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Effects of Hydraulic Retention Time, Soluble Carbon Source and Substrate Media on Nitrate Removal Efficiency of Column Denitrification Bioreactors

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Abstract

In New Zealand, dairy farming is a major contributor to nitrogen (*N*) contamination of the aquatic environment, due mainly to nitrate leaching from cow urine patches and intensive cropping. This enrichment of the aquatic environment affects groundwater and surface water quality and their dependent freshwater ecosystems by promoting eutrophication. It is therefore important to better understand and mitigate critical flows of nutrient losses from agricultural lands to receiving waterways. In response to this environmental challenge, denitrifying bioreactors have emerged a novel edge-of-field practice, which can reduce nitrate levels in agricultural drainage waters by facilitating the conversion of dissolved nitrate to nitrogen gases using a carbon source in microbial denitrification process under anaerobic conditions.

Woodchips, a common carbon media of bioreactors, are favoured for their simplicity, cost-effectiveness, and efficiency in nitrate removal. Their use is growing worldwide, particularly at the edges of agricultural fields to minimise farming disruption and effectively mitigate nitrate losses in drainage waters. However, as woodchips age, the availability of dissolved organic carbon (*DOC*) decreases, potentially limiting denitrification rates over time in woodchip bioreactor. However, soluble carbon dosing is proposed as a solution to enhance nitrate removal by maintaining a consistent supply of organic carbon. Although carbon dosing has predominantly been studied in woodchip bioreactors, it has also shown success in non-woodchip bioreactors that utilise a range of substrates including soil, sediment, sand, pumice stone, and vermiculite, supplemented with ethanol and methanol as soluble carbon sources. These systems have proven effective in reducing nitrate levels in aquaculture and underground water systems. However, the use of ethanol as an external carbon source in non-woodchip bioreactors for agricultural drainage systems has not been well researched.

This study investigated the use of soluble carbon dosing in denitrifying bioreactor to enhance nitrate removal, comparing its effectiveness across woodchip, pumice, and sedimentary rock gravel a substrate media. The study used a set of nine (*9*) small-scale replicated column bioreactors. The specific research objectives were to investigate the effect of varying hydraulic retention time (*HRT*), soluble carbon source (methanol and ethanol) and compare different low-cost bioreactor media (woodchips, sedimentary gravel, and pumice gravel).

The first experiment assessed the impact of different HRTs on the nitrate removal performance in woodchip column bioreactors. Three HRTs (6.6, 10, and 20 hours) and average inflow nitrate concentration of 19.6 mg N L^{-1} were used to measure effects of HRT's on nitrate removal efficiency (% reduction in nitrate concentration) and nitrate removal rate (quantified as g NO_3^--N removed per m^3 of woodchips per day). The inflow water temperature varied from 19.6^{o}C to 20.4^{o}C, with an average of 20^{o}C. However, extending the HRT from 6.6 to 20 hours, increased nitrate removal efficiency from an average of 35% to 71%, but decreased nitrate removal rate from an average 13.6 to 9.4 g NO_3^--N m^{-3} day^{-1}, respectively.

The second set of experiments (two) evaluated the effects of methanol and ethanol dosing on nitrate removal efficiency in woodchip column bioreactors, operated with an HRT of 6.6 hours, an average inflow water nitrate concentration at 19.5 mg N L^{-1}, and average water temperature of 16.4^{o}C from both experimental sets. A soluble carbon dosing at a C:N ratio of 1:1 significantly enhanced nitrate removal efficiency. The ethanol-dosed treatment achieved a nitrate removal efficiency of 66-68%, compared to 55-57% for the methanol-dosed treatment and 33-38% for the control (non-dosed) treatment.

In the third experiment, the efficacy of ethanol dosing for nitrate removal was assessed across different column bioreactor media (woodchips, pumice, and sedimentary rock gravel). The experiment used ethanol dosing at a 1:1 C:N ratio, a 6.6-hour HRT, an average inflow water nitrate concentration at 19.6 mg N L^{-1}, and average water temperature of 13.4^{o}C. The ethanol dosing was effective particularly achieving an average of 97% reduction in the inflow NO_3^- concentration in the woodchip bioreactors columns, compared to 72% for pumice and 75% for gravel used as substrate media. However, the increased outflow total organic carbon levels in woodchip bioreactors suggest a higher release of organic carbon from both fresh woodchips and added ethanol, providing a higher carbon source for enhanced denitrification in the woodchip bioreactor columns. This was also evidenced by consistently low outflow NO_3^--N concentrations in the ethanol-dosed woodchip bioreactor columns.

The bioreactor columns experiment results in terms of measured nitrate removal efficiency were finally applied to construct a comparative economic-opportunity cost analysis of ethanol-dosed (C:N ratio of 1:1) woodchip and gravel bioreactors (assuming 200 m^3 bioreactor size) for informing further development of practical cost-effective bioreactor design for agricultural drains. . A lifespan of either 10 or 15 years was sued for the woodchip bioreactor and of 30

years for the gravel bioreactor. When both media (woodchips and gravel) were assumed to have a porosity of 50%, the cost-effectiveness of nitrate removal using woodchip bioreactor was calculated at NZ$5.90 per kg N removed for a 10-year lifespan scenario, and NZ$4.30 per kg N removed for 15 years lifespan scenario, while for the gravel bioreactor it was NZ$8.80 per kg N removed for 30 years lifespan scenario. A gravel bioreactor, despite its higher annual costs, due to their longer lifespan without the need for media replacement offers practical advantage, but requires active management of external carbon-dosing system.

In summary, this thesis has developed a comprehensive dataset that enhances our understanding the potential of ethanol dosing to improve the performance of denitrification bioreactors, while also exploring alternative media such as gravel and pumice compared to woodchips. The accompanying cost analysis reveals that although woodchip bioreactor appears more cost-effective in the short term, due to lower initial and maintenance costs, gravel bioreactor may have potential to be cost effective and practical in the long-term. However, longer term field testing is needed to assess the actual relative performance of these two media over time to better assess their cost-benefits and practicality in their potential applications in real-world conditions.

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Chapter 1 Introduction, research objectives and thesis outline

1.1 Introduction

In New Zealand, dairy farming and other intensive land use has been identified as a major contributor to losses of nitrogen (*N*) to the aquatic environment. The cow urine patch is a source of excessive nitrate concentrations, which is the main source of nitrate leaching from these farming systems (Monaghan & Smith, 2012; Scarsbrook & Melland, 2015). The increasing use of nitrogen fertilisers has also contributed to increased nitrate leaching (Stevens, 2019). As nitrates leach from the soil into groundwater and surface waters, they degrade the quality of freshwater ecosystems (McDowell et al., 2021; Moghaddam, 2022a), causing serious environmental problems such as algal blooms and eutrophication (Cui et al., 2020; Sutton et al., 2011; Zhang et al., 2015). Therefore, targeted and effective measures are required to mitigate losses of nitrate from agricultural lands to receiving waters. A denitrifying bioreactor is considered an effective system for reducing nitrate levels in agricultural drainage water through the denitrification process. This engineered system directs high-nitrate water through a trench filled with carbon sources where microbes then use the carbon to primarily convert nitrates into dinitrogen gas (N_2) (Christianson & Schipper, 2016; Schipper et al., 2010a; Schipper et al., 2010b; Warneke et al., 2011b).

Woodchips are the preferred carbon source for bioreactors globally due to their straightforward design, low maintenance, cost efficiency, and effective nitrate removal (Christianson et al., 2021; Robertson et al., 2009). In practical applications, woodchip bioreactors have demonstrated efficiency in removing 8% to 99% of nitrate loads from synthetic drainage systems, supported by multiple studies (Christianson et al., 2012a; Hassanpour et al., 2017; Hoover et al., 2016; Jaynes et al., 2008; Rivas et al., 2020; Van Driel et al., 2006a; Woli et al., 2010). However, a decrease in the available carbon in woodchips over time can lead to reduced effectiveness of denitrifying bioreactors in nitrate removal, posing challenges to achieving sufficient nitrate reduction required (Abusallout & Hua, 2017; Cameron & Schipper, 2010;

Lopez-Ponnada et al., 2017; Nordström & Herbert, 2019; Rivas et al., 2020; Warneke et al., 2011b). For example, in a meta-analysis of 26 peer-reviewed journal articles, Addy et al. (2016) reported that the average nitrate removal rate in the first year of operation was 9.2 g N m^{-3} day^{-1}. By the second year, this rate had significantly dropped to 2.8 g N m^{-3} day^{-1}. At the field-scale level, Christianson et al. (2021) found an average nitrate removal rate of 20% across 37 bioreactors, noticeably lower than the 30 to 50% reduction achieved by constructed wetlands reported by Tanner et al. (2012). This difference was attributed to the inadequate carbon input from woodchips in the bioreactors.

Carbon dosing can play a crucial role in enhancing nitrate removal by providing a consistent source of carbon for denitrification reactions (Xiong et al., 2021; Zhang et al., 2024). Substances such as methanol (Hartz et al., 2017; Moghaddam, 2022a), ethanol (Jansen et al., 2019), acetate (Feyereisen et al., 2020; Feyereisen et al., 2023; Herbert Jr et al., 2014; Palomo et al., 2013; Roser et al., 2018) and glucose (Warneke et al., 2011a) have been used successfully in denitrifying bioreactors to improve nitrate reduction, with positive results observed in both laboratory and field settings. Hartz et al. (2017) investigated the impact of consistent methanol dosing on nitrate removal and found that it significantly increased denitrification rates, achieving complete nitrate removal in both mesocosm experiments and field-scale bioreactors operating with a 2-day Hydraulic Retention Times (*HRT*). In bioreactors dosed with methanol at a consistent 1.4:1 C:N ratio and under a water temperature of 17°C, a nitrate removal efficiency exceeding 95% was observed in 2014 laboratory research at University of California Agriculture and Natural Resources. This was significantly higher compared to the control woodchip bioreactors without methanol dosing, which demonstrated an approximate 6% nitrate removal efficiency. Moghaddam et al. (2023b) found that the daily addition of methanol (with a C:N ratio of 1.48) increased nitrate removal to 8.6 g N m^{-3} d^{-1} in 2020, compared to only 0.67-1.60 g N m^{-3} d^{-1} in 2017-2018 in a woodchip bioreactor without dosing in Waikato, New Zealand. When the methanol dose was halved in 2021, the rate decreased to 5.1 g N m^{-3} d^{-1}. A later mesocosm study showed that methanol dosing with C:N ratio of 1.48 improved the nitrate removal rate by four times, from 7 to 27 g N m^{-3} day^{-1}, outperforming a woodchip control group (Moghaddam et al., 2022b). Jansen et al. (2019) also studied the effectiveness of using ethanol as an external carbon and electron source for nitrate removal in reactor at a farm in Noordhoek, the Netherlands. Their findings revealed significant differences in nitrate removal efficiencies between reactor types. Specifically, a vessel reactor dosed with ethanol at a 2.4-hour HRT achieved up to 95% nitrate removal efficiency. In contrast, woodchip

bioreactors operating at a 5-day HRT reached a maximum of 80% nitrate removal efficiency. This demonstrates a notable improvement in performance with ethanol dosing in the flow-through reactor.

Some studies have also focused on the effectiveness of carbon dosing in nitrate removal in combination of aggregates in aquaculture systems. Pungrasmi et al. (2013) examined substrates such as soil, sand, pumice stone, and vermiculite, using methanol to enhance denitrification in a denitrification tank for a recirculating aquaculture system at Chulalongkorn University, Thailand. They reported use of pumice stone and methanol, dosed at a C:N ratio of 5:1, as most effective, facilitating bacterial growth due to its high surface area and leading to an 85% reduction in nitrate concentration. However, limited studies have explored the use of aggregates and soluble carbon dosing for the purpose of treating agricultural drainage water. Particularly, the economic advantages and performance efficacy of potentially alternative substrates have not been thoroughly compared to woodchips, which currently dominate denitrifying bioreactor designs for treating agricultural drainage waters. In addition, while the use of methanol as a carbon source for denitrification bioreactors has been widely research, studies evaluating ethanol are less common. Ethanol is produced as a by-product of the dairy industry in New Zealand, whereas methanol is made from natural gas. Natural gas is a fossil fuel and a declining resource in New Zealand and elsewhere. Therefore, ethanol has potential to be a more sustainable source of soluble carbon dosing in denitrifying bioreactors. However, this gap in existing knowledge underscores the need for comprehensive experimental data to compare methanol and ethanol as carbon sources. Additionally, it is essential to evaluate alternative substrate media, such as gravel and pumice, as readily available solid, porous matrices for hosting denitrifying bacteria, as well as long-lasting, cost-effective, and efficient replacements for woodchips in agricultural drainage bioreactors. By exploring different media types and their interaction with carbon sources, the research presented in this thesis aims to provide valuable insights that could lead to design and implementation of practical and cost-effective denitrifying bioreactors to treat nitrate in agricultural drainage waters.

1.2 Research objectives

The main aim of this study is to investigate and improve the nitrate removal performance of denitrification bioreactor systems used for treating agricultural drainage water. The study uses

a series of small-scale column bioreactor experiments to achieve specific research objectives, as follows:

1. Investigate the effect of varying hydraulic residence time (*HRTs*) on nitrate removal efficiency of woodchip bioreactor media;
2. Compare the effectiveness of methanol and ethanol, as soluble carbon sources, on the nitrate removal efficiency of woodchip bioreactor media; and
3. Evaluate the nitrate removal efficiency of ethanol as a carbon source when used in combination with different low-cost bioreactor media: sedimentary rock gravel and pumice gravel, as compared to the woodchip media.

1.3 Thesis outline

Chapter 1 outlines the background and state the research objectives of the thesis. The next Chapter 2 provides a comprehensive review of existing relevant literature focused on the environmental impact of nitrogen, the denitrification process, and the use of denitrifying bioreactors, such as woodchip bioreactors to remove nitrate from drainage water. This chapter also reviews key variables that influence denitrifying bioreactor performance including HRT, flow rate, dissolved oxygen, drainage water nitrate concentrations, and substrate characteristics. Existing studies on economics of carbon dosing are also reviewed. This review has informed design and implementation of experiments conducted in this thesis.

Chapter 3 describes the column bioreactors experimental set-up, experiments conducted, and methods used to study the effects of different HRTs on nitrate removal, and to compare the effectiveness of methanol versus ethanol and evaluates how ethanol performs with different bioreactor media (woodchip, gravel, and pumice) to enhance nitrate removal efficiency.

Chapter 4 describes and discusses the experimental results. It quantifies the nitrate removal efficiency and rate under different column bioreactor experimental treatments. Putting in the connect of existing literature, the experimental results are discussed further validating the information and offering new insights in potential performance of different design and operation (substrate dosing, bioreactor media and hydraulic residence time) denitrification bioreactors for agricultural drainage waters. Finally, Chapter 5 provides the conclusions of research, offering practical recommendations derived from the study's findings.

Chapter 2 Literature review

2.1 Nitrogen's role in agriculture and its challenges

Nitrogen (*N*) is a fundamental element found throughout the atmosphere, lithosphere, and hydrosphere (Fageria, 2014). As a vital macronutrient, it is essential for plant growth, development, and reproduction, being a key component of many biomolecules (Leghari et al., 2016; Miller & Cramer, 2005). Even though plants require nitrogen for growth, obtaining sufficient amounts is often challenging in natural and agricultural ecosystems, limiting crop yields and quality globally (Dong et al., 2012; Ueda et al., 2017). To address the low nitrogen concentrations in soils, nitrogen fertilisation approaches are commonly implemented (Fradgley et al., 2021; Grandy et al., 2022). With the global population projected to increase from 8.1 billion (Worldometer, 2024) to nearly 10 billion by the mid-21st century, the demand for nitrogen fertilisers is anticipated to rise to meet the expanding food requirements (Penuelas et al., 2023; Vollset et al., 2020). Remarkably, in the past thirty years, the utilisation of N fertiliser has increased significantly, largely driven by the advancements in intensive farming methods (Luo et al., 2020; Sun et al., 2020).

In New Zealand, dairy farming is a major contributor to nitrogen contamination of the aquatic environment. The cow urine patch is a source of excessive nitrate concentrations, which is the main source of nitrate leaching from these farming systems (Monaghan & Smith, 2012; Scarsbrook & Melland, 2015). The increasing use of nitrogen fertilisers has also contributed to increased nitrate leaching (Stevens, 2019). Over recent decades, this surge in nitrogen inputs has led to water contamination through drainage and nitrate leaching, degrading both surface water quality and downstream ecosystems (McDowell et al., 2021; Moghaddam, 2022a). Excessive nitrate leaching can trigger environmental issues, particularly eutrophication, where nutrient influx promotes algal blooms. As these algal blooms decompose, they deplete oxygen levels, posing a threat to aquatic life (Cui et al., 2020; Sutton et al., 2011; Zhang et al., 2015). Nitrate leaching not only impacts ecosystems but also threatens surface and drinking water quality. Elevated nitrate levels in drinking water supplies raise health concerns, particularly an

increased risk of colorectal cancer (Espejo-Herrera et al., 2016; Schullehner et al., 2018). Given these significant ecological and health risks, it is crucial to implement more sustainable nitrogen management strategies in agriculture to protect the environment and ensure safe water sources (Cameron et al., 2013; Sutton et al., 2011).

2.2 Denitrification process and denitrifying bioreactors to reduce nitrate loss

Agricultural drainage can be a critical flow pathway for nitrate losses from poorly drained lands, particularly those enhanced for agricultural land use with surface and subsurface drainage systems to remove excess water from the soil profile (Randall & Goss, 2008). However, a practical approach to treating agricultural drainage at field edges involves heterotrophic denitrification bioreactor, using a microbial process in which nitrate (NO_3^--N) is converted to nitrogen gases (N_2O and N_2) using a carbon source as an electron donor (Feyereisen et al., 2023). This reaction occurs in oxygen-limited (anaerobic) conditions, where bacteria and archaea use NO_3^- as an electron acceptor in soil and water (Firestone, 1982; Knowles, 1982; Seitzinger et al., 2006). A denitrifying bioreactor is an engineered structure that directs water high in nitrates through a trench lined with solid carbon sources. These sources are used by microorganisms as fuel to transform nitrate into nitrogen gases, primarily dinitrogen gas (N_2) (Christianson & Schipper, 2016; Schipper et al., 2010a).

2.2.1 Type of denitrifying bioreactor

Column bioreactor

Lab-scale column denitrification bioreactors are commonly constructed as packed-bed reactors, a subtype of plug-flow reactors. These reactors are filled with a carbon source material, such as woodchips, and the solution is directed through the reactor via either an upflow or downflow current (Hoover et al., 2016; Nordström & Herbert, 2017). In an ideal plug-flow reactor, all particles have an identical transit (hydraulic residence) time, meaning the theoretical retention time matches the actual retention time. As a result, particles exit the reactor

in the same order they entered, maintaining the sequence and integrity of the process (Metcalf, 2003).

These lab-scale and/or pilot-scale column bioreactors play a crucial role in simulating the conditions of full-scale bioreactors, providing a controlled environment for parameter manipulation and the study of nitrate removal efficiencies and rates. These smaller-scale systems are invaluable for collecting experimental data and validating their performance model accuracy. They facilitate the replication of specific treatments or the exploration of various treatment options concurrently, thereby enhancing our understanding of bioreactor performance under different operational scenarios (Hoover, 2012).

Field-scale bioreactor

Field-scale denitrifying bioreactors can be mainly categorised into three types according to their hydrological configuration: denitrification walls, denitrification beds, and denitrification layers (Schipper et al., 2010b).

Denitrification beds are commonly used to intercept subsurface discharge, with the flow path running along the length of the bed. These beds are essentially large containers, often in the form of pits or trenches below the soil surface. They are filled with wood by-products such as wood chips or sawdust, which act as a carbon source for denitrifying bacteria. Wood by-products are chosen for their high C:N ratio, affordability, commercial availability, high permeability, and durability. Water containing concentrated NO_3^- enters these beds through pipes, which are typically connected to subsurface tile drains or wastewater outlets (Christianson et al., 2021; Robertson & Anderson, 1999; Robertson & Merkley, 2009; Schipper et al., 2010b; Woli et al., 2010).

Denitrification walls are strategically installed across groundwater flow paths, typically parallel to and adjacent to streams or drainage ditches. These walls are designed to intercept shallow groundwater by positioning their cross-sections perpendicular to the flow direction (Barkle et al., 2008; Robertson et al., 2000; Schipper & Vojvodić-Vuković, 1998). Denitrification walls can be constructed entirely from carbon materials or a mixture of carbon materials and soil. Unlike denitrification beds, which handle higher flow rates, denitrification walls are intended for lower flow rates. For optimal efficiency and to prevent flow short-

circuiting beneath the barrier, it is recommended to embed the base of the porous wall into a less permeable soil layer (Christianson et al., 2021; Schipper & Vojvodić-Vuković, 1998; Schipper et al., 2010b)

Denitrifying layers are horizontal covers composed of solid carbon substrates. These layers are designed to reduce nitrate concentrations in leachate, which is the liquid that drains or 'leaches' from a landfill or other sources, preventing its migration into groundwater (Schipper et al., 2010b). These installations are commonly placed beneath weeping tiles in septic tank drainage fields (Robertson & Cherry, 1995) or under effluent-irrigated topsoil (Schipper & McGill, 2008).

2.3 Performance of woodchip bioreactor

Woodchip is the most commonly used carbon media for bioreactors. Countries worldwide are increasingly adopting woodchip bioreactors for conservation, due to their simple design, low maintenance, cost-effectiveness, and efficient nitrate removal (Christianson et al., 2021; Robertson et al., 2009). A primary design consideration is tailoring the bioreactor's size to fit the available space at the edge of agricultural fields, balancing cost-effectiveness with minimal disruption to farming while ensuring effective nitrate mitigation (Hoover et al., 2016).

Numerous studies have evaluated woodchip bioreactors, focusing on their nitrate removal rates (*NRR*) and/or nitrate removal efficiency (*NRE*) in treating drainage water. These studies have extensively explored various bioreactor designs, including laboratory columns and mesocosm setups (Cameron & Schipper, 2010; Christianson et al., 2017; Chun et al., 2009; Gibert et al., 2008; Hackshaw, 2018; Healy et al., 2015; Healy et al., 2012; Healy et al., 2006; Hoover et al., 2016; Nordström & Herbert, 2017), denitrification bed-style (Christianson et al., 2012a; Christianson et al., 2013; Christianson et al., 2011b; Christianson et al., 2011a; David et al., 2016; Elgood et al., 2010; Lepine et al., 2016; Pluer et al., 2016; Rivas et al., 2020; Robertson & Merkley, 2009; Robertson et al., 2009; Schipper et al., 2010a; Warneke et al., 2011c; Warneke et al., 2011a), and denitrifying walls (Moorman et al., 2010; Schipper & Vojvodić-Vuković, 2000; Schmidt & Clark, 2012a). In practice, woodchip bioreactors have consistently demonstrated the ability to achieve nitrate removal efficiencies up to 99% in treating drainage water.

A meta-analysis by Addy et al. (2016) examined denitrifying woodchip bioreactors and found that while nitrate removal rates in bed and laboratory column designs showed no significant differences, both had higher rates compared to denitrification walls. Specifically, among the 27 units of denitrification bed and laboratory column bioreactors analysed, denitrification beds demonstrated nitrate removal rates ranging from 2.9 to 7.3 g N m^{-3} d^{-1}, while laboratory column bioreactor designs had rates between 2.4 to 4.9 g N m^{-3} d^{-1}. In contrast, the three units of denitrification walls examined had notably lower nitrate removal rates, ranging from 0.2 to 2.4 g N m^{-3} d^{-1}. This observation is consistent with the findings of Schipper et al. (2010b), who studied various designs of denitrifying bioreactors and reported that denitrification walls typically exhibited lower nitrate removal rates (0.01–3.6 g N m^{-3} d^{-1}) compared to dentification beds (2–22 g N m^{-3} d^{-1}). One possible explanation for this difference is that denitrification walls are often utilized in environments with limited nitrogen availability. As a result, they generally have lower influent nitrogen concentrations and longer hydraulic retention times (*HRT*) than denitrification bed designs. Despite these variations, woodchip bioreactors remain a reliable and effective method for reducing nitrate levels, particularly in agricultural settings where managing nutrient runoff is critical for both environmental protection and water quality improvement (Christianson et al., 2012a; Hassanpour et al., 2017; Rivas et al., 2020).

2.4 Factors influence the performance of a denitrifying bioreactor system

Even though woodchip bioreactors are considered low-maintenance, cost-effective, and efficient at nitrate removal, their operational efficiency is influenced by important factors such as HRT, flow rate, temperature, woodchip age, and particle size.

2.4.1 Hydraulic residence time (*HRT*) and flow rate

Design inflow rate and size (porosity) of woodchip bioreactors control hydraulic residence time for treatment effects in the bioreactors. Hydraulic residence time (*HRT*) and flow rate stand as cornerstone parameters profoundly influencing the efficiency and functionality of bioreactors. HRT specifically defines the average time water spends inside the bioreactor (Roy & Das, 2016). It is observed that longer HRT provide microorganisms more time to convert nitrates

into nitrogen gas (Addy et al., 2016; Song et al., 2020), while also allowing carbon source such as woodchips to release more organic carbon. This additional carbon source supports microbial activity, leading to improved nitrate removal efficiency (Brink, 2023; Lin et al., 2011). In contrast, shorter HRTs can cause a quicker washout of biomass from the carbon substrate, which reduces the contact between denitrifying bacteria and the substrate. This reduced interaction may limit denitrification activity and result in lower nitrate removal efficiency (Damaraju et al., 2015; Hoover, 2012; Yusoff et al., 2010).

A study by Rivas et al. (2020) on a field denitrification bed in Waikato region of New Zealand in 2017 found that shorter HRT of less than 4 days resulted in a nitrate removal efficiency of approximately 43%, while longer HRTs of more than 5 days achieved over 50% efficiency. Similarly, Hoover (2012) examined woodchip bioreactor columns with HRTs of 2, 4, 8, 12, 18, and 24 hours in Iowa, USA. They reported minimal nitrate reduction at shortest HRT (2 hours). However, extending the HRT from 4 hours (7.8% efficiency) to 24 hours (54.9% efficiency) led to a 47% increase in nitrate removal efficiency. Further research by Hoover et al. (2016) expanded on these findings, showing that increasing the HRT from 1.7 to 21.2 hours improved nitrate removal efficiency from 7% to 55%. A study by Christianson et al. (2017) at the University of Illinois Urbana-Champaign, USA, demonstrated that in a paired-column configuration, comparing a 7.2-hour HRT with a 51-hour HRT showed a significant increase in nitrate removal efficiency, from approximately 18% to over 90%.

However, the relationship between varying HRT and flow rates on nitrate removal rates shows mixed results. A meta-analysis of woodchip bed-type bioreactors found that water retained in the bioreactor for less than 6 hours resulted in significantly lower nitrate removal rates, averaging just 0.7 g N m^{-3}. In contrast, woodchip bioreactors with HRTs between 6 and 20 hours achieved an average removal rate of 4.4 g N m^{-3}, while those with HRTs exceeding 20 hours showed an even higher average of 6.7 g N m^{-3} (Addy et al., 2016). However, a laboratory experiment by Hua et al. (2016) demonstrated that reducing the bioreactor's HRT from 24 hours to 6 hours increased the nitrate removal rate from 18.9 g N m^{-3} d^{-1} to 21.6 g N m^{-3} d^{-1}, suggesting an inverse relationship between HRT and nitrate removal rate. Additionally, Greenan et al. (2009) demonstrated in their laboratory column study that woodchip bioreactors operating at higher flow rates (6.6–13.6 cm d^{-1}) achieved nitrate removal rates ranging from 4.01 to 4.51 g N m^{-3} d^{-1}, while the lowest flow rate (2.9 cm d^{-1}) resulted in a reduced nitrate removal rate of 2.94 g N m^{-3} d^{-1}. However, despite the lower removal rate, a nitrate-N removal

efficiency of 30% was observed at the highest flow rate of 13.6 cm d^{-1} over an HRT of 9.8 days, while a 100% efficiency was achieved at the lowest flow rate of 2.9 cm d^{-1} over 2.1 days. Although other research has shown that nitrate removal rates can increase or decrease with rising flow rates, Hoover et al. (2016) found that, while nitrate removal efficiency improved with longer HRTs, the nitrate removal rate remained relatively consistent as HRT increased from 1.7 to 21.1 hours.

It is suggested that the increase in nitrate removal rates observed with shorter HRTs may be due to the larger volumes of nitrate passing through the system within a given time, accounting for higher nitrate removal rates. As flow rates increase, more nitrate is processed by the bioreactors, leading to greater nitrate removal rates. Therefore, while shorter HRTs yield higher nitrate removal rates, longer HRTs are generally more effective at improving overall nitrate removal efficiency (Hackshaw, 2018; Lepine et al., 2016). However, the differences in nitrate removal rates observed across studies may be due to variability in bioreactor design, substrate composition, or microbial community structure, which could contribute to the differences in performance between studies.

2.4.2 Temperature

The performance of woodchip bioreactors is strongly influenced by water temperature due to its impact on microbial processes. Denitrification, wherein bacteria transform NO_3^- to N_2, is temperature-sensitive (Christianson et al., 2012a; David et al., 2016; Hoffmann et al., 2019). Especially during the primary drainage season (winter), when NO_3^- loads in agricultural drains are high, colder temperatures can inhibit bacterial activity, leading to a marked decline in nitrate removal efficiency (Andersen et al., 2006; Jin & Sands, 2003). Research has shown that cold temperatures, approximately 5°C in winter, can reduce microbial activity in the woodchip bioreactor, leading to a decline in nitrate removal efficiency to approximately 10-20% (Feyereisen et al., 2018; Ghane et al., 2015; Hoffmann et al., 2019; McDowell et al., 2021). In addition, A meta-analysis by Addy et al. (2016) of nitrate removal across various environmental and design conditions in 27 denitrification bed experiments revealed that nitrate removal rates varied with temperature. Denitrification beds with temperatures below 6°C showed a mean nitrate removal rate of 2.1 g N m^{-3} d^{-1}, which was notably lower than the rate observed at intermediate temperatures (6–16.9°C), averaging 5.7 g N m^{-3} d^{-1}. Beds with

temperatures above 16.9°C demonstrated the highest removal rate, averaging 8.6 g N m^{-3} d^{-1}. However, despite the high nitrate removal rates observed in the highest temperature range, the difference from the intermediate category was not statistically significant, likely due to sample variability.

Studies indicates that bioreactors can achieve higher nitrate removal rates, and even enhance NO_3^- removal efficiency, through bioaugmentation. For example, a research by Schipper et al. (2010b) indicated that, at an average nitrate removal rate of 2 g N m^{-3} d^{-1} at temperatures below 6°C, a denitrification bed with a 12-hour HRT can reduce nitrate N concentrations by 1g N m^{-3}. The significance of these removal rates in reducing nitrate flow will vary by location. It necessitates an assessment of the distribution of input nitrate-N concentrations and water movement to the bioreactor under colder conditions. Moreover, studies of Jéglot et al. (2021) and Jéglot et al. (2022) emphasised the effectiveness of bioaugmentation, which involves introducing pre-cultivated microbial cultures to enhance specific processes, in improving woodchip microbiomes during low-temperature denitrification. Their pilot-scale research at Aarhus University in Denmark showed that by establishing a microbial community at 5°C, nitrate removal rates in woodchips from an operational full-scale bioreactor increased by 27-37%. It is suggested that the enhanced nitrate removal efficiency at colder temperatures may be attributed to the careful selection and addition of cold-adapted denitrifying bacteria.

2.4.3 Age of woodchips

In woodchip bioreactors, wood particles have been the preferred carbon source for field trials, demonstrating a sustained ability to remove NO_3^- over an extended period ranging from 5 to 15 years (Blowes et al., 1994; Cooke & Bell, 2014; Fahrner, 2002; Schipper et al., 2005). However, as the carbon sources age, the dissolved organic carbon (*DOC*) availability diminishes, potentially constraining denitrification rates over time (Abusallout & Hua, 2017; Lopez-Ponnada et al., 2017; Nordström & Herbert, 2019; Rivas et al., 2020).

A meta-analysis research by Addy et al. (2016) indicated a clear relationship between bioreactor age of carbon sources and nitrate removal efficiency. The meta-analysis studied 27 bioreactor bed units and found that woodchip bioreactors exhibited higher labile carbon availability during the initial months of operation, which enhanced nitrate removal. Over the

first 13 months, the mean nitrate removal rate was recorded at 9.2 g N m^{-3} day^{-1}. Specifically, during the first 13 months of operation, the mean nitrate removal rate was observed at 9.2 g N m^{-3} day^{-1}. However, by the second year, this rate declined substantially to an average of 2.8 g N m^{-3} day^{-1}. There was minimal variance in removal rates between denitrification beds aged 13 to 24 months and those older than 24 months, with rates at 2.8 g N m^{-3} d^{-1} and 2.6 g N m^{-3} d^{-1}, respectively, indicating a levelling off in removal efficacy over time. At the field-scale level, the limited availability of carbon poses challenges, potentially constraining the rate of denitrification (Cameron & Schipper, 2010; Warneke et al., 2011b). This was reflected in findings by Christianson et al. (2021), who reported an average nitrate removal efficiency of 20% across 37 bioreactors in USA. This subdued performance, when compared to other mitigation methods, can be attributed to the insufficient carbon input from woodchips.

It is considered that bioreactors exhibit enhanced nitrate removal rates within their first year of construction, primarily attributed to the carbon contribution from new woodchips (Hoover et al., 2016; Robertson, 2010). Research conducted by Hoover et al. (2016) in the USA and Robertson (2010) in Canada suggested that this early increase in efficiency is often driven by the flushing of dissolved DOC and total organic carbon from the fresh woodchips, providing a readily available carbon source for denitrifying bacteria. However, these initial high removal rates should be approached with caution, as they may not accurately represent the bioreactor's long-term performance After this initial phase, the removal rates usually stabilize, giving a clearer indication of the bioreactor's consistent effectiveness over time (Addy et al., 2016).

To maximise woodchip bioreactors performance, several techniques have been proposed. One prominent method involves cycling the bioreactor through an aerobic and anaerobic phases, prompting a more pronounced release of carbon from woodchips (Maxwell et al., 2019a, 2019b; McGuire et al., 2021). Additionally, the integration of soluble organic substances like ethanol (Jansen et al., 2019; Ortmeyer et al., 2021), methanol (Hartz et al., 2017; Moghaddam, 2022a; Moghaddam et al., 2023a; Moghaddam et al., 2023b; Moghaddam et al., 2022b), acetate (Feyereisen et al., 2020; Feyereisen et al., 2023; Herbert Jr et al., 2014; Roser et al., 2018; Wunderlich et al., 2012) and glucose (Jiang et al., 2022; Warneke et al., 2011a; Warneke et al., 2011b) has been recognised as a promising approach to amplify nitrate removal rates. This is further reviewed in the Section 2.5 below.

2.4.4 Particle size

Particle size of woodchips is also considered as influencing effectiveness of woodchip bioreactors in nitrate removal in drainage waters. Despite its critical role, various studies have been conducted on woodchip particle size in denitrification bioreactors and have revealed mixed outcomes (Cameron & Schipper, 2010; Peterson et al., 2015).

A study by Cameron and Schipper (2010) indicated that there was no significant difference in nitrate removal rates across five different grain sizes of softwood media, nor between softwood and hardwood media, suggesting a broad non-dependence of nitrate removal on the woodchip particle size. This observation aligns with other studies (Greenan et al., 2006; Robertson et al., 2000; Van Driel et al., 2006b), suggesting that the size of woodchips by itself is not the main factor in their effectiveness at removing nitrates in woodchip bioreactors. However, a closer examination of specific experimental conditions within the same study reveals more detailed insights. Cameron and Schipper (2010) found that under certain conditions, notably at a lower temperature of 14°C, larger woodchip particles (15 mm) demonstrated a greater nitrate removal rate compared to smaller particles (4 mm), between 10 and 23 months of their study. The observed increase in nitrate removal rate with larger particle size may be attributed to a longer HRT associated with coarser grain-sized softwood media. Nevertheless, the specific roles of porosity and particle size in the observed trends from this study remain unclear, requiring further investigation to better understand their impact on nitrate removal efficiency.

In contrast, Peterson et al. (2015) explored how woodchip size affects denitrification bioreactor performance from a different angle. Their findings revealed that smaller woodchips are more effective in nitrate removal than larger ones. This enhanced performance is attributed to the fact that smaller chips offer a greater surface area per unit of weight, allowing for the extensive growth of microorganism. However, the influence of particle size on denitrification efficiency in woodchip bioreactors remains an area with limited clarity. A deeper investigation is essential to uncover the precise role of particle size in enhancing bioreactor performance, which is pivotal for optimising nitrate removal strategies.

2.5 Carbon dosing and their effects on nitrate removal

Carbon dosing is one of the key approaches to optimising nitrate removal, ensuring a steady supply of organic carbon to fuel denitrification reactions (Xiong et al., 2021; Zhang et al., 2024). Incorporating soluble organic carbon sources into denitrification bioreactors has demonstrated an enhancement in nitrate removal in both field and lab-experiment settings. Nonetheless, the efficiency of this removal varies based on the chosen carbon type and the size of the bioreactor system (Feyereisen et al., 2020; Feyereisen et al., 2023; Hartz et al., 2017; Jansen et al., 2019; Jiang et al., 2022; Moghaddam, 2022a; Moghaddam et al., 2023a; Moghaddam et al., 2023b; Moghaddam et al., 2022b; Roser et al., 2018; Warneke et al., 2011a; Warneke et al., 2011b).

2.5.1 Carbon dosing in woodchip bioreactor

Many research efforts have delved into understanding how the addition of low molecular weight organic substances, such as methanol (Hartz et al., 2017; Moghaddam, 2022a; Moghaddam et al., 2023a; Moghaddam et al., 2023b; Moghaddam et al., 2022b), ethanol (Jansen et al., 2019), or acetate (Feyereisen et al., 2020; Feyereisen et al., 2023; Roser et al., 2018), can enhance nitrate removal in dentification bioreactors.

A study conducted by Hartz et al. (2017) found that consistent methanol dosing with a C:N ratio of 1.4:1 greatly enhanced denitrification rates in 2014 at University of California Agriculture and Natural Resources, USA. This resulted in complete nitrate removal in both mesocosm experiments and field-scale bioreactors, which operated over a 2-day HRT with elevated nitrate inflow concentrations ranging between 150-193 mg N L^{-1}. Denitrification bioreactors with methanol dosing achieved nitrate removal rates close to 36 g N m^{-3} d^{-1}, which was significantly higher compared to the rates of around 9 g N m^{-3} d^{-1} observed in control bioreactors without the methanol addition. Further supporting these findings, a field study by Moghaddam et al. (2023b) indicated that the addition of 14.4 L of methanol (with a C:N ratio of 1.48) solution daily resulted in nitrate removal rates of 8.6 g N m^{-3} d^{-1} in 2020, which reduced to 5.1 g N m^{-3} d^{-1} in 2021 when the methanol dose was halved in a pilot woodchip bioreactor in Waikato region of New Zealand. These rates were notably higher than the 0.67–1.60 g N m^{-3} d^{-1} observed in 2017 and 2018 when no methanol was added. Moreover, a subsequent mesocosm experiment by Moghaddam et al. (2022b) revealed that methanol

dosing, with a C:N ratio of 1.48, resulted in a nitrate removal rate four times higher than the woodchip control, increasing from 7 to 27 g N m^{-3} day^{-1}.

Several studies have explored the use of acetate as a carbon source to enhance nitrate removal in denitrification bioreactor systems. Roser et al. (2018) evaluated how sodium acetate dosing (with a C:N ratio of 2.33:1) influenced nitrate removal rates in mesocosm-scale bioreactors under controlled hydrological conditions in Minnesota, USA. Their findings showed that acetate dosing significantly increased nitrate removal in woodchip bioreactors to peak rates of 121 g N m^{-3} day^{-1}, a substantial improvement from the 7.1 g N m^{-3} day^{-1} observed in control groups without carbon dosing, under the same operating conditions. Research conducted by Feyereisen et al. (2020) and Feyereisen et al. (2023) through both column and field experiments in Minnesota, USA demonstrated the effectiveness of acetate dosing, with a C:N ratio of 2.33:1 similar to Roser et al. (2018), in reducing nitrate loads in studies conducted in the same region. The column experiments revealed that nitrate-N removal rates increased significantly with a 2-hour HRT compared to a 12-hour HRT without carbon dosing, suggesting that shorter HRTs, facilitated by the addition of carbon, effectively improve nitrate removal efficiency without necessarily leading to an increase in N_2O production. A field experiment evaluated the effectiveness of acetate dosing (*biostimulation*) in enhancing nitrate removal from agricultural runoff using woodchip bioreactors under cold conditions. The biostimulation with acetate led to 66% nitrate-N load removal, substantially higher than the 21% and 18% removal rates observed for bioaugmentation and control treatments, respectively under cold conditions. The nitrate-N removal rate for biostimulation was 15.0 g N m^{-3} d^{-1}, markedly greater than the 5.8 and 4.4 g N m^{-3} d^{-1} rates for bioaugmentation and control. These findings demonstrated that acetate dosing not only facilitates increased nitrate removal rates in both column and field experiments but also proved particularly effective under cold conditions where microbial activity is reduced. However, Herbert Jr et al. (2014) reported that at long 15-hour HRT and with the addition of acetate, NO_3^- and NO_2^- were removed to below detection levels, achieving a NO_3^- removal rate of 5-10 g N m^{-3} day^{-1} in a field experiment at the Malmberget iron ore mine in northern Sweden. This result contrasts with the column experiment insights provided by Feyereisen et al. (2023), where shorter HRTs with carbon addition were found to be beneficial.

Glucose, another important carbon source, has been investigated for its impact on denitrification rates as an external carbon dosage. A laboratory experiment conducted in New

Zealand by Warneke et al. (2011a) investigated the effectiveness of using glucose among other amendments to identify whether denitrification rates were limited by C and/or NO_3^-. It was found that adding glucose enhanced the denitrification rates by about 85% compared to unamended controls. This finding demonstrates that glucose dosing can indeed help increase nitrate removal by providing an essential carbon source for denitrification, particularly in environments where carbon is the limiting factor for microbial denitrification processes.

Ethanol is considered as an effective external carbon and electron source for stimulating denitrification, acting as a fast electron donor with a short lifespan. In contrast, carbon sources such as woodchips provide slower electron donation but offer a longer lifespan, presenting an alternative approach to enhancing the denitrification process (Jansen et al., 2019). Ethanol was used in previous studies and showed significantly reduced NO_3, NO_2, and chemical O_2 demand in groundwater, achieving positive results in biological denitrification (Cao et al., 2014; Grassi et al., 2007). Jansen et al. (2019) also explored the potential benefits of using ethanol as an external carbon and electron source for nitrate removal in field-scale denitrifying woodchip bioreactors at a farm in Noordhoek, the Netherlands. They compared a substrate-free bioreactor with passive ethanol dosing to a larger woodchip bioreactor without dosing. The ethanol-augmented reactor achieved nitrate removal rates of 50 g N m^{-3} d^{-1}, significantly outperforming the woodchip bioreactor's rate of 1 g N m^{-3} d^{-1}. Furthermore, a vessel reactor dosed with ethanol and operating at a 2.4-hour HRT demonstrated superior nitrate removal efficiencies, achieving up to 95% compared to 80% in woodchip bioreactors with a 5-day HRT, marking a significant improvement.

2.5.2 Carbon dosing in non-woodchip bioreactor

Research of use of non-woodchip bioreactor is very limited in treating agricultural drainage waters. However, in exploring aquaculture systems, significant research have focused on effect of organic carbon sources and concentrations on nitrate removal in denitrification tanks specially designed for recirculating aquaculture system. Pungrasmi et al. (2013) conducted a study to compare various bottom substrates such as soil, sand, pumice stone, and vermiculite, using methanol as a carbon source (a C:N ratio of 5:1) to enhance denitrification in a denitrification tank for a recirculating aquaculture system at Chulalongkorn University, Thailand. They found that pumice stone was the most effective due to its high surface area, which facilitates bacterial growth. This configuration not only optimised the environmental

conditions for microbial activity but also achieved impressive nitrate removal efficiencies, with the best results showing an 85.2% reduction, equivalent to an average removal rate of 6311 ± 945 mg N/m² tank bottom area per day. Extending this research, Pungrasmi et al. (2016) further explored the use of pumice stone in a recirculating system and discovered that under aerobic conditions with same methanol dosing at C:N ratio of 5:1, pumice stone effectively served as a nitrification biofilter. This setup successfully maintained ammonia and nitrite levels below 1 mg N L^{-1} and nitrate levels under 50 mg N L^{-1}, significantly lower than the 352 ± 9.7 mg N L^{-1} observed in the control tank. This significant reduction in nitrate levels demonstrates the potential of alternative substrates over traditional carbon sources such as woodchips. Playchoom et al. (2011) conducted a similar experiment at Chulalongkorn University, Thailand, to assess the effects of organic carbon sources on nitrate removal by comparing treatment tanks with pumice rock and methanol or molasses (with C:N ratio of 5:1) to control tanks without organic carbon. The results indicated that the maximum denitrification rates for methanol and molasses were 4,531 and 4,094 mg N m^{-2} day^{-1}. However, tanks with molasses showed a higher risk of ammonia accumulation and hydrogen sulfide production; therefore, methanol was considered a suitable carbon source for this study.

2.5.3 Side effects of carbon dosing in denitrifying bioreactor

Introducing soluble carbon to denitrifying bioreactors has shown promise in enhancing nitrate removal rates. However, limited studies have provided quantitative data to inform potential adverse effects from using such carbon additions. It is considered that excess soluble carbon can lead to increased DOC leaching from bioreactors, posing risks to surrounding ecosystems (Abusallout & Hua, 2017). Elevated DOC can increase the biological oxygen demand in nearby water bodies, potentially harming aquatic life (Chambers et al., 2000).

In addition, a study by Warneke et al. (2011b) observed that nitrate removal rate in woodchip bioreactors increased with a glucose amendment in their glasshouse at Karaka, New Zealand. However, this was coupled with adverse effects, including the production of N_2O and methane (CH_4), as well as the release of total organic carbon, especially in experimental barrels. Denitrification may not fully consume the added carbon, leading to potential carbon loss from the bioreactor. Another concern is the impact of added soluble carbon, which can stimulate microbial biomass growth. This increased microbial activity could potentially accelerate biological clogging, leading to rapid changes in bioreactor hydrology (Christianson et al., 2016;

Ma et al., 2021). Moreover, Christianson and Schipper (2016), indicate that increased biological oxygen demand inputs can influence bioreactor hydrodynamics. Therefore, bioreactors in agricultural areas with fluctuating water inputs might experience pronounced reductions in hydraulic efficiency due to carbon dosing. Proper dosing is essential to prevent these side effects and boost nitrate removal.

2.6 Cost analysis of carbon dosage

While the effectiveness of this approach is well-established (Hartz et al., 2017; Jansen et al., 2019; Moghaddam et al., 2023b; Roser et al., 2018), understanding its cost implications are crucial for stakeholders considering the implementation of carbon dosing strategies, as they underline the balance between cost efficiency and nitrate removal efficacy. Many studies have undertaken a comprehensive cost analysis of carbon dosing strategies within denitrifying bioreactors (Hartz et al., 2017; Jiang et al., 2022; Moghaddam, 2022a; Zhang et al., 2024)

The research conducted by Moghaddam (2022a) explored the effectiveness of external carbon dosing, specifically using methanol, to enhance nitrate removal rates in denitrifying bioreactors in Waikato, New Zealand. Methanol dosing was found to significantly increase seasonal nitrate removal rates from 1 g N m^{-3} day^{-1} in the undosed bioreactor to 8 g N m^{-3} day^{-1} in 2020 and 5 g N m^{-3} day^{-1} in 2021 with a halved methanol dosing rate. This demonstrated its potential not only to enhance bioreactor performance efficiently but also to provide a cost-effective solution. Over a 70-day period during the 2020 drainage season, the analysis calculated the operational costs of methanol dosing, which amounted to a total expenditure of NZ$346. This figure was derived from a daily consumption rate of methanol, priced specifically at NZ$4.90, not including the costs for setting up or maintaining the dosing system. The calculation of the cost per kilogram of nitrate removed, determined to be $NZ186/kg N, was based on dividing the total cost of methanol by the total nitrate reduction. The investigation underscored a key strategy for enhancing methanol dosing methods, recommending that the dosing rates be more precisely adjusted according to actual nitrate concentrations to significantly lower operational costs.

Similarly, Zhang et al. (2024) conducted an in-depth cost analysis of using acetate for carbon dosing in a denitrifying woodchip bioreactor in Tompkins County, New York State, USA. The

research showed that using acetate costed US$86 or NZ$135 for every kilogram of nitrate removed, highlighting acetate as the main expense driver in the real-time control approach. The findings suggested that while acetate dosing can significantly enhance denitrification, the approach's cost-effectiveness was contingent upon optimising acetate utilisation and exploring alternatives for reducing overall expenses. It was observed that woodchips absorbed a portion of the acetate, which meant not all the added acetate directly contributed to denitrification, thereby affecting the cost-to-benefit ratio. Therefore, organisation considering carbon dosing must evaluate their specific operational contexts, potential for optimising acetate utilisation, and the broader economic implications of integrating such a system into their nitrate removal strategies.

Hartz et al. (2017) estimated that for a 200-acre farm in northern Monterey County, USA, producing 65,000 gallons of tile drainage daily, a woodchip bioreactor (without dosing) sized 200 feet long, 55 feet wide, and 6 feet deep is required to achieve the environmental target of less than 10 mg L^{-1} NO_3^--N in discharge water. The cost for this system over a 10-year lifespan is estimated at US$92,000 or NZ$144,836, equating to US$1.50 or NZ$ 5.20per kg of nitrogen denitrified. Alternatively, a dentification bioreactor employing carbon enrichment, which allows for handling fluctuating nitrate loads and reduces the size requirement, was also considered. Such a system measuring 100 feet by 30 feet by 6 feet could manage similar drainage volumes and achieve complete denitrification within 1.7 days. The estimated cost for this enriched bioreactor is around US$33,000 or NZ$51,950 over 10 years, resulting in a cost of US$0.60 or NZ$2.07 per kg of nitrogen denitrified, not including carbon source expenses. The price of methanol, a common carbon source, varies between NZ$4.85 (US$1.40) and NZ$5.95 (US$1.70) per kg of nitrogen denitrified, depending on oil prices. While carbon enrichment may seem more expensive per unit of nitrogen removed compared to passive operation, it offers a practical approach to efficiently handle high and fluctuating nitrate levels.

In addition, Jiang et al. (2022) conducted an experiment at Anhui University in China to examine efficiency and denitrification rates of carbon dosing with methanol, sodium acetate, and glucose along with their cost analysis. The findings indicated that methanol performed slightly better than sodium acetate, while glucose demonstrated the lowest nitrate removal rate in this study. For the denitrification biofilters (*DNBFs*), the cost analysis suggested that the potential cost for nitrate removal using sodium acetate was CNY ¥22.56 or NZ$5.13 per kg N, which is higher than that for methanol (CNY ¥15.15 or NZ$3.45/kg N) but lower than for

glucose (CNY ¥20.63 or NZ$4.69 /kg N). This implied that while acetate and glucose dosing were effective in removing nitrate, their cost compared to using methanol needs to be considered.

However, there exists a significant gap in understanding the economic implications and long-term viability of carbon dosing strategies, particularly those employing ethanol. Given its biodegradable nature and lower toxicity (Sharma et al., 2017), ethanol presents a promising alternative that merits comprehensive financial analysis. The lack of detailed cost-benefit evaluations underscores the need for research focused on ethanol's potential to make water treatment processes economically sustainable.

2.7 Conclusion

Denitrification bioreactors are considered a promising solution for mitigating nitrate contamination in agricultural drainage waters, given their simplicity, cost-effectiveness, low maintenance demands, and commendable nitrate removal efficiency. However, their performance can be influenced by various factors, such as the availability of carbon, temperature, HRT and media particle size. The introduction of external carbon sources has been proposed to optimise nitrate reduction in denitrification bioreactors, considering that carbon availability can decrease over time. Several studies have demonstrated that carbon dosing in woodchip bioreactors achieves higher nitrate removal rates and efficiencies compared to those without dosing, evident in both field and laboratory scales. Additionally, research on carbon dosing in non-woodchip bioreactor substrates in aquaculture treatment systems, such as pumice, sand, and soil, has shown nitrate removal efficiencies above 80%.

However, there is a lack of research on using ethanol as an external carbon source in denitrification bioreactors with non-wood-based solid substrates for reducing nitrate in agricultural subsurface or tile drainage. A comparative analysis of carbon dosing in woodchip and non-woodchip bioreactors, such as those using pumice or gravel, could provide valuable insights into alternatives that offer longer lifespans than woodchip substrates in bioreactors.

Given the crucial role of carbon dosing in the denitrification process within bioreactor systems, a thorough evaluation of the cost-benefit dynamics of this strategy is essential. This assessment

is vital to confirm the economic viability and sustainability of carbon dosing methods. A detailed analyses of these economic aspects should be conducted to ensure that the ongoing integration of carbon dosing into bioreactor systems remains a feasible and effective approach for enhancing water quality over the long term. Therefore, future research should compare the effectiveness of carbon dosing in woodchip bioreactors and non-woodchip bioreactors with long-lasting solid substrates, such as gravel or pumice, considering their potential for long-term use.

Chapter 3 Materials and Methods

We conducted a lab-scale study to investigate the influence of hydraulic retention time (*HRT*), different carbon sources, and various bioreactor media on nitrate removal rate and efficiency in treating synthetic drainage waters. The experimental setup involved the construction and operation of woodchip column bioreactors under controlled conditions in a workshop at Massey University.

3.1 Design of woodchip column bioreactor experimental set-up

The experimental setup for the woodchip column bioreactor was based on methodologies outlined in prior studies (Christianson et al., 2014; Hoover et al., 2016; Moghaddam, 2022a; Roser et al., 2018) with some modifications. A total of 9 polyvinyl chloride (*PVC*) columns, each 43 cm tall with an internal diameter of 15 cm, were utilised. Inside these columns, a set of three plates, each separated by 10 cm, were installed in a cross-sectional layout to optimise the even distribution of water within the columns. Each column was filled with approximately 2100 g of wet pine woodchips, which had particle sizes ranging from 1 to 7 cm. About 5 grams of moist topsoil (collected from a dairy farm at Massey University No.1 dairy farm) was scattered on top of the packed woodchips in each column to inoculate with soil bacteria (Figure 3-1b).

Two of 200 litter tanks (main tanks) were used to supply the NO_3^- solution to the columns with the target inflow concentrations of 20 mg L^{-1}. This concentration was chosen based on the typical nitrate levels observed in early winter drainage water from dairy farms in New Zealand, which generally range from 15 to 30 mg L^{-1} (Houlbrooke et al., 2004). Nitrate solution was prepared using 99% of nitrate potassium (KNO_3). The influent nitrate (NO_3^-) was then added to the columns at the top, where its gravity-fed through the woodchip media. Outflow tubes were strategically installed at the bottom of the columns and raised to the column's height, facilitating saturation in the woodchips in the columns encouraging plug flow through the media and passive effluent collection for the sampling (Figure 3-1a). The woodchip porosity,

calculated at 52%, was determined by using ratio between the volume of the outflow drained from the column after 24-hour soak with the total column volume.

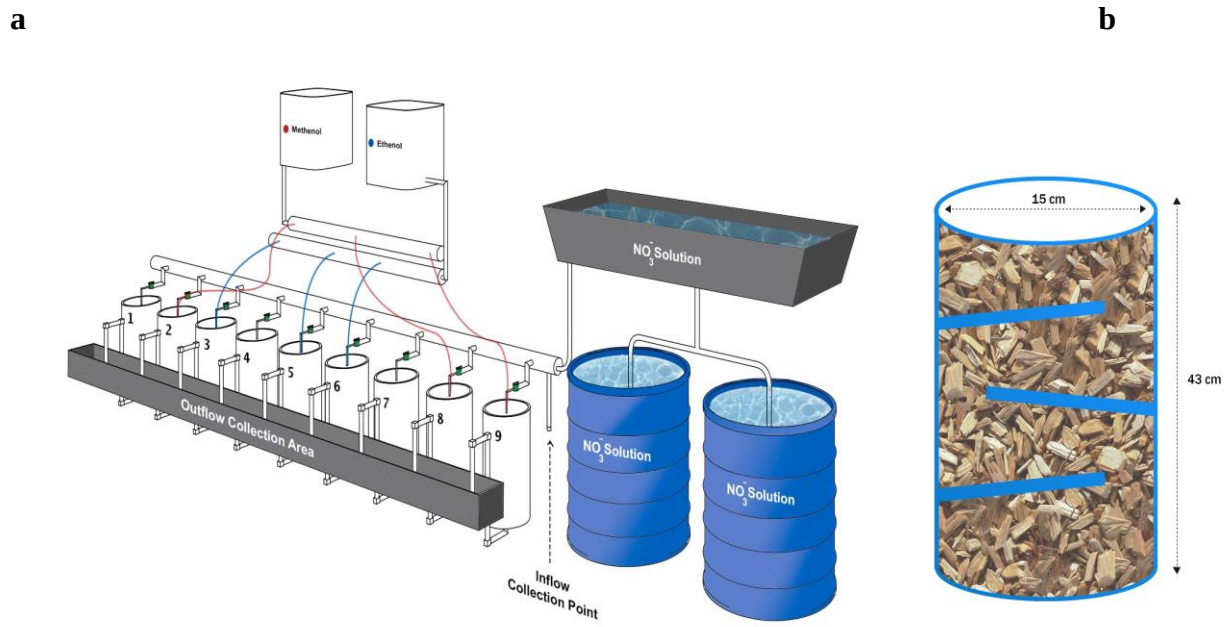

Figure 3-1. (a) Experimental setup of column woodchip bioreactors with ethanol (blue pipes) and methanol (red pipes) dosing systems. (b) design of woodchip column.

3.2 Experiment design

3.2.1 Experiment 1: Evaluation of effects of different hydraulic resident times (HRT) on NO_3^- removal performance of woodchip column bioreactors

The first experiment was conducted to measure potential impacts different *HRT* on nitrate removal in the woodchip column bioreactor. The flow rates through the columns were controlled to achieve three different HRTs including 6.6, 10 and 20 hours in the columns.

However, in December 2023, an initial setup phase of 14 days was undertaken to ensure the growth of denitrifying microorganisms within the columns and to address any potential design issues. Through this period, it was determined that the columns exhibited similar and consistent performance levels. A total of nine (*9*) columns were then evaluated, receiving NO_3^- solution at a flow rate of 9.3 ml min^{-1}, resulting in HRT of approximately 6.6 hours. The inflow and outflows sampling were conducted three days after the evaluation commenced. Outflow samples were collected daily for the first five days and then every two days for the following six days. This resulted in a total of eight samples being collected per column.

Following the initial setup phase, two additional evaluations were conducted to assess the performance of the bioreactor columns under varying flow rates and HRTs. The first evaluation aimed to compare the NO_3^- removal rates at HRTs of 6.6 (9.3 ml min^{-1}) and 10 hours (6.2 ml min^{-1}). This evaluation took place during December 2023 to January 2024 for 17 days. Nine columns were divided into two groups: Group A, consisting of 6.6 HRT (*5* columns), and Group B, consisting of 10 HRT (*4* columns). Outflow samples were collected every two days, starting seven (*7*) days after the evaluation began, resulting in a total of five (*5*) samples per column.

During January to February 2024, the second evaluation was carried out over a period of 12 days to assess the performance of HRTs of 6.6 (9.3 ml min^{-1}) and 20 hours (3.1 ml min^{-1}). The nine (*9*) columns were divided into two groups, Group A consisted of five (*5*) columns with an HRT of 6.6 hours, while Group B consisted of four (*4*) columns with an HRT of 20 hours. Outflow samples were collected every two days, starting two (*2*) days after the evaluation commenced, resulting in five (*5*) samples per column. However, only the sampling data from the last three sampling days in each trial were used for analysis, as these samples were the most stabilized and representative. The sampling data outputs from all five sampling days during the initial evaluation phase were provided in Appendix 3 to demonstrate the stabilisation observed in the last three sampling days. The results from all evaluations served as a baseline for determining the conditions for subsequent experiments.

3.2.2 Experiment 2: Comparison the effects of methanol and ethanol C sources on NO_3^- removal performance of bioreactor

The second experiment was conducted in March 2024, with a HRT of 6.6 hours selected based on the findings of the previous evaluation (the Experiment 1 as described above). The inflow solution was consistently maintained at 20 mg N L^{-1}. Nine woodchip columns were allocated among three different treatments: a control group (*WC*) without additional soluble carbon sources, and two experimental groups with ethanol dosage (*WE*) and methanol dosage (*WM*), respectively. Each treatment was replicated three times using the sets of three woodchip bioreactor columns each.

Methanol (99.9%) and ethanol (absolute ethanol 98% with 2% methanol) were selected as the soluble carbon additions. A C:N ratio of 1:1 (molar ratio by weight) was chosen for carbon dosing in this experiment(Romero Ramírez, 2024). Ethanol was dosed at a rate of 12 mg L^{-1}, providing a carbon content of 52%, while methanol was dosed at a rate of 16.6 mg L^{-1}, providing a carbon content of 37.5%. Each addition was diluted in a 10 L container containing NO_3^- solution, maintaining the same concentration as the main tanks used for inflows to the woodchip columns. This approach ensured consistency in the NO_3^- concentration of the solution entering the columns. To facilitate solution delivery into the columns, 10 L containers were connected to the tops of the column bioreactors via tubing equipped with fluid regulators, ensuring a gravity-fed process. The fluid regulators were monitored daily to maintain the required solution dripping rate (Figure 3-1a).

Experiment 2 consisted of two continuous sets of observations to confirm the reliability and stabilisation of bioreactor performance. et 1 was conducted over 13 days, with bi-daily sampling beginning on Day 4 (designated as Day 1 of sample collection) and continuing for 10 days, resulting in five samples per column. Set 2 was conducted over 11 days, with bi-daily sampling starting 1 day after the experiment's onset and continuing for 10 days, producing five samples per column. The sampling data from the last three sampling days were used for analysis, as these were the most stabilised. The sampling data outputs from all five sampling days of Set 1 were provided in Appendix 3.

3.2.3 Experiment 3: Comparative analysis of ethanol dosing on nitrate removal efficiency in woodchip, pumice, and gravel column bioreactors

The Experiment 3 study was conducted in May 2024, maintaining a consistent influent nitrate concentration of 20 mg L^{-1}, as used in the Experiment 2. However, in this experiment, nine column bioreactors were equally distributed among three treatments of different media material: woodchip (*WB*), gravel (*GB*), and pumice (*PB*). This experiment is first-time conducted experiment to investigate if soluble carbon dosing in inert media could deliver nitrate reduction, as compared to the traditional woodchip column bioreactors, in drainage waters.

The bioreactor media were repacked with particle sizes ranging from 5-10 mm to maintain uniformity across the treatments. The HRT was maintained at 6.6 hours for the woodchip columns treatment, corresponding to a flow rate of 8.2 ml min^{-1} and a porosity of 46%. To maintain consistency in the experimental design, the same flow rate of 8.2 ml min^{-1} was applied to all three materials (*WB*, *GB*, and *PB*). However, by using a uniform flow rate, all treatments received the same amount of nitrogen, facilitating a direct comparison of nitrate removal efficiency across different media bioreactor columns.

All nine (9) column bioreactors were initially operated without ethanol dosing to evaluate a baseline effectiveness of woodchip, pumice, and gravel column bioreactors. The initial evaluation period lasted 8 days, beginning with bi-daily sampling on the second day after the start of the experiment and continuing for 6 days. Ethanol was then dosed at a ratio of 1:1 C:N, consistent with the previous experiment 2. Ethanol concentrations and flow rate in the effluent were monitored daily to ensure consistent dosing and to study its impact on nitrate removal. The study continued for 16 days, with bi-daily sampling beginning 2 days after the experiment's onset and continuing for 14 days. A total of seven samples were collected from each column during this sampling schedule. For data analysis, the stabilised samples from the final three days of the experiment were used, while the data outputs from all sampling days were presented in Appendix 3.

3.3 Analysis

The inflow and outflow samples were sampled and analysed for water temperature, total organic carbon and nitrate concentrations during the experiments. Water temperature was measured using an In-Situ smarTROLL device. NO_3^- concentration and total organic carbon

were measured using a TriOS Optical Sensor. The accuracy of the TriOS Optical Sensor was verified by comparing its nitrate readings with a set of standard samples prepared in the laboratory. The nitrate sensor testing involved measuring a series of standard nitrate-N solutions with concentrations ranging from 0.5 to 10 mg L^{-1}. The TriOS Optical Sensor readings closely matched the standard values, with minimal deviations (Appendix 1). For instance, the sensor measured 0.58 mg L^{-1} for a 0.5 mg L^{-1} standard and 9.69 mg L^{-1} for a 10 mg L^{-1} standard. The high correlation coefficient (R^2 = 0.9999) from the linear regression analysis indicated excellent concordance between the standard nitrate-N concentrations and the sensor measurements, validating the sensor's accuracy and reliability for the experimental setup (Appendix 1).

3.3.1 Removal efficiency and rates

Performance of woodchip column bioreactors was quantified and analysed in terms of the nitrate removal efficiency (*NRE*) and nitrate removal rate (*NRR*), comparing the measured inflow and outflow nitrate fluxes through the coumn bioreactors.

NRE was determined as the percentage of nitrate reduction by assessing the difference between the nitrate load entering (N_{inflow}) and exiting the bioreactor ($N_{outflow}$) columns during the experiment periods (Moghaddam, 2022a), as follows:

$$\text{NRE \%: } \frac{N_{inflow} - N_{outflow}}{N_{inflow}} \text{ X } 100$$

The nitrate removal rate (NRR) for Experiment 1 was calculated in units of g m^{-3} day^{-1}, using the following equation (Moghaddam et al., 2022b), as follows:

$$\text{NRR: } \frac{N_{inflow} - N_{outflow} \ X \ Q}{V}$$

where $\boldsymbol{Q}$ represents the flow rate (m^3/day), and $\boldsymbol{V}$ is the saturated volume of woodchips within the bioreactor (m^3).

3.3.2 Cost analysis

The cost analysis utilised results from this column bioreactor experiment as a baseline to project costs for a larger, field-scale bioreactors. This analysis specifically compared woodchip bioreactors, without ethanol dosing, to gravel bioreactors with ethanol dosing (C:N ratio of 1:1), with each targeted to a scale of 200 m^3.

The analysis considered two scenarios: Scenario A mirrored the study conditions with woodchip porosity at 50% (flow rate of 4.2 L s^{-1}) and gravel porosity at 30% (flow rate of 2.5 L s^{-1}); and Scenario B adjusted both woodchip and gravel porosity to 50%. It was to test potential effects of different gravel particle sizes (porosity) on its cost-effectiveness. The estimated lifespan for the woodchips was set at 10-15 years, while gravel bioreactor was projected to last 30 years. This configuration enabled a comparative analysis of the cost-effectiveness, measured in New Zealand dollars (*NZD*) per kilogram of nitrate removal rate, across the different lifespan projections: 10 and 15 years for woodchip, and 30 years for gravel.

Capital costs encompassed elements such as piping, PVC liners, field excavation work, and labour. The specific expenses for the woodchip bioreactor included the cost of the woodchip material itself, while the capital costs for the gravel bioreactor also covered expenditures for washed gravel (3-5 mm) and ethanol. The total annual costs were calculated considering a 6% opportunity (interest) cost over the estimated lifespans of the bioreactors: 10 and 15 years for the woodchip, and 30 years for the gravel. Details of these calculations are provided in Appendix 2.

3.3.3 Statistic analysis

The collected column bioreactors samples and their water analysis results were recorded and prepared for subsequent statistical analysis. Linear regression was conducted to assess the effects of HRT and temperature as independent variables (the Experiment 1) on nitrate outflow concentrations (dependent variable). One-way ANOVA was conducted to evaluate the impact of various treatments on nitrate removal efficiency, nitrate removal rate, and nitrate outflow concentrations. These treatments included different HRTs (6.6-hour, 10-hour, and 20-hour) in Experiment 1, carbon sources such as methanol and ethanol in Experiment 2, and different bioreactor media—woodchip, gravel, and pumice—in Experiment 3. When significant

treatment effects were identified, the Tukey-Kramer post-hoc test was applied to compare nitrate reduction among the various treatments. Additionally, paired t-tests were performed for post-hoc pairwise comparisons (woodchip vs. gravel, woodchip vs. pumice, and gravel vs. pumice) on specific sampling days to identify significant differences when interaction effects between the treatments and dependent variables were observed.

Chapter 4 Results and Discussion

4.1 Results

4.1.1 Experiment 1: Woodchip column bioreactor evaluation under different HRTs

The initial evaluation of the woodchip column bioreactors focused on assessing nitrate removal efficiency and nitrate removal rate at a 6.6-hour HRT. In this experiment all nine (*9*) columns were tested. During the final three days of the experiment, the average water temperature was 19.6 ± 1.2℃. Over the last 3 samplings (during 8 – 10 days of running the experiment), the woodchip columns decreased the inflow nitrate concentrations from an average of 19.5 ± 0.2 mg NO_3^--N L^{-1} to the outflow nitrate concentrations ranging between 12.7 and 13.1 mg NO_3^--N L^{-1} (Figure 4-1). This reduction corresponded to an average nitrate removal efficiency of 34%, with a calculated nitrate removal rate of 13.2 g m^{-3} day^{-1} (Table 4-1).

The first evaluation in the Experiment 1 assessed potential effects of both the 6.6-hour HRT and 10-hour HRT on nitrate removal in the woodchip columns. The woodchip columns received an average inflow nitrate concentration of 19.5 ± 0.4 mg NO_3^--N L^{-1}, with an average water temperature of 20.4 ± 1.9℃ (Figure 4-2). The 6.6-hour HRT resulted in the outflow nitrate concentrations ranging from 12.3 to 14.2 mg NO_3^--N L^{-1}, whereas the 10-hour HRT achieved slightly lower outflow nitrate concentrations, between 11.4 and 12.6 mg NO_3^--N L^{-1}. The average nitrate removal efficiency was 31% for the 6.6-hour HRT and 39% for the 10-hour HRT. However, despite the higher nitrate removal efficiency observed in the 10-hour HRT, the nitrate removal rate was greater in the 6.6-hour HRT, at 12.0 g m^{-3} day^{-1}, compared to 10.2 g m^{-3} day^{-1} for the 10-hour HRT (Table 4-1). This suggested that while the 10-hour HRT showed a higher nitrate removal efficiency, the difference was not substantial, and the 6.6-hour HRT demonstrated a higher nitrate removal rate.

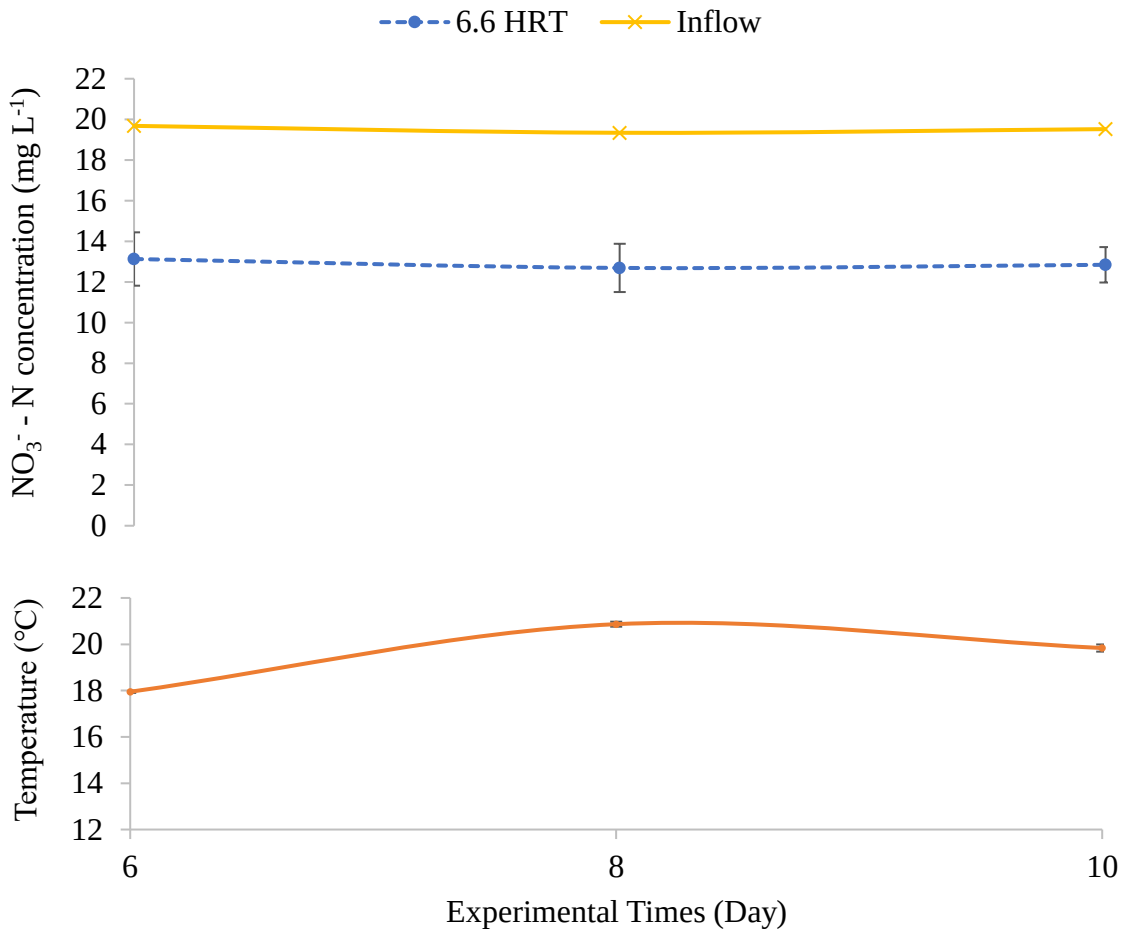

Figure 4-1. A comparison of inflow and outflow NO_3^--N concentrations and temperature over the last three sampling days during the initial evaluation of woodchip column bioreactors for 6.6-hour hydraulic residence time (*HRT*) treatment. Error bars indicate standard error of the mean.

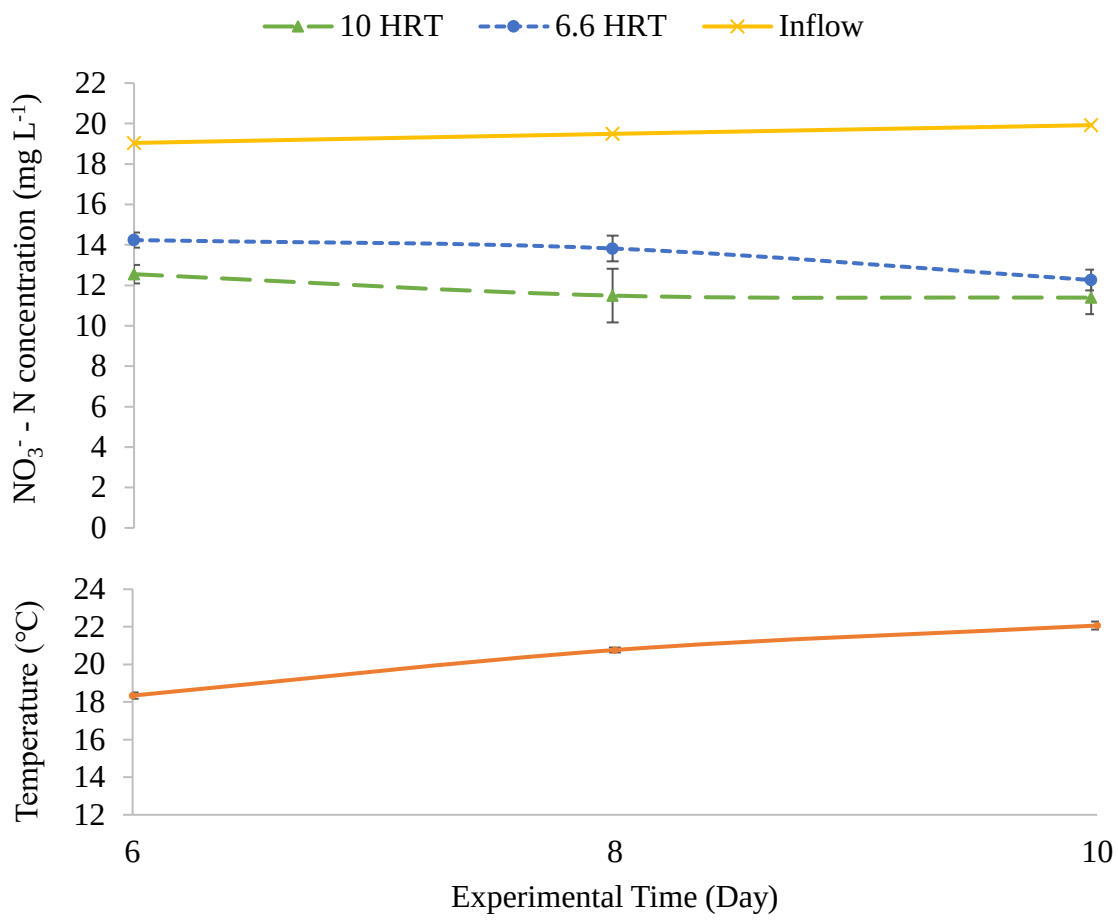

Figure 4-2. A comparison of inflow and outflow NO_3^--N concentrations and temperature over the last three sampling days during the first evaluation of woodchip column bioreactors comparing 6.6-hour and 10-hour hydraulic residence time (*HRT*) treatments. Error bars indicate standard error of the mean.

The second evaluation in the Experiment 1 investigated the differences in nitrate removal efficiencies between the 6.6-hour HRT and the 20-hour HRT. In this test, the woodchip column bioreactors received an average inflow nitrate concentration of 19.8 ± 0.3 mg NO_3^-N L^{-1} and maintained an average water temperature of 19.9 ± 1.6℃. For the 6.6-hour HRT, the outflow nitrate concentrations varied between 11.5 and 12.1 mg NO_3^-N L^{-1}. In contrast, the 20-hour HRT achieved significantly lower outflow nitrate concentrations, which fell between 5.4 and 5.8 mg NO_3^-N L^{-1} (Figure 4-3). The average nitrate removal efficiency was quantified at 39% for the 6.6-hour HRT, while the 20-hour HRT demonstrated a markedly higher efficiency of 72%. However, the nitrate removal rate differed, with the 6.6-hour HRT calculated at 15.5 g

m^{-3} day^{-1} and the 20-hour HRT at a lower rate of 9.4 g m^{-3} day^{-1} (Table 4-1). While the 20-hour HRT significantly enhanced nitrate removal efficiency, it did not correspondingly increase the nitrate removal rate. This is attributed to relatively higher flow rates associated with relatively shorter HRT through the woodchip columns. This indicated that longer HRTs are more effective in enhancing nitrate removal efficiency, rather than in boosting the overall nitrate removal rate.

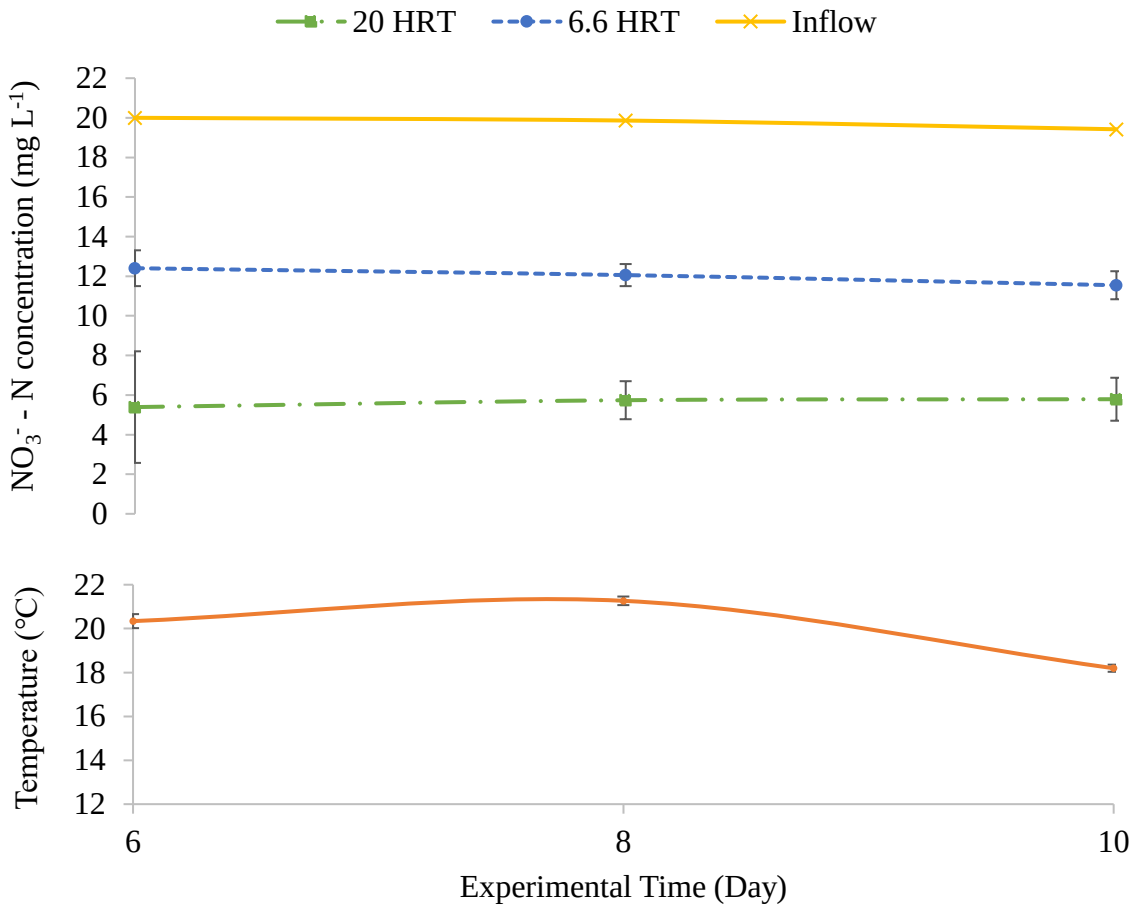

Figure 4-3. A comparison of inflow and outflow NO_3^--N concentrations and temperature over the last three sampling days during the second evaluation of woodchip column bioreactors comparing 6.6-hour and 20-hour hydraulic residence time (*HRT*) treatments. Error bars indicate standard error of the mean.

When comparing the three HRTs across all sets, the 20-hour HRT demonstrated the highest nitrate removal efficiency at 72%, followed by the 10-hour HRT at 39%, and the 6.6-hour HRT, which exhibited efficiencies of 32%, 31%, and 39% across different test sets. Notably, the

nitrate removal rate of the 6.6-hour HRT was greater than both the 10-hour and 20-hour HRTs. The 6.6-hour HRT achieved nitrate removal rates varying from 12.1 to 15.5 g m^{-3} day^{-1}, with an average of 13.6 g m^{-3} day^{-1}. In comparison, the 10-hour HRT recorded a nitrate removal rate of 10.2 g m^{-3} day^{-1}, while the 20-hour HRT had the lowest rate at 9.4 g m^{-3} day^{-1} (Table 4-1). HRT has a statistically significant negative effect on outflow nitrate concentration ($P < 0.01$), indicating that longer HRTs result in lower outflow nitrate concentrations. Conversely, although temperature varied slightly across all sets, it did not show a statistically significant impact on outflow nitrate concentration ($P > 0.05$).

However, while the 20-hour HRT resulted in significantly lower outflow nitrate concentrations compared to both the 10-hour HRT and 6.6-hour HRT ($P < 0.01$), the comparison between the 10-hour HRT and 6.6-hour HRT did not show a statistically significant difference in outflow nitrate concentrations ($P > 0.05$). Particularly, nitrate removal efficiency of the 6.6-hour HRT (39%) in the second evaluation matched that of the 10-hour HRT (39%) in the first evaluation, indicating that increasing from 6.6 HRT to 10 HRT did not significantly enhance nitrate removal efficiency.

Table 4-1.Summary of Mean (± *SD) temperature, inflow and outflow nitrate-nitrogen NO_3^--N concentration, and NO_3^--N removal efficiency and rate from three HRT experiment sets**

Experiment set (HRT)	Temperature (°C)	Inflow NO_3^--N concentration (mg L^{-1})	Outflow NO_3^--N concentration (mg L^{-1})			NO_3^--N removal rate (g N m^{-3} day^{-1})			NO_3^--N removal efficiency (%)		
			6hrs - HRT	10hrs - HRT	20hrs - HRT	6hrs - HRT	10hrs - HRT	20hrs - HRT	6hrs - HRT	10hrs- HRT	20hrs- HRT
6.6	19.6 ± 1.2	19.5 ± 0.2	12.9 ± 0.2			13.2[a]			34.0 ± 0.6[a]		
6.6 vs 10	20.4 ± 1.9	19.5 ± 0.4	13.4 ± 1.0	11.8 ± 0.6		12.0[a]	10.2[b]		31.0± 6.8[a]	39.3 ± 4.6[a]	
6.6 vs 20	19.9 ± 1.6	19.8 ± 0.3	12.0 ± 0.4		5.6 ± 0.2	15.5[a]		9.4[b]	39.2 ± 1.3[a]		71.4 ± 1.5[b]

*SD stands for standard deviation. Same subscript letter means there is not significantly different ($P \geq 0.05$)

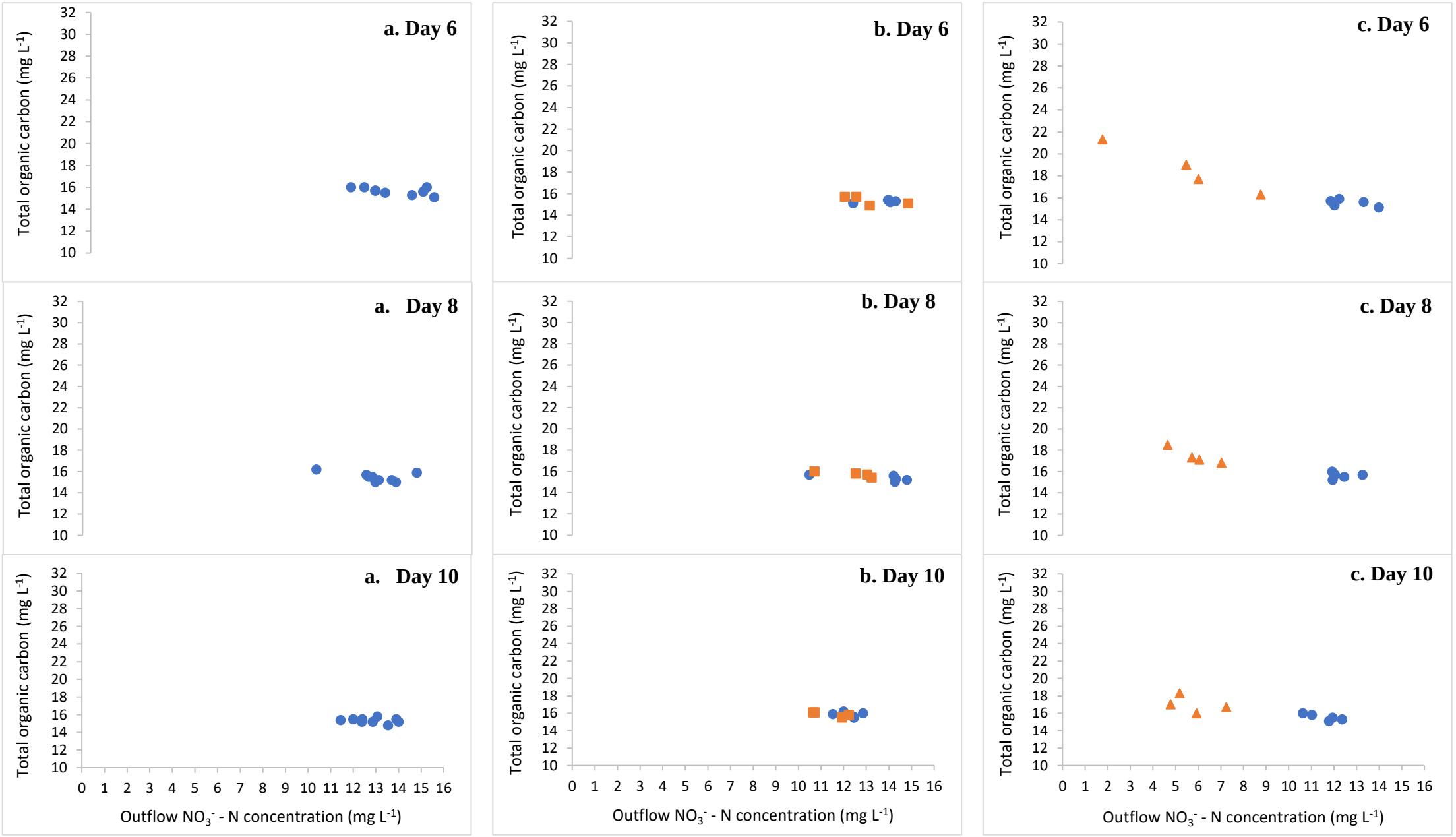

Figure 4-4. Relationship between outflow total organic carbon and outflow NO_3^--N concentrations for sampling on Day 6, 8, and 10 in Experiment 1. (a) Woodchip bioreactor evaluation under 6.6-hour HRT. (b) Woodchip bioreactor evaluation under 6.6-hour vs. 10-hour HRT. (c) Woodchip bioreactor evaluation under 6.6-hour vs. 20-hour HRT. "O" represents 6-hour HRT "□" represents 10-hour HRT. "Δ" represents 20-hour HRT.

To further explore the impact of increasing HRT from 6.6-hour to 10-hour on nitrate removal efficiency, it is essential to examine the overall performance of the column bioreactor in terms of outflow TOC levels and outflow NO_3^--N concentrations across all three sets of experiments for daily sampling (

Figure 4-4). The inflow NO_3^--N and inflow TOC levels were consistent across both the 6.6-hour HRT and the 6.6-hour versus 10-hour HRT evaluations. For the 6.6-hour HRT, inflow NO_3^--N spanned from 19.0 to 20 mg L^{-1} (

Figure 4-1,

Figure 4-2, Figure 4-3), with TOC levels between 13.5 and 14.8 mg L^{-1} (

Figure 4-4). Similarly, in the 6.6-hour versus 10-hour HRT, inflow NO_3^--N fluctuated from 19.04 to 19.92 mg L^{-1} (

Figure 4-2), and TOC varied from 13.5 to 14.2 mg L^{-1} (

Figure 4-4).

When analysing the results for the 6.6-hour HRT during the initial and first evaluation experiment sets, both the outflow NO_3^--N concentrations and outflow TOC levels were consistent. In the initial evaluation of the woodchip columns, the outflow NO_3^--N concentrations ranged from 10.1 to 14.9 mg L^{-1}, with TOC levels varying from 14.8 to 16.2 mg L^{-1} (
Figure 4-4a). Similarly, in the first evaluation experiment set, the outflow NO_3^--N concentrations ranged between 10.5 and 14.9 mg L^{-1}, while the TOC levels varied from 15.1 to 16.2 mg L^{-1}. In contrast, the outflow NO_3^--N for the 10-hour HRT in the first evaluation set ranged from 10.3 to 13.2 mg L^{-1}, and the outflow TOC ranged from 14.9 to 16.1 mg L^{-1} (
Figure 4-4b). When comparing the 6.6-hour and 10-hour HRT scenarios, it was observed that although the 10-hour HRT resulted in slightly lower outflow NO_3^--N concentrations, the TOC levels did not show a significant difference between the two HRTs. This suggests that increasing the HRT from 6.6 to 10 hours did not significantly enhance the overall performance of the column bioreactor in terms of TOC utilisation.

However, the 20-hour HRT significantly reduced outflow NO_3^--N concentrations (average reduction from 19.8 to 5.6 mg L^{-1}) compared to the 6.6-hour HRT (average reduction from 19.8 to 12 mg L^{-1}) (Table 4-1), with a marked increase in outflow TOC levels. Specifically, the TOC levels in the outflow for the 20-hour HRT had an overall average of 17.9 mg L^{-1}, ranging from 16.0 to 21.3 mg L^{-1}, compared to an average of 15.6 mg L^{-1}, ranging from 15.1 to 16.3 mg L^{-1} for the 6.6-hour HRT (
Figure 4-4c). The higher outflow TOC levels observed in the 20-hour HRT indicated that the column bioreactor released additional organic carbon, which facilitated more effective nitrate removal.

4.1.2 Experiment 2: Comparison the effects of methanol and ethanol C sources on NO_3^- removal performance of woodchip column bioreactors

To assess the impact of dosing with different soluble carbon sources on nitrate removal efficiency, a 6.6-hour HRT was selected due to its lower efficiency compared to 10 and 20-hour HRTs (Table 4-1). In this experiment, methanol and ethanol were added to the woodchip bioreactor columns at a C:N ratio of 1:1. Two experimental sets were conducted to confirm the reliability and consistency of the nitrate removal performance under different carbon source treatments. The average temperature, inflow and outflow nitrate concentrations, and nitrate removal efficiency for each treatment are presented in Table 4-2.

As the experiment progressed, significant differences in the outflow nitrate concentrations were observed among the treatment groups ($P < 0.01$) (Figure 4-5). The ethanol treatment resulted in significantly lower outflow nitrate concentrations compared to both the control ($P < 0.01$) and methanol treatments ($P < 0.05$). Similarly, methanol treatment demonstrated significantly lower outflow nitrate concentrations compared to the control ($P < 0.05$).

Table 4-2. Summary of Mean (± *SD) temperature, inflow and outflow nitrate concentrations, and nitrate removal efficiency for control, methanol and ethanol treatments.**

Experiment set	Temperature (°C)	Inflow Nitrate-N (mg L^{-1})	Outflow Nitrate-N (mg L^{-1})			Nitrate-N removal efficiency (%)		
			Control	Methanol	Ethanol	Control	Methanol	Ethanol
Set 1	16.9 ± 0.6	19.4 ± 0.4	12.1 ± 0.6^{a}	8.4± 0.4^{b}	6.7 ± 1.3^{b}	37.6 ± 3.2^{a}	56.7 ± 1.4^{b}	65.7 ± 6.3^{b}
Set 2	15.8 ± 0.5	19.6 ± 0.4	13.2 ± 0.4^{a}	8.9 ± 0.8^{b}	6.4 ± 0.6^{b}	33.0 ± 1.7^{a}	54.7 ± 4.8^{b}	67.5 ± 2.5^{b}

*SD stands for standard deviation. Same subscript letter means there is not significantly different ($P \geq 0.05$).

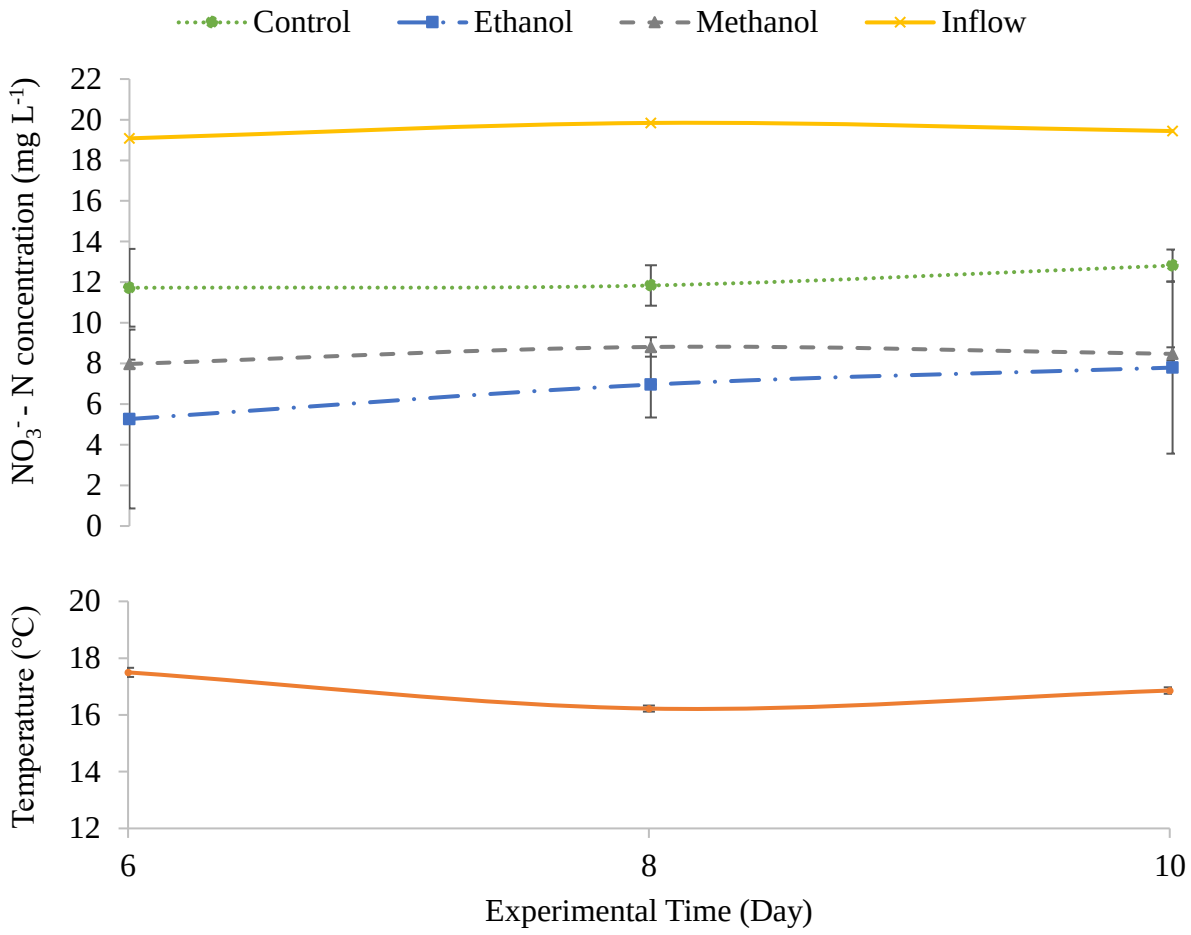

Figure 4-5. A comparison of inflow and outflow NO_3^--N concentrations and temperature over the last three sampling days during the first experiment set comparing control, methanol, and ethanol treatments. Error bars indicate the standard error of the mean.

In the first experimental set, the ethanol treatment reduced NO_3^--N concentrations in the outflows ranging from 5.3 to 7.8 mg NO_3^--N L^{-1}, which were lower than those observed in the methanol treatment group (7.9 to 8.8 mg NO_3^--N L^{-1}) and the control group (11.7 to 12.8 mg NO_3^--N L^{-1}) (Figure 4-5). The nitrate removal efficiency was quantified for the ethanol group at 66%, followed by 57% for the methanol group, and 38% for the control group (Table 4-2). To validate these findings, the second experimental set was conducted, and it further confirmed the superior performance of the ethanol treatment (Table 4-2, Figure 4-6). In this set, the ethanol-treated outflows showed NO_3^--N concentrations between 5.8 and 6.9 mg NO_3^--N L^{-1}, moderately lower than those in the methanol-treated (8.3 to 9.7 mg NO_3^--N L^{-1}) and control (12.9 to 13.7 mg NO_3^--N L^{-1}) groups (Figure 4-6). The corresponding nitrate removal

efficiencies were quantified at 68% for the ethanol group, 55% for the methanol group, and 33% for the control group (Table 4-2).

The addition of carbon sources to the woodchip column bioreactor significantly enhanced nitrate removal efficiency compared to the control, which had no carbon source addition. These results consistently indicate that the ethanol treatment group outperformed the methanol group in both experimental sets, achieving nearly double the nitrate reduction level of the control group. This consistent performance across both experimental sets highlights the potential of ethanol as a more effective carbon source for nitrate removal in woodchip column bioreactor systems.

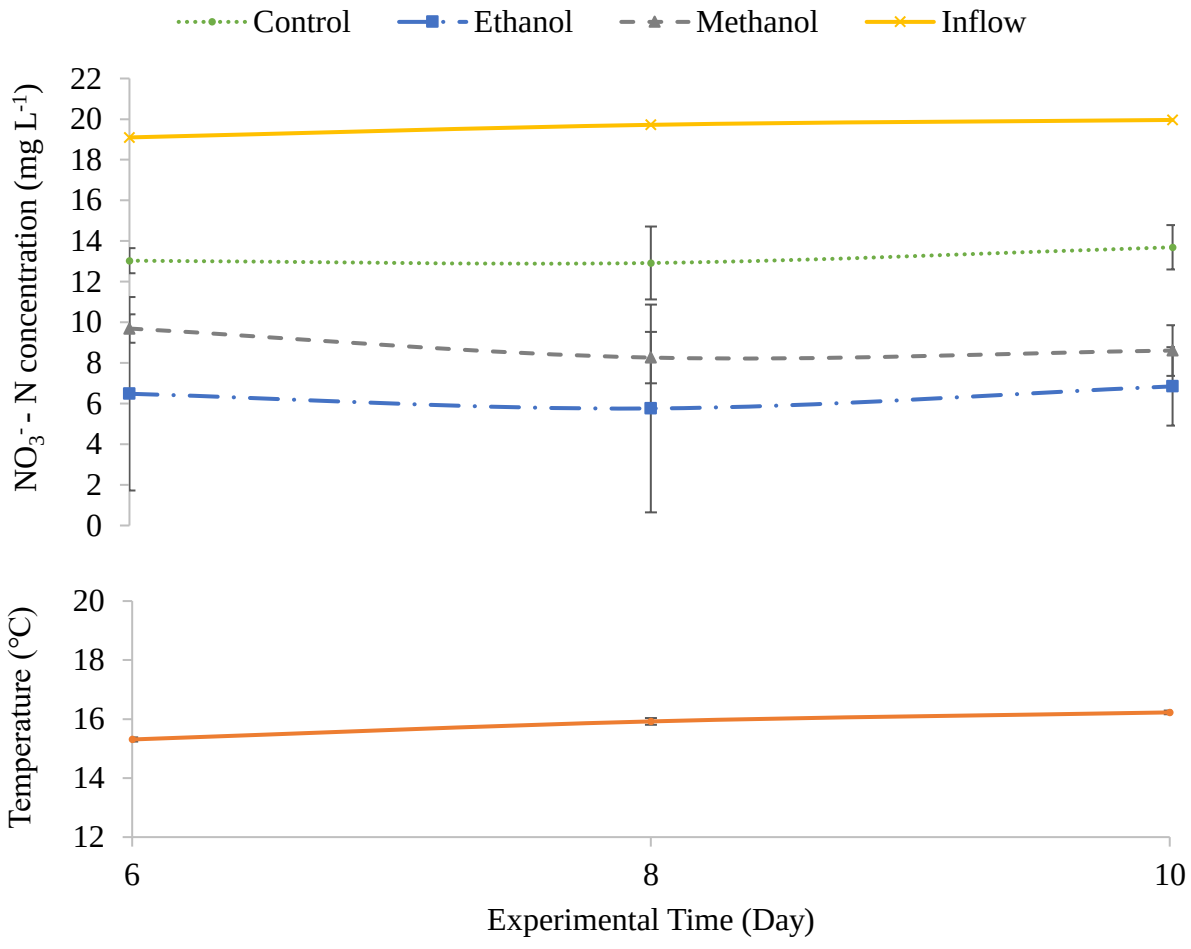

Figure 4-6. A comparison of inflow and outflow NO_3^--N concentrations and temperature over the last three sampling days during the second experiment set comparing control, methanol, and ethanol treatments. Error bars indicate the standard error of the mean.

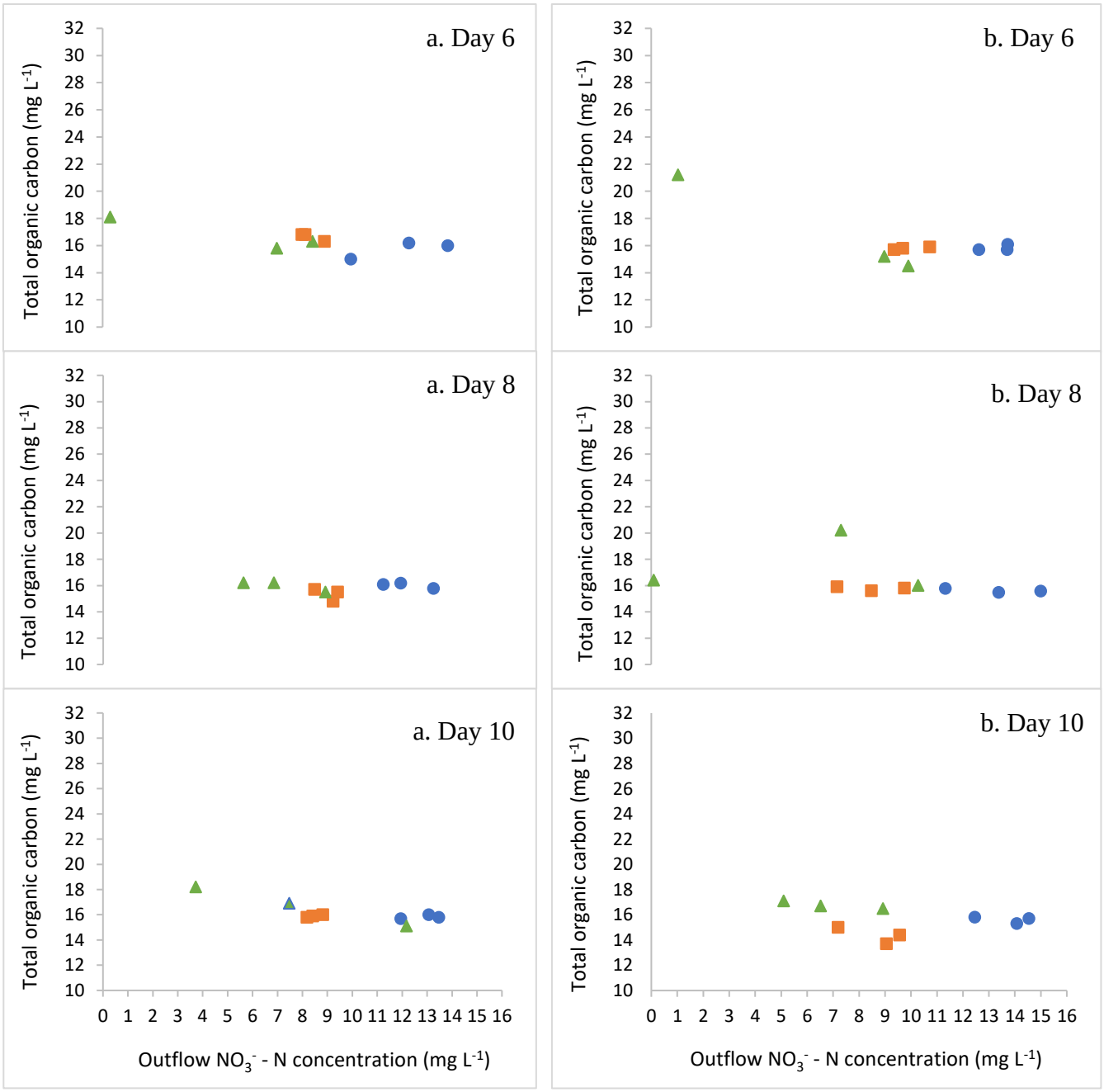

Figure 4-7. Relationship between outflow total organic carbon and outflow NO_3^--N concentrations for sampling on Day 6, 8, and 10 in Experiment 2. (a) Comparison the effects of methanol and ethanol dosing on NO_3^- removal in Experimental set 1. (b) Comparison the effects of methanol and ethanol dosing on NO3- removal in experimental set 2. "O" represents control treatment. "□" represents methanol treatment. "Δ" represents ethanol treatment.

To comprehensively assess the effectiveness of carbon dosing on bioreactor performance, it is critical to analyse the relationship between outflow TOC and outflow NO_3^--N concentrations. The inflow NO_3^--N concentrations were measured, ranging from 19.1 to 20.0 mg L^{-1}, (Figure 4-5, Figure 4-6) and TOC levels ranged from 14.2 to 14.8 mg L^{-1} across both experimental sets (Figure 4-7).

The control columns, which did not receive any additional carbon dosing, demonstrated the least effective nitrate removal performance. The outflow NO_3^--N concentrations for the control columns varied between 12.0 and 14.6 mg L^{-1}, while TOC levels in the outflow ranged from 15.0 to 16.2 mg L^{-1}. In contrast, methanol consistently reduced NO_3^--N concentrations in both experimental sets, resulting in NO_3^--N outflow concentrations between 5.5 and 10.5 mg L^{-1}. The outflow TOC levels from the methanol-dosed columns fluctuated from 14.4 to 16.8 mg L^{-1}, indicating a modest increase compared to the inflow levels and control group. Meanwhile, ethanol dosing demonstrated superior nitrate removal efficiency compared to both the methanol-dosed and the control columns. The outflow NO_3^--N concentrations for ethanol-dosed columns were significantly lower, with values ranging from 0.3 to 10.0 mg L^{-1}. The outflow TOC levels from the ethanol-dosed columns were measured slightly higher, ranging from 14.5 to 21.2 mg L^{-1} (Figure 4-7).

The findings showed a relationship between increased outflow TOC concentrations in the methanol-dosed and ethanol-dosed column bioreactors and decreased outflow NO_3^--N levels. Overall, the outflow concentrations in the ethanol-dosed group were not significantly different from those in the methanol-dosed and control groups. However, a few ethanol-dosed columns showed elevated levels of organic carbon on Day 10 (TOC: 18.1 mg L^{-1}) in the experimental set 1; and on Day 6 (TOC: 21.2 mg L^{-1}) and Day 8 (TOC: 20.2 mg L^{-1}) in the experimental set 2. This indicated variability in carbon dosing consistency in the columns. Biofilm formation was also observed in two columns on Day 6 and Day 8, suggesting a potential link between elevated outflow TOC levels and microbial growth dynamics. Despite the high outflow TOC concentrations in these ethanol-dosed columns, only one column on Day 6 in the experimental set 2 (TOC: 21.2 mg L^{-1}) achieved a lower NO_3^--N concentration of 1.0 mg L^{-1}.

4.1.3 Experiment 3: Comparative analysis of ethanol dosing on nitrate removal efficiency in woodchip, pumice, and gravel column bioreactors

In Experiment 3, the column bioreactors without ethanol dosing were initially operated to assess the baseline effectiveness of woodchip, pumice, and gravel column bioreactors. During the last three days of this undosed phase (Days 2 to 6 of the experiment), the inflow NO_3^--N concentration fluctuated between 19.4 and 20 mg NO_3^--N L^{-1} (mean 19.8 mg NO_3^--N L^{-1}), with temperatures varying between 13.3 and 13.9℃ (mean 13.6℃). Both the gravel and pumice column bioreactors showed minimal nitrate reduction, with outflow NO_3^--N concentrations spanning from 18.4 to 19.1 mg NO_3^--N L^{-1} (mean 18.7 mg NO_3^--N L^{-1}) for the gravel columns, and from 18.7 to 18.9 mg NO_3^--N L^{-1} (mean 18.8 mg NO_3^--N L^{-1}) for the pumice columns. In contrast, the woodchip column bioreactors exhibited more effective nitrate reduction, with outflow NO_3^--N concentrations falling between 14.1 and 17.1 mg NO_3^--N L^{-1} (mean 15.6 mg NO_3^--N L^{-1}) (Figure 4-8). The pumice and gravel column bioreactors both achieved a nitrate removal efficiency of 5%, whereas the woodchip columns demonstrated a threefold increase, achieving a 19% nitrate removal efficiency.

To compare the effects of ethanol dosing on nitrate removal efficiency in woodchip, pumice, and gravel media bioreactors, a 1:1 C:N ratio was used under a 6.6-hour HRT. During the last three days of the experiment (i.e., 10 to 14 days in the experiment run), the inflow NO_3^--N concentration ranged from 19.3 to 19.8 mg NO_3^--N L^{-1} (mean 19.6 mg NO_3^--N L^{-1}), and the temperature ranged from 13.1 to 13.6℃ (mean 13.4℃) (Table 4-3, Figure 4-9).

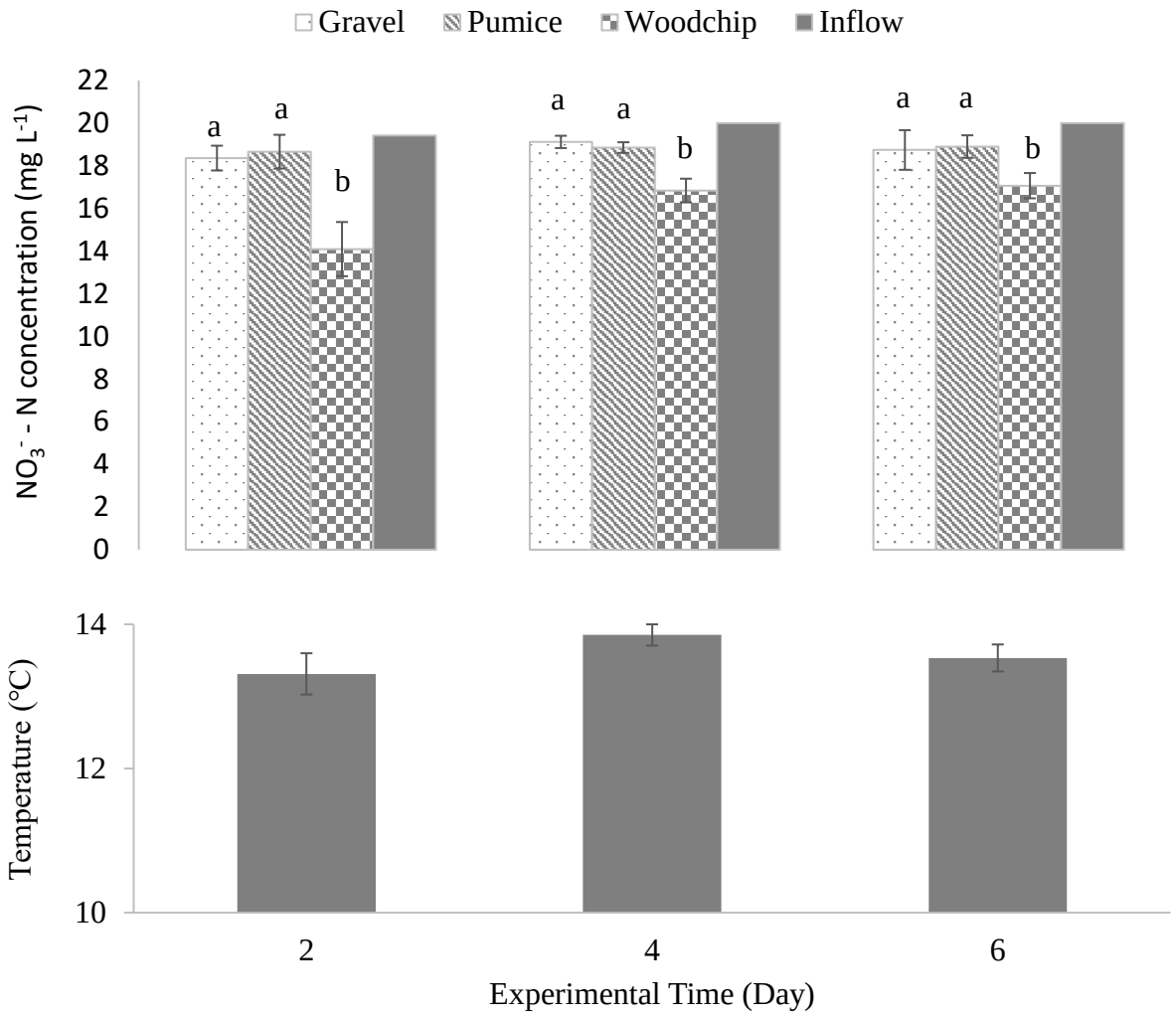

Figure 4-8. Comparison of the inflow and outflow NO_3^--N concentrations and temperature during the three sampling days without ethanol dosing for woodchip, pumice, and gravel column bioreactors. Error bars indicate the standard error of the mean. Same subscript letter means there is not significantly different ($P \geq 0.05$).

The woodchip column bioreactor demonstrated exceptionally high performance in reducing nitrate concentrations, significantly outperforming both the gravel and pumice column bioreactors (Figure 4-9). The average outflow NO_3^--N concentration for the woodchip column bioreactors was measured as 0.54 mg NO_3^--N L^{-1}, with a range from 0.31 to 0.97 mg NO_3^--N L^{-1}. In contrast, the gravel column bioreactor had an average outflow NO_3^--N concentration of 4.9 mg NO_3^--N L^{-1} (range 3.2 to 6.7 mg NO_3^--N L^{-1}), and the pumice column bioreactor had an average outflow NO_3^--N concentration of 5.6 mg NO_3^--N L^{-1} (range 5.2 to 6.2 mg NO_3^--N L^{-1}) (Figure 4-9).

All three treatments of different media (woodchip, gravel, and pumice) combined with ethanol dosing achieved nitrate removal efficiencies above 70% (Table 4-3), a significant improvement compared to the undosed column bioreactors, which attained only 5% for pumice (average 18.8 NO_3^--N L^{-1}) and gravel (average 18.7 NO_3^--N L^{-1}) and 19% (average 16 NO_3^--N L^{-1}) for woodchip column bioreactors (Figure 4-8). The woodchip column bioreactors dosed with the ethanol demonstrated an exceptionally high nitrate removal efficiency of 97%, nearly eliminating the inflow NO_3^--N concentration (Figure 4-9). The gravel and pumice column bioreactors also showed high nitrate removal efficiencies, achieving 75% and 72% in the outflow NO_3^-N concentrations, respectively (Table 4-3).

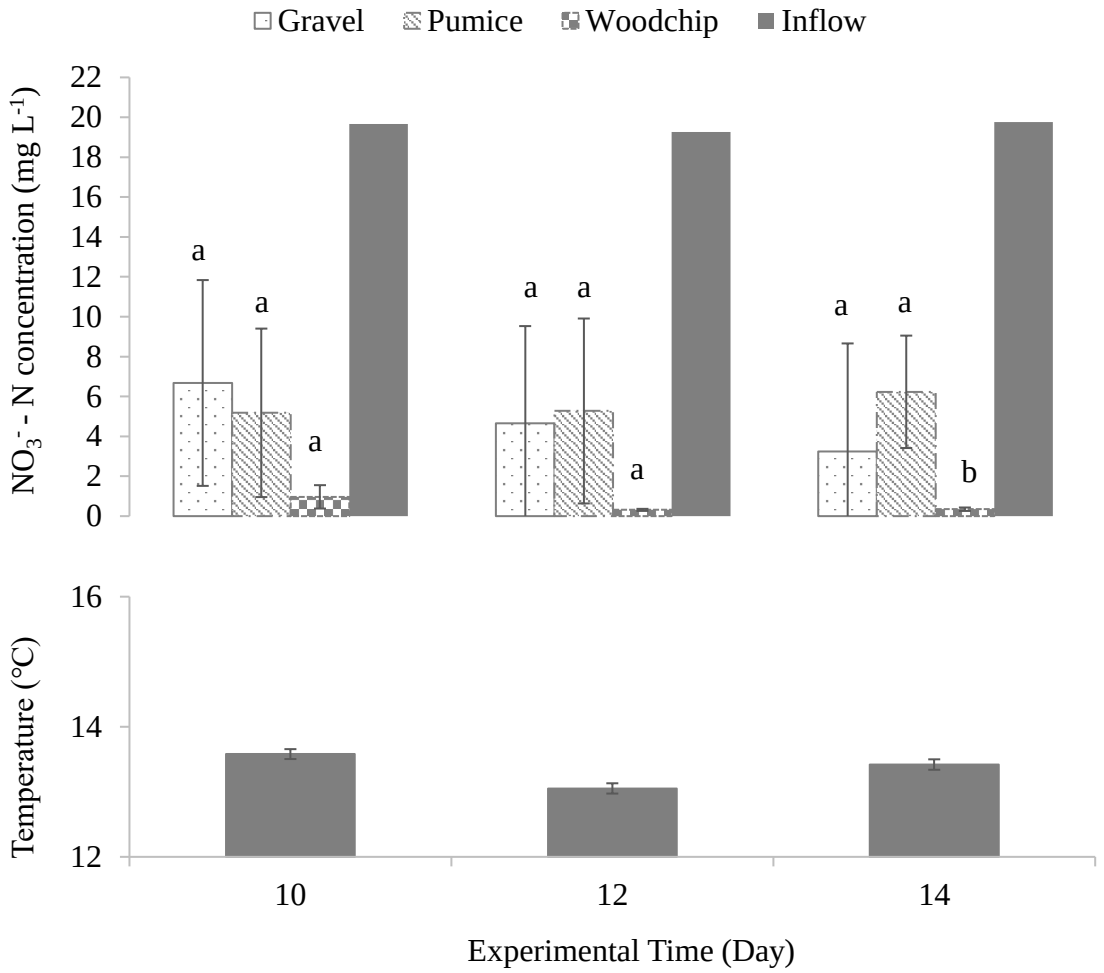

Figure 4-9. Comparison of the inflow and outflow NO_3^--N concentrations and temperature during the last three sampling days for ethanol-dosed woodchip, pumice, and gravel column bioreactors. Error bars indicate the standard error of the mean. Same subscript letter means there is not significantly different ($P \geq 0.05$)

Table 4-3. Summary of Mean (± *SD*), temperature, inflow nitrate concentration, outflow nitrate concentration and nitrate removal efficiency for gravel, pumice and woodchip bioreactors

Experiment day	Temperature (°C)	Inflow NO_3^-N (mg L^{-1})	Outflow NO_3^-N (mg L^{-1})			NO_3-N removal efficiency (%)		
			Gravel	Pumice	Woodchip	Gravel	Pumice	Woodchip
10	13.6 ± 0.08	19.7	6.7 ± 5.2[a]	5.2 ± 4.2[a]	0.97 ± 0.59[a]	66.0	73.7	95.1
12	13.1 ± 0.08	19.3	4.6 ± 4.9[a]	5.3 ± 4.6[a]	0.31 ± 0.05[a]	75.9	72.6	98.4
14	13.4 ± 0.08	19.8	3.3 ± 5.4[a]	6.2 ± 2.8[a]	0.35 ± 0.09[b]	83.6	68.5	98.3
Mean	**13.4 ± 0.27**	**19.6 ± 0.3**	**4.9 ± 1.7[a]**	**5.6 ± 0.6[a]**	**0.54 ± 0.36[b]**	**75.2 ± 8.8[a]**	**71.6 ± 2.8[a]**	**97.3 ± 1.2[b]**

± stands for standard deviation (*SD*). Same subscript letter means there is not significantly different ($P \geq 0.05$)

Table 4-4. Summary of Mean (± *SD*), outflow nitrate concentration and ANOVA analysis for gravel, pumice and woodchip column bioreactors

Experiment	Mean outflow NO_3^-N (mg L^{-1})			*P-value*			
day	**Gravel**	**Pumice**	**Woodchip**	**Between treatments**	**WB and GB**	**WB and PB**	**GB and PB**
10	6.7 ± 5.2	5.2 ± 4.2	0.97 ± 0.59	0.25	0.25	0.43	0.89
12	4.6 ± 4.9	5.3 ± 4.6	0.31 ± 0.05	0.31	0.41	0.33	0.98
14	3.3 ± 5.4	6.2 ± 2.8	0.35 ± 0.09	0.003**	0.23	0.03*	0.11
Three days	**4.9 ± 1.7**	**5.6 ± 0.6**	**0.54 ± 0.36**	**0.009****	**0.03***	**0.01***	**0.90**

WB = Woodchip Bioreactor, GB = Gravel Bioreactor, PB = Pumice Bioreactor; ± SD = Standard Deviation.

***P* <0.01 indicates a highly significant difference; **P* <0.05 indicates a significant difference.

The outflow NO_3^--N were statistically significantly different among the different media column bioreactor types ($P < 0.01$). The woodchip column bioreactor had significantly lower outflow NO_3^--N concentrations compared to both the gravel ($P < 0.05$) and pumice ($P < 0.05$) columns bioreactors. However, no significant difference was observed between the gravel and pumice bioreactors ($P > 0.05$). While the Days 10 and 12 did not show statistically significant differences in the outflow NO_3^--N concentrations among the bioreactor columns ($P > 0.05$), the results for the Day 14 indicated that the pumice column bioreactors had significantly higher outflow NO_3^--N concentrations compared to the woodchip column bioreactor ($P < 0.05$). However, there were no statistically significant differences in the outflow NO_3^--N concentrations between the gravel and woodchip bioreactors, nor between the gravel and pumice column bioreactors on Day 14 ($P > 0.05$) (Table 4-4). These findings highlight the overall superior performance of the woodchip column bioreactors in nitrate removal across the experimental period, with consistent reductions in nitrate levels outperforming the other media.

However, the daily fluctuations in outflow NO_3^--N and outflow TOC levels during the last three sampling days indicated inconsistencies in ethanol dosing, particularly in the gravel and pumice column bioreactors, where significant variability was observed. The outflow NO_3^--N concentrations for the gravel column bioreactors varied from 4.0 to 10.4 mg L^{-1} on Days 10 and 12, with TOC levels between 14.5 and 17.6 mg L^{-1} (Figure 4-10a and b). Conversely, on Day 14, the gravel column bioreactors displayed inconsistency in ethanol dosing. One column showed an unusually high TOC concentration, reaching a peak of 30.3 mg L^{-1}, the highest observed across all experimental days. This elevated TOC level of 30.3 mg L^{-1} led to a sharp reduction in the outflow NO_3^--N concentration to a minimal level of 0.1 mg L^{-1}. In contrast, gravel column No.1 had outflow TOC concentration of 13.9 mg L^{-1} on Day 14, which was below the inflow TOC level (ranging from 14.2 to 14.8 mg L^{-1}). This insufficient carbon availability resulted in significantly higher outflow NO_3^--N concentrations, with an outflow NO_3^--N level of 9.5 mg L^{-1} (Figure 4-10c).

The pumice columns exhibited outflow NO_3^--N concentrations ranging from 0.15 to 9.34 mg L^{-1} and outflow TOC levels between 14.7 and 21 mg L^{-1} over the last three sampling days. On Day 10, the pumice columns bioreactors showed better nitrate removal efficiency than the gravel columns bioreactors, but were still less effective than the woodchip column bioreactors (Figure 4-10a). On Days 12 and 14, the pumice columns were outperformed by the gravel

columns in nitrate removal (Figure 4-10b and c). Analysing the relationship between outflow TOC levels and nitrate removal efficiency in the pumice columns revealed that when the outflow TOC concentrations of pumice (14.7 to 16.2 mg L^{-1}) were close to the inflow TOC (14.5 to 14.8 mg L^{-1}), the outflow NO_3^--N concentrations remained above 5 mg L^{-1}. However, when the outflow TOC levels increased from 14.7 to 16.2 mg L^{-1} to 17 mg L^{-1} on Day 14, 19.8 mg L^{-1} on Day 10, and 21 mg L^{-1} on Day 12, the outflow NO_3^--N concentrations dropped significantly to 3.8 mg L^{-1}, 0.40 mg L^{-1}, and 0.15 mg L^{-1}, respectively (Figure 4-10). The outflow TOC variability observed in one pumice column implied slight inconsistency in the ethanol dosing within the pumice bioreactors.

Overall, the woodchip columns achieved significantly lower outflow NO_3^--N concentrations (average 0.54 mg L^{-1}) compared to both pumice (average 5.6 mg L^{-1}) and gravel (average 4.9 mg L^{-1}) bioreactor columns across all three sampling days (Table 4-4). The results demonstrated a correlation between higher outflow TOC concentrations and lower outflow NO_3^--N concentrations, indicating effective nitrate removal when outflow TOC levels were elevated. Specifically, the woodchip columns consistently exhibited outflow NO_3^--N concentrations below 2 mg L^{-1} over the three sampling days, while outflow TOC concentrations ranged from 19.8 to 25.8 mg L^{-1}, markedly higher than the inflow TOC concentrations (14.5 to 14.8 mg L^{-1}) (Figure 4-10).

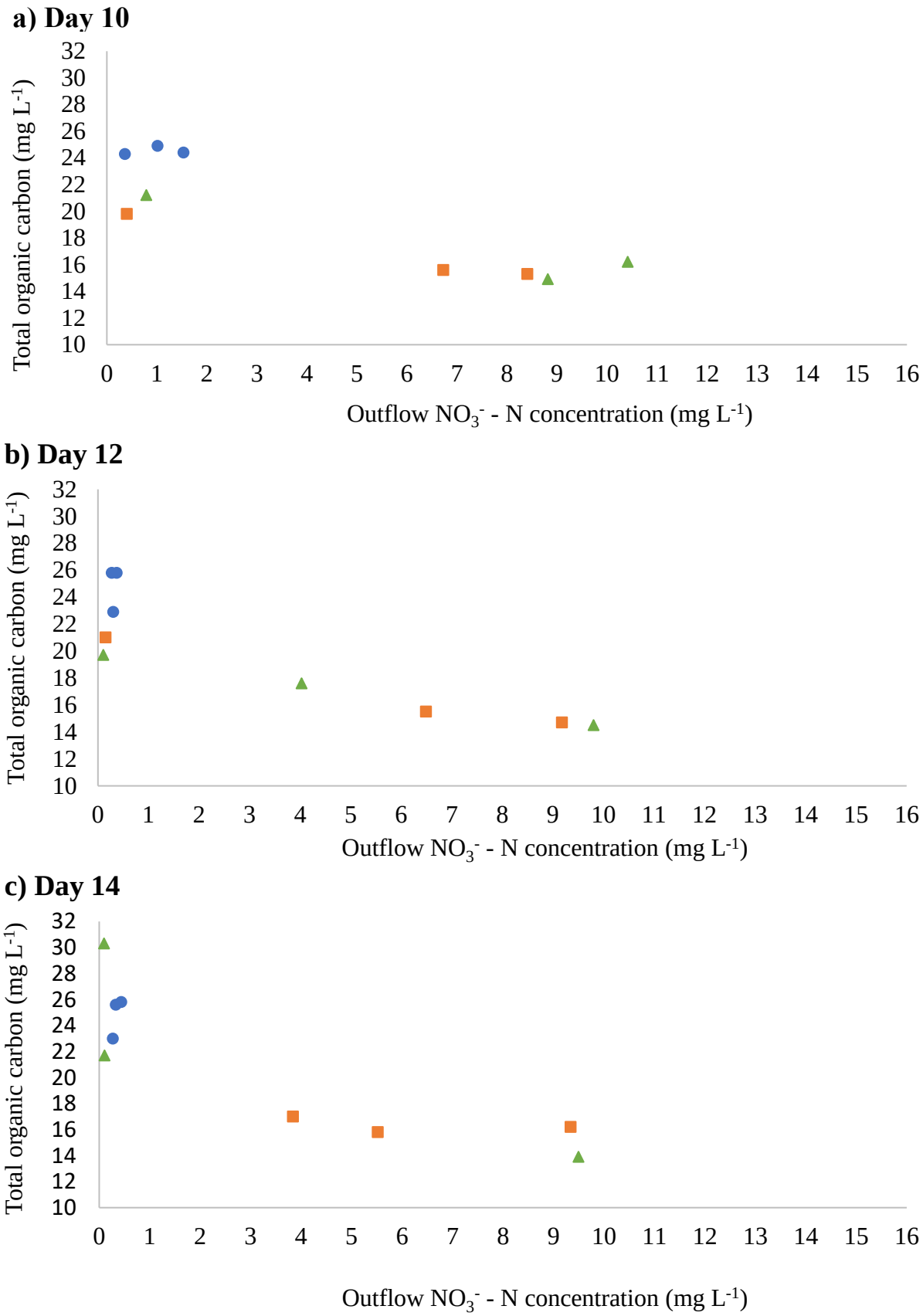

Figure 4-10. Relationship between outflow total organic carbon and outflow NO_3^--N concentrations for the sampling on Days 10, 12, and 14 in Experiment 3. "O" represents woodchip media treatment. "O" represents woodchip media treatment. "□" represents pumice media treatment. "Δ" represents gravel media treatment.

4.1.4 Cost comparison of ethanol-dosed gravel bioreactors and non-dosed woodchip bioreactors

The cost-effectiveness projection, shown in New Zealand dollars (*NZD*) per kilogram per year of nitrate removal, was derived by analysing the lifespan projections of different bioreactors: 10 and 15 years for woodchip bioreactors, and 30 years for gravel bioreactors. The analysis accounted for shared costs between the bioreactors, including piping, PVC liners, field excavation, and labour. Additionally, the woodchip bioreactor incurred specific expenses for the woodchip material, whereas the capital costs for the gravel bioreactor included expenditures on washed gravel and ethanol.

In Scenario A, which adhered to the Experiment 3 settings, woodchip bioreactors with a porosity of 50% and gravel bioreactors with a porosity of 30% were evaluated. The annual nitrate removal rate (*NRR*), calculated for the drainage season covering half a year, showed that the woodchip bioreactor achieved an estimated 466 kg N removed per year in both the 10-year and 15-year lifespan scenarios, while the gravel bioreactor was projected to achieve 599 kg N removed per year in the 30-year scenario. In this cost analysis, both woodchip and gravel bioreactors were scaled to 200 m³. The capital expenditure for the woodchip bioreactor primarily included the cost of the woodchip material itself, amounting to NZ$35 per m³, which totals an annual cost of NZ$7000 ($NZ35 m^{-3} x 200 m^3). In comparison, the capital costs for the gravel bioreactor included NZ$9940 ($NZ49.7 m^{-3} x 200 m^3) for washed gravel and an additional NZ$4223 for ethanol, with ethanol consumption calculated at NZ$23 day^{-1} ($NZ2.75 L^{-1} x 8.39 L Day^{-1}), over the 183-day drainage season. The comprehensive annual costs of both bioreactors also accounted for a 6% opportunity cost (interest) calculated over their projected lifespans.

The annual cost for the woodchip bioreactor in Scenario A was estimated at NZ$2,727 for a 10-year lifespan and NZ$2,023 for a 15-year lifespan. The gravel bioreactor's annual cost over a 30-year lifespan was projected at $NZ5,984 (Appendix 2). When assessing cost-effectiveness based on the nitrate removal rate, the woodchip bioreactor achieved $5.9 NZD per Kg N removed for the 10-year lifespan and NZ$4.3 per kg N removed for the 15-year lifespan, while the gravel bioreactor achieved NZ$10 per kg N removed over 30 years (Figure 4-11).

In Scenario B, the porosity for both woodchip and gravel bioreactors were uniformly adjusted to 50%. However, during the drainage season, the nitrate removal rates were assumed consistent with those observed in Scenario A: the woodchip bioreactor achieved 466 kg N removed per year for both the 10-year and 15-year lifespan scenarios. Meanwhile, for this scenario, the gravel bioreactor, assessed over a 30-year lifespan, achieved a higher nitrate removal rate of 998 kg N removed per year compared to Scenario A. This improvement was attributed to the higher flow rate achieved with 50% porosity compared to 30%, with the HRT fixed at 6.6 hours. The increased porosity enhanced flow distribution and water-media contact, resulting in greater nitrate reduction efficiency. The capital expenditure for the woodchip bioreactor remained the same as in Scenario A, with an annual cost of NZ$7000 for the woodchip. However, the capital costs for the gravel bioreactor in this scenario were higher than in Scenario A due to increased ethanol requirements based on the flow rate. Ethanol consumption was calculated at NZ$23 per day (NZ$2.75 L^{-1} x 13.99 L day^{-1}), accumulating to NZ$7038 over the 183-day drainage season. The cost of washed gravel remained consistent with Scenario A, amounting to NZ$9940. With a 6% opportunity cost (interest), the annual cost for the woodchip bioreactor remained consistent with Scenario A, at NZ$2,727 for a 10-year lifespan and NZ$2,023 for a 15-year lifespan. The annual cost for the gravel bioreactor, projected over a 30-year lifespan, was NZ$8,800, as detailed in Appendix 2. In terms of cost-effectiveness based on nitrate removal, the woodchip bioreactor maintained its performance with costs of NZ$5.9 per kg N removed over 10 years and NZ$4.3 per kg N removed over 15 years. The gravel bioreactor, projected for 30 years, achieved a rate of NZ$8.8 per kg N removed, indicating a lower cost compared to the gravel bioreactor in Scenario A (Figure 4-11).

It is evident that gravel bioreactors in Scenario B, costing NZ$8.8 per kg N removed, are less expensive than those in Scenario A, which cost NZ$10 per kg N removed. However, a comparative analysis of woodchip and gravel bioreactors over various lifespans reveals that the woodchip bioreactors for 10-year and 15-year lifespan consistently outperforms the gravel bioreactor in both scenarios. Specifically, the woodchip bioreactor achieves the lowest cost of NZ$4.3 per kg N removed for a 15-year lifespan, demonstrating superior cost-effectiveness in both Scenario A and Scenario B. This assumes that the nitrate removal rate for woodchip bioreactors is maintained steadily at an average of 466 kg N removed per year across their 10- to 15-year lifespan, which is lower than that of gravel bioreactors over a 30-year lifespan (998 kg N removed per year).

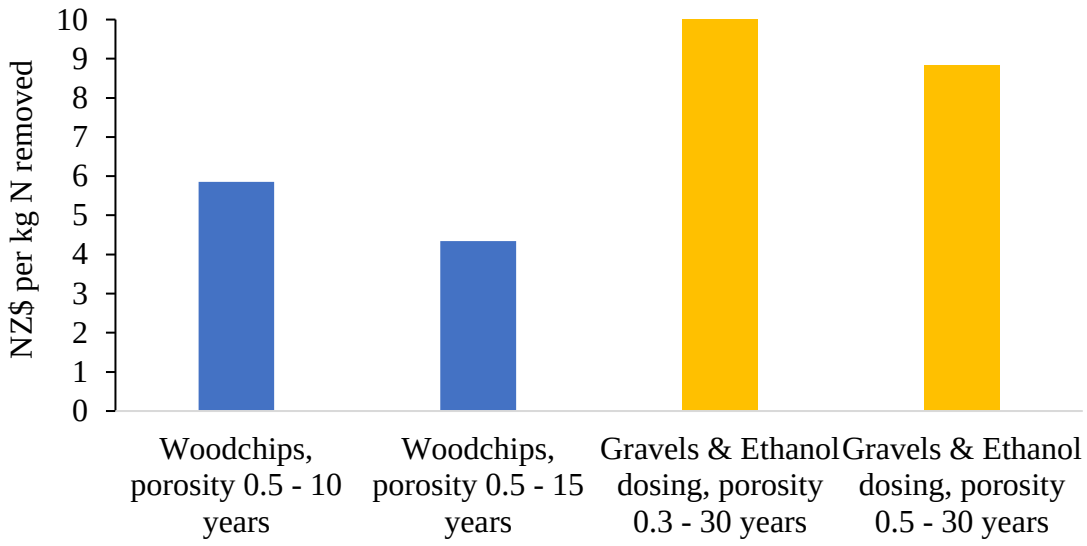

Figure 4-11. a comparative analysis of the cost-effectiveness, measured in New Zealand dollars (*NZD*) per kilogram of nitrate removal rate, across lifespan projections of 10 and 15 years for woodchip, and 30 years for gravel, with porosity indicated as 0.5 (50%) and 0.3 (30%).

4.2 Discussion

4.2.1 Potential effects of different HRT on nitrate reduction in woodchip column bioreactor

Experiment 1 demonstrated that longer HRTs improved nitrate removal efficiency in the column woodchip bioreactors. Extending the HRT from 6.6 hours (with nitrate removal efficiencies between 31% and 39%) to 20 hours (achieving a 71% nitrate removal efficiency) resulted in a substantial increase in nitrate removal efficiency, ranging from 32% to 40%.

The correlation between longer HRTs and improved nitrate removal efficiency observed in this study is consistent with findings from previous column experiments that tested a wide range of HRTs (Addy et al., 2016; Christianson et al., 2017; Hoover, 2012; Nordström & Herbert, 2017;

Pluer et al., 2016). Hoover (2012) examined woodchip column bioreactor study with HRTs of 2, 4, 8, 12, 18, and 24 hours at a constant temperature of 20°C, which was similar to the temperature range of the current study (19.55-20.39°C). Their outcomes reported minimal nitrate removal efficiency at a 2-hour HRT, while extending the HRT from 4 hours (7.8%) to 24 hours (54.9%) resulted in a 47% increase in nitrate removal efficiency. Nordström and Herbert (2017), found that at higher HRTs, nitrate removal efficiency exceeded 95% at temperatures of 22°C, 12°C, and 5°C, compared to approximately 50% at lower HRTs in laboratory column experiment. Similarly, Christianson et al. (2017) revealed that when comparing a 7.2-hour HRT to a 51-hour HRT, nitrate removal efficiency increased significantly from approximately 18% to over 90% in a paired-column configuration study, with an influent water temperature of 17.6°C.

However, it has been suggested that shorter HRTs lead to a faster washout of microbial communities, particularly denitrifying bacteria, from the woodchips, reducing the physical interaction between these bacteria and the woodchip surfaces. This reduction in contact may constrain denitrification activity in the bioreactor, resulting in lower nitrate removal efficiency (Damaraju et al., 2015; Hoover, 2012; Yusoff et al., 2010). On the other hand, the significant improvement in nitrate removal efficiency observed under the 20-hour HRT in this column study is accompanied by higher TOC levels in the outflow compared to 6.6-hour and 10-hour HRTs. These findings suggest that longer HRTs, such as 20 hours, allow more time for woodchips to release organic carbon, enhancing denitrification by providing a greater carbon source for microbial activity and thus improving nitrate removal efficiency (Brink, 2023; Lin et al., 2011). From the perspective of denitrification in our study, a longer HRT of 20 hours proved to be a more suitable operating condition for the woodchip column bioreactor compared to a 6.6-hour HRT, achieving 71% nitrate removal efficiency and a nitrate removal rate of 9.39 $g\ m^{-3}\ day^{-1}$.

Even though increasing HRTs in bioreactors has been proven to improve nitrate removal efficiency (Christianson et al., 2017; Hoover, 2012; Nordström & Herbert, 2017; Pluer et al., 2016), Experiment 1 indicated no significant difference in nitrate removal efficiency between the 6.6-hour HRT (ranging between 31% and 39%) and the 10-hour HRT (39%). The findings for nitrate removal efficiency at the 6.6-hour HRT align with Brink (2023)'s column bioreactor study, which reported a median nitrate removal efficiency of 30% at a 6-hour HRT in woodchip

column bioreactors when compared with other substrate types, including peanut shells and gravel. Similarly, Pluer et al. (2016) conducted a controlled laboratory experiment comparing nitrate removal efficiencies at various HRTs (2, 4, 6, 8, 10, and 12 hours). Their study revealed a 33% nitrate removal efficiency at the 6-hour HRT, which is consistent with the Experiment 1 findings. However, when comparing the 6-hour and 10-hour HRTs, their results showed a higher efficiency of 50% at the 10-hour HRT, compared to 39% efficiency achieved at the same HRT in the Experiment 1. The difference in nitrate removal efficiency might be due to potential variability in bioreactor design, substrate composition, or microbial community structure, which could contribute to the differences in performance between the studies. A close examination of the Experiment 1 data reveals that the outflow TOC concentrations for the 6.6-hour and 10-hour HRTs remained similar. This suggests that increasing the HRT from 6.6 to 10 hours did not significantly enhance TOC utilisation or release, which may explain the similar nitrate removal efficiencies of 39% observed at 6.6-hours and 10-hours HRTs in the Experiment 1. The comparable TOC levels imply that the increased retention time from 6.6 to 10 hours in the Experiment 1 was insufficient to promote additional carbon release from the woodchips, thus limiting further enhancement in denitrification efficiency.

Nevertheless, the Experiment 1 findings observations that the 6.6-hour HRT, with the highest flow rate of 9.3 ml min^{-1}, achieved the highest nitrate removal rate, ranging from 12.03 to 15.46 g m^{-3} day^{-1}. In comparison, the 10-hour HRT, with a lower flow rate of 6.2 ml min^{-1}, resulted in a slightly reduced nitrate removal rate of 10.2 g m^{-3} day^{-1}. The lowest flow rate of 3.1 ml min^{-1} at the 20-hour HRT corresponded with a nitrate removal rate of 9.39 g m^{-3} day^{-1}. This indicates that while longer HRTs enhance nitrate removal efficiency, they may also reduce the overall nitrate removal rate due to less flow rate through the bioreactor. Several studies have confirmed that extending HRTs often results in reduced nitrate removal rates, a pattern consistent with the outcomes observed in this study (Hackshaw, 2018). In a mesoscale laboratory experiment conducted by Hackshaw (2018), the woodchip bioreactor demonstrated that longer HRTs increased nitrate removal efficiency from 52% at a 4-hour HRT to 81% at a 12-hour HRT. However, this improvement in nitrate removal efficiency was accompanied by a decline in nitrate removal rates, which decreased from 7.8 g N m^{-3} d^{-1} at the 4-hour HRT to 4.5 g N m^{-3} d^{-1} at the 12-hour HRT. Additionally, Lepine et al. (2016) observed similar results in their field study of denitrifying woodchip bioreactors treating recirculating aquaculture system effluent. Their findings revealed that while the shortest HRT (12 hours) had the lowest nitrate removal efficiency (45%) compared to the longest HRT (55 hours) with a nitrate

removal efficiency of 99%. However, the 12-hour HRT exhibited a significantly higher nitrate removal rate (39 g N m^{-3} d^{-1}) than the longest HRT (55 hours), which achieved only 18 g N m^{-3} d^{-1}. Greenan et al. (2009) also demonstrated in their laboratory column study that woodchip bioreactors with higher flow rates (6.6–13.6 cm d^{-1}) achieved nitrate removal rates between 4.01 and 4.51 g N m^{-3} d^{-1}, while the bioreactor operating at the lowest flow rate (2.9 cm d^{-1}) recorded a reduced nitrate removal rate of 2.94 g N m^{-3} d^{-1}. It is suggested that the increase in nitrate removal rates observed with shorter HRTs (6.6-hour and 10-hour) in Experiment 1 can be attributed to the larger volumes of nitrate passing through the system within a given time, which accounts for the higher removal rates. As the flow rate increases, more nitrate is processed by the bioreactors, leading to greater removal rates (Hackshaw, 2018; Lepine et al., 2016). Therefore, shorter HRTs yield higher nitrate removal rates, even though longer HRTs are generally more effective at improving overall nitrate removal efficiency.

While other studies (Damaraju et al., 2015; Hackshaw, 2018; Hoover, 2012; Hoover et al., 2016) have demonstrated a stepped increase in nitrate reduction with relative high temperature in their column or laboratory bioreactor experiments, these studies typically compared temperature variations ranging from 5°C to 15°C. In contrast, Experiment 1 in this study experienced a minimal temperature variation of less than 1°C throughout the three experimental HRT sets. As a result, temperature did not have a significant impact on nitrate removal within the woodchip columns bioreactors used in Experiment 1.

4.2.2 Comparison of the potential effects of methanol and ethanol dosing on nitrate removal performance in woodchip column bioreactors

Experiment 2 demonstrated that soluble carbon dosing significantly enhanced nitrate reduction in woodchip columns bioreactors. In two experimental runs, both ethanol and methanol-dosed columns bioreactors exhibited substantial improvements compared to the non-dosed control woodchip columns bioreactor in the Experiment 2. Several studies have explored the effectiveness of ethanol (Dos Santos et al., 2004; Jansen et al., 2019; Ortmeyer et al., 2021) and methanol (Hartz et al., 2017; Moghaddam, 2022a; Moghaddam et al., 2023a; Moghaddam et al., 2023b; Moghaddam et al., 2022b) dosing on nitrate reduction in woodchip bioreactors.

Hartz et al. (2017) performed laboratory research on the effects of carbon enrichment using methanol as an additional carbon source at a 1.4:1 C:N ratio, achieving more than 95% nitrate removal efficiency compared to only 6% in the control treatment (no dosing) within a 48-hour HRT.

Moghaddam et al. (2023b) conducted a series of field experiments to evaluate the effectiveness of methanol dosing in a woodchip bioreactor for improving nitrate removal from agricultural tile drainage in Waikato region New Zealand. The results indicated that daily addition of methanol solution at C:N rate of 1.48 led to nitrate removal rates of 8.6 g N m^{-3} d^{-1} in 2020 and 5.1 g N m^{-3} d^{-1} in 2021, compared to the 0.67–1.60 g N m^{-3} d^{-1} observed in 2017 and 2018, when the 53 m^3 chips woodchip bioreactor was not dosed. Specifically, full methanol dosing (14.4 L of 8% methanol solution) increased nitrate removal by approximately 7.5 times, while half methanol dosing (7.2 L of 8% methanol solution) increased it by about 4.5 times compared to not dosing. Additionally, Moghaddam et al. (2022b) conducted a mesocosm-scale woodchip bioreactor experiment and examined the nitrate removal rate and efficiency of methanol-dosed denitrifying bioreactors. The study reported that methanol dosing increased nitrate removal efficiency to between 84% and 90%, compared to 22.4% to 34% without dosing (Moghaddam et al., 2022b). The nitrate removal efficiency in Experiment 2 in the current study was significantly lower than in other studies (Hartz et al., 2017; Moghaddam et al., 2022b), however this might be due to differences in dosing conditions, bioreactor scales, and inlet nitrate concentrations, making direct comparisons challenging. For instance, Hartz et al. (2017) used an influent concentration of 160 mg L^{-1}, while the current study used only 20 mg L^{-1}. It is suggested that bioreactors with higher influent N concentrations (>30 mg L^{-1}) tend to achieve higher nitrate reduction efficiencies than those with intermediate (10-30 mg L^{-1}) and low (<10 mg L^{-1}) concentrations (Addy et al., 2016).

However, considering all findings, ethanol is the most effective additional carbon source, consistently surpassing methanol and more than doubling the nitrate removal efficiency compared to the control no dosing woodchip columns bioreactors in Experiment 2. Jansen et al. (2019) also conducted two field experiments to compare different organic matter sources and dosing strategies by evaluating nitrate reduction in a woodchip bioreactor without dosing (489 x 280 m in size) and an ethanol-dosed reactor at a farm in Noordhoek, the Netherlands. The findings indicated that ethanol-dosed vessel reactor at a 2.4-hour HRT achieved significantly higher nitrate removal efficiencies compared to woodchip bioreactors at a 5-day

HRT. The data showed that while the woodchip bioreactor achieved a nitrate removal efficiency of 80%, the flow-through reactor with ethanol dosing reached up to 95%, demonstrating a significant improvement. There is limited research directly comparing ethanol and methanol for nitrate reduction rates or removal efficiency across various HRTs and C:N ratios. However, Dos Santos et al. (2004) conducted a lab-scale batch reactor to compare methanol, ethanol, and methane as electron donors for denitrification using apparent kinetic parameters. With a C:N ratio of 1.0, consistent with this study, they found that the ethanol-dosed reactor achieved 100% nitrate removal in 50 minutes, whereas the methanol-fed reactor took 120 minutes. These findings support our results, highlighting ethanol as a more effective carbon source, compared to methanol, for enhancing nitrate reduction efficiency in denitrifying bioreactors.

Modest increases in outflow TOC levels in the methanol-dosed columns, compared to the control columns (Figure 4-7), suggests effective utilisation of methanol as a carbon source for denitrification. On the other hand, biofilm formation was observed in two ethanol-dosed columns on Day 6 and Day 8, corresponding to spikes in outflow TOC levels (Figure 4-7). The significantly higher outflow TOC levels in some ethanol-dosed columns indicate potential inconsistencies in the dosing system. These inconsistencies may have led to excessive carbon dosing, resulting in the overgrowth of microbial communities, which can aggregate and form biofilms on surfaces within these bioreactor columns (Anderson et al., 2020; Christianson et al., 2016; Gibert et al., 2008; Moghaddam et al., 2023a).

4.2.3 Evaluation of ethanol dosing for nitrate removal in woodchip, pumice and gravel column bioreactors

Experiment 3 demonstrated that ethanol dosing markedly improved the nitrate removal efficiency to above 70% in all bioreactor media, including the sedimentary rock gravels and pumice. The ethanol dosing in the woodchip columns bioreactors was particularly effective, nearly removing all of the inflow NO_3^- concentration and outperforming both pumice and gravel media in this column study. Due to the limited studies available on ethanol dosing in woodchip bioreactors, drawing direct comparisons with our results is difficult. However, Jansen et al. (2019) reported a nitrate removal efficiency reaching 95% in a flow-through passive mechanism bioreactor dosed with ethanol, even without a solid substrate to promote

microbial growth, under a 0.1-day HRT. Despite differences in experimental settings and HRT conditions, their findings are consistent with our results, demonstrating the effectiveness of ethanol dosing with or without a solid substrate.

Interestingly, when comparing the results of Experiments 2 and 3, both of which employed ethanol dosing in woodchip column bioreactors with the same C:N ratio of 1:1 and the same HRT, notable differences in nitrate removal efficiency were observed. The woodchip bioreactor in Experiment 2 achieved only 66-68% nitrate removal efficiency, while those in Experiment 3 reached 97%. This discrepancy, despite the same experimental settings, may be attributed to the difference in woodchip particle size. Experiment 3 used smaller woodchip particles (5-10 mm), compared to the larger particles (70-100 mm) in Experiment 2. Smaller woodchips are considered more effective in nitrate removal due to their greater surface area per unit of weight, which facilitates extensive microbial growth and enhances denitrification efficiency (Peterson et al., 2015). Moreover, in Experiment 3, the bioreactor columns were repacked with new woodchips, while Experiment 2 utilized the same woodchips that had been in use since Experiment 1, for a duration of four months. The age of the woodchips is a critical factor that can significantly influence nitrate removal efficiency (Addy et al., 2016). The new woodchips in Experiment 3 presumably provided a more abundant supply of carbon sources, which are essential for denitrifying bacteria. In contrast, slightly older woodchips in Experiment 2 may have experienced a depletion of readily available dissolved organic carbon, as much of it could have been flushed out of the bioreactors over time, leading to diminished denitrifying activity (David et al., 2016; Robertson, 2010; Schipper et al., 2010b). Additionally, the introduction of ethanol in Experiment 3 supplied an extra source of carbon, enhancing the carbon availability provided by the new woodchips. This combination of fresh woodchips and supplemental ethanol likely contributed to the higher nitrate removal efficiency observed in Experiment 3 compared to Experiment 2.

In this study, ethanol proved to be an effective electron donor, allowing the gravel and pumice bioreactors to reach nitrate removal efficiencies above 70%, even in the absence of woodchips. However, there is no research on using ethanol as an external carbon source in reactors with gravel or pumice as solid substrates for reducing nitrate in agricultural subsurface or tile drainage. Most existing studies on solid substrates focus on nitrate removal in groundwater or aquaculture treatment systems. A circulation column study by Ortmeyer et al. (2021) was conducted to examine nitrate reduction for groundwater treatment using various liquid carbon

sources, including ethanol, with sediment as the solid substrate in the reactor. The finding revealed that ethanol is an effective electron donor for denitrification in groundwater. With continuous ethanol dosing, the reactor achieved a nitrate removal efficiency of up to 55% at room temperature (21.5°C) and a remarkable 97% efficiency at a low temperature of 10°C.

Several studies had evaluated effect of organic carbon sources on nitrate removal in denitrification tanks specially designed for recirculating aquaculture system. Pungrasmi et al. (2013) compared various bottom substrates, including soil, sand, pumice stone, and vermiculite, using methanol as a carbon source to enhance denitrification in a recirculating aquaculture system. Their study found pumice stone to be the most effective substrate due to its high surface area, which promotes bacterial growth and optimises conditions for microbial activity, achieving up to 85% nitrate removal efficiency. Pungrasmi et al. (2016) further explored the use of pumice stone in a recirculating system with methanol. This setup significantly facilitated denitrification, reducing nitrate levels in the recirculating tank to seven times lower than those in the control tank without dosing. Although the type of dosing differed from our study, these findings align with our results, highlighting the potential of gravel or pumice as alternative substrates to woodchips.

The elevated outflow TOC levels in woodchip column bioreactors indicate increased organic carbon release from both woodchips and ethanol dosing, providing a rich carbon source for denitrification. This results in effective nitrate removal, as evidenced by consistently low outflow NO_3^--N concentrations, in the ethanol dosed woodchip column bioreactors (Figure 4-9). The performance of gravel and pumice column bioreactors exhibited variability, largely influenced by the consistency of ethanol dosing. In some gravel columns, outflow TOC levels were observed to be lower than the inflow levels (e.g., 13.9 mg L^{-1} compared to an inflow range of 14.2 to 14.8 mg L^{-1}). This insufficient carbon availability led to significantly higher outflow NO_3^--N concentrations, reaching 9.5 mg L^{-1}, compared to other gravel columns, which maintained a much lower outflow NO_3^--N concentration of 0.1 mg L^{-1} on Day 14. Conversely, on the same day, one gravel column exhibited an unusually high outflow TOC concentration of 30.3 mg L^{-1}, the highest recorded across all experimental days. This sharp increase in TOC corresponded with a drastic reduction in outflow NO_3^--N concentration to 0.1 mg L^{-1} (Figure 4-9c). Similarly, in the pumice column bioreactors, elevated outflow TOC levels were associated with decreased NO_3^--N concentrations. As outflow TOC levels increased from 14.7

to 16.2 mg L^{-1}, and further to 17.0 mg L^{-1} on Day 14, 19.8 mg L^{-1} on Day 10, and 21.0 mg L^{-1} on Day 12, the outflow NO_3^--N concentrations dropped significantly to 3.8 mg L^{-1}, 0.4 mg L^{-1}, and 0.15 mg L^{-1}, respectively (Figure 4-9). These observations clearly demonstrate that higher outflow TOC levels, which indicate increased ethanol dosing, are closely linked to enhanced nitrate removal efficiency in both gravel and pumice column bioreactors. This relationship highlights the potential for improved nitrate removal when ethanol dosing is appropriately increased. Although there were inconsistencies in the ethanol dosing system, the findings suggest that by optimising ethanol dosing, the nitrate removal performance of these bioreactor systems can be significantly improved.

However, the presence of high organic carbon in water bodies requires careful consideration due to its potential negative impacts. Elevated TOC levels can lead to excessive consumption of dissolved oxygen, ultimately resulting in water quality deterioration. According to U.S. Environmental Protection Agency (1998) the rule for disinfectants and disinfection by-products mandates TOC removal from source water if levels exceed 2 mg L^{-1}. In addition, the World Health Organization (2017) guidelines for drinking water recommend that TOC levels should not exceed 0.5 mg L^{-1} to minimise risks such as biofilm growth in water distribution systems. In this study, TOC levels consistently exceeded 2 mg L^{-1}, highlighting the necessity for treatment by surface water facilities. Balancing the benefits of enhanced nitrate removal with the need for optimised TOC removal is essential for maintaining water quality and ensuring compliance with international standards.

4.2.4 Cost analysis

The comparative analysis of woodchip and sedimentary rock gravel bioreactors underscores crucial economic and operational factors that influence their long-term utility. When considering woodchip and gravel bioreactors over different lifespans, the woodchip bioreactor demonstrates greater cost-effectiveness at NZ$4.3 kg N removed for the 15-year lifespan.

Even though the annual costs for the gravel bioreactor are approximately twice as high for a 30-year lifespan in both scenarios (Scenarios A and B), it offers the convenience of not requiring replacement during this period. Specifically, in Scenario B, the gravel bioreactor's cost-effectiveness is estimated at $NZ8.8 N removed. Although this figure is higher than the

costs for the woodchip bioreactor over the 10- and 15-year spans, it is important to consider that the woodchip bioreactor would require replacement two or three times over the same 30-year period. Consequently, the cumulative cost of maintaining and replacing woodchip bioreactors might equal or exceed that of maintaining a gravel bioreactor over 30 years.

Notably, gravel bioreactors, despite their higher initial and annual costs over a 30-year lifespan, including labour costs for operation and additional expenses for maintaining an ethanol-dosing system, have significant potential to offer long-term advantages. No requirement for replacement within this extensive period presents a compelling argument for their use in applications where long-term stability and reduced maintenance are prioritised. This aspect is particularly beneficial for owners, as it eliminates the annual labour costs associated with replacing woodchips annually and demonstrates its importance in environments where frequent maintenance and replacement are logistically challenging or financially untenable.

This analysis highlights the necessity of considering lifecycle costs and the operational demands of bioreactor technologies. While woodchip bioreactors might be more cost-effective in the short term, the long-term resilience of gravel bioreactors can be more advantageous for sustained nitrate removal in larger-scale or more permanent installations. Future research should thus not only focus on optimising the cost and efficiency of these systems but also on enhancing their durability and adaptability to different operational contexts.

Chapter 5 Conclusion and Recommendations

5.1 Conclusion

The woodchip column bioreactors experiments, conducted in this study, demonstrated that longer HRTs significantly improve nitrate removal efficiency, with a 20-hour HRT achieving a 71% removal rate compared to 31-39% at a 6.6-hour HRT and 39% at a 10-hour HRT. The enhanced nitrate removal at longer HRTs is attributed to increased carbon release from woodchips, which supports more effective denitrification. However, the nitrate removal efficiency was similar for both the 6.6-hour and 10-hour HRTs, each reaching a maximum of 39%. This suggests that increasing the HRT from 6.6 to 10 hours does not enhance TOC utilisation or release, as indicated by the comparable TOC levels. The short retention time increase in this column experiment might be insufficient to promote additional carbon release from the woodchips, thereby limiting further improvements in denitrification efficiency. However, it is observed that the 6.6-hour HRT achieved the highest average nitrate removal rate of 13.6 g m^{-3} day^{-1}. In comparison, the 10-hour HRT resulted in a slightly lower rate of 10.2 g m^{-3} day^{-1}, while the 20-hour HRT, with the lowest flow rate, corresponded to a nitrate removal rate of 9.4 g m^{-3} day^{-1}. These results suggest that while longer HRTs improve nitrate removal efficiency, they may reduce the overall nitrate removal rate due to less flow rate through the bioreactor.

Soluble carbon dosing significantly enhances nitrate reduction in the woodchip column bioreactors at a shorter HRT (6.6 hours). Ethanol dosing at a C:N ratio of 1:1 achieved significantly higher nitrate removal efficiency (66-68%) compared to methanol dosing at the same C:N ratio (55 -57%), and about twice the efficiency of the no-dosing woodchip columns (33-38%). However, a biofilm formation was observed in the last two sampling days in ethanol-dosed columns, which corresponded to spikes in out TOC levels, indicating potential dosing inconsistencies and risks of overdosing. Continuous dosing improves nitrate reduction; however, it may lead to lower redox potentials and potential clogging issues over time. Careful management is required to optimise microbial activity and maintain effective denitrification processes.

Ethanol dosing significantly enhanced nitrate removal efficiency in the column bioreactors with different media types, with the woodchip column bioreactors achieving up to 97% efficiency, surpassing both pumice (72%) and gravel (75%) media. The higher nitrate removal efficiency observed in woodchip column bioreactors in Experiment 3, compared to Experiment 2, despite using the same C:N ratio, HRT, and dosing rate, can be attributed to the greater effectiveness of smaller woodchip particles, highlighting the importance of surface area for microbial growth and denitrification. As observed in some column bioreactors, a higher outflow TOC levels, potentially indicating increased ethanol dosing, are strongly linked to improved nitrate removal efficiency in both gravel and pumice bioreactors. Despite inconsistencies in the ethanol dosing system, the results suggest that optimising ethanol dosing can significantly enhance nitrate removal performance. Use of gravel media with ethanol dosing demonstrated promising results in nitrate removal, achieving higher nitrate removal efficiencies than pumice. It demonstrates potential of using river gravel as a reliable alternative to woodchip column bioreactors. Although its performance was slightly lower than that of woodchips, gravel was commendable due to its balance of practical effectiveness, availability, and long-term stability.

The cost analysis shows that while woodchip bioreactors offer cost advantages in the short term, due to lower initial and maintenance expenses, gravel bioreactors may be economically viable over the long-term. Despite higher initial and annual cost of nitrate removal rate, the longevity and reduced maintenance requirements of gravel bioreactors could potentially offset these expenses, making them an advantage where frequent replacements are logistically challenging. This comprehensive analysis underscores the need to consider both nitrate removal performance metrics and lifecycle costs when selecting bioreactor materials, ensuring that the choice aligns with the specific operational and budgetary goals.

5.2 Recommendations for future research

This thesis highlights the potential of ethanol dosing to reduce nitrate concentrations in denitrifying bioreactors and examines the use of alternative media, such as gravel or pumice, which may offer greater long-term stability compared to woodchips for agricultural subsurface or tile drainage. It recommends further research related to employing ethanol as an added soluble carbon source used in bioreactors with inert media, as follows:

1. A long-term study, over multiple years, using a pilot-scale bioreactors in the field is needed to compare the effectiveness of woodchip and gravel bioreactors with ethanol dosing to determine if their relative performance changes over time.

2. Evaluate the effectiveness of ethanol dosing in gravel bioreactors under various HRTs, to gain a better understanding of the relationship between HRT and nitrate removal efficiency in these systems.

3. Assess the environmental impacts of using ethanol as a carbon source, particularly focusing on the potential release of TOC into the environment, as compared to the woodchip bioreactors and agricultural drainage waters. Assessing the effects on water quality and ecosystem health will be crucial for developing sustainable nitrate reduction practices and mitigating potential adverse impacts.

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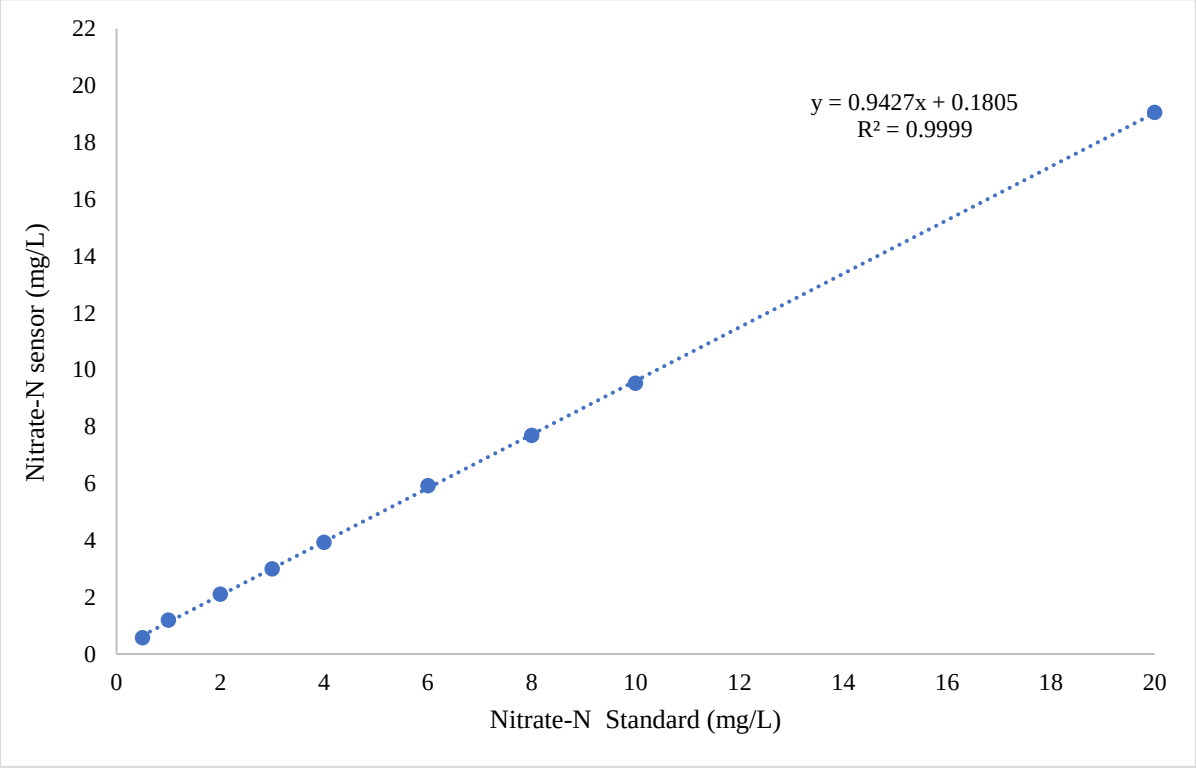
Appendix

Appendix 1. Concordance between the standard nitrate-N concentrations and the sensor measurements

Standard	Nitrate-N (mg/L)	Nitrate-N sensor (mg/L)
S1	0.5	0.57
S2	1	1.19
S3	2	2.11
S4	3	3
S5	4	3.93
S6	6	5.92
S7	8	7.69
S8	10	9.53
S9	20	19.06



Appendix 2. Financial Comparison Between Ethanol-Dosed Gravel Bioreactors and Non-Dosed Woodchip Systems

Scenario	Woodchip		Gravel
	10 years	15 years	30 years
NRC* ($NZD/Kg N removed) (Scenario A)	5.9	4.3	10
NRC* ($NZD/Kg N removed) (Scenario B)	5.9	4.3	8.8

NRC* refers to nitrate removal rate.

Scenario A indicates that the porosity of woodchip is 0.5 and the porosity of gravel is 0.3.

Scenario B specifies that the porosity of both woodchip and gravel is 0.5.

Scenario A: Assumed the porosity of woodchip is 0.5 and the porosity of gravel is 0.3

Variable	Type of bioreactor (200 m^3)		
	Woodchip (10 years)	Woodchip (15 years)	Gravel with ethanol dosing (30 years)
Flow rate (L/s)	4.2	4.2	2.5
Inflow (mg/L)	20	20	20
NRE (%)	35	35	75
Outflow (mg/L)	13	13	5
NRR (g/day)	2545	2545	3273
NRR (kg/half year - drainage season)	466	466	599
NRR ($NZD/Kg N removed)	**5.9**	**4.3**	**10**

Items	Woodchip over 10 years ($NZD)	Woodchip over 15 years ($NZD)	Gravel with ethanol dosing ($NZD)
Pipe	1000	1000	1000
PVC liner	500	500	500
Excavation field work	8000	8000	8000
Labour fee	4000	4000	4000
Woodchip ($35/m^3)	7000	7000	
Washed gravel (3-5mm) ($49.7/m^3)			9940
Capital cost	20500	20500	23440
Ethanol required @ 1:1 C:N ratio (L per day)			8.39
Ethanol cost ($ per day)			23
Ethanol cost (operate for half a year drainage season)			4223
Total annual cost for 10 years, with 6% opportunity (interest) cost	**2727**	**2023**	
Total annual cost for 30 years, with 6% opportunity (interest) cost			**5984**

Scenario B: Assumed the porosity of both woodchip and gravel is 0.5.

Variable	Type of bioreactor (200 m^3)		
	Woodchip (10 years)	Woodchip (15 years)	Gravel with ethanol dosing (30 years)
Flow rate (L/s)	4.2	4.2	4.2
Inflow (mg/L)	20	20	20

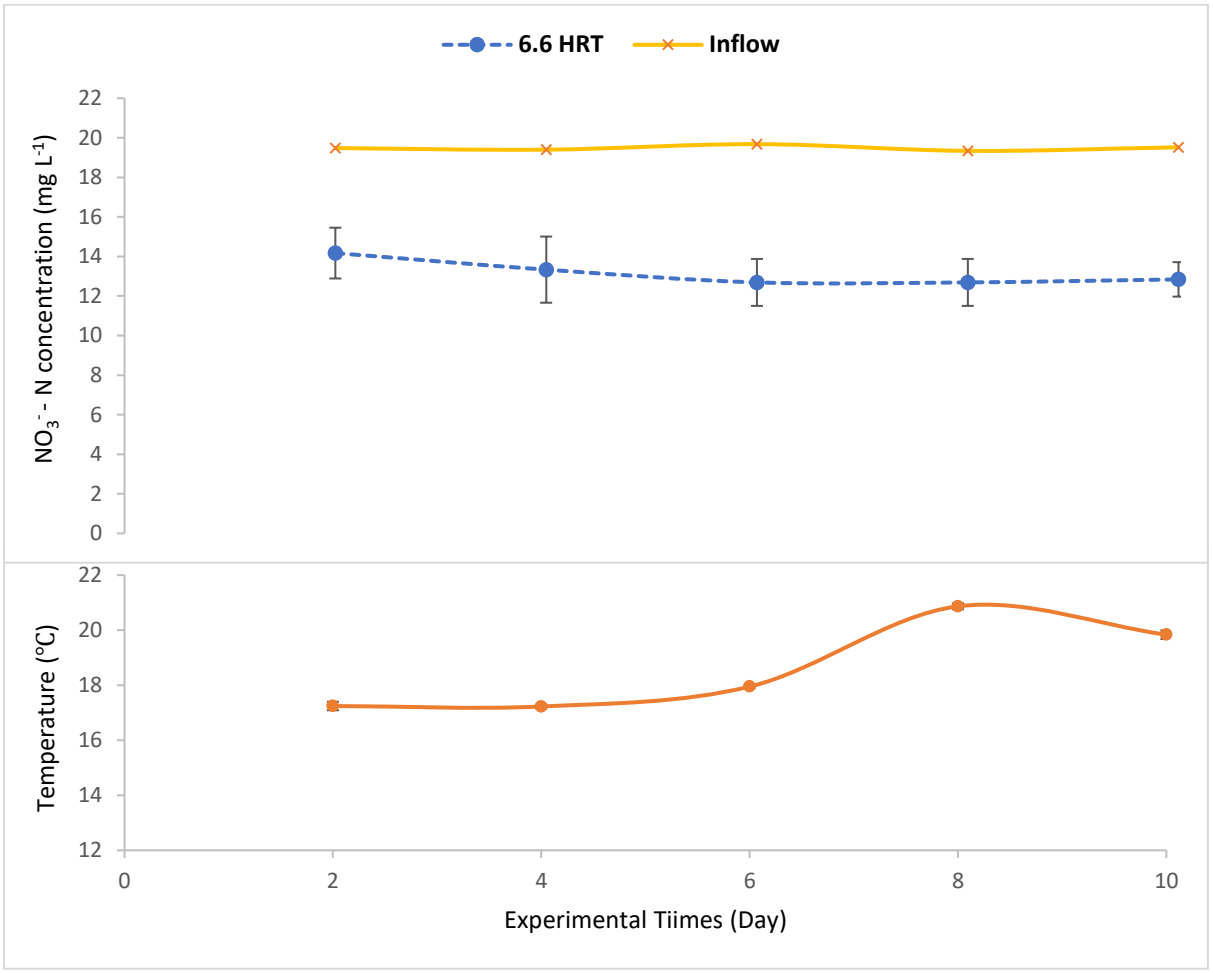
NRE (%)	35	35	75
Outflow (mg/L)	13	13	5
NRR (g/day)	2545	2545	5455
NRR (kg/half year - drainage season)	466	466	998
NRR ($NZD/Kg – N removed)	**5.9**	**4.3**	**8.8**

Items	Woodchip over 10 years ($NZD)	Woodchip over 15 years ($NZD)	Gravel with ethanol dosing ($NZD)
Pipe	1000	1000	1000
PVC liner	500	500	500
Excavation field work	8000	8000	8000
Labour fee	4000	4000	4000
Woodchip ($35/m3)	7000	7000	
Washed gravel (3-5 mm) ($49.7/$m^3$)			9940
Capital cost	20500	20500	23440
Ethanol required @ 1:1 C:N ratio (L per day)			13.99
Ethanol cost ($ per day)			38
Ethanol cost (operate for half a year drainage season)			7038
Total annual cost for 10 years, with 6% opportunity (interest) cost	**2727**	**2023**	

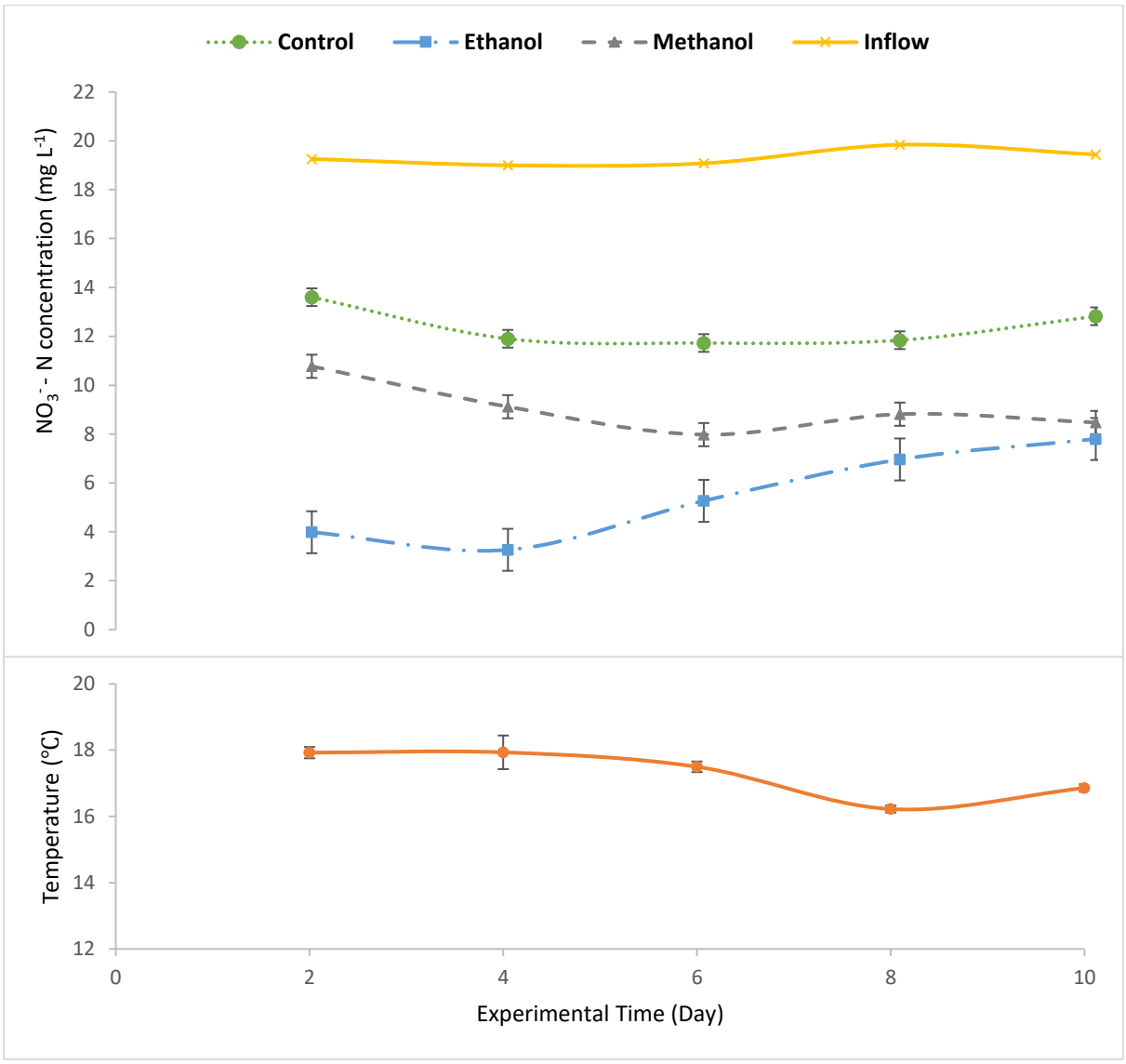
Total annual cost for 30 years, with 6% opportunity (interest) cost			**8800**

Appendix 3. Sampling Data Outputs from All Sampling Days of Experiment 1, Experiment 2, and Experiment 3

Experiment 1: Initial evaluation phase (6.6-hour HRT)



Experiment 2: Set 1 of Experiment 2



Experiment 3: