining the AHRT with the nominal (theoretical) hydraulic retention time (derived from bioreactors drainable porosity (49%), flowrate (1.05 L h^{-1}), and saturation volume (26 L)) as follows:

$$e = \frac{AHRT}{THRT} \qquad \text{Equation 5-8}$$

The drainable porosity of the mesocosm bioreactors was calculated by dividing the volume of water drained out by the volume of woodchips in the bioreactors.

Short-circuiting in the bioreactors was suggested by e < 1, whereas plug flow through the medium was suggested by e = 1.

5.2.5.5 Tanks-in-series model

The theoretical number of CSTRs in the tanks-in-series model (N) was used to determine the extent of ideal (plug) flow and/or mixed flow regimes through the mesocosm bioreactors (Holland et al., 2004; Persson, 2000; Persson et al., 1999; Persson and Wittgren, 2003):

$$N = \frac{(AHRT)^2}{\sigma^2} \qquad \text{Equation 5-9}$$

Where N was the number of tanks in the tanks-in-series model and σ^2 was the RTD curve's variance (h^2). The variance of the RTD curve σ^2 indicated how much the RTD curve deviated from the AHRT (centroid of the RTD curve) and was computed as follows (Guo et al., 2017; Persson, 2000; Persson and Wittgren, 2003):

$$\sigma^2 = \sum_{i=1}^{n-1} (t_i - AHRT)^2 \times \frac{A_i}{A} \qquad \text{Equation 5-10}$$

where i is the time step at each observation point, n represents the total number of observations, A_i represents the area contained by the RTD of two consecutive observations, and A represents the overall area of the RTD curve.

5.2.5.6 Hydraulic efficiency

Two hydraulic efficiency measures, λ_e (hydraulic efficiency based on effective volume) and λ_P (hydraulic efficiency based on peak time), are frequently used to measure the hydraulic efficiency of porous media, including bioreactors. The hydraulic efficiency based on effective volume (λ_e) was calculated as follows (Guo et al., 2017; Holland et al., 2004; Persson et al., 1999; Persson and Wittgren, 2003):

$$\lambda_e = e(1 - \frac{1}{N}) \qquad \text{Equation 5-11}$$

where λ_e was hydraulic efficiency based on effective volume (ideally close to 1), e was the effective volume ratio, and N was the number of tanks in the tanks-in-series model.

The hydraulic efficiency based on peak time (λ_P, ideally close to 1) was estimated using the equation below (Guo et al., 2017):

$$\lambda_{\mathrm{p}} = \frac{t_p}{t_n} \qquad \text{Equation 5-12}$$

where λ_P denoted hydraulic efficiency based on peak time, t_p denoted the time corresponding to the greatest RTD value, and t_n denoted the theoretical HRT.

5.2.6 Statistical Analysis and Modelling

The R programming language, as well as the R external packages Tidyverse (Wickham et al., 2019) and Rstatix (Kassambara, 2020), were used for all statistical analyses, data modelling, and visualizations. The Shapiro-Wilk normality tests were employed to determine whether the residuals of the data were normally distributed. We also conducted the Q-Q (quantile-quantile) plot to visually assess the assumption of data normality (results not shown). We utilized the student T-test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data.

5.3 Results

5.3.1 Flow regimes

The mean Reynolds number in the field bioreactor ranged from 0.063 to 0.67 during different drainage seasons, indicating laminar flow (Ghane et al., 2014; Ghane et al., 2016)(Table 5-1).

Table 5-1 Reynolds numbers of flow through the field bioreactor during three drainage seasons

Drainage season	Mean RN*	RN 95% CI*
2018	0.123	[0.122, 0.125]
2020	0.65	[0.637, 0.665]
2021	0.065	[0.063, 0.067]

*RN: Reynolds number, CI: confidence interval

5.3.2 Forchheimer coefficient

Forchheimer's coefficient (α) of the field bioreactor ranged from 9.4×10^{-6} to 5.3×10^{-5} ($day^2\ m^{-2}$), suggesting that linear flow occurred in the bioreactor throughout all the drainage seasons, including both drainage seasons with methanol dosage (Ghane et al., 2014; Ghane et al., 2016; Sobieski and Trykozko, 2014) (Table 5-2). Across drainage seasons, the bioreactor's Forchheimer hydraulic conductivity ranged between 1298 and 4519 m day^{-1} (Table 5-2).

Table 5-2 Cumulative flow, Forchheimer coefficients, and RSE for the bioreactor during three drainage seasons.

Drainage season	Cumulative flow (m^3)	*α	*K_f	*RSE
2018	909	8.8×10^{-6}	3414	1.7×10^{-5}
2020	130	9.4×10^{-6}	4519	6.9×10^{-5}
2021	235	5.3×10^{-5}	1298	6.6×10^{-5}

*α: Forchheimer's coefficient ($day^2\ m^{-2}$) K_f: Forchheimer hydraulic conductivity (m day^{-1}), RSE: Residual standard error

5.3.3 Darcy hydraulic conductivity

Specific discharge through the field bioreactor ranged from 0 to 15 m day^{-1} in 2018 (non-dosed year). With methanol dosing, specific discharge through the bioreactor ranged from 0 to 25 m day^{-1} in 2020, and from 0 to 12 m day^{-1} in 2021.

The mean hydraulic conductivity of the field bioreactor was 4518 m day^{-1} for the 2018 drainage season, 3079 m day^{-1} for 2020, and 1567 m day^{-1} for 2021 (Table 5-3 and Figure 5-2). This represented a 31% reduction in bioreactor hydraulic conductivity from 2018 to 2020 and a 49% reduction from 2018 to 2021. Across drainage seasons, a Wilcoxon signed-rank test demonstrated a statistically significant (P-value < 0.01) decline in hydraulic conductivity between, with, and without carbon dosing. There were no undosed circumstances in the 2020 and 2021 drainage seasons to separate out the methanol contribution to the field bioreactor's decreasing hydraulic conductivity.

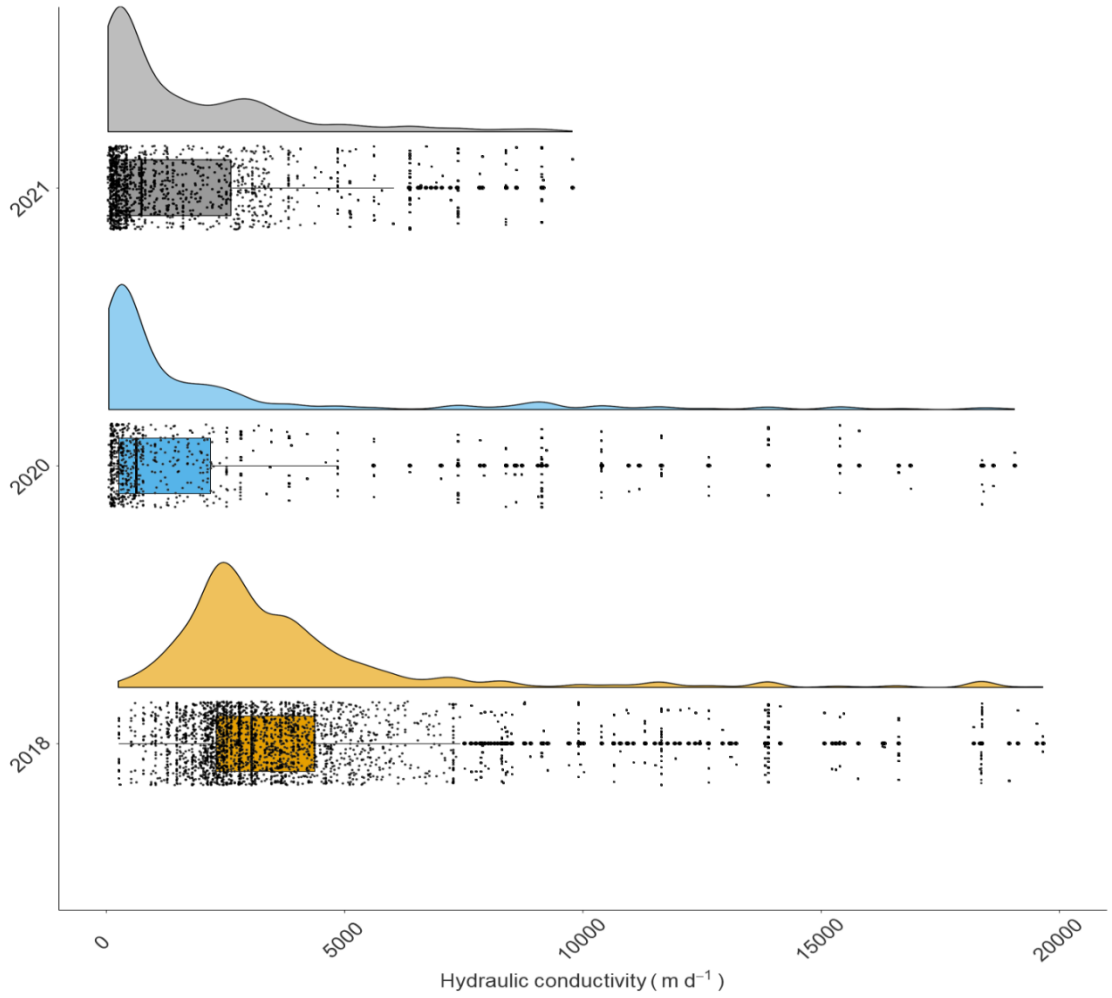

Figure 5-2 Split half violins, scatter plots, and boxplots depicting the distribution of hydraulic conductivity in the field bioreactor over three drainage seasons. The scatters are 5-minute measurements of the bioreactor's hydraulic conductivity. The split-half violins represent the hydraulic conductivity kernel density.

Table 5-3 Hydraulic conductivity of the field bioreactor during three drainage seasons

Drainage season	Mean HC*	HC 95% CI*	SE*
2018	4518	[4462, 4574]	28.5
2020	3079	[2943, 3215]	69.3
2021	1567	[1536, 1597]	15.5

*HC: hydraulic conductivity (m day⁻¹), CI: confidence interval, and SE: Standard error

5.3.4 Tracer tests

The mean and standard error (SE) of the hydraulic parameters obtained from the mesocosm tracer tests (Figure 5-3) are presented in Table 5-4. For example, the BN treatment bioreactors showed the longest AHRT of 13.2 h in comparison to the BNM treatment's 10 h, which had the shortest AHRT. In comparison to the 17.2 L of the BNM treatment, which displayed the lowest effective volume utilization, the BN treatment bioreactors had the highest effective volume utilization of 23.1. Statistical analysis of treatment effects on any hydraulic parameters in BN, BM, and BNM treatment bioreactors revealed no statistically significant differences (P-value > 0.1) (Table 5-4).

Table 5-4 Mean and standard error of hydraulic indicators in BM, BN, and BNM treatment bioreactors and Statistical comparisons of the hydraulic indicators in BN, BNM, and BM treatment bioreactors. The data of the BM and BNM treatments were merged to form the BMM treatment, which included observations of methanol-dosed bioreactors in the presence and absence of nitrate.

Treatment(s)	AHRT (h)		Effective volume (L)		λ_p		λ_e		N		Morrill Dispersion Index (t_{90}/t_{10})	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
BM	12.4	1.7	19.9	2.3	0.67	.2	.79	.05	2.5	.05	4.5	.35
BN	13.2	1.8	23.1	1.7	.56	.23	.86	.01	2.7	.9	6.8	2.1
BNM	10	2.3	17.2	3.3	.48	.1	.65	.14	4.8	1.6	4.2	1.23
BMM	11.22	1.3	18.7	1.8	.56	.1	.77	.07	3.9	1.04	4.3	0.57
	P-value											
BM/BN	0.77		0.31		0.74		0.58		0.87		1	
BM/BNM	0.4		0.55		0.45		0.46		0.29		0.85	
BN/BNM	0.3		0.21		0.76		0.31		0.32		0.36	
BN/BMM	0.42		0.12		0.96		0.23		0.4		0.38	

*BNM: methanol + sulfate + nitrate fed bioreactors (nitrate prevailing); BM: methanol + sulfate fed bioreactors (nitrate limiting); BN: sulfate + nitrate fed bioreactors; BMM: methanol fed bioreactors (observations from both BN and BNM treatment bioreactors)

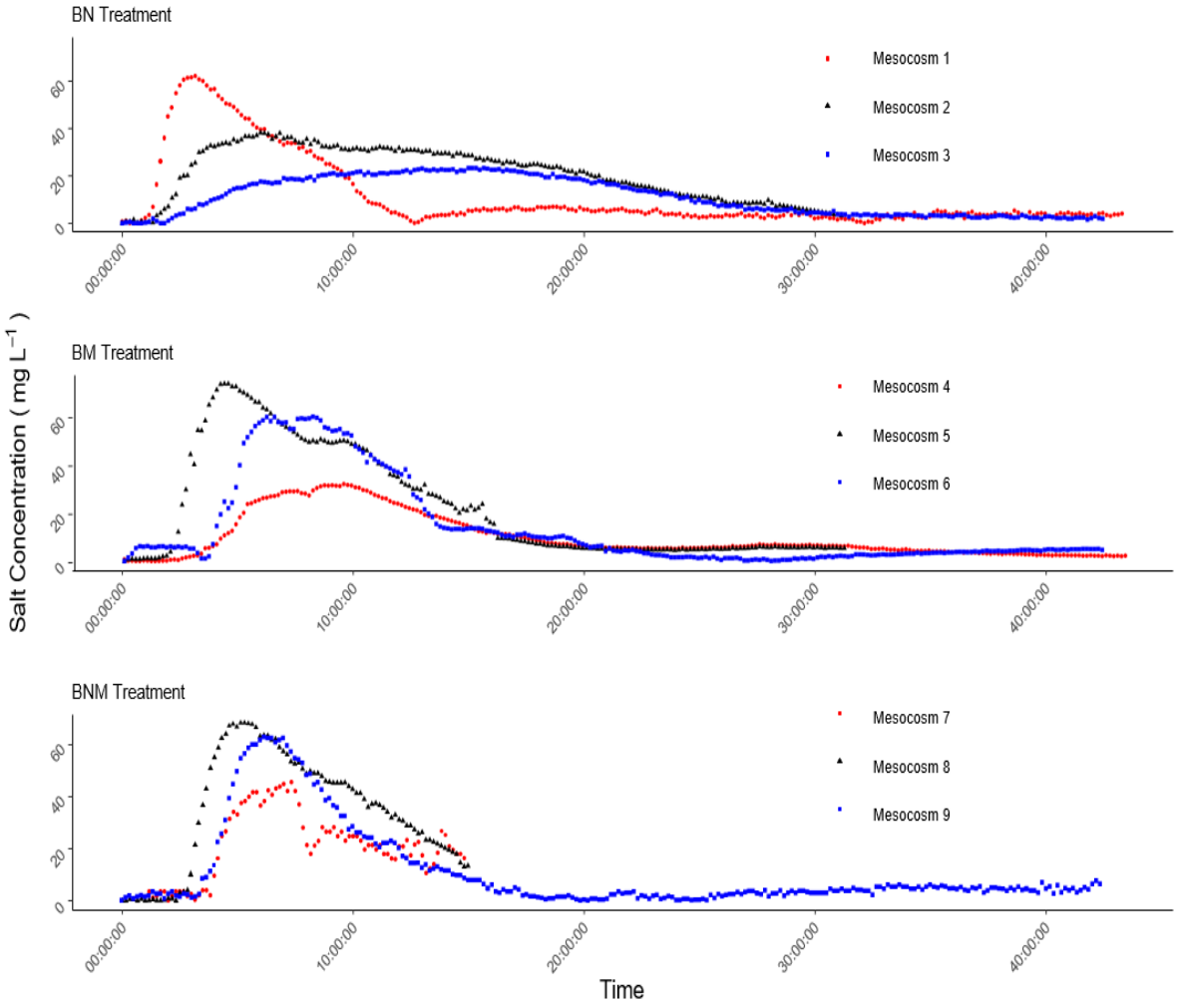

Figure 5-3 Tracer curves for the different treatment mesocosm bioreactors. "BN treatment" was nitrate fed (80 mg NO_3^--N L^{-1}) bioreactors (carbon limiting), and the "BM treatment" was methanol fed (60 mg CH_3OH-C L^{-1}) bioreactors (nitrate limiting), and the "BNM treatment" for methanol + nitrate fed bioreactors. All bioreactors were dosed with sulfate, which was already present in the tap water (8 mg SO_4^{2-}-S L^{-1}). The replications of each treatment are distinct bioreactors that were subjected to the same treatment.

5.4 Discussion

5.4.1 Hydraulic conductivity

Bioreactors, as porous mediums, are prone to clogging over time for physical and biological reasons (Christianson et al., 2013a; Christianson et al., 2020; Christianson et al., 2016; Lepine et al., 2020; Ma et al., 2021). The hydraulic performance of the bioreactors may become an even more important characteristic in carbon-dosed bioreactors, as some evidence suggests that increased microbial activity due to increased available carbon in the bioreactor input result in a more rapid change in the hydraulic performance of the bioreactors (e.g., Christianson et al. (2016)).

In our study, the mean hydraulic conductivity of the aged woodchips in the field bioreactor (i.e., 2018, 2 years of operation) was 4518 m day^{-1} 95% CI [4462 m day^{-1}, 4574 m day^{-1}] (Table 5-3 and Figure 5-2). The hydraulic conductivity of the field bioreactor in 2018 was within the range reported by Ghane et al. (2016) of between 9590 to 2764 m day^{-1} conducting a permeability test on aged woodchips. Ghane et al. (2016) assumed nonlaminar flow in the bioreactors and calculated hydraulic conductivity using the Forchheimer equation, by fitting a quadratic flow regime that included inertial and viscous forces. In our work, we evaluated the Forchheimer hydraulic conductivity of different drainage seasons using real-time field data (inlet and outlet water levels, matching outflow rates, and bioreactor dimensions) in comparison to Ghane et al. (2016), who conducted their analysis under controlled laboratory permeameter conditions. In our study, the Forchheimer coefficient (indicative of nonlinear flow) was low (2018: 8.8×10^{-6} (day^2 m^{-2})), resulting in hydraulic conductivity close to Darcy's estimation (for instance, Darcy's hydraulic conductivity in 2018 was 4518 m day^{-1} compared to Forchheimer's hydraulic

conductivity of 3414 m day^{-1}). The difference in Darcy's conductivity and Forchheimer's conductivity in our study could be explained by the fact that Forchheimer's conductivity was derived by fitting a quadratic flow equation to the entire flow data to estimate the equation parameters, whereas Darcy's conductivity was applied to each measurement of flow through the bioreactor, averaging conductivity across the whole drainage season, resulting in more confidence in the estimations. The low Reynolds numbers (< 2) of the bioreactor across different drainage seasons also suggested laminar viscous flow with no need to account for the turbulent flow interactions. The field observations in our study were conducted in a highly flashy characteristic of the inflow, which may have influenced the estimation of Reynolds numbers and hydraulic conductivity.

Overall, using Darcy's law to model hydraulic conductivity in bioreactors yielded satisfactory results in our bioreactor, without needing to take into consideration non-linear flow interactions. Under carbon dosing, the bioreactor had a mean hydraulic conductivities of 3079 m day^{-1} 95% CI [2943 m day^{-1}, 3215 m day^{-1}] and 1567 m day^{-1} 95% CI [1536 m day^{-1}, 1597 m day^{-1}] (Table 5-3 and Figure 5-2) in 2020 (higher methanol dosing rate) and 2021 (lower methanol dosing rate), respectively. The hydraulic conductivities of bioreactors under carbon dosing were within the range of the hydraulic conductivity of 2764 m day^{-1} reported by Ghane et al. (2016) and 252 to 4320 m day^{-1} reported by Robertson (2010). The hydraulic conductivities of the field bioreactor with methanol dosing were also consistent with a hydraulic conductivity of 2000–10,000 m day^{-1}, reported by Cameron and Schipper (2010), who investigated several solid substrates, including woodchips throughout a two-year experiment, and also reported a temporal decline in hydraulic conductivity. Cameron and Schipper (2010) attributed the decrease in hydraulic conductivity to gas bubbles (caused by denitrification, a biological element of clogging) and the tendency of woodchips to

settle on their flat surface (compaction, physical factors). While the hydraulic conductivity of the bioreactor showed a steady decline between drainage seasons (approximately 1000 m day^{-1} decline per drainage season), the similarity of the hydraulic conductivity of the methanol-dosed bioreactor with other non-dosed studies implies that methanol dosing at the relatively low rates utilized in the field bioreactor (Moghaddam et al., 2023) should not have a major impact on the bioreactors functioning and ability to accept hydraulic loads from the field. However, the observations were limited to relatively short drainage seasons, suggesting that longer trials are required.

Even though the effects of carbon dosing did not result in hydraulic failure or bypass or backflow from the pilot-scale bioreactor, the field observations did not address the impact of dosing on internal hydraulic parameters of the bioreactor, including AHRT, which would be relevant for design purposes. Non-ideal flow regimes in the full-scale bioreactors have been found in a few studies, including Christianson et al. (2013a) and Cameron and Schipper (2011), which contributed to the bioreactors' poor nitrate removal efficacy. The preferred hydraulic performance would be characterized as ideal flow and employing a greater volume of the bioreactor by inflow (Christianson et al., 2013a; Persson et al., 1999). Furthermore, the field observations with methanol dosing were conducted with relatively low added carbon inputs without a control experiment, making the precise contribution to the dropped hydraulic conductivity unclear. More research is also needed, particularly in the field, to determine whether higher dosing loads have a more important adverse impact on hydraulic performance.

5.4.2 Tracer tests

Inadequate design (bioreactor size, shape, and slope) and non-ideal positioning of input and outflow structures are two potential causes of non-ideal flow regimes in

bioreactors without external carbon dosing (Christianson et al., 2013a; Christianson et al., 2016; Lepine et al., 2016). Methanol dosage in bioreactors, on the other hand, may exacerbate this suboptimal hydraulic functioning by increasing microbial activity and subsequent EPS and biofilm production, as well as the formation and entrapment of gas bubbles due to accelerated denitrification.

Although we observed lower use of bioreactor media volume in methanol-dosed bioreactors in the presence and absence of nitrate, the differences were not statistically significant ($p > 0.05$). In the BNM treatment bioreactors, the mean λe (the hydraulic efficiency based on the effective volume ratio) was 0.48, which was lower than 0.56 of the BN treatment bioreactors. The lower effective use of bioreactor volume by flow in carbon-dosed bioreactors compared to the control highlighted the potential impact of external carbon dosing on bioreactor media utilization. Increased heterotrophic microbial activity because of the added carbon source could explain the difference in effective media utilization between the BNM mesocosm bioreactors and the BN treatment bioreactors. This was in agreement with the visible gas bubbles, presumably consisting of dinitrogen and carbon dioxide as gaseous by-products of accelerated denitrification, and might also have contributed to the altered hydrology (Supplementary video 1).

BNM treatment bioreactors, on the other hand, experienced more plug flow than BN and BM treatment bioreactors, according to N values (Table 5-4), which are indicators of the extent of plug flow regimes. For instance, the mean N in the BNM treatment bioreactors was 4.8, which was higher than 2.7 in the BN treatment bioreactors. The higher Ns of the BNM treatment, which served as a measure of the degree of plug flow, suggested that the dosage of methanol may have led to more plug and/or lower mixing flow regimes compared to the BN treatment.

Overall, methanol dosing resulted in a lower effective volume in the bioreactor media but more plug flow, demonstrating the contrasting effects of methanol dosing on internal hydraulic performance in terms of the extent of the plug flow and effective utilization of the bioreactor media. Overall, the altered hydraulic characteristics of BNM treatment bioreactors did not result in overflow or even an increase in the mesocosm bioreactor head, implying that the flow, nitrate, and methanol input loads tested in mesocosm bioreactors would not cause hydraulic failure.

5.4.3 Innovative design approach with carbon dosing

Most bioreactor designs have relied on volumetric nitrogen removal rates from solid carbon substrates to calculate the amount of media required to remove a certain load of nitrate (Addy et al., 2016; Christianson and Schipper, 2016; Schipper et al., 2010; Warneke et al., 2011a). The nitrogen removal rates calculated in several bioreactor studies have been primarily based on the assumption that the bioreactor was carbon-limited rather than nitrogen-limited (Addy et al., 2016; Cameron and Schipper, 2010; Christianson et al., 2021; Warneke et al., 2011b). The assumption of carbon limitation might be reasonable in bioreactors with aged woodchips and no exogenous carbon dosing. In our field trial, however, we demonstrated previously that methanol dosage resulted in rapid nitrogen removal in the early parts of the bioreactor, leaving a significant portion of the downstream sections unused for nitrogen removal (Moghaddam et al., 2023). Because of the improved nitrate removal in carbon-dosed bioreactors, assuming carbon-limited conditions for nitrogen removal rates may no longer be reasonable, and other environmental and operational parameters, such as temperature and hydraulic conductivity, might limit the efficacy of bioreactors. A gradual decline in the hydraulic conductivity of the bioreactors caused by a variety of physical and biological factors may eventually cause a failure in bioreactor function,

rendering the bioreactor inoperable (Christianson et al., 2020). The hydraulic conductivity threshold might be determined using the peak flow input to the bioreactors as well as the shape and volume of the bioreactors.

Designing new bioreactors with external carbon dosing may necessitate a novel design technique to address this operational constraint, specifically hydraulic conductivity. Creative design techniques, such as wider bioreactors or the use of a more lasting porous medium in place of organic sources like woodchips, may address the hydraulic restrictions of carbon-dosed bioreactors. For instance, Maxwell et al. (2022) tested a wide bioreactor with a length-to-width ratio of one as opposed to the traditional bioreactors' length-to-width ratio above five and found that the wider bioreactors could capture more of the flow from the field but at the expense of a shorter HRT and a lower nitrate removal efficiency. Carbon dosage may overcome the carbon and/or HRT constraint of nitrate removal in wider bioreactor designs while catching more flow and so increasing overall removal efficiency. Furthermore, the inherent degradation of organic solid substrates such as woodchips may need the use of non-organic solid porous mediums such as plastic biofilter media to maintain microbial growth, which could be addressed in the future.

Conclusion

The continuous decline in available carbon from the solid substrate may impede nitrate removal in bioreactors. This decline in nitrate removal is also accompanied by a decrease in the hydraulic performance of the bioreactor, reducing the bioreactor's ability to accept higher flow. This issue might be more severe in carbon-dosed bioreactors, where increased microbial activity and subsequent biofilm and EPS production brought on by extremely reduced conditions hasten bioclogging. Our field observations demonstrated an approximately 1000 m day^{-1} per drainage season

decrease in hydraulic conductivity of the full-scale bioreactor over three years from 2018 to 2021, with the last two years involving exogenous carbon dosing. There was no backflow or bypass in the bioreactor, suggesting that the hydraulic conductivity was still sufficient to allow the bioreactor to operate satisfactorily. However, field observations were unable to quantify the contribution of added carbon to the internal hydraulic parameters of bioreactors. By conducting tracer studies in mesocosm bioreactors under hydrologically steady-state conditions, we demonstrated a statistically insignificant impact of methanol dosing on the internal hydraulic parameters of bioreactors. Methanol dosing reduced bioreactor medium utilization in both the presence and absence of nitrate compared to woodchips control treatment. Surprisingly, methanol dosage enhanced the degree of the plug flow through the medium in the methanol-dosed bioreactors. However, the alterations in internal hydraulic parameters were not statistically significant when compared to control bioreactors with no carbon dosing, presumably due to a small number of observations and high variability among replicates. Because conventional carbon limiting constraints do not apply to carbon-dosed bioreactors, other environmental and operational parameters, such as hydraulic conductivity, may become limiting. As a result, these limiting factors should be considered when determining the size and shape of carbon-dosed bioreactors.

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Chapter 6

Summary, Conclusion, and Recommendations

The goal of this thesis was to evaluate carbon dosing as a feasible approach to increase nitrate removal rates in denitrifying bioreactors. The thesis also quantified some of the adverse effects of carbon dosing on bioreactors or the downstream aquatic environment. More specifically, the thesis sought to (1) determine whether and to what extent external carbon dosing of bioreactors increases nitrate removal rates, and (2) quantify some of the potentially detrimental effects of carbon dosing, i.e., accelerated hydraulic deterioration, added carbon losses, and sulfate reduction.

6.1 Summary and Conclusions

Figure 6-1 depicts the main research questions and findings. The thesis's first study designed and evaluated a simple carbon dosing strategy to improve nitrate removal in a full-scale bioreactor under transient flow conditions. This study demonstrated a substantial increase in nitrate removal from 1 g N m^{-3} day^{-1} of the undosed bioreactor to 8 g N m^{-3} day^{-1} and 5 g N m^{-3} day^{-1}, in the 2020 and 2021 drainage seasons, respectively. This study demonstrated the bioreactor's effectiveness in attenuating added carbon under nitrate-limiting conditions, achieving methanol removal rates of 106 g CH_3OH-C m^{-3} day^{-1}.

The second study of the thesis examined the effects of methanol dosage on the performance of bioreactors in mesocosms operating under steady-state conditions to determine the removal of nutrients and added methanol more accurately.

This study demonstrated that methanol dosage resulted in an approximately fourfold increase in nitrate removal rates from 7 to 27 g N m^{-3} day^{-1} when compared to the control treatment. Methanol dosage significantly increased sulfate removal rates from

1.5 g SO_4^{2-}-S m^{-3} day^{-1} in the presence of nitrate to 5.5 g SO_4^{2-}-S m^{-3} day^{-1} under nitrate-limiting conditions. Added methanol was removed at the rate of 23 g CH_3OH-C m^{-3} day^{-1} under nitrate-prevailing conditions compared to 18 g CH_3OH-C m^{-3} day^{-1} when nitrate was limiting.

The third study aimed to quantify the effects of methanol dosing on bioreactors' hydraulic performance by combining observations from field and mesocosm experiments. The flow analysis of the field bioreactor revealed a 1000 m day^{-1} per drainage season decline in hydraulic conductivity after the addition of external carbon. Mesocosm tracer tests were performed as a follow-up to quantify the added carbon effects on the internal hydraulic parameters of bioreactors such as effective utilization of media volume. Methanol dosing did not significantly affect any of the internal hydraulic parameters of the bioreactors (P-value > 0.05). To illustrate, the tracer study revealed a mean hydraulic efficiency based on effective volume utilization (λ_e) of 0.77 in methanol-dosed bioreactors (comprising observations from both nitrate limiting and prevailing bioreactors) compared to 0.86 in control bioreactors; however, this difference was not statistically significant (P-value > 0.05).

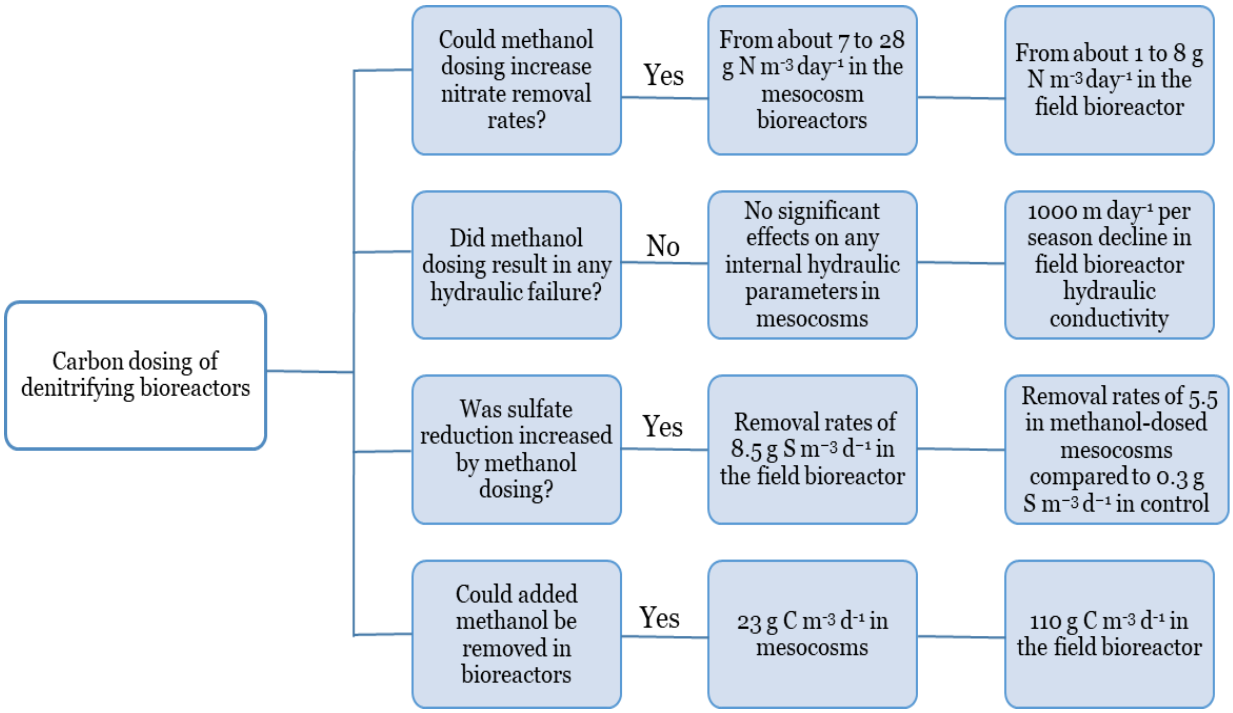

Figure 6-1 The thesis's diagram, including the primary research questions and conclusions

6.2 A back-of-envelope cost analysis of methanol use

The cost analysis of methanol used in the 2020 drainage season of the field bioreactor, as part of the conclusion of the thesis, revealed a total cost of 346 NZD for the 70-day period. The calculation was based on the daily usage of 1.1 liters of 99.8% pure methanol, which had a cost of 4.9 NZD per day based on prices in February 2023.

It's crucial to note that the initial expenses for setting up the methanol dosing system, including the pumping system, were not taken into account in this analysis. To provide a clearer picture, each day, one liter of 99.8% pure methanol costs $4.5 NZD, which translates to a daily cost of $4.95 NZD ($4.5 NZD x 1.1 liters). Over the course of a 70-day drainage season, the cost of methanol usage accumulates to $346 NZD. The efficiency of using methanol as a bioreactor additive was determined by dividing this total cost by the cumulative nitrate load reductions of 1.859 kg N, yielding a cost per

kg N reduction of $186 NZD/kg N. It's important to keep in mind that the overall cost estimation incorporates instances when the added methanol exceeded the nitrate presence, which could have resulted in a lower cost if the methanol addition was precisely matched with the nitrate input.

6.3 Recommendations for the future work

This thesis sheds light on the potential of carbon dosing to improve nitrate removal rates in denitrifying bioreactors, as well as the adverse effects of this approach on bioreactors and the downstream aquatic environment. Some research topics, however, remain unexplored that might improve the efficiency of carbon dosing as well reducing the adverse impacts of this approach on either bioreactors or downstream aquatic environments. The following sections of this chapter outline the potential research topics about carbon dosing in bioreactors.

c. Carbon dosing of bioreactors with extremely high nitrate inputs

The nitrate inputs to the field bioreactor ranged from 10 to 30 mg N L^{-1}, which is typical of agricultural drainage nitrate concentrations (Addy et al., 2016; Christianson et al., 2012; Christianson et al., 2013b; Rivas et al., 2020a). The rapid removal of nitrate in the bioreactor's early compartments demonstrated the effectiveness of methanol dosing in accelerating denitrification rates. As a result of the improved nitrate removal, a large portion of the bioreactor became nitrate limited. Bioreactors have been tested in the treatment of some wastewaters with overwhelmingly high nitrate loads, such as glasshouse effluent, where nitrate removal is carbon limited. For example, Schipper et al. (2010a) demonstrated that carbon was limiting nitrate removal in a massive bioreactor (176 m × 5 m × 1.5 m) treating glasshouse effluent with 250 mg N L^{-1} input nitrate concentrations. Carbon dosing could be used to increase nitrate removal rates in bioreactors with extremely high nitrate inputs. Excess availability of both methanol

and nitrate at concentrations far above the limiting ranges might be investigated to determine nitrate and methanol removal rates as well as some potential adverse effects such as bioclogging in elevated concentrations of both methanol and nitrate.

d. Effects of carbon dosing on dissimilatory nitrate reduction to ammonium

Dissimilatory nitrate reduction to ammonium (DNRA), in addition to denitrification, anaerobic ammonium oxidation (ANAMMOX), and biomass assimilation, may contribute to nitrate removal in bioreactors (Kraft et al., 2011; Kuypers et al., 2018; Lam and Kuypers, 2011; Rambags et al., 2019). However, it has been generally demonstrated that denitrification is the dominant nitrate removal pathway, with the other microbial nitrate removal pathways being less important (Gibert et al., 2008; Nordström and Herbert, 2018; Schipper et al., 2010b).

Even though it is minor, the DNRA and subsequent ammonium generation are regarded as an unfavorable mechanism for nitrate removal as ammonium oxidation consumes oxygen (via nitrification) and it can be harmful to aquatic inhabitants (Li et al., 2020; Xu et al., 2021). However, higher C:N ratios in influents have been correlated with a higher prevalence of DNRA, especially when nitrate is limiting denitrification in bioreactors (Hartfiel et al., 2022; Nordström and Herbert, 2018). Even though the extent of DNRA contribution to nitrate removal has been reported as minor (Gibert et al., 2008; Nordström and Herbert, 2018; Schipper et al., 2010b), external carbon dosing might trigger this microbial pathway by potentially imposing higher C:N ratios. The effects of external carbon dosing and the higher C:N ratio it imposes could be studied on DNRA and subsequent ammonium production as an adverse effect of external carbon dosing.

e. Carbon dosing of bioreactors operating at low temperatures

Temperature is a critical environmental factor that regulates the rate of biological reactions (Schipper et al., 2014). The temperature has been found to positively correlate with the rate of nitrate removal in bioreactors (Canion et al., 2014; Christianson et al., 2013a; David et al., 2016; Schipper et al., 2010b; Warneke et al., 2011). Bioreactors operating at lower temperatures have been shown to have lower nitrate removal rates than those operating at higher temperatures. In a meta-analysis of various field scale bioreactors, for example, Addy et al. (2016) found that bioreactors operating at temperatures lower than 6°C had significantly lower nitrate removal rates of 0.9 g N m^{-3} d^{-1} compared to 3.7 g N m^{-3} d^{-1} of bioreactors operating at temperatures higher than 16.9°C.

Both DOC generation from woodchips and nitrate removal are microbial processes that are regulated by environmental temperature (Nordström and Herbert, 2017; 2019). Carbon dosing could be investigated in a cold climate to determine how added carbon affects nitrate removal rates.

f. Testing different organic carbon compounds as external carbon sources

Previous dosing studies focused on the use of low molecular weight synthetic organic compounds, such as methanol and acetate, to improve nitrate removal rates in bioreactors (Hartz et al., 2017; Herbert Jr et al., 2014; Jansen et al., 2019; Roser et al., 2018). Even though these carbon substrates have been shown to increase nitrate removal rates, there may be local constraints for storing and applying these compounds in the field, such as on dairy farms, due to toxicity and handling concerns. Agricultural byproducts such as molasses are found effective carbon sources for denitrification (Fu et al., 2022; Horova et al., 2020; Quan et al., 2005). Liquid carbon-containing industrial wastes could be utilized as potentially more affordable external

carbon sources while fostering a circular economy (Kiani et al., 2020). However, these effluents may contain elevated levels of nitrogen and phosphorus, potentially increasing the rate of aerobic fungal/bacterial biofilm growth and increasing the risk of clogging at the point of adding carbon sources (Le Coustumer et al., 2012; Palmonari et al., 2020; Parnaudeau et al., 2008). Elevated nitrogen and phosphorus availability in agricultural effluents may promote undesirable anoxic microbial pathways such as fermentation in bioreactors, which competes with denitrification for added carbon, and subsequent biomass synthesis, which exacerbates clogging. The effects of liquid agricultural wastes as external carbon sources on nitrate removal in bioreactors, as well as potential adverse effects such as clogging and added carbon losses from bioreactors, could be investigated further.

g. Evaluating denitrifiers' acclimation to the new substrate

Microbial communities begin to acclimate to a new carbon substrate as soon as they are exposed to them (Badia et al., 2021; Paradis et al., 2022). The acclimated microbial community may function differently with altered rates in removing nutrients such as nitrate and sulfate (Aalto et al., 2022; Paradis et al., 2022). Microbe populations in anoxic bioreactor environments are diverse, including fungal communities (which degrade woody organic materials), denitrifying microbes, and sulfate-reducing bacteria (Aalto et al., 2022). Using exogenous carbon as a dominant electron donor may result in the establishment of a new microbial community in bioreactors. Understanding the acclimatized microbial populations is critical for studying the mechanistic effects of new substrates on nutrient removals such as nitrate and sulfate. A better understanding of acclimatized microbial populations and their roles in different nutrients and added carbon removal could aid in the design of more efficient

carbon dosing while also minimizing the potential adverse effects of dosing such as sulfate reduction and DNRA.

h. Designing more hydraulic efficient bioreactors

Enhanced nitrate removal because of carbon dosing would imply either designing new bioreactors with smaller spatial footprints but treating the same flow and nitrate loads, or treating higher nitrate and flow loads in existing bioreactors. However, the latter may be limited by the hydraulic capacity of the bioreactors. Most bioreactor designs have used length: width ratios ranging from 2.0 to 10 (long bioreactors design approach) to achieve adequate HRT and encourage plug flow, both of which improve nitrate removal rates (Christianson et al., 2012; Maxwell et al., 2022; Rivas et al., 2020b). Long bioreactors (higher length: width ratios), however, have lower flow capacity and a potential increase in bypass flow when compared to bioreactors with lower length: width ratio designs (David et al., 2016; Maxwell et al., 2022). Designing bioreactors with higher flow capacity, such as wider bioreactors (Maxwell et al., 2022), can overcome the hydraulic shortcomings of bioreactors and, when combined with carbon dosing, can overcome the trade-off of short HRT of wider bioreactor designs to maintain high nitrate removal efficiency. Future research could include carbon dosing of wide bioreactors, determining nitrate and added carbon removal, and assessing the risk of added carbon losses from the wide bioreactor's outlet.

Overall conclusion

Methanol dosing was demonstrated in this thesis to be an effective approach for increasing nitrate removal in denitrifying bioreactors. This thesis designed and demonstrated a straightforward constant methanol dosing approach for improving the nitrate removal performance of bioreactors with transient nitrate inputs. The thesis also showed that carbon dosing had no significant effects on the internal hydraulic

performance of bioreactors. It is suggested that more studies be conducted to design and test more hydraulically efficient bioreactors with external carbon dosing to maintain higher nitrate removal efficiency by reducing bypass flow. Carbon dosing could also be assessed in bioreactors with extremely high nitrate inputs, such as glasshouse effluents. Understanding the new microbial populations acclimatized to new carbon substrates is also recommended to gain insight into the impact of external carbon dosing on the internal microbial community as well as outflow microbial content. It is also advised to investigate the effect of carbon dosing on alternative nitrate removal pathways in bioreactors such as DNRA and anammox.

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Appendix A – Co-Authorship Forms

THE UNIVERSITY OF WAIKATO
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Co-Authorship Form

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

The third chapter of the thesis "A Simple and Effective Carbon Dosing Method for Improving Nitrate Removal in a Full-Scale Denitrifying Bioreactor" has been submitted to Ecological Engineering for publication.

Nature of contribution by PhD candidate	Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization, Data analysis
Extent of contribution by PhD candidate (%)	90

CO-AUTHORS

Name	Nature of Contribution
Greg Barkle	Conceptualization, Validation, Writing - Review & Editing
Aldrin Rivas	Conceptualization, Validation, Writing - Review & Editing
Dorisel Torres-Rojas	Conceptualization, Validation, Writing - Review & Editing
Louis Schipper	Conceptualization, Validation, Writing - Review & Editing, Funding acquisition, Project administration, Supervision

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Greg Barkle		5/9/22
Aldrin Rivas		7/9/22
Dorisel Torres-Rojas		2/9/22
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The fourth chapter of the thesis "Enhanced Nitrate Removal and Side Effects of Carbon Dosing in Denitrifying Bioreactors" has been submitted to Ecological Engineering for publication.

Nature of contribution by PhD candidate	Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization, Data analysis
Extent of contribution by PhD candidate (%)	90

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Name	Nature of Contribution
Dorisel Torres-Rojas	Conceptualization, Validation, Writing - Review & Editing
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Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
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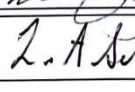
Louis Schipper		1/9/22

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The fifth chapter of the thesis "Flow analysis and hydraulic performance of denitrifying bioreactors under different carbon dosing treatments" has been submitted to Journal of Environmental Management for publication.

Nature of contribution by PhD candidate	Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization, Data analysis
Extent of contribution by PhD candidate (%)	90

CO-AUTHORS

Name	Nature of Contribution
Greg Barkle	Conceptualization, Validation, Writing - Review & Editing
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