

CRANFIELD UNIVERSITY

Ayisha Affo Souleymane

Solid-phase Denitrification for Tertiary Nitrate Removal from  
Municipal Wastewater

School of Water, Energy and Environment  
MSR Water

Master by Research in Water  
Academic Year: 2022 - 2023

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Associate Supervisor: Dr Luca Alibardi  
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## **ABSTRACT**

Elevated levels of nitrate in surface water and groundwater can lead to eutrophication, algal blooms, and depletion of dissolved oxygen, causing harm to aquatic ecosystems. Solid-phase denitrification (SPD) offers a promising approach for removing nitrate from wastewater sources using carbon-based materials to promote the activity of microorganisms that convert nitrate into nitrogen gas. This study evaluated the suitability of six common cheap and readily available materials as external carbon sources for denitrification in SPD systems. The materials tested were woodchips, barley straw, spruce wood bark, beech sawdust, and polycaprolactone (PCL). The study focused on the leaching rates of organic compounds and their impact on denitrification efficiency, with particular attention to the potential for secondary pollution. Results indicated that woodchips and wood bark exhibited a good balance between carbon content and secondary pollution risk, while barley straw posed the highest risk for secondary pollution and potential for the rapid depletion of organic compounds. PCL showed promise as a carbon source for nitrate removal and released minimal nitrate or ammonium-nitrogen. The low concentrations of metals found in the media suggest that they would not inhibit denitrification or pose significant health risks. The method was transferred to a pilot scale to evaluate the potential of woodchips in removing nitrate from wastewater sources and the performance of four columns for solid-phase denitrification using woodchip media, based on the results of the first part of the study, was studied for a period of 168 days. The nitrate removal efficiency was observed to be between 62-75% with the best performance seen in the hardwood seeded with sludge column. The addition of sludge from a wastewater treatment plant helped to speed up the start-up process. The results suggest that higher removal rates can be expected with higher influent nitrate concentrations. Over time, significant variability in the performance was observed. However, the variability in the results could not be clearly explained. In conclusion, all tested media have the potential to be used as supporting carbon sources for SPD, but their efficacy and potential for secondary pollution must be evaluated on a case-by-case basis.

**Keywords:**

Woodchips, Barley straw, Sawdust, Polymer, Wood bark, Leaching, Solid-phase denitrification, Nitrate, Wastewater

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## LIST OF ABBREVIATIONS

|         |                                                                     |
|---------|---------------------------------------------------------------------|
| ANAMMOX | Anaerobic Ammonium Oxidation                                        |
| ANOVA   | Analysis of Variance                                                |
| ASP     | Activated Sludge Process                                            |
| BNR     | Biological Nutrient Removal                                         |
| BOD     | Biological Oxygen Demand                                            |
| BSt     | Barley Straw                                                        |
| BSw     | Beech Sawdust                                                       |
| COD     | Chemical Oxygen Demand                                              |
| DEFRA   | Department for Environment, Food and Rural Affairs                  |
| DO      | Dissolved Oxygen                                                    |
| DOC     | Dissolved Organic Carbon                                            |
| EBCT    | Empty Bed Contact Time                                              |
| FLFW    | Food Fermentation Liquid                                            |
| FRW     | Wastewater From Food-Waste Recycling Industries                     |
| H       | Hardwood Only                                                       |
| HGS     | Hardwood And Gravel Mixture Innoculated With Sludge                 |
| HS      | Hardwood Innoculated With Sludge                                    |
| MBBR    | Moving Bed Biofilm Reactor                                          |
| MLOW    | Mixed Liquor Waste from the Manufacturing Process of 1,4-Butanediol |
| NOB     | Nitrite Oxidising Bacteria                                          |
| PCL     | Polycaprolactone                                                    |
| PHB     | Polyhydroxy Butyrate                                                |
| RE      | Removal Efficiency                                                  |
| SBR     | Sequencing Batch Reactor                                            |
| SPD     | Solid-Phase Denitrification                                         |
| SS      | Softwood Innoculated With Sludge                                    |
| STW     | Sewage Treatment Works                                              |
| SWB     | Spruce Wood Bark                                                    |
| WFD     | Water Framework Directive                                           |
| WWTP    | Waste Water Treatment Plant                                         |

# 1 Introduction

## 1.1 Background

The presence of nutrients in water is essential for the growth and maintenance of aquatic flora and fauna. However, high concentrations of these nutrients pose ecological and health risks to these organisms, and subsequently to humans. Nitrogen (N) and phosphorus (P) are of particular concern. Although nitrogen is naturally present in the environment, high concentrations are a result of anthropogenic activities such as fertilizer application, livestock, landfill leachate and release of sewage (Moghaddam & Sargolzaei, 2013). Overstimulation of the growth of aquatic plants and algae can result from the presence of excess nitrogen. The excessive development of algae can then block water intakes, block light from reaching deeper waters, and deplete dissolved oxygen leading to anoxic conditions, a condition known as eutrophication (Kurosu, 2001; Mayo & Abbas, 2014; European Environment Agency, 2020; Cooper et al., 2022). Eutrophication of lakes and water bodies is a problem that can lead to the death of aquatic flora and fauna, unsightly algal scums on the water's surface, and even the "death" of a lake by depriving it of oxygen completely (Water Science School, 2018; O'Connor, 2020).

One of the most significant point sources of waterways nutrient pollution and a major contributor to freshwater eutrophication is sewage effluent from wastewater treatment plants (WWTPs) (Bowes et al., 2012; Cooper et al., 2022). In the United Kingdom, there are over 9000 WWTPs that treat and release approximately 11 billion L of treated effluent into the environment every day (DEFRA, 2002). This effluent can be rich in biologically usable forms of N and P, which are currently discharged at quantities higher than allowed for water bodies to achieve "good" ecological and chemical status under the EU Water Framework Directive (WFD) (2000/60/EC) (Yates et al., 2019). "Good" status is defined by four evaluations: ecological and chemical status of surface waters, and quantitative and chemical status of groundwater. The ecological assessment rates overall ecosystem health using biological quality elements such as phytoplankton, macrophytes, invertebrate fauna and fish. If "good" status is still not achieved, further

assessments are done on nutrients, oxygen, temperature, and salinity among other parameters. The EU WFD focuses majorly on environmental status rather than sewage works but have the ability to influence the consents for discharge of treated sewage into water bodies. Accordingly, the EU Urban Wastewater Treatment Directive (91/271/ EC) has set water quality parameters for effluent discharging into a surface waterbody at 2.0 mg/L for total phosphorus (TP), 15.0 mg/L for total nitrogen (TN), and 50.0 mg O<sub>2</sub>/L for BOD for WWTPs with a size of 10,000 to 100,000 population equivalent (p.e.). For facilities servicing beyond 100,000 p.e. these values drop to 1.0 mg/L and 10.0 mg/L. Smaller facilities serving up to 10,000 p.e. have no legal duty to fulfil such requirements. However, the concentrations in effluent discharges of up to 20 mg/L and 100 mg/L have previously been reported, making even small WWTPs a significant eutrophication risk (Yates et al., 2019; Cooper et al., 2022). Wastewater is processed through several stages at WWTPs to reduce the environmental impact of sewage effluent. These stages include pre-treatment (screening to remove coarse material), primary treatment (settling tanks to encourage the sedimentation of suspended fines), and secondary treatment (biological degradation of organics and nutrients). However, after treatment, the effluent can still contain a significant level of nutrients and usually needs a tertiary treatment to reduce the risk of eutrophication and to meet the increasingly stringent regulations (GOV.UK, 2019; Cooper et al., 2022).

Nitrogen removal usually involves a 2-step process known as nitrification/denitrification. Nitrification involves oxidizing ammonia (NH<sub>4</sub><sup>+</sup>) into nitrites (NO<sub>2</sub><sup>-</sup>) by autotrophic ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) then oxidize the nitrites into nitrates (NO<sub>3</sub><sup>-</sup>) in the presence of oxygen (O<sub>2</sub>). In denitrification, denitrifying bacteria reduce nitrates to nitrites and then to nitrogen gas (N<sub>2</sub>) consuming biodegradable organics (Pelaz et al., 2018). Because the denitrifying bacteria are usually heterotrophic in nature, they would require an external source of biodegradable organic matter.

Regardless of the size of the wastewater treatment facility, the fundamentals of nitrate removal through nitrification and denitrification are the same. However,

depending on the facility's size and capacity, the type and volume of influent wastewater, and the treatment goals, different techniques may be adopted (Ni et al., 2017). Larger wastewater treatment facilities may adopt more sophisticated technologies like activated sludge, membrane bioreactors or moving bed biofilm reactors to accommodate larger volumes of wastewater and generate higher treatment levels. Although more complicated and expensive, these technologies offer a better level of treatment efficiency and can manage larger volumes of wastewater (Newton, 2023). In contrast, due to lower influent wastewater flow rates and pollutant loads, smaller facilities may use less complex and costly technology than bigger ones. To achieve nitrification and denitrification, for instance, a small municipal wastewater treatment facility may use an extended aeration method, a type of activated sludge process (Jamal, 2017). This is a less expensive and relatively straightforward technique, takes up less space, and needs less maintenance than more complex treatment systems. Many smaller WWTPs in the UK only apply nitrification of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  either in suspended or attached growth biological systems. This usually involves the use of aeration tanks, trickling filters, and rotating biological contactors (aerobic zones) (Jamal, 2017). Despite their effectiveness at removing nitrogen from wastewater, these technologies used in smaller facilities may not always be enough to comply with the required discharge restrictions or to address particular water quality issues. Further post-treatment or tertiary nitrate removal may be required in such circumstances. This means that efficient alternatives that meet these expectations at the post-treatment tertiary level and cost-effective/profitable to the industry are needed since it is difficult to fit new steps in existing systems or convert systems for example to an activated sludge system in existing WWTPs.

Numerous studies have investigated heterotrophic denitrification, a process in which bacteria use organic materials as external carbon sources under anoxic conditions (Lee et al., 2013). There must be enough carbon sources available for the process to succeed. Carrera et al. (2004) mentioned that a C/N ratio of above 7.1 is needed for complete denitrification to occur. The carbon-nitrogen ratios (C/N) of tertiary-stage sewage, however, are often poor and the carbon sources,

insufficient. Sun et al. (2010) described wastewater with ratios of less than 8.0 as low C/N ratio wastewaters. Therefore, when the C/N in wastewater is low, it is necessary to use an external carbon source as an electron donor to ensure efficient denitrification (Zhang et al., 2016b). This technology has been applied using liquid carbon sources such as methanol but have been limited due to the cost and high-level maintenance required to operate the systems (Lee et al., 2013) and their ability to inhibit microbe growth due to the accumulation of nitrites (Glass & Silverstein, 1998; Lee et al., 2013) despite the high removal efficiencies reported. Companies are being forced to invest in finding alternative carbon sources which will provide the benefits of cost-efficiency, stability, and homogeneity of operations, along with a reasonable level of removal efficiency (Kaplan et al., 1987; Lee et al., 2013). Studies have also investigated the use of carbon sources other than methanol with varying results reported. For example, nutrient broth, anaerobic sludge digestate or liquor, sweet whey (Kaplan et al., 1987), mixed liquor manufacturing waste (Lee et al., 2013), food fermentation waste (Zhang et al., 2016a, 2016b) have been tested. Leverenz et al. (2010) reported the use of solid-phase denitrification (SPD) in which a solid media is used as both a biofilm carrier and electron donor for the process. For this reason and its reported affordability, this alternative is gaining more attention. SPD avoids the risk of over-dosing and secondary pollution associated with liquid sources, which is undesirable, especially in the tertiary treatment stage (Boley et al. 2000). Materials such as woodchip (Damaraju et al., 2015; Von Ahnen, et al., 2016a, 2016b; Von Ahnen et al., 2018b), a combination of woodchip and non-carbonaceous materials such as sand or gravel, sawdust (Hu et al., 2019; Yang et al., 2020) corn cobs (He et al., 2016; Feng et al., 2017), and rice straw and hull (Lu et al., 2017; Luo et al., 2018) have been tested. Also, biodegradable plastics (Yamada et al., 2013; Li et al., 2016) including the reuse of waste biodegradable snack-ware (Wang & Wang, 2013) have been investigated. These are expected to provide a more reliable and controllable source of carbon but will be expensive and to date are not readily available on the market. SPD has generally been applied in fixed bed reactor, either in columns with the water flowing upwards or

in horizontal bed filter as seen for the large-scale implementation of woodchip systems (Von Ahnen et al., 2018b).

Evaluation of the performance reported in the literature have shown that the different types of systems with the different types of carbon sources can all provide significant removals of nitrate with the majority achieving more than 80% removal of the nitrate. Interestingly, as a cheap and readily available material, woodchip has been extensively tested with water sources such as aquaculture (Von Ahnen et al., 2016; Lepine et al., 2020; Lindholm-Lehto et al., 2020; Pulkkinen et al., 2021), synthetic wastewater (Hoover et al., 2016; Zhao et al., 2018, 2020) and groundwater (Manca et al., 2020; Díaz-García et al., 2021) up to full scale treatment but there is limited understanding of its application to municipal wastewater. Also, there is generally a lack of understanding on the life of these materials with the longest trials reported in the literature limited to a maximum of 2 years. Overall, the diversity in system configuration and options for carbon sources combined to the high performance reported highlight the potential for tertiary denitrification but further evaluations are required to determine the applicability in real conditions for tertiary municipal wastewater treatment. Another limitation is the lack of detailed reporting on the start-up phase as only a few works have reported on the start-up period (Von Ahnen, et al., 2016; Pulkkinen et al., 2021) The gaps and limitations above will be addressed in this work as it explores the use of SPD, under different startup conditions in ambient weather conditions while also mimicking real-life WWTP conditions at pilot scale with real wastewater.

## **1.2 Aims and Objectives**

The overall aim of this project is to establish the efficacy of the novel solid-phase denitrification technology using low-cost solid media for tertiary removal of nitrates from municipal wastewater. To deliver the overall aim, the following objectives were set:

**Objective 1-** To evaluate current knowledge through the review of existing literature on the application of liquid and solid external carbon sources

for denitrification in municipal wastewater treatment and understand their application at the tertiary treatment stage.

**Objective 2-** To determine the potential of a range of low-cost solid media as carbon sources for denitrification.

**Objective 3-** To assess the effects of different operating and start-up conditions on the efficacy of woodchip based solid-phase denitrification.

### **1.3 Thesis Plan**

This thesis is presented in chapters formatted as journal papers. All papers were written by the primary author, Ayisha Affo Souleymane and edited by Dr. Marc Pidou and Dr. Luca Alibardi. The experimental work was designed and completed by the primary author at the Cranfield University's sewage treatment works and within the laboratories. In Table 1-1 and Figure 1.1, the links between the objectives and different chapters are addressed.

In chapter 2 (paper 1), the focus was on nitrate removal in wastewater. The review briefly highlights the different technologies currently used for nitrate removal and the potential limitations associated with them, as well as their potential applications at the tertiary treatment stage. The focus of the literature review is then on heterotrophic denitrification, especially solid-phase denitrification as this has proven to be a cost-effective technology and suitable for use at the tertiary step in a wastewater treatment train.

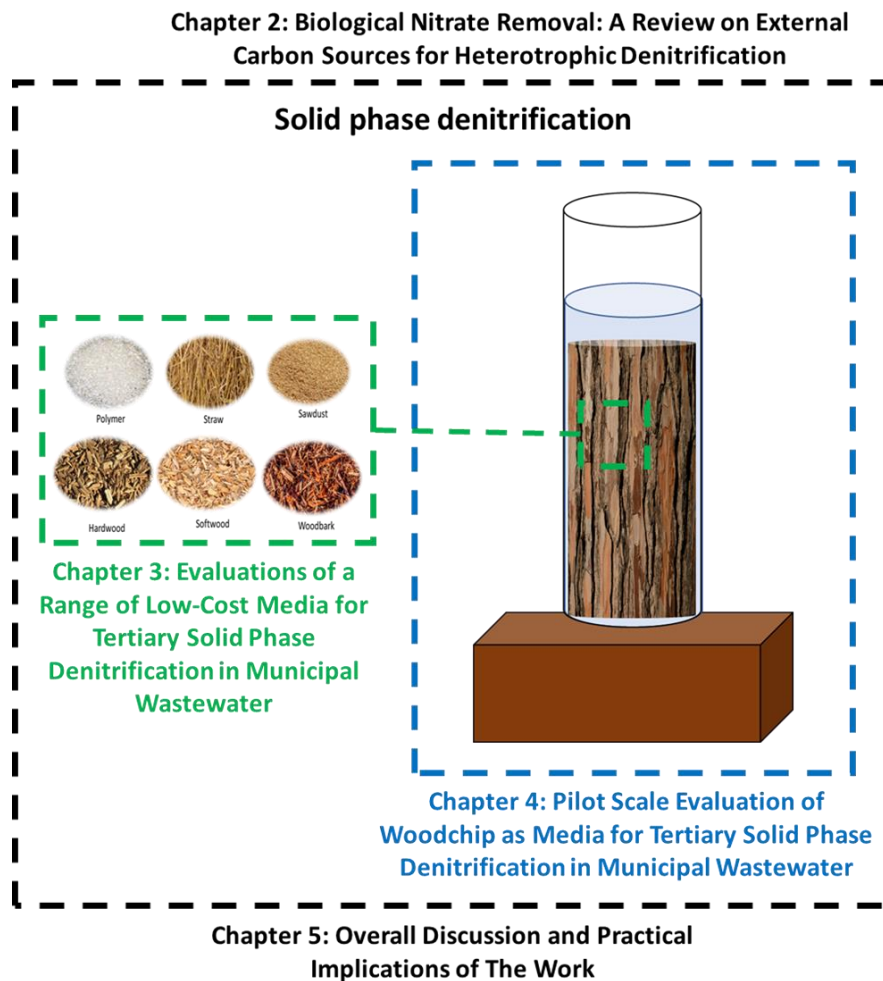
The experimental work was divided in two sections. In the first section of the experimental work presented in chapter 3 (paper 2), leaching tests to study the potential of a range of low-cost media for tertiary solid-phase denitrification in municipal wastewater were conducted. The findings from chapter 2 influenced the media selected for the leaching tests. At lab scale, leaching tests were conducted on woodchips, and other materials including bark, saw dust and polycaprolactone beads to assess the organics leached, their leach rates and, the suitability of the different materials as external carbon sources for denitrification. Samples from these tests were analysed for organics each time.

The second section presented in chapter 4 (paper 3) was focused on a pilot scale evaluation of woodchip as media for tertiary solid-phase denitrification in municipal wastewater. A trial was set to simulate a full-scale WWTP comprising four columns of plexiglass. Columns HS and H were filled with hardwood, HGS with a mixture of hardwood and washed gravels, and SS with softwood. Columns HS, HGS and SS were inoculated with seed sludge from a municipal WWTP. Final effluent from the Cranfield University WWTP was used as influent to the pilot. This was to assess the effects of different operating and start-up conditions on the efficacy of woodchip based solid-phase denitrification.

The technical chapters were then integrated to discuss the implications of the work by evaluating what the different media can do, appropriate system designs and operating conditions, and suitable applications from an economic and technical perspective. The closing chapter summarizes the key conclusions and areas for further work to improve the SPD technology.

**Table 1-1 Thesis structure and corresponding objectives.**

| Chapter | Paper | Title                                                                                                                               | Objective Met |
|---------|-------|-------------------------------------------------------------------------------------------------------------------------------------|---------------|
| 1       |       | Introduction                                                                                                                        |               |
| 2       | 1     | Literature Review                                                                                                                   | 1             |
| 3       | 2     | Pilot scale evaluation of woodchip as media for tertiary solid-phase denitrification in municipal wastewater                        | 2             |
| 4       | 3     | Leaching tests to study the potential of a range of low-cost media for tertiary solid-phase denitrification in municipal wastewater | 3             |
| 5       | 4     | Overall discussions and implications of the work                                                                                    | 1,2,3         |
| 6       |       | Conclusions and further work                                                                                                        | 1,2,3         |



**Figure 1.1 Graphical representation of objectives and thesis structure**

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## 2 Biological Nitrate Removal: A Review on External Carbon Sources for Heterotrophic Denitrification

Affo Souleymane, A., Alibardi, L., and Pidou, M.

Cranfield University, Bedfordshire, MK43 0AL, UK

**Abstract:** Nitrogen removal from wastewater is an essential process for protecting aquatic life and preserving water quality. Supporting carbon sources, such as methanol and acetate, have been widely used to enhance denitrification rates in biological treatment processes. However, the use of alternative carbon sources, including food fermentation broth and molasses, has also shown promise in promoting microbial activity. In this review, the use of different supporting carbon sources in the sustainable performance of denitrification systems were explored. This review provides an overview of the types of wastewaters and bioreactor setups that have been trialed with supporting carbon sources, highlighting variations in results reported. The effects of different operating conditions on the performance of denitrification systems, including temperature, pH, and hydraulic retention time are also discussed. Furthermore, potential risks associated with the use of certain carbon sources, including the growth of unwanted organisms and the presence of toxic compounds are highlighted. Overall, this review provides insight into the current state of knowledge regarding the use of supporting carbon sources in denitrification systems and identifies areas for further research.

**Keywords:** biological nitrate removal, solid-phase denitrification, liquid-phase denitrification.

## 2.1 Introduction

The current regulations for wastewater discharge to the environment not only require ammonium removal but expect the removal of total nitrogen including nitrites and nitrates to very low levels (8 – 10 mg/L) on some sites, rendering some of the current infrastructures incapable of meeting the required consents (European Environment Agency, 2020; GOV.UK, 2019). Indeed, some facilities were originally designed for ammonia removal and with the evolution in regulations around total nitrogen removal. The water utilities are then now tasked with finding alternatives that can meet these expectations, and in most cases the adaptations have to be implemented at the tertiary treatment stage since it is almost impossible to retrofit a new step within the existing treatment trains. Additionally, these alternatives should not only be efficient at removing nitrogen but must also make economic and business sense to the companies installing them. This has become a major challenge for WWTPs across the UK.

Nitrogen removal from wastewater is typically achieved through a 2-step biological process known as nitrification and denitrification, with ammonification usually preceding nitrification (Moghaddam & Sargolzaei, 2013). In this process, organic nitrogen is converted into ammonia through hydrolysis while traveling through sewer pipes. It takes about 4.5 mg of oxygen to fully oxidize 1 mg of ammonia. Therefore, even small concentrations of ammonia can cause significant deterioration to aquatic flora and fauna if not removed from wastewater before discharge (Moghaddam & Sargolzaei, 2013). Nitrification involves oxidizing ammonia ( $\text{NH}_4^+$ ) into nitrites ( $\text{NO}_2^-$ ) and nitrates ( $\text{NO}_3^-$ ) in the presence of oxygen ( $\text{O}_2$ ) and in denitrification, microorganisms reduce nitrates to nitrites and then to nitrogen gas ( $\text{N}_2$ ) consuming biodegradable organics (Pelaz et al., 2018). A reduction in total alkalinity may occur during nitrification because a significant amount of bicarbonate is consumed, with about 7.14 mg/L of bicarbonate ( $\text{HCO}_3^-$ ) used for each mg/L of ammonia oxidized. Reductions in alkalinity can cause reductions in buffering capacity, directly affecting pH stability and corrosivity of water toward lead and copper (Evans & Sober, 2023). Denitrification occurs when oxygen levels are depleted, and nitrate becomes the primary source of oxygen for the microbes (Moghaddam & Sargolzaei, 2013; Pelaz et al., 2018). This process is performed under anoxic conditions when  $\text{NO}_3^-$  is broken

down by microbes to nitrous oxide ( $\text{N}_2\text{O}$ ), and in turn to nitrogen gas ( $\text{N}_2$ ) which has low solubility and hence escapes into the atmosphere as bubbles. More specifically, nitrate is used by denitrifying bacteria as an electron acceptor, and organic and inorganic materials are used as electron donors and sources of energy to support microbial growth. This is the critical contributor in the ecosystem's biogeochemical nitrogen cycle and satisfies the energy needs of many denitrifying microorganisms. Denitrification generally follows the hierarchy of electron-accepting processes based on energy generated; i.e.  $\text{O}_2 > \text{NO}_3^- > \text{Mn}^{4+} > \text{Fe}^{3+} > \text{SO}_4^{2-} > \text{CO}_2$ . Conditions affecting removal efficiency are nitrate concentration, anoxic conditions, presence of organic matter, pH, temperature, alkalinity, and trace metals. To maintain the anoxic conditions, dissolved oxygen (DO) should be kept below 0.3 mg/L while optimum pH values for denitrification range between 6.5 to 8.5 (Wang & Chu, 2016) and optimum temperatures range from 5 to 30°C (Pelaz et al., 2018). Because the nitrification/denitrification process is microbe-mediated, organic matter is needed as a source of carbon for the microorganisms and is an essential aspect of the nitrate removal process (Chai et al., 2021). Organic matter may be present in the wastewater to be treated or supplemented if found to be limited in the wastewater under consideration.

There are a variety of biological processes available to companies and municipalities used for the treatment of wastewater, either for disposal or reuse (Frankel, 2019). One of such biologically aerated processes (BAP) is the activated sludge process (ASP) in which influent entering an aeration tank is injected with extra oxygen, allowing microbes to multiply to decompose the organic matter in the water and convert ammonia to nitrate. An anoxic zone can be implemented at the front of the system to enable denitrification and achieved total nitrogen removal. The water from the aeration tank then moves into a clarifier, where the microorganisms present in biological flocs settle to the bottom of the tank as sludge, resulting in water that is cleaner. This cleaner water flows out of the settling tank, and part of the sludge at the bottom is recycled back into the aeration tank, where it works as seed for further treatment of wastewater. As this process continues, the number of microbes in the tank increases, allowing for more efficient treatment if there is constant supply of oxygen to the microbes (Gerardi, 2003). ASP has proven to be flexible enough to treat different load types and a reliable

method of treatment overall. It is also more familiar to many operators than the newer innovations. While ASP may be one of the most widely used form of biological wastewater treatment, it is not the most effective or efficient. Some of the cons associated with the process are the inconsistencies in the final effluent qualities, the maintenance requirement to operate an efficient ASP and the space required to set up (Genesis Water Technologies, 2019). Similar to ASP, is the sequencing batch reactor (SBR) technology. The basic difference is that this system uses one tank for all the treatment steps, as opposed to different pieces of equipment for each stage of the procedure (Mace & Mata-Alvarez, 2002). The SBR system is designed to use a timed sequence to complete sub-processes such as equalization, aeration, and sedimentation in the same tank. Again, a period of anoxic conditions can be included in the cycle to achieve denitrification. It does so through a 5-step set cycle. In the SBR process, the tank is filled with influent, after which the aeration process and microorganisms begin the chain of biological reactions. After this, the sludge is settled and the treated effluent is pumped out from the tank, and the wasted sludge removed. Once the SBR system has reached this stage, it can restart, adding a fresh batch of influent to the tank and repeating the series of sub-processes that convert wastewater into treated effluent (Mace & Mata-Alvarez, 2002; Dela Calzada, 2022). Some advantages of using SBR include its ability to work with some automated controls and easy adaptation to different volumes (Frankel, 2019). Compared to the ASP, it also requires much less space to operate. On the other hand, it also requires more maintenance than conventional systems and is prone to problems like discharging sludge at the wrong times and clogging aeration devices (Frankel, 2019). Another important BAP is the moving bed bioreactor (MBBR) which is mainly used for organic pollutant removal but also plays a role in the implementation of nitrification and denitrification processes (Moghaddam & Sargolzaei, 2013). Unlike processes such as ASP, there is no need for a settling tank, and the treated effluent tends to be cleaner (Frankel, 2019). In the MBBR process, influent enters an aeration tank full of plastic media which maximize the surface area they provide for microorganisms to grow on. An aeration grid also helps the media mix in the tank and provides a continuous supply of oxygen. Like other aerobic methods, oxygen in the tank nourishes the microbes, enabling them to effectively break down the organic matter and also deliver the

nitrification step in the water (Andreottola et al., 2000). MBBR, however, requires continuous monitoring, though it requires minimal intervention and can attract insects with specific system models not designed to keep them out (Frankel, 2019). The methods mentioned above require constant sludge recycling and oxygen to work. Even though they work well for nitrogen removal, they work best at the secondary treatment stage.

A more recent technology known as Autotrophic Anaerobic Ammonium Oxidation (Anammox), in which ammonium is directly oxidized to nitrogen gas by anaerobic ammonia-oxidizing bacteria has been employed by some sewage facilities. Anammox utilizes nitrite as an electron acceptor rather than oxygen, making nitrite an important part of the process. In the use of this method, oxygen consumption is limited, hence, the elimination of the need for carbon sources (Moghaddam & Sargolzaei, 2013). In their work, Bertino (2010) and Hu et al. (2013), mentioned that the Anammox process consists of two steps, with the first being a partial nitrification of ammonium to nitrite by ammonia-oxidizing bacteria. The ammonium and resulting nitrites are then converted to nitrogen gas with less than 15% nitrite left in the system (Kartal et al., 2010; Hu et al., 2013). According to Bertino (2010), the advantages of Anammox are that it eliminates the need for an organic carbon source for nitrification, lowers energy demand for aeration, produces less residual sludge, and emits lesser CO<sub>2</sub> than traditional nitrification/denitrification in activated sludge systems (Bertino, 2010; Hu et al., 2013). On the other hand, it has the disadvantages of limited knowledge, which is a problem particularly because of the requirements associated with high maintenance and skilled operation. If the Anammox process replaces traditional nitrification/denitrification in treatment plants, construction prices will dramatically increase, which again defeats the concept of making economic and business sense for companies.

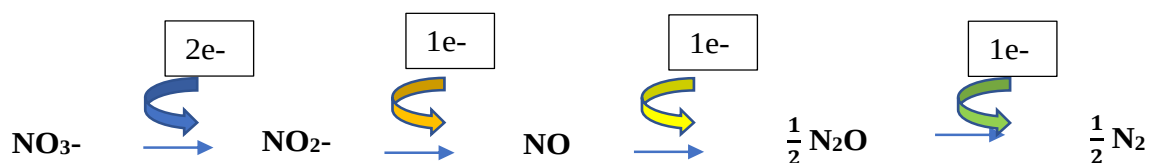
In other circumstances, algae ponds have been used for nitrogen removal from wastewater. Most often, nitrogen is still present in treated wastewaters in the form of nitrate, nitrite, and ammonia/ammonium. These nitrogen sources, as well as phosphorus, CO<sub>2</sub>, and light, are necessary for the photosynthetic process, which is the method by which microalgae grow autotrophically (Chai et al., 2021). The carbon

supply for this process can be found in the CO<sub>2</sub> that is present and is transformed by the carbonic anhydrase enzyme into carbonate ions (HCO<sub>3</sub><sup>-</sup>) (Nozari & Karabeyoğlu, 2015). An important benefit of using this microalgae property in wastewater treatment is that no secondary contamination is produced when the biomass is harvested, and nutrients are recycled effectively. The adaptation of microalgae to any nutrient environment, from municipal to industrial, is another important benefit of biotreatment using microalgae. Microalgae are also generally safe compared to other biological choices like bacteria, which may be hazardous for some species (Abinandan & Shanthakumar, 2015). However, the presence of toxins, predators, or competing species may reduce the effectiveness of microalgal biotreatment, which may have a detrimental impact on the growth of microalgae to some degree (Chen et al., 2015). Nitrogen removal by algae pond includes direct and indirect removal. Nutrient uptake in algal cells represents direct algal removal and photosynthesis, resulting in removal of ammonium (Chai et al., 2021). This method of nitrogen removal could work as a tertiary treatment step for WWTPs with sufficient space to set it up.

Finally, there are some that solely target ammonia/ammonium removal, and a key one is the use of high-siliceous zeolites, usually clinoptilolite as a medium for ion exchange. The porous nature of zeolites allows them to carry a range of cations, including Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>. The mineral's two-dimensional channel system enables it to serve as a molecular sieve, giving it a high capacity for sorption and ion exchange, ion exchange selectivity, catalytic activity, and structural stability up to 700–750°C (Moghaddam & Sargolzaei, 2013; Capodaglio et al., 2015; Climate Policy Watcher, 2022). Clinoptilolite removes ammonium using cationic ion exchange as its primary mechanism. However, ammonia can also be eliminated via adsorption in the zeolite's structural pores. Temperature has a significant impact on ammonia removal efficiency, the higher the temperature, the greater the removal efficiency. When it comes to pH, clinoptilolite's removal efficiency is relatively stable between 4 and 8, drastically declining outside of this range (Zabochnicka-Świątek & Malińska, 2010). The inconsistencies and expenses associated with ion exchange operation (e.g. regeneration) make the technology less appealing to companies despite its ability to remove and recover ammonium without converting to nitrites or nitrates (Capodaglio et al., 2015).

With the limitations associated with the existing technologies described above, alternatives are still needed. Biological denitrification remains a key technology to achieve efficient nitrogen removal but more efficient and sustainable options have to be established. There are two basic types of biological denitrification: heterotrophic and autotrophic, which differ in the various carbon sources needed for microbial growth (Karanasios et al., 2010). Inorganic substances such as hydrogen, reduced sulphur compounds, ferrous iron, iron sulphides, arsenide, and manganese are used by autotrophic denitrification as electron donors (Pang & Wang, 2021). The most prevalent denitrifying bacteria in nature are heterotrophic species that use organic carbon as a carbon source (Van Rijn et al., 2006). The most popular and efficient denitrification process, according to research, is heterotrophic denitrification. It benefits from low treatment costs, great efficiency, and being more practical (Wang & Chu, 2016). In the process known as heterotrophic denitrification, microorganisms turn nitrate and nitrite into gas and release it into the atmosphere when the ecosystem is anoxic. The carbon source is a critical part of heterotrophic denitrification and has a direct impact on the process' pace and outcome. An external carbon source is required to increase denitrification efficiency if there are no sufficient organic carbon sources already present in the system (Della Rocca et al., 2006; Fu et al., 2022). The current domestic sewage typically exhibits poor influent carbon-nitrogen ratios (C/N) and insufficient carbon sources (Zhang et al., 2016b). This has led to the release of nitrate in sewage treatment plant effluent, which is worse during the winter months. It is therefore needed to use an external carbon source as an electron donor to ensure smooth and effective denitrification, to boost the system's denitrification rate and lower the concentration of nitrate-nitrogen in the effluent. (Zhang et al., 2016b). Figure 2.1 shows the hypothetical denitrification pathway for heterotrophic denitrification, outlining four consecutive steps, where nitrates are reduced to nitrites and so on until they escape as nitrogen gas from the system. Since DO might inhibit the development of the denitrification enzymes, anoxic conditions are necessary for denitrification (di Capua et al., 2019). Nitrite reductase and nitrous oxide reductase are more sensitive to O<sub>2</sub> than nitrate reductase and their inhibition at high DO concentrations results in the accumulation of denitrification intermediates, such as NO<sub>2</sub> and N<sub>2</sub>O (Oh & Silverstein, 1999; Sabba et al., 2017). Lower temperatures may limit the denitrification

rate and efficiency, while temperatures around 30 °C are often optimal for denitrifying bacteria (Hu et al., 2013; Hoover et al., 2016; Soupir et al., 2018). Acidic pH levels also slow down denitrification and make toxic intermediates like N<sub>2</sub>O more abundant (Sabba et al., 2017). Water hardness and excessive pH increase can both impact bacterial metabolism and result in mineral precipitation (Karanasios et al., 2010).



**Figure 2.1 Hypothetical pathway of heterotrophic denitrification**

(adapted from di Capua et al., 2019)

Varied materials can be employed as external carbon sources for the denitrification process, according to existing research. The external carbon source might be classified as a solid, liquid, or gas depending on its physical form (Fu et al., 2022). Methanol (Kaplan et al., 1987; Lee et al., 2013), acetic acid (Zhang et al., 2016b ; Xu et al., 2018), and various low molecular weight sugars are common liquid carbon sources. Although methanol provides high efficiency and removal, it has the ability to inhibit microbe growth due to the accumulation of nitrites (Glass & Silverstein, 1998; Lee et al., 2013). Moreover, due to the large quantities needed to reduce high nitrate influent loads and the accompanying expenses, many companies have been forced to investigate finding alternative carbon sources which will provide the benefits of cost-efficiency, stability, and homogeneity of operations (Kaplan et al., 1987; Lee et al., 2013). These sources include methane, biodegradable polymers (Boley et al., 2000; Chu & Wang, 2013, 2017a; Xu & Chai, 2017 ; Lan et al., 2020), natural cellulose materials (Xu et al., 2009; Von Ahnen, et al., 2016 ; Sun et al., 2019; Zhao et al., 2020 ; Díaz-García et al., 2021; Domingos et al., 2022;), industrial wastewater (Lee et al., 2013), sludge fermentation broth (Cao et al., 2013; Xu et al., 2020), and food waste fermentation broth (Zhang et al., 2016a, 2016b ; Kim et al., 2017; Qi et al., 2020, 2021). Other media such as woodchip (Damaraju et al., 2015; Von Ahnen et al., 2016 and 2018) also combined with non-carbonaceous materials such as sand and/or gravel, corn cobs (He et al., 2016; Feng et al., 2017), sawdust (Hu et al., 2019; Yang et al., 2020) and rice straw and hull (Lu et al., 2017; Luo et al., 2018) have been evaluated.

Also, biodegradable plastics (Yamada et al., 2013; Li et al., 2016) including the reuse of waste biodegradable snack ware (Wang & Wang, 2013a) have been trialed.

To illustrate, Kaplan et al. (1987) conducted a study to evaluate the effectiveness of various organic compounds for treating effluent. The compounds they used were reagent grade methanol, nutrient broth, anaerobic sludge digest from a municipal wastewater treatment plant (WWTP), corn steep liquor, acid whey, sweet whey, and sugar beet molasses. Another study by Lee et al. (2013), investigated the use of mixed liquor waste from the manufacturing process of 1,4-butanediol (MLOW). Products such food fermentation liquid (Zhang et al., 2016a, 2016b; Qi et al., 2021), food-recycling wastewater (Kim et al., 2017), glucose (Zhang et al., 2016a; Xu et al., 2018), sodium acetate (Zhang et al., 2016a; Xu et al., 2018), pig manure hydrolysate (Yang et al., 2019), ethanol (Domingos et al., 2022), and sludge fermentation broth (Cao et al., 2013) have all been trialed. A study by Fernández-Nava et al. (2010) also tested the use of candy-factory wastewater, the residue from a soft drink factory and dairy-plant residue as external carbon sources for denitrification. The media used in all the studies mentioned above can be categorized as liquid-based carbon sources.

In other studies, however, solid substrates have been used as a steady supply of denitrification and provided a platform for microbial biomass development in recent years, giving rise to the phrase “solid-phase denitrification.” In this type of denitrification, the solids act simultaneously as biofilm carrier and a carbon source for microbial activities, unlike the conventional method which requires dosing of a soluble carbon source and an inert biofilm carrier for microbe attachment (Boley et al., 2000). Indeed, materials such as biodegradable polymers have been tested (Boley et al., 2000; Chu & Wang, 2013, 2017b; Shen et al., 2015; Zhang et al., 2016a; Xu & Chai, 2017; Lan et al., 2020; He et al., 2021) , as well as biodegradable plastics (Wang & Wang, 2013). Plant-based products such as woodchip (Damaraju et al., 2015; Hoover et al., 2016; Von Ahnen et al., 2018b; Zhao et al., 2018, 2020; Nordström & Herbert, 2019; Burberry et al., 2020; Audet et al., 2021; Díaz-García et al., 2021; Domingos et al., 2022; Maxwell et al., 2022) woodchips combined with non-organic materials such as sand or gravel (Wei et al., 2014; Šereš et al., 2019), sawdust (Xu et al., 2009; Hu et al., 2019; Yang et al., 2020) corn cobs (He et al., 2016; Feng et al., 2017; Sun et

al., 2019) and rice straw and hull (Lu et al., 2017; Luo et al., 2018) have also been trialed in different studies.

As shown above, a wide range of media have been explored in different works and on varying scales. There has however not been consistent reporting of the results of these studies, hence different parameters have been reported over time, giving a wide range of information to be examined. There have also been significant variability in the types of media, scale, operational conditions and types of water being treated with quite limited information on the use of these systems at the tertiary treatment stage for municipal wastewater. It is important therefore, for these different reporting parameters to be grouped into comparable data for a better understanding of the current state of the technology and to then identify where there might be gaps in knowledge and room for improvement.

This chapter then aims to ascertain the potential and feasibility of heterotrophic biological denitrification as a tertiary treatment step in a typical municipal wastewater treatment facility through the review of existing literature. For this, the following objectives will then be addressed:

1. To understand the use of external carbon sources for denitrification in municipal wastewater treatment plants (WWTP).
2. To investigate the potential for the use of different organic liquids as carbon sources for biological denitrification at the tertiary treatment step.
3. To understand the use of different solid media as carbon sources and biofilm carrier for simple and sustainable tertiary biological denitrification.
4. To synthesize the results of the existing studies and draw conclusions on the best practices and future directions for solid-phase denitrification in wastewater treatment.

## **2.2 Literature Review Methods**

A 5-step literature review was used in this study, comprising the development of a review protocol, literature search, evaluation and quality assessment of literature, evaluation of inclusion criteria to determine relevant literature, review of the literature, and meta-analyses of extracted data (Figure 2.2).

### 2.2.1 Development of Review Protocol

As postulated by Boland et al. (2017), a systematic review protocol was developed for the purpose of this study. The protocol involved establishing the scope of this study, statements to be evaluated, objectives of the literature review, literature search strategies, inclusion criteria, quality assessment, data extraction and management methods, data analysis and synthesis. The inclusion criteria comprised date of study, language, study design, industry/system, scale of study, carbon sources, and geographic location. Table 2-1 below provides details on the specified inclusion criteria for this study.

**Table 2-1 Inclusion Criteria for literature review.**

|    | <b>Criteria</b> | <b>What/Who to be included</b>                                                                            | <b>Justification of the selected inclusion criteria</b>                                                           |
|----|-----------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| 1. | Date of Study   | Open                                                                                                      | No date criterion was set for this study. This was to expand the possibility of finding relevant literature       |
| 2. | Language        | English                                                                                                   | Primary language of this study is English and set in the context of an English-speaking country                   |
| 3. | Study Design    | Descriptive: meta-analysis, qualitative studies<br><br>Analytic; experimental and observational analytics | There are varied literature and so no limits were set on study design for maximum coverage of existing literature |
| 4. | Industry/System | All WWTPs and systems                                                                                     | This criterion was set to cover the application of the technology in consideration to WWTPs treating all          |

|    |                     |              |                                                                                                                                                                                                                                                                             |
|----|---------------------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                     |              | types of wastewaters and with different operational setups                                                                                                                                                                                                                  |
| 5. | Scale of Study      | All          | To expand the reach of the literature review, all scales of operations were considered: laboratory, pilot, and full scales                                                                                                                                                  |
| 6. | Carbon Sources      | All external | This criterion was set to only include studies that utilised external carbon sources to get a good understanding of the potential of different materials to be used in heterotrophic denitrification, and how the new C/N ratios impact denitrification efficiency and rate |
| 7. | Geographic location | Open         | This criterion was open to studies conducted all over the world, while paying specific attention to carbon sources that are applicable to the UK                                                                                                                            |

### 2.2.2 Literature Search

Scopus, Google Scholar, and ResearchGate were the main databases from which literature was sourced. Keyword searches were conducted in the databases using, “heterotrophic denitrification”, “external carbon sources”, and “solid-phase denitrification” as keywords. The searches yielded tens of thousands of papers, which were narrowed down by adhering to the inclusion criteria set for this study in Table 2-1 above. The final search was narrowed down to 174 publications and further relevance checks were done by reading the abstracts and conclusions to further filter the literature search results. The sources of literature were classified as follows: Primary Sources (peer-reviewed journal articles, books, research reports), Secondary Sources (conference papers, guidelines, policy documents, government reports and websites,

trusted and endorsed websites), and regulations such as EU Urban Wastewater Directive (91/271/ EC) and EU Water Framework Directive (2000/60/EC).

### 2.2.3 Data Extraction and Analysis

A data extraction table was constructed in Microsoft Excel, which captured critical information from the selected literature found through the searches. The table included: names of author(s), title, abstracts, details of experimental conditions, and relevant outcomes. Content analysis and data extraction were made by fully reading all relevant publications three consecutive times to minimise errors in the data extraction process.

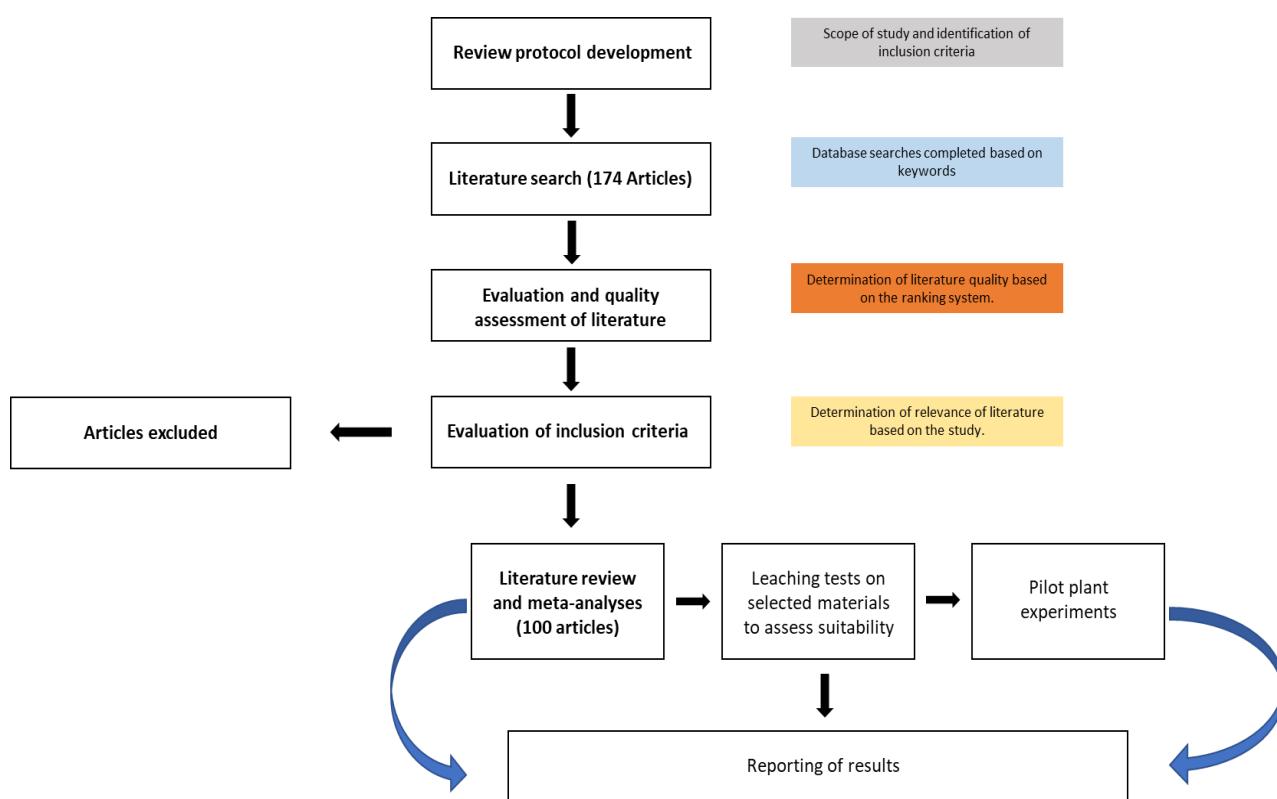


Figure 2.2 Overview of methodology

## 2.3 Results

### 2.3.1 Liquid-Phase Carbon Sources for Denitrification

Several studies have investigated and reported the use of different carbon sources other than the more commonly used methanol (Table 2-2). In the work by Kaplan et al. (1987), for example, the use of reagent grade methanol, nutrient broth, anaerobic

sludge digest from a municipal WWTP, corn steep liquor, acid whey, sweet whey, and sugar beet molasses for the treatment of industrial effluents with nitrate loads of up to 285 mg/L of  $\text{NO}_3\text{-N}$  were investigated. The experiments were run as single-step denitrification systems using continuous flow, and the efficiencies of the carbon sources compared. with the minimum C/N at which at least 95% denitrification occurred. Methanol, sweet and acid whey, corn steep liquor, nutrient broth, and sugar beet molasses all achieved 95% denitrification efficiency at C/N of between 1.1 and 1.7, except for sugar beet molasses which had C/N of up to 3.6. Although these C/N ratios are lower than the suggested theoretical value of 8.0 needed to achieve denitrification, they have been reported to achieve substantially high denitrification efficiencies. This is likely to be as result of the addition of these C/N values to the values already present in the water. Acid hydrolysed sewage sludge digest also maintained a 95% efficiency at C/N of 2.1 until it reaches 2.8, where it started to decline (Kaplan et al., 1987). As expected, Methanol showed good efficiency of 87.1% and  $\text{NO}_3\text{-N}$  reduction from approximately 32.5 mg/L to 3.9 mg/L (Pelaz et al., 2018). Domingos et al. (2022) evaluated the use of ethanol as an external carbon source. The results showed 90% denitrification efficiency and a reduction from an average  $\text{NO}_3\text{-N}$  of 20.9 mg/L to about 2 mg/L. Xu et al. (2018) further proved the efficiency of liquid-phase denitrification by assessing that of glucose and acetate, in which he reported denitrification efficiency of 92% and 79%, respectively. Glucose reduced  $\text{NO}_3\text{-N}$  from 50.6 mg/L to 2.38 mg/L and acetate, from 49.1 mg/L to 7.96 mg/L. According to Lee et al. (2013), mixed liquor waste from the manufacturing process of 1,4-butanediol (MLOW) was assessed as an external carbon source to treat wastewater from a stainless-steel manufacturing company with  $\text{NO}_3\text{-N}$  influent load of up to 100 mg/L. This was however done at the secondary stage under anoxic conditions, with an aeration tank and clarifier stages following the denitrification. In their setup, two pilot plants were used with MLOW supplied to one tank and methanol to the other to compare denitrification efficiencies. After the pilot experiment, MLOW was applied to a full-scale plant, where it was injected in doses to acclimatise the microbes to methanol. MLOW was used alone from week seven, when the microbes had fully adapted. The study concluded that the denitrification capacity of the MLOW was comparable to that of methanol, while providing an added benefit of lower COD

than methanol. In this work, at a C/N of 4.01 and 4.89 for methanol and MLOW, respectively, the rates of denitrification were measured at 304 and 312 mg/g/d.

Wastewater from food-waste recycling industries (FRW) contains high organic matter levels and so could be used as an external carbon source for heterotrophic denitrification. To investigate the use of food waste, the work undertaken by Qi et al. (2021) was reviewed where it showed promising results for the use of food fermentation liquid (FLFW) as an external carbon source at secondary stage in a full scale WWTP. In this work, it was reported that the FLFW did not affect effluent COD and  $\text{NO}_3\text{-N}$  reduced from 15 to about 0.6 mg/L after addition. Prior to the application of FLFW in full-scale, Qi et al. (2021) conducted a denitrification test in which it was reported that acetic, butyric, and lactic acids led to residual  $\text{NO}_3\text{-N}$  of 0.7, 0.3 and 7.5 mg/L, respectively, compared to FLFW which attained residual of 1.7 mg/L. It was concluded that FLFW had a higher denitrification rate than lactic acid, and a slightly lower rate than acetic and butyric acids. These results have also further indicated the ability of wastewater from food to be used as organic matter for heterotrophic denitrification. A review of the work done by Kim et al. (2017) on a full-scale sewage WWTP, aimed at investigating denitrification performance of FRW as an external source of carbon for denitrifying microbes. The WWTP used in this study treated effluent from steel-making industries. Like the previous study by Lee et al. (2013), this work was also done as a secondary stage treatment in a facility designed as Anaerobic Anoxic Aerobic ( $\text{A}_2\text{O}$ ) process, but with a slightly different setup. The diluted FRW used in this work had an average COD of 51.7 g/L. and recorded an average denitrification efficiency of 97.2%, which later increased to 99.3% after day 98. In another study by Zhang et al. (2016a), FLFW was again used as an external carbon source. Prior to the start of the experiment, sludge from an  $\text{A}_2\text{O}$  facility was used as seed for 2 days. At a C/N of 6, the effluent reached optimal levels of COD and nitrogen, achieving efficiency as high as 98%, similar to sodium acetate although the former required a longer time to reach stability. In the same study, glucose was also used, yielding an efficiency of only 39.6%. It can be concluded that liquids from food waste can successfully be used as external carbon sources for denitrification. In other works, different liquid-phase external carbon sources were evaluated with various results. Fernández-Nava et al. (2010) assessed the use of candy-factory wastewater, residue

from a soft drink factory, and residue from a dairy plant with feed  $\text{NO}_3\text{-N}$  of up to 700 mg/L, where residual concentrations of  $<0.05$  mg/L were observed for both candy-factory wastewater and residue from a soft drink factory. The dairy-plant residue reduced  $\text{NO}_3\text{-N}$  to a level of 0.4 mg/L. The three carbon sources mentioned above had C/Ns of 6.5, 5.5 and 4.6, respectively. Yang et al. (2019) also used pig manure hydrolysate in their study and reported a C/N of 6.5, at which  $\text{NO}_3\text{-N}$  levels were reduced from 93.76 mg/L to  $<5$  mg/L, indicating a denitrification efficiency of 90.2%. A denitrification efficiency of 92.2% was reported in the work conducted by Cao et al. (2013), using sludge fermentation broth to treat synthetic wastewater in combination with Anammox.

**Table 2-2 Nitrate removal efficiencies for liquid-based carbon sources for denitrification.**

| <b>Carbon Source</b>            | <b>% Removal</b> | <b>Nitrate In</b> | <b>Nitrate Out</b> | <b>Reference</b>              |
|---------------------------------|------------------|-------------------|--------------------|-------------------------------|
| Mixed liquor organic waste      | 99               | 100               | 0.1                | (Lee et al., 2013)            |
| Acid hydrolysed sludge digest   | 95               |                   |                    |                               |
| Anaerobic sludge digest         | 70               | -                 | -                  | (Kaplan et al., 1987)         |
| Nutrient broth                  | 95               |                   |                    |                               |
| Fish condensates                | 70               |                   |                    |                               |
| Food waste recycling wastewater | 99.3             | -                 | -                  | (Kim et al., 2017)            |
| Candy factory wastewater        | 99.9             |                   | <0.05              | (Fernández-Nava et al., 2010) |
| Soft drinks residue             | 99.9             | 700               |                    |                               |
| Dairy plant residue             | 98               |                   | 0.4                |                               |
| Food waste fermentation broth   | 87.4             | 15                | 0.6                | (Qi et al., 2020)             |
| Food waste fermentation broth   | 98               | 48                | 3                  | (Zhang et al., 2016a, 2016b)  |
|                                 | 90.1             | 40                | 3                  |                               |
| Corn husk hydrolysate           | 96.9             | 300.6             | 9.2                | (He et al., 2016)             |
| Pig manure hydrolysate          | 90.2             | 9376              | 5                  | (Yang et al., 2019)           |
| Methanol                        | 99               | 100               | 0.1                | (Lee et al., 2013)            |

### 2.3.2 Solid Media for Denitrification

Solid substrates have also been used as a steady supply organic matter for denitrification and also provide a platform for microbial biomass development, giving rise to the phrase “solid-phase denitrification.” In this type of denitrification system, the solids act simultaneously as biofilm carrier and a carbon source for microbial activities, unlike the conventional method which requires dosing of a soluble carbon source and an inert biofilm carrier for microbe attachment (Boley et al., 2000). In recent years, there has been an increase in research on solid-phase denitrification for wastewater treatment which has led to a better understanding of the key factors that affect the efficiency of the process, including the type of solid substrate used, the type of denitrifying bacteria, and the environmental conditions (Yuan et al., 2016). One of the key findings from recent research is the importance of the type of solid substrate used. Different substrates, such as sawdust, woodchips, and straw, have different properties, including porosity, water-holding capacity, and surface area, which can affect the efficiency of denitrification (Gupta and Sharma, 2013). Research has shown that the most effective substrates are those with high surface area and good water-holding capacity.

To illustrate, the use of biodegradable polymers as media for heterotrophic denitrification have been investigated. Boley et al. (2000) applied biodegradable polymers in their work on recirculated aquaculture systems where they reported denitrification after 8 days of operations. In this work, polyhydroxy butyrate (PHB), polycaprolactone (PCL) and bionolle were used, with PCL and bionolle achieving nitrate concentrations below the detection limit. PHB however reached only 18 mg/L and this was attributed to operational issues in the bioreactor. Chu & Wang, (2013) investigated the use of PCL for nitrate removal from groundwater and reported final effluent  $\text{NO}_3\text{-N}$  at <3.7 mg/L and a total denitrification efficiency of up to 95%. In 2017, Chu & Wang conducted another study using polybutylene succinate (PBS) as the carbon source for treating groundwater. In this work, effluent nitrate levels went down to between 3.3 and 8.8 mg/L and achieved 88 – 97% denitrification efficiency. Rates of 0.12 g/L/d to 0.35 g/L/d were reported for different temperatures. He et al. (2021), Shen et al. (2015) and Zhang et al. (2016) also reinforced the potential of polymers to

be used as media for biofilm growth and carbon sources by testing the efficiency of PCL under different conditions, with above 98% removal efficiency for (He et al., 2021), 98.3 – 100% removal efficiency for (Zhang et al., 2016a). Shen et al. (2015) reported a removal rate of 0.17 mg/g/d for PCL, 0.53 and 0.3696 mg/g/d for starch/PCL blends. Despite the promising results obtained from some of the studies, there is the limitation of availability on a large scale if the technology is to be replicated on full-scale WWTPs.

The limitations and/or costs associated with a lot of potential external carbon sources have led researchers and organizations to look into using agricultural/wood-based products which are more readily available and relatively cheap. To illustrate, Feng et al. (2017) tested and compared a variety of solid media including corn cob, rice straw and hull among others on synthetic wastewater. Using a hydraulic retention time (HRT) of 24 hours, nitrogen removal efficiencies of >90% for corn cob and rice straw. An efficiency of >50% was found when rice hull was used. In the same study, the three materials were also treated with acid/alkaline to determine how that affected the performance of the media. Agricultural-based products are known as lignocellulosic materials and have the tendency to be recalcitrant, hence the use of pre-treatment methods to reduce this characteristic has become desirable. Pre-treatment methods include size reduction, treatment with hot water or chemicals (acid/alkaline), and biological treatments to improve the accessibility of microorganisms to lignocellulosic biomass and improve digestibility (Brar et al., 2016; Elumalai et al., 2016; Feng et al., 2017). The pre-treatment with acid/alkaline slightly increased the removal efficiencies in this study for corncob and rice straw, except for alkaline pre-treated rice straw which fell from 91.2 to 85.1%. The treatment for rice hull increased the performance from 56.4 to 77.3% and 81.3% when treated with acid and alkaline, respectively. In another study, pre-treated sawdust was tested on synthetic wastewater, resulting in nitrate removal efficiencies of >99%, compared to the 56.64% recorded for untreated sawdust in the same study. Zhang et al. (2012) used sawdust, wheat straw and biodegradable polymer in their study. The carbon sources in this study were pre-treated by washing with distilled water and sterilization and tested using synthetic groundwater with nitrate concentrations up to 50 mg/L for 45 days. Sawdust demonstrated removal efficiencies of between 85 – 90% in the first 15 days and dropped to 71% which coincided with the

drop in COD concentrations measured in that period. Biodegradable polymer on the other hand, maintained removal efficiency of up to 99% throughout the study while wheat straw ranged between 89 – 95% at different times in the experimental period. The study associated the decrease in sawdust removal efficiency to high amounts of lignin contained in it, which is not easily degradable by microbes. It has been reported that lower lignin content in carbon sources results in better biodegradability and higher bacterial activity, which then increases nitrate removal potential (Gibert et al., 2004) (Table 2-3).

Other lignocellulosic materials that have been used in many studies are woodchips. In these works, the woodchips are usually classified as softwood or hardwood based on the type of wood used. A few studies have categorically named the trees used in generating these woodchips, such as eucalyptus, birch, spruce, or pine, but most studies have just broadly classified them. To explore the use of woodchips as external carbon sources for nitrate removal, Von Ahnen et al. (2016) used freshly chopped willow woodchips to treat water from a recirculating aquaculture system. In their work, nitrate removal rates of 5.20 to 8.98 g/m<sup>3</sup>/day and efficiency averaging 98% were reported (Table 2-3). Citrus woodchip has also been used to treat brine from groundwater desalination plants with influent nitrate concentrations ranging between 40 – 50 mg/L. This study reported nitrate removal rates of 15 – 25 g/m<sup>3</sup>/day and 5 -10 g/m<sup>3</sup>/day. These different ranges coincided with the drop in temperatures in the operation period (Díaz-García et al., 2021). Lopez-Ponnada et al. (2017) reported on the use of softwood and hardwood to treat stormwater, with 75.2 and 63% removal efficiencies for softwood and hardwood, respectively. In treating groundwater, Manca et al. (2020) also used softwood and a hardwood mix, with average removal rates of 2.0 and 1.6 g/m<sup>3</sup>/day for softwood and hardwood, respectively (Table 2-3). A mixture of hardwood and softwood (~70/30) was also tested by Maxwell et al. (2022) to treat surface drainage in which removal efficiency of >80% was reported for average influent nitrate concentration of 6.4 mg/L. In another study by Von Ahnen et al. (2018), the performances of full-scale hardwood bioreactors treating aquaculture effluent were evaluated, with reported removal rates of averagely 4.8, 4.5 and 7.8 g/m<sup>3</sup>/day for the 3 sites evaluated and corresponding nitrate removal values of 84, 84 and 92%, respectively (Table 2-3).

In addition to the use of woodchips, a few other studies have combined woodchips with non-organic materials. This is because, it has been suggested that this combination, usually with sand or gravels, has proven to add to the stability of the bioreactor systems, while maintaining a similar range of performance as those reported for the systems operated using woodchips only. To illustrate, Šereš et al. (2019), evaluated the use of woodchips (birch and spruce) mixed with gravels to treat synthetic wastewater. In this work, removal rates of 1.4 and 1.2 g/m<sup>3</sup>/day for the two woodchips, and average removal efficiency values of 96 and 85%, respectively. Woodchip and quartz sand has also been used by Zhao et al. (2018) to treat synthetic water with a nitrate removal success of about 95% (Table 2-3). See 6.2Appendix B for full table of carbon materials used in the literature review.

**Table 2-3 Nitrate removal rates for solid-based carbon sources for SPD**

| Carbon Source                 | % Removal | Nitrate In  | Nitrate Out | Removal Rate (mg/L/d) | Reference                  |
|-------------------------------|-----------|-------------|-------------|-----------------------|----------------------------|
| Cotton wool                   | 98        | 137.16      | 2.08        |                       | (Aloni & Brenner, 2017)    |
| Wheat straw                   | 91        | 100         | 9           |                       | (Aslan & Türkman, 2005)    |
| Pine bark                     | 59.7      |             | 24.2        | 0.09                  | (Capodici et al., 2014)    |
| Sawdust                       | 77.7      | 60          | 13.4        | 0.37                  |                            |
| Cork                          | 80.8      |             | 11.5        | 0.28                  |                            |
|                               | 99.66     | 30          |             | 0.023                 | (Capodici et al., 2018)    |
| Olive nut                     | 99.47     | 50          |             | 0.034                 |                            |
|                               | 76.3      | 75          |             | 0.039                 |                            |
| Wine industry by-products     |           | 12.04       |             |                       | (Carrey et al., 2018)      |
| Cotton                        |           |             |             | 190                   | (Della Rocca et al., 2006) |
| Cotton + Steel wool           |           | 110         |             | 236                   |                            |
| Citus Woodchip                | 80 -95    | 40 -50      | 10          | 18.9                  | (Díaz-García et al., 2021) |
| Maple/birch                   | 88        | 7.6         | 6.68        |                       | (Ergas et al., 2010)       |
| Corn cob                      | 92.5      |             |             | 4                     | (Feng et al., 2017)        |
| Acid pre-treated corn cob     | 94.1      |             |             | 4.2                   |                            |
| Alkaline pre-treated corn cob | 93.3      | 3.88 - 4.79 |             | 4.2                   |                            |
| Rice straw                    | 91.2      |             |             | 4                     |                            |

|                                 |       |      |       |       |                        |
|---------------------------------|-------|------|-------|-------|------------------------|
| Acid pre-treated rice straw     | 95    |      |       | 4.2   |                        |
| Alkaline pre-treated rice straw | 85.1  |      |       | 4.3   |                        |
| Rice hull                       | 56.4  |      |       | 2.2   |                        |
| Acid pre-treated rice hull      | 77.3  |      |       | 3.2   |                        |
| Alkaline pre-treated rice hull  | 81.3  |      |       | 4.1   |                        |
| Mulch                           | 89.7  | 32.2 | 0.066 |       |                        |
| Softwood                        | 98.7  |      | 0.067 | 0.067 |                        |
| Softwood                        | 96    | 50   | 0.022 | 0.034 |                        |
| Hardwood                        | 98.7  |      | 0.035 | 0.035 |                        |
| Coniferous                      | 95.1  |      | 0.048 | 0.048 | (Gibert et al., 2008)  |
| Willow                          | 86.3  | 32.2 | 0.056 | 0.026 |                        |
| Leaves                          | 93.9  |      |       | 0.217 |                        |
| Compost                         | 92.7  |      |       | 0.026 |                        |
| Cornstalks                      | 90    | 405  | 39.4  | 2.88  |                        |
| Cardboard                       | 93    | 150  | 10.1  | 1.05  | (Greenan et al., 2006) |
| Woodchips                       | 78.6  | 75   | 16    |       |                        |
| Fresh hardwood                  | 39    | 30   |       | 15.6  |                        |
| Weathered hardwood              | 52    |      |       | 21    | (Hoover et al., 2016)  |
| Untreated sawdust               | 56.64 |      |       | 29.3  |                        |
| Lime treated sawdust            | 100   |      |       | 131.9 | (Hu et al., 2019)      |

|                                       |              |      |            |                               |
|---------------------------------------|--------------|------|------------|-------------------------------|
| Peracetic-acid treated sawdust        | 100          |      | 140.6      |                               |
| Sand coated oil                       |              | 20   | 21         | (Hunter, 2001)                |
| Wheat straw                           |              |      | 53         |                               |
| Wheat straw                           |              | 22.6 | 0.31       | (Ine et al., 1998)            |
| Biodegradable plastic                 |              | 50   | 40.27      | (Zhang et al., 2012a)         |
| Brewery spent grain                   | 95           |      |            |                               |
| Hydrated soluble potato solids        | 95           |      |            | (Kaplan et al., 1987)         |
| Softwood                              | 75.2         | 33.4 | 5.84       |                               |
| Hardwood                              | 63           | 11.6 | 3.45       | (Lopez-Ponnada et al., 2017b) |
| Hardwood                              |              | 2.3  | 2.29       | (Lynn et al., 2015)           |
| Hardwood Mix                          |              |      | 1.6        | (Manca et al., 2020)          |
|                                       | 9.0 ± 3.69   |      | 9.0 ± 4.01 |                               |
| Hardwood                              | 32.1 ± 5.18  |      | 8.5 ± 2.42 | (Martin et al., 2019)         |
|                                       | 53.8 ± 17.55 |      | 7.4 ± 2.52 |                               |
| a ~70/30 mixture of hardwood/softwood | >80          | 6.4  | 0.3        | (Maxwell et al., 2022)        |
| PHBV                                  |              | 80   |            | (Mergaert et al., 2001)       |
| Softwood                              | 52           |      | 1.44       |                               |
| Maple                                 | 38           | 3    | 1.86       | (Peterson et al., 2015)       |
| Wild cherry                           | 64           |      | 1.08       |                               |

|                                  |         |            |           |                            |                                 |
|----------------------------------|---------|------------|-----------|----------------------------|---------------------------------|
| Oak                              | 38      |            | 1.86      |                            |                                 |
| Beech                            | 73.3    |            | 0.96      |                            |                                 |
| Polycaprolactone                 | 94      | 31.66      | 0.94      | 29.7                       | (Shen et al., 2020)             |
| Polycaprolactone                 | 96.1    | 48.8       | 0.5       | 19.3                       | (Zhang et al., 2016a)           |
| Woodchips                        | 78.3    | 4.8        | 1.04      |                            | (Robertson & Merkley, 2009)     |
|                                  | 74      | 1.2        | 0.2       | 0.7                        |                                 |
| Waste cellulose solids           | 58      | 4.8        | 2         |                            | (Robertson et al., 2000)        |
|                                  | 80      | 57         | 11.6      | 2.6                        |                                 |
| Crab shell chitin                |         | 22.7       |           | 2.4                        | (Robinson-Lora & Brennan, 2009) |
|                                  |         | 22.7       |           | 24.5                       |                                 |
| Woodchips                        |         | 53         |           | 1.4                        | (Schipper et al., 2010)         |
| Sawdust                          |         | 50         |           | 1.4                        | (Schipper et al., 2005)         |
| Washed gravels + Birch woodchips | 96 ±6   |            |           | 1.4 ± 0.7                  | (Šereš et al., 2019)            |
| Washed gravel + Spruce woodchips | 85 ± 20 |            |           | 1.2 ±0.7                   |                                 |
| Activated sludge                 |         | 15         |           |                            | (Sun et al., 2017)              |
| Wood particles                   |         | 11.8       | 3.9       |                            | (Van Driel et al., 2006)        |
| Fresh willow woodchips           | 98      |            |           | 5.20 ± 0.02 to 8.96 ± 0.19 | (Von Ahnen et al., 2016)        |
| Hardwood                         | 84      | 5.3 ± 0.2  | 1.0 ± 0.5 | 4.8 ± 2.3                  | (Von Ahnen et al., 2018b)       |
| Hardwood                         | 84      | 10.5 ± 3.5 | 7.5 ± 2.6 | 4.5 ± 1.9                  |                                 |

|                                                       |       |                 |                |           |                     |
|-------------------------------------------------------|-------|-----------------|----------------|-----------|---------------------|
| Hardwood                                              | 92    | 9.5 ± 2.8       | 4.9 ± 2.5      | 7.8 ± 2.1 |                     |
| Biodegradable snack ware                              | 100   | 50              | 0.01           |           | (Wang & Wang, 2011) |
| Corncoobs                                             | 87    | 25.3            | 3.5            | 203       | (Xu et al., 2009)   |
| Polycaprolactone                                      | 87    | 50              |                |           | (Shen et al., 2015) |
| Starch/PCL                                            | 97    | 50              |                |           |                     |
| Starch/PCL                                            |       | 15              |                | 640       | (Shen et al., 2013) |
| Starch/Polylactic acid                                |       | 50              |                | 607       |                     |
| Poly (butanediol succinate)                           |       | 50              | 1.92           | 600       | (Shen et al., 2016) |
| poly(3-hydroxybutyrateco-3-hydroxyvalerate)<br>(PHBV) | 92.36 | 9.53            | 0.73 ±<br>0.86 |           | (Yang et al., 2020) |
| PHBV- Rice Hull                                       | >95   |                 | 0.99 ±<br>0,95 |           |                     |
| PHBV-Sawdust                                          | 96.64 |                 | 0.32 ±<br>0.38 |           |                     |
| Woodchip and quartz sand                              | 95.4  | 50.57 ±<br>1.32 | <5.00          |           | (Zhao et al., 2020) |

## 2.4 Discussion

The carbon sources reported in the literature have been tested on different types of wastewaters, with for example, synthetic wastewater (Hoover et al., 2016; Feng et al., 2017; Zhao et al., 2018), aquaculture (Von Ahnen et al., 2016a, 2016b; Von Ahnen et al., 2018a, 2018b), drainage (Martin et al., 2019; Maxwell et al., 2022), stormwater (Lopez-Ponnada et al., 2017) and brine from groundwater desalination (Díaz-García et al., 2021), which could account for some of the variations in results reported. However, there is scarce information on the use of this technology for municipal wastewater (Table 2-4), even though this is one of the common application for biological nutrients removal.

**Table 2-4 Types of wastewaters trialed with SPD.**

| <b>Wastewater Type</b> | <b>Reference</b>                                                                                                   |
|------------------------|--------------------------------------------------------------------------------------------------------------------|
| Synthetic Wastewater   | (Aslan & Türkman, 2005; Della Rocca et al., 2006; Capodici et al., 2014; Hoover et al., 2016 ; Feng et al., 2017;) |
| Brine                  | (Díaz-García et al., 2021)                                                                                         |
| Spiked Groundwater     | (Greenan et al., 2006 ; Gibert et al., 2008)                                                                       |
| Stormwater             | (Lopez-Ponnada et al., 2017)                                                                                       |
| Agricultural Drainage  | (Robertson & Merkley, 2009)                                                                                        |
| Septic Sewage          | (Robertson et al., 2000)                                                                                           |
| Effluent from WWTP     | (Sun et al., 2017; Yang et al., 2020)                                                                              |
| Aquaculture            | (Von Ahnen et al., 2016; Von Ahnen et al., 2018b)                                                                  |
| Surface Drainage       | (Maxwell et al., 2022)                                                                                             |

Also, different bioreactor setups have been tested, and although it makes the comparison of the data reported difficult, this leaves room to explore different designs based on the type of water to be treated, the conditions and space allocation. Systems such as batch and continuous flows (Hu et al., 2013; Lopez-Ponnada et al., 2017), up-flow packed bed (Hoover et al., 2016), vertical down-flow (Von Ahnen et al., 2018b), continuous flow reactors (Yang et al., 2020), trenches (Manca et al., 2020), and horizontal flow bioreactors (Von Ahnen et al., 2018b) have all been tested for SPD (Table 2-5).

**Table 2-5 Different system configurations for deploying SPD.**

| <b>Reactor System Type</b> | <b>Reference</b>                                                                 |
|----------------------------|----------------------------------------------------------------------------------|
| Batch                      | (Greenan et al., 2006; Gibert et al., 2008; Shen et al., 2015 ; Hu et al., 2019) |
| Column/ Continuous Flow    | (Xu et al., 2009; Wang & Wang, 2011; Peterson et al., 2015)                      |
| Packed Bed                 | (Robertson et al., 2000; Shen et al., 2015 ; Shen et al., 2016)                  |
| Layer                      | (Robertson et al., 2000)                                                         |
| Horizontal Flow            | (Von Ahnen et al., 2016)                                                         |
| Vertical Flow              | (Von Ahnen et al., 2018b)                                                        |
| Others                     | (Hunter, 2001; Quan et al., 2005; Sun et al., 2017 ; Maxwell et al., 2022)       |

Moreover, some papers have reported evidence of the effects of different operating conditions on the performance of the denitrification systems, while others have reported seeing no apparent effect of some of these conditions on the performance. There is a wide range of information available on conditions such as temperature, pH,

HRTs and microbiology of this technology (Hoover et al., 2016; Soupir et al., 2018; Zhang et al., 2019; Griebmeier et al., 2019; Aalto et al., 2020). Most of the studies reviewed for this work tested the technology under regulated temperatures ranging from 15 – 30 ° C (Kaplan et al., 1987; Yang et al., 2015; Zhang et al., 2016a; Hoover et al., 2016; Kim et al., 2017; Feng et al., 2019; Shen et al., 2020; Díaz-García et al., 2021) and pH of 5.5 – 8 (Yang et al., 2015; Díaz-García et al., 2021).

Part of the variations in existing studies are the materials used as carbon sources. Liquid carbon sources such as methanol and acetate which are chemical based have been used. Newer options such food fermentation broth or molasses have also proven to successfully act as supporting carbon source, where slightly higher metabolic rates for microbes were detected in sludge samples from the addition of fermented food waste than for acetate (Zhang et al., 2016b). However, part of the risks associated with such sources is the presence of other nutrients such as proteins, amino acids, and vitamins that can lead to the growth of other unwanted organisms (Qi et al., 2020). Industrial waste streams used in denitrification such as brewery waste have shown to work well but due to the wide variation in compositions of these streams, they may contain compounds that are toxic to microbes. Some waste streams may contain elevated levels of organic compounds, such as ethanol or organic acids, which can be readily used by denitrifying bacteria, while others may contain compounds that require additional treatment steps to make them suitable for use in the denitrification process. Some studies have however used these industrial waste streams as supporting carbon sources without any reports of toxicity (Rodríguez et al., 2007; Swinarski et al., 2009; Lee et al., 2013). It can then be concluded that liquid-based carbon sources can be successfully used as external carbon sources for denitrification, provided the risks can be controlled or mitigated. To minimize the risk of unwanted microbial growth, it is important to carefully monitor and control the conditions in the SPD system. This may include maintaining the pH and temperature in the appropriate range for the organisms.

Lignocellulosic materials such as straw and corn cobs as carbon sources are relatively inexpensive, widely available (Feng et al., 2017; Sánchez et al., 2020) and have been

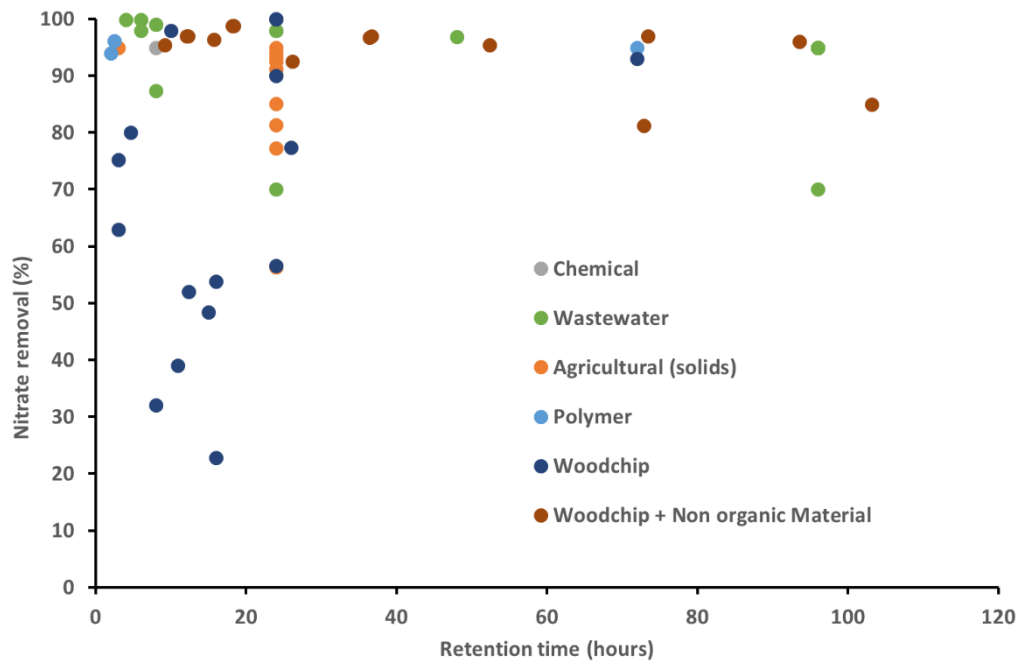
successfully trialed as external carbon sources on different scales. However, they may require pre-treatment, such as chemical or enzymatic hydrolysis, to break down the complex carbohydrates into simpler sugars that can be readily used by microorganisms. Additionally, lignocellulosic materials may contain lignin, which is a complex polymer that is difficult to degrade and can inhibit microbial growth in SPD systems (Brar et al., 2016; Feng et al., 2017). On the other hand, biodegradable polymers as a carbon source are typically more readily biodegradable than lignocellulosic materials and may require less pre-treatment before use in SPD systems. However, the cost of producing biodegradable polymers can be relatively high, and they may not be as widely available as lignocellulosic materials or other carbon sources (Table 2-6) (Chu & Wang, 2013; Yamada et al., 2013; Zhang et al., 2016a).

Of the materials tested as carbon sources in the different studies, woodchip has shown to be the most versatile, allowing for different configurations and applicability (Figure 2.3). Woodchips can be an interesting support media to explore for solid-phase denitrification due to several potential benefits. For example, woodchips are a readily available and renewable resource that can be easily obtained from the forest industry or other sources. This can make them a more environmentally sustainable alternative to other support media such as plastic or ceramic materials and may also be more affordable than other support media, as they can be obtained at a lower cost (Table 2-6) and can be used as a by-product from other industrial processes (Sreevani, 2018).

**Table 2-6 Estimated prices of different carbon sources for denitrification.**

Sources: IBISWorld - Industry Market Research, Reports, & Statistics, Global Market Research Reporting and Business Intelligence Consulting - Euromonitor.com, Bloomberg.com, American Chemistry Council, Home - Biomass Power Association ([usabiomass.org](http://usabiomass.org))

| <b>Material</b>                  | <b>Woodchip</b> | <b>Methanol</b> | <b>Acetate</b> | <b>Straw</b> | <b>Sawdust</b> | <b>Polycaprolactone</b> |
|----------------------------------|-----------------|-----------------|----------------|--------------|----------------|-------------------------|
| <b>Estimated cost (US\$/ton)</b> | 100 - 200       | 300 - 400       | 500 – 800      | 80 - 120     | 70 - 100       | 3,000 – 8,000           |



**Figure 2.3 Nitrate removal efficiencies reported in literature with various external carbon sources.**

Woodchips can also provide a high surface area and a porous structure that can support the growth and proliferation of denitrifying bacteria (Lepine et al., 2020) . This can enhance the efficiency of the denitrification process. Woodchips are known to harbour a diverse range of bacterial communities, including denitrifying bacteria. This can increase the potential for successful denitrification and reduce the need for introducing and establishing the bacteria in the system. They can also be compatible with a wide range of wastewater types and conditions, making them suitable for use in different applications (Wang & Chu, 2016). Woodchips have been barely explored for their potential in treating domestic sewage through solid-phase denitrification even though they have been shown to be compatible with various types of wastewaters. Further research is needed to fully evaluate the potential of woodchips as a support medium for solid-phase denitrification in domestic sewage treatment.

The most variation in results reported also are in the studies on woodchip, leaving room for more exploration to be done in this area to understand why this is so and to optimise the system. This coupled with the apparent lack of information on the application of this treatment technology on municipal wastewater makes the evaluation of woodchip-based solid-phase denitrification a subject of interest and further studies.

## **2.5 Conclusions**

In conclusion, the use of alternative carbon sources other than methanol for biological denitrification has been investigated and reported in various studies. These studies have shown that alternative carbon sources such as waste materials including nutrient broth, corn steep liquor, acid and sweet whey, sugar beet molasses, mixed liquor waste from the manufacturing process of 1,4-butanediol, food fermentation liquid, and diluted food waste can be effective in denitrification processes. These alternative carbon sources have been found to have comparable or even higher denitrification efficiency compared to methanol, while also providing the benefits of cost-efficiency, stability, and homogeneity of operations. The results of these studies have particularly highlighted the ability of wastewater from the food industry to be used as organic matter for heterotrophic denitrification due to the high content in biodegradable organics. However, further research is needed to determine the optimal conditions for the application of these alternative carbon sources in various wastewater treatment plants and to explore their potential as sustainable solutions to traditional carbon sources in the field of biological denitrification. Indeed, if waste materials are to be used as external carbon sources, it will also be critical to understand if they may lead to secondary contamination of the treated water due to the presence of other pollutants.

Solid-phase denitrification, on the other hand, is a promising technology for removing nitrogen from various types of wastewaters. This process is a form of biological denitrification that occurs on a solid substrate, such as woodchips, which serve as a support medium for the growth of denitrifying bacteria. The performance of this technology is dependent on several factors such as the type of wastewater, reactor system design, and operating conditions. One important consideration in SPD is the

availability of an external carbon source to support the growth of denitrifying bacteria. Chemicals such as methanol, ethanol, and acetate have commonly been used as external carbon sources in SPD systems. However, due to their high cost, researchers have evaluated the potential of waste materials as external carbon sources, such as food waste and agricultural by-products. Woodchips have been identified as a versatile and sustainable support medium for solid-phase denitrification due to their high surface area and ability to support the growth of denitrifying bacteria. They are a cost-effective and readily available substrate that has been successfully used in various types of SPD systems. The use of woodchips can also help to reduce the environmental impact of wastewater treatment by providing a sustainable use for this waste material. In addition to woodchips, other support media, such as polycaprolactone, straw, and corncobs, have also been investigated for use in solid-phase denitrification. These media offer unique advantages, such as higher selectivity for nitrate removal or the ability to remove other contaminants, but they also have limitations, such as higher cost or lower availability.

The available literature on the application of tertiary denitrification of municipal wastewater is limited, but a range of technologies applied for water denitrification and applicable at the tertiary treatment stage have been identified. Most studies reported in the literature are research studies at laboratory and pilot scales, with limited full-scale applications. Some of the more common configurations applied to SPD are batch and column bioreactors.

Evaluation of the performance reported in the literature has generally shown that the different types of systems with the different types of carbon sources can all provide significant removals of nitrate, with the majority achieving more than 80% removal of the nitrate. Overall, the diversity in system configuration and options for carbon sources, combined with the high performance reported, highlight the potential for tertiary denitrification. However, further evaluations are required to determine the applicability in real conditions for tertiary municipal wastewater treatment.

In conclusion, solid-phase denitrification is a promising technology for removing nitrogen from various types of wastewaters. The use of woodchips as a support

medium and waste materials as external carbon sources can help to make this process more sustainable and cost-effective. Further research is needed to optimize the use of different support media and external carbon sources, and to explore the effects of different operating conditions on the performance of SPD systems.

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### 3 Evaluations of a Range of Low-Cost Media for Tertiary Solid-phase Denitrification in Municipal Wastewater

Affo Souleymane, A., Alibardi, L., and Pidou, M.

Cranfield University, Bedfordshire, MK43 0AL, UK

**Abstract:** This study evaluated six common materials found in the UK for their suitability as external carbon sources for denitrification in biological nutrient removal (BNR) systems. The study focused on the leaching rates of organic compounds and their impact on denitrification efficiency, with particular attention to the potential for secondary pollution. The materials tested were woodchips, barley straw, spruce wood bark, beech sawdust, and polycaprolactone (PCL). Results indicated that woodchips and wood bark exhibited good balance between carbon content and secondary pollution risk, while barley straw posed the highest risk for secondary pollution and the potential for rapid depletion of organic compounds. PCL showed promise as a carbon source for nitrate removal and released minimal nitrate or ammonium-nitrogen over the course of the trial. The release of phosphorous from all media was observed, with barley straw leaching much higher quantities (3 – 7.6 mg/L) than the others. The study suggests that pre-treatment techniques such as washing may minimize the quantities of organics leached. The low concentrations of metals found in the media suggest that they would not inhibit denitrification or pose significant health risks to aquatic organisms or humans. In conclusion, all tested media have the potential to be used as supporting carbon sources for BNR, but their efficacy and potential for secondary pollution must be evaluated on a case-by-case basis. Further research is needed to determine the suitability of wood bark as a carbon source and the impact of different types of lignocellulosic material and plant species on BNR efficiency.

**Keywords:** solid-phase denitrification, straw, woodchip, sawdust, polycaprolactone, wood bark, leaching

### 3.1 Introduction

The discharge of nitrogen-containing sewage into the environment poses a risk of eutrophication, general decline in water quality and potential hazards to flora and fauna (Mayo & Abbas, 2014; Cooper et al., 2022). Biological Nitrogen Removal (BNR) processes have been proven to remove nitrogen compounds from wastewater by conversion into nitrogen gas, which is subsequently released into the atmosphere through the nitrification and denitrification processes (Sun et al., 2010). For municipal wastewaters, low carbon-nitrogen ratios ( $C/N < 8.0$ ) can be a limiting factor for BNR because higher organic carbon to nitrogen ratios are often required for the effective conversion of nitrate into nitrogen gas (Carrera et al., 2004; Sun et al., 2010). Municipal wastewater will typically contain a mix of organic and inorganic compounds. However, the carbon content of the sewage can vary depending on factors such as wastewater source and the treatment processes used. Municipal sewage from sources such as households, commercial buildings and hospitals typically exhibits poor influent carbon-nitrogen ratios ( $C/N$ ) and insufficient biodegradability (Zhang et al., 2016b). Specifically, after the secondary treatment stage, when the levels of organics are depleted, the introduction of an external carbon source will be required to ensure good denitrification (Della Rocca et al., 2006; Fu et al., 2022). Alternatively, when  $C/N$  ratios are too high, the performance of the system is affected by the transfer of dissolved organics into the final treated effluent (Schmidt & Clark, 2013). Chemicals such as methanol, ethanol and acetate have commonly been used as external carbon sources with reported nitrate removal efficiencies (RE) of up to 99% (Lee et al., 2013; Pelaz et al., 2018; Domingos et al., 2022). However, owing to their high cost (Table 2-6), numerous studies have also evaluated the potential of organic waste materials as external carbon sources (Procurement Resource, 2022). Indeed, organic rich liquids and wastewaters from a range of industries such as food and beverages (RE >98%) (Fernández-Nava et al., 2010; Zhang et al., 2016b; Kim et al., 2017; Qi et al., 2020), chemical production (RE <90%) (Lee et al., 2013) and municipal sludge processing (RE >92%) (Cao et al., 2013) have been used. Solid substrates have also been

explored in recent years as a steady supply. For this purpose, products such as woodchips (Damaraju et al., 2015; Von Ahnen et al., 2018b) have been used with reported removal efficiencies ranging from approximately 50% (Hoover et al., 2016) to 98% when combined with sulphur granules and seashells (Von Ahnen et al., 2018a). Studies have also reported a removal efficiency of up to 96 – 100% when woodchips were combined with non-organic materials such as soil (Healy et al., 2006; Greenan et al., 2009) or gravel (Šereš et al., 2019), suggesting that the reduction in the quantity of media used does not directly affect denitrification and may even provide added benefits of stability to the systems. Other materials such as untreated sawdust (RE >50%), lime/peracetic-acid treated saw dust (RE >99%) (Hu et al., 2019), corn cobs (RE >90%) (He et al., 2016; Feng et al., 2017) and rice straw and hull (RE 56 – 95%) (Feng et al., 2017; Luo et al., 2018) have been trialed. In addition, biodegradable plastics, and polymers (Yamada et al., 2013; Li et al., 2016; Feng et al., 2019) have been explored, including the reuse of waste biodegradable snack ware (Wang & Wang, 2013). Most of these materials were chosen based on meeting the carbon content required to successfully use them as media in BNR and the reported removal efficiencies in the existing literature.

However, in addition to removal efficiency, longevity of nitrate removal, pollution swapping (increase in one pollutant in an attempt to remove another), and the risk of secondary pollution are factors that must also be considered when selecting solid carbon sources for SPD, although not much attention has been paid to the compounds released by these materials prior to their use as the carbon sources (Zhang et al., 2012a). In most cases, slow release of organics from the decomposition of solid carbon sources is required to support nitrate removal. Thus, the selected carbon sources should have relatively slow degradation rates, low concentrations of released compounds, and lead to high denitrification rates (Zhang et al., 2012a). Other factors to be considered are cost and material safety. The costs involved are usually associated with the purchasing costs of the carbon sources and sludge management (Ramírez-Godínez et al., 2015).

A few studies have been carried out to identify substances that may be leached from some of these popular carbon sources, including organics and metals, the leaching rates, and the potential of these substances to cause further pollution. For example, the behaviour of wheat straw, sawdust, and biodegradable plastics (Zhang et al., 2012b), pine woodchips, cardboard, pine needles and barley straw (Healy et al., 2012), barley grains and peanut shells (Ramírez-Godínez et al., 2015) have been tested as exogeneous carbon materials for the application of BNR. In another study by Yang et al. (2015), eight carbonaceous media including corncob, peanut shell, *retinervus luffae fructus* (*Si Gua Luo*), wheat straw, cotton stalk, rice straw, rice husk and reed were tested for functional suitability as carbon sources. In this study, rice straw was concluded to be the most effective in controlling COD concentrations in the final effluent while achieving good denitrification. In the work done by Ramírez-Godínez et al. (2015), leachate analyses indicated that woodchips and peanut shells had the potential to be used in BNR because of the low concentrations of released organic matter. Peanut shells however had the downside of easy disintegration, causing it to significantly increase turbidity and suspended solids. The major appeal of SPD technology is its relatively low cost, stemming from the affordability and availability of external carbon sources. Because most of the proposed sources are agriculturally based, it is important to examine the availability of these materials at given geographical locations where the technology is to be implemented. A significant number of the studies have been conducted in Asia and other parts of Europe, but little information is available on materials that can be easily sourced from the UK. Barley straw for instance is a by-product of UK's agricultural industry, which recorded production of up to 6.9 million tons of barley in 2021. Cereal straw production was generally estimated at 8.6 million tons in 2021, making it a readily available carbon material for use in biological denitrification (DEFRA, 2022).

Analyses have been performed on different lignocellulosic materials to determine their elemental compositions. The results from these studies provide a good baseline for anticipating the elements that are likely to leach out of these materials when used as supporting media for solid-phase denitrification. One of such studies was conducted

by Witczak et al. (2015) on different woody, agricultural and fruity materials using high-temperature combustion, column separation and potentiometric titration. Deciduous sawdust, similar to what was used in this study was analyzed and percentage compositions of 50.3, 6.2, 0.1, 0.02 and 0.03% were reported for carbon, hydrogen, nitrogen, sulfur, and chlorine, respectively. Wood bark used in this study yielded the following result: carbon (52.9%), hydrogen (5.7%), nitrogen (0.9%), sulfur (0.1%), and chlorine (0.02%). In this same study, cereal straw was analysed, and the following results reported: carbon (47.7%), hydrogen (6.7%), nitrogen (1.0%), sulphur (<0.01%), and chlorine content of 0.3%. Although the specific type of straw was not mentioned, it can be used as a general reference point for straw materials. Gündüz et al. (2016) also performed elemental analyses on 3 different types of wood pellets with the following results reported: carbon (49.45 – 53.37%), hydrogen (<5.0%), nitrogen (0.27 – 0.34%), and chlorine (0.03 – 0.05%). All the materials showed a significantly higher composition of carbon compared to the other elements. Part of the reasons for the potential application of these materials as supporting media is because of the carbon content and these percentage compositions are expected to reflect in the analysis of the leached materials.

This study aimed to investigate the leaching behaviour of plant-based carbon sources in wastewater treatment and their impact on water quality, and nitrate removal efficiency. Specifically, the study aimed to identify and quantify the types and amounts of leachates released from different types of plant-based carbon sources available in the UK and to evaluate the impact of leaching on water quality parameters. The study also aimed to look at comparing the performance of different plant-based carbon sources in nitrate removal, including an evaluation of their availability and sustainability. Overall, this research will provide insights into the use of plant-based carbon sources in wastewater treatment and to identify best practices for optimizing their effectiveness while minimizing their impact on water quality.

## **3.2 Materials and Methods**

### **3.2.1 Types of carbon materials**

In this study, different carbon-containing materials directly sourced from the UK were tested as possible external carbon sources for heterotrophic denitrification. The materials used in this study were hardwood and softwood chips (10 – 30 mm) (CPA Horticulture, Llanelli, UK), beech sawdust (BSw; 10- 30 µm) (Weber-Stephen Products, London, UK), barley straw (BSt; 10 – 40 mm) (Sam Turner & Sons, Yorkshire, UK), spruce wood bark (SWB; 10 – 30 mm) (Anything 4 Home Ltd. Stoke-on-Trent, UK), and polycaprolactone beads (PCL; 3 mm) (Ingevity, Virginia, USA) as a benchmark using the same method and laboratory analyses. The biodegradable polymer, polycaprolactone (PCL), is prepared by the chemical treatment of saccharides through conversion to ethanol and acetic acid by fermentation. This is then converted to cyclohexane using chromic acid, and further oxidized to caprolactone and is usually commercially produced as small beads (Rani & Sharma, 2022).

### **3.2.2 Leaching Tests and physical-chemical analyses**

In the tests, 100 g of media was put in 1 L of deionised water and kept on a Stuart Orbital Shaker SSL1 (Cole-Parmer, St Neots, UK) at 165 rpm for 24 hours (Figure 3.1). For the tests with Barley Straw, 50 g of material was used in 1 L of deionized water due to the volume occupied by the straw. After 24 hours, the water was drained, and samples of the water were collected. Fresh water was added again, and the process was repeated another 3 times corresponding to samples at 48, 72 and 96 hours. Each time, the water was analysed for their content in nitrate, total nitrogen, ammonium-nitrogen, total phosphorous, and chemical oxygen demand (COD) with cell tests (Merck Life Science UK Limited, Watford, UK) and measured in a Merck Spectroquant NOVA 60. The samples were also analysed for pH with a Jenway 3510 pH meter (Cole-Parmer, St Neots, UK), for dissolved oxygen (DO) with a LE621-IP67 DO meter (Mettler Toledo, Leicester, UK), metals (NexION 350D ICP Mass Spectrometer), and for dissolved organic carbon (DOC) using a Shimadzu TNM-1

Total Organic Carbon Analyzer. For quality control, all tests were carried out in triplicates and the averages reported.



**Figure 3.1 Leaching tests done on variety of solid organic media.**

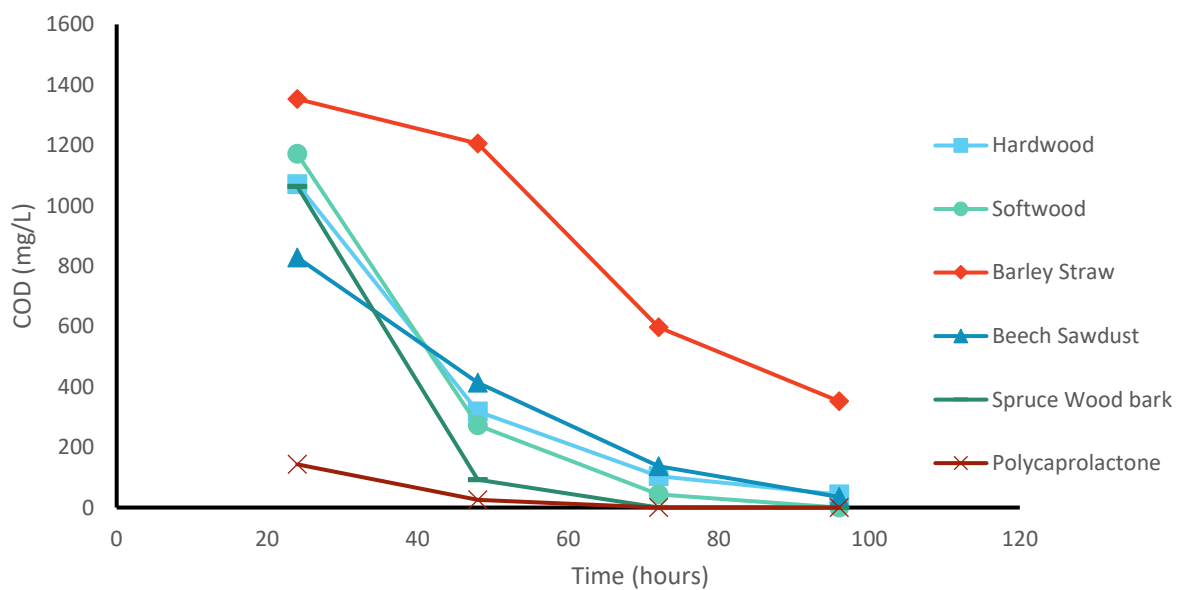
### 3.2.3 Statical Analysis

The leaching test data was subjected to both qualitative and quantitative analysis methods. Qualitative analysis involved examining the data for trends, themes, and patterns across the datasets. For quantitative analysis, the Kruskal-Wallis test, with a confidence level of 95%,  $\alpha = 0.05$ , was used to compare the medians of the datasets. Minitab software was used for conducting the statistical analyses. The decision to use the Kruskal-Wallis test was based on the sample sizes being less than 5 each, which is too small for the Analysis of Variance test (ANOVA). The null hypothesis stated that the medians of leached organics were equal for all the materials, while the alternative hypothesis posited a significant difference in the medians of organics leached from the different materials. The Kruskal-Wallis test was employed to calculate individual p-values for the various organics observed across the different materials. A multiple comparison test was then used to identify which materials were significantly different from the control, PCL.

### 3.3 Results and Discussion

#### 3.3.1 Organics Leaching from Media

As stated earlier, a suitable level of organics in the form of carbon is required for the denitrification process and the media trialed here, woodchip, was expected to provide some of this organic matter. As indicated by the dark colour of the leachate after 24 and 48 hours during the trial (Figure 3.1), high levels of organics were found in the samples (Figure 3.2) confirming the leaching of materials from the media. Healy et al. (2012) confirmed this pigmentation as an indication of COD release. For the hardwood and softwood media, COD levels of over 1000 mg/L were measured over the first 24 h (Table 3-1). In the subsequent days of the leaching tests, COD levels decreased significantly, suggesting that the media contained some easily leachable materials that were released initially and then stopped over time. To illustrate, in the first 24 h, COD measurements of 1071 mg/L and 1171 mg/L were measured for hardwood and softwood, respectively, which sharply decreased to 318 and 273 mg/L after 48 h, 104 and 43 mg/L after 72 h, and 43 mg/L and <25 mg/L after 96 h, respectively (2).



**Figure 3.2 COD measurements over 96 hours.**

In the first 24 of hours of the study, Spruce wood bark (SWB) measured COD concentrations of 1063 mg/L, quite similar to the concentrations measured for

woodchips. This however quickly fell to 92 mg/L on the second day, and to undetectable limits by the fourth day (Figure 3.2). This seems to indicate that all the easily leachable material was released within 24 hours of the trial. Compared to the woodchips, there was a faster decline in COD measurements and given that the woodchips exhibited similar traits in the pilot trial to what was observed in the leaching tests, it can be assumed that the same would happen for SWB if used on a larger scale as carbon source for SPD. To the knowledge of the authors, as this is the first report of the use of wood bark in this context, direct comparison to other studies is not possible but the characteristics are expected to be similar to other wood-based materials such as woodchip. Beech sawdust (BSw) on the other hand, recorded a COD reading of 828 mg/L in the first 24 hours and 414, 137 and 35 mg/L in 48, 72 and 96 hours, respectively (Figure 3.2). Although this is a more stable decline in release compared to SWB, the readings still seem to suggest an exhaustion of organic carbon by the fourth day of the trial. The sawdust has much smaller particles than the other media used. Smaller particles would have a much greater surface area available for contact with the water which would explain greater rate of release over time than the other media. The BSt material exhibited a similar trend in COD but at elevated levels. In the first 24 hours, a COD reading of 1353 mg/L was recorded with only a slight reduction to 1205 mg/L by 48 hours (Table 3-1). This further declined to 597 and 392 mg/L at 72 and 96 hours, respectively (Figure 3.2). BSt appears to have more easily leachable organic matter than the other media used in the trials, given the mass of media used and the levels of COD measured. This could be attributed to the softness of the media and its porosity, which could also mean the organics are likely to completely run out at a point, leaving the media without any soluble organic matter. PCL exhibited quite low readings for all the parameters tested for in this study. The COD readings in the first 24 hours was at 143 mg/L and undetectable limits by day 4 (Figure 3.2).

DOC concentrations of 3.309 and 2.506 mg/g (Table 3-1) were recorded for hardwood and softwood at 24 hours compared to the reported DOC concentration of 45.5 mg/g for pine woodchips by Ramírez-Godínez et al. (2015), and most of the other measured parameters, also decreased over the subsequent period to levels of 0.449 and 0.235

mg/g, respectively. This tends to further support the COD trends mentioned previously, indicating a release of easily leachable organics at the start of operations, declines in these concentrations, and subsequent microbial degradation of the media (Feng et al., 2017). Relatively low C/N ratios measured post-bioreactors have been documented (Christianson & Schipper, 2016; Ghane et al., 2018; Christianson et al., 2020). These studies reported carbon consumption, increasing effluent nitrate concentrations, and a corresponding decline in C/N ratios over time. However, this change in carbon content did not directly indicate the need for media replacement, even though one may be tempted to associate reduced C/N ratios with carbon exhaustion for denitrification. This is illustrated by the tests performed on 9-year post-bioreactor woodchips, which demonstrated that despite the apparent decline in carbon quality, the media was still capable of supporting denitrification (Christianson et al., 2020), and many materials that demonstrate low C/N ratios have been proven to support denitrification and even provide better results in certain instances than high-carbon materials. For example, Christianson et al. (2012) reported average removal rates of 7.5- 8.4 g/m<sup>3</sup>/d using yard wastes compared to the 3.8 – 5.6 g/m<sup>3</sup>/d measured for woodchip in the same study. The use of this material is however not encouraged for long-term bioreactors due to its relatively short life span as external carbon source for denitrification. Several other studies have also used biodegradable polymers, with low C/N ratios, and reported removal efficiencies of up to 99% (Zhu et al., 2015; Chu & Wang, 2017; Xiong et al., 2019). This suggests that these materials may contain carbon that is more readily available to microbes for nitrate removal, and that microbes may not be using leachable carbon in the denitrification process but rather localised activities on the media itself (Wang & Chu, 2016). Zhang et al. (2012b) used wheat straw in their study and reported COD concentrations of 506.4 mg/L in the first 24 hours, 8.0 mg/L of ammonium which rapidly decreased to <0.50 mg/L by day 28, and a nitrate concentration of 3.23 mg/L in the first 24 hours. Healy et al. (2012) and Hashemi et al. (2010) used barley straw in their study, however there is no report on the values of organics measured in the leaching phase for either of them. For softer materials such as straw, hydrolysis is usually strong enough to result in higher DOC concentrations than needed, leading to high effluent DOC concentrations and a shorter life span

(Zhong et al., 2020a). COD concentrations of 24.0 to 81.5 mg/L have also been reported in other studies which have used PCL (Zhang et al., 2012a). Despite this, there are several different studies that have reported nitrate removal of up to 99% with the use of this media as a carbon source for denitrification in the presence of microorganisms (Shen et al., 2015; Li et al., 2016; Jiang et al., 2020; Yu et al., 2021). Comparing to the 321.6 mg/L of COD reported by Zhang et al. (2012b), this study recorded a much higher reading for COD for sawdust in the first 24 hours. It was also reported that COD readings fell to <50.0 mg/L in 15 days, compared to the 35 mg/L measured in this work by day 4. This difference may be as result of wood used for the sawdust as this was not specified in the study.

The results indicate that the quantities of COD measured for the materials tested in this study (woodchips, sawdust, wood bark and straw) are significantly high when no microorganisms are present, suggesting a potential to cause unwantedly high effluent COD concentrations in the start-up phase of BNR but long-lasting effects are not expected due to the rapid decrease, over 3 - 4 days, of the easily leachable materials. The biodegradability of organic matter is crucial to the performance of wastewater treatment systems, as it determines the efficiency of microbial degradation and the overall treatment efficacy. The commonly used indicators of biodegradability, BOD/COD (>0.3 indicates biodegradability) and COD/DOC ratios, have been shown to provide valuable insight into the biodegradability of organic matter in wastewater. A high biodegradability ratio is desirable for wastewater treatment processes, as it indicates that the organic pollutants in the wastewater can be effectively removed by microbial activity (Lakhlifi et al., 2017). However, the relationship between these ratios and the availability of carbon substrate to the microbes may not always be straightforward. Polycaprolactone indicated the highest biodegradability amongst the media tested during the leaching tests with COD/DOC ratios between 0.6 and 1.7 (Table 3-1). This may explain the denitrification removal efficiencies reported for PCL despite the low organic matter measured. Spruce wood bark demonstrated the least biodegradability with COD/DOC values between 1 and 7. The other materials had varying biodegradabilities measured (Table 3-1).

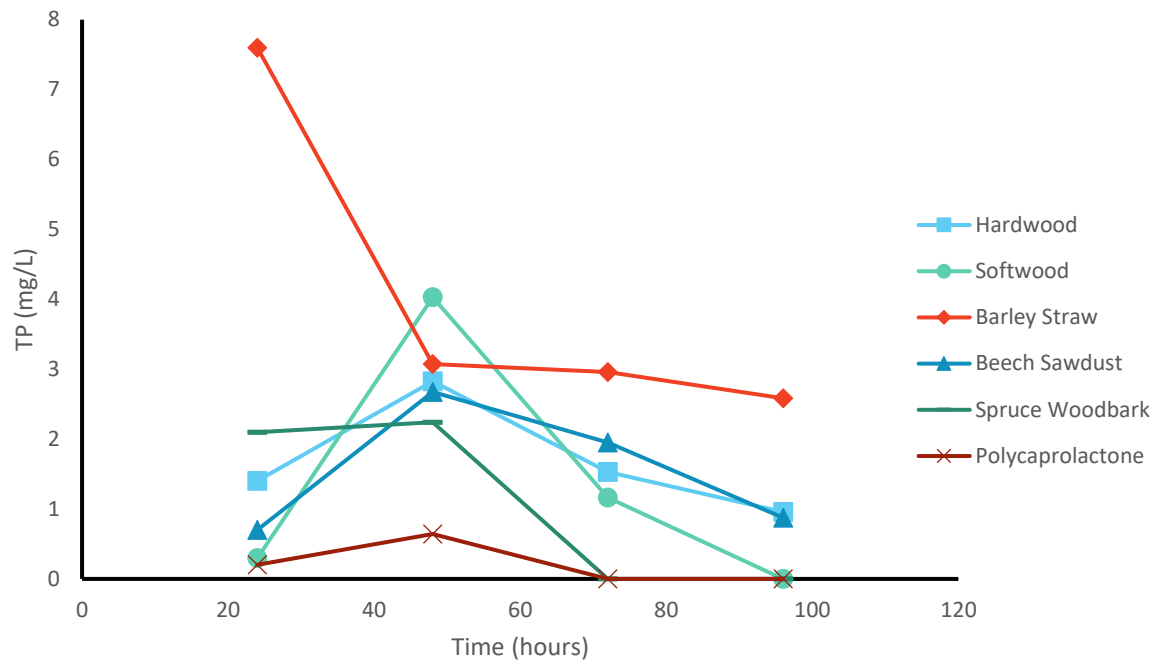
### 3.3.2 Nutrients Leaching

There was an average total phosphorous reading of 0.2 mg/L over the trial duration and almost no release of nitrate or ammonium-nitrogen at all for PCL (Figure 3.3). The tests also confirmed the release of total phosphorous (TP) from other media (Figure 3.3) where high levels were found with the hardwood releasing a concentration of 1.4 mg/L (14 mg/kg woodchip) after 24 hours. In the case of softwood, the release was relatively stable for the four days of the leaching tests at a concentration of about 0.3 mg/L. The readings from the tests with BSw indicated a relatively stable total phosphorous release of between 0.5 - 0.7 mg/L while SWB leached relatively high total phosphorous levels of 2.1 mg/L in the first 24 hours of the trials and eventually falling to 0.4 mg/L. The total phosphorous levels of barley straw were significantly higher than all the media again. A reading of 7.6 mg/L was recorded for day 1 and fell to 3 mg/L by the next reading and 0.8 by the final day of the trial. This is considered very significant, especially now that there are stricter consents for total phosphorous removal on some sites (0.25 mg/L) based on the Water Framework Directive and would have to be considered prior to implementation (Environment Agency, 2019).

The results for woodchips were found to be similar to the study by Healy et al. (2012), in which ortho-phosphate concentration of 1.1 mg/L was measured for pine woodchips in the initial leaching period of their work. In a similar way to the COD, the release of total phosphorous decreased over time. TP leaching from woodchip was also observed in another study using hardwood and deionized water as control (Sharrer et al., 2016). The study reported TP concentrations of 17.1 mg/kg of dry woodchip after 24 hours, quite similar to the 14 mg/kg measured for this study. For hardwood, following the trend in decreasing total phosphorous, it can be assumed that once implemented, the total phosphorous concentrations will be almost similar to the influent concentrations after a few days of operation (Chapter 4). The result from this study contrasts the barely detectable concentrations of total phosphorous measured by Ramírez-Godínez et al. (2015).

For nitrate, an 11 mg/L concentration was recorded in the first 24 hours for hardwood and reduced to 2.7 mg/L at 48 and 72 hours. It was below detectable limit (1 mg/L) at

96 hours. However, these concentrations are likely to be removed once denitrification is triggered in the systems. Softwood on the other hand did not seem to contribute any nitrate to the system over the whole trial period. Ammonium-nitrogen leaching was negligible with the highest concentration measured (0.19 mg/L) at 48 hours for hardwood, suggesting that both nitrate and ammonium-nitrogen concentrations should not be affected by the woodchips when used as media for solid-phase denitrification. The nitrate and ammonium-nitrogen levels in the leachate started at 6.2 mg/L and 0.61 mg/L, for BSw and SWB, respectively and ran out by the end of the test. The nitrate concentration was <1.0 mg/L by day 4 and <0.2 mg/L for ammonium-nitrogen (Table 3-1). This is similar to the nitrate value of 5.13 mg/L measured in a study by Zhang et al. (2012b), which fell to <0.6 mg/L by day 20 and stayed the same till the end of the study. In contrast however, the reported ammonium concentration in the first 24 hours (3.84 mg/L) was significantly higher than what was measured for this study, fell to 0.66 mg/L in 8 days and no detectable concentration by the end of the study. PCL biodegradable polymer has demonstrated the least risk of secondary pollution if used as a carbon source (Zhang et al., 2012b), reporting between 0.20 and 0.66 mg/L of ammonium-nitrogen leaching. However, one limitation of this media may be its exhibited fast degradation rate when exposed to air and the issues associated with availability of bulk quantities for application in wastewater treatment (Rani & Sharma, 2022).



**Figure 3.3 Total P measurements over 96 hours.**

Similar to COD and total phosphorous, there were higher concentrations of nitrate and ammonium-nitrogen recorded for BSt compared to the other media (Table 3-1) But again, this is expected to be removed during denitrification. The release of both organics and nitrate into a water with low organics and significant levels of nitrate to be removed, may again generate an imbalance in C/N ratio. Schipper et al. (2010) mentions that mitigation measures such as pre-washing of carbon media may be necessary to reduce the high leaching rates of organics measured in the initial phase of bioreactors.

### 3.3.3 pH and Other Parameters

pH was generally unaffected by all these media with the range staying between 6 – 7. This is considered a good characteristic when considering the use of these media as supporting carbon sources on large scale. A major part of the costs of wastewater treatment is the adjustment of pH and eliminating the need to do this again, especially at the tertiary treatment stage is cost saving for wastewater treatment facilities (PRWater, 2023). Since the measured value falls within the pH needed for biological denitrification to happen (6.5 – 8.5), the apparent lack of change in pH is a desirable

quality of all the media (Wang & Chu, 2016). Another observation that was made with this trial was the change in colour of the water. All the media except PCL caused a dark reddish/brownish colour change in the water in the first 24 h (Figure 3.1). However, this declined over the subsequent days and was clear by the final day of the experiments (96 h). Hence, the change in colour is not expected to be an issue after the start-up phase in a real application.

### **3.3.4 Metals**

An understanding of the elemental metal contents of the materials used as supporting carbon for SPD is essential for ensuring environmental and human health, as well as the effectiveness of the denitrification processes. The elemental concentrations are summarized in mg/g (Table 3-2). Of the results obtained from the study, few metal concentrations were of significant values, with BSt recording most of the high values. Over the 96-hour period, Na (0.45 – 0.03 mg/g), Mg (0.2 – 0.02 mg/g), and K (14.5 – 0.7 mg/g) were recorded for barley straw. The other materials however, only showed significant K concentrations; SWB (0.43 – 0.07 mg/g), Hardwood (0.46 – 0.06 mg/g), softwood (0.2 – 0.02 mg/g) and BSw (0.6 – 0.01 mg/g), over the 96-hour trial period. K, Na and Mg are mostly required for several enzymatic activities and so the addition of these lignocellulosic materials might promote microbial activities in the denitrification reactor (Yang et al., 2015). Other elements such as Al, Ca, Fe, Ni, Cu and Zn were observed in much lower quantities (µg/g) than the inhibitory concentration. These elements have been shown to stimulate bacterial activity and may act as electron donors, providing them with additional energy sources for denitrification activities (Labbé et al., 2003).

**Table 3-1 Concentrations of organics measured over 96 hours.**

| Sample                        | Time | COD    |        | dCOD | NO <sub>3</sub> -N |        | NH <sub>4</sub> -N | TN  | DOC    |        | COD/DOC | TP     |        |
|-------------------------------|------|--------|--------|------|--------------------|--------|--------------------|-----|--------|--------|---------|--------|--------|
|                               |      | (mg/L) | (mg/g) |      | (mg/L)             | (mg/g) |                    |     | (mg/L) | (mg/g) |         | (mg/L) | (mg/g) |
| <b>Barley Straw (BSt)</b>     | 24   | 1353   | 27.06  | 1315 | 28.6               | 0.572  | 6.42               | 117 | 1352   | 27.0   | 1.0     | 7.6    | 0.152  |
|                               | 48   | 1205   | 24.1   | 355  | 8.4                | 0.168  | 5.61               | 44  | 392.2  | 7.     | 3.1     | 3      | 0.06   |
|                               | 72   | 597    | 11.94  | 289  | 5.2                | 0.104  | 2.55               | 19  | 201.8  | 4.0    | 3.0     | 1      | 0.02   |
|                               | 96   | 352    | 7.04   | 161  | 4.8                | 0.096  | 2.3                | <1  | 136.2  | 2.7    | 2.6     | 0.8    | 0.016  |
| <b>Beech Sawdust (BSw)</b>    | 24   | 828    | 8.28   | 354  | 6.2                | 0.062  | 0.61               | <1  | 322.4  | 3.2    | 2.6     | 0.7    | 0.007  |
|                               | 48   | 414    | 4.14   | 123  | 3.2                | 0.032  | <0.2               | <1  | 154.8  | 1.5    | 2.7     | 0.5    | 0.005  |
|                               | 72   | 137    | 1.37   | 68   | 1.4                | 0.014  | 0.02               | <1  | 70.23  | 0.7    | 2.0     | 0.5    | 0.005  |
|                               | 96   | 35     | 0.35   | 25   | 0.6                | 0.006  | <0.2               | <1  | 39.97  | 0.4    | 0.9     | 0.2    | 0.002  |
| <b>Spruce Wood Bark (SWB)</b> | 24   | 1063   | 10.63  | 198  | 5.7                | 0.057  | 0.19               | 13  | 148.5  | 1.5    | 7.2     | 2.1    | 0.021  |
|                               | 48   | 92     | 0.92   | 37   | <0.5               | <0.005 | <0.2               | 1   | 41.04  | 0.4    | 2.2     | 0.5    | 0.005  |

|                                   |    |      |       |     |      |        |      |    |       |     |     |     |       |
|-----------------------------------|----|------|-------|-----|------|--------|------|----|-------|-----|-----|-----|-------|
|                                   | 72 | <25  | <0.25 | <10 | 0.5  | 0.005  | <0.2 | <1 | 23.92 | 0.2 | 1.0 | 0.4 | 0.004 |
|                                   | 96 | <25  | <0.25 | <10 | <0.5 | <0.005 | <0.2 | <1 | 15.88 | 0.2 | 1.6 | 0.1 | 0.001 |
| <b>Hardwood</b>                   | 24 | 1071 | 10.71 | 338 | 11   | 0.11   | 0    | 10 | 330.9 | 3.3 | 3.2 | 1.4 | 0.014 |
|                                   | 48 | 318  | 3.18  | 170 | 2.7  | 0.027  | <0.2 | <1 | 112.5 | 1.1 | 2.8 | 0.5 | 0.005 |
|                                   | 72 | 104  | 1.04  | 56  | 2.7  | 0.027  | <0.2 | <1 | 68.03 | 0.7 | 1.5 | 0.6 | 0.006 |
|                                   | 96 | 43   | 0.43  | 36  | <0.5 | <0.005 | <0.2 | <1 | 44.9  | 0.4 | 1.0 | 0.2 | 0.002 |
| <b>Polycaprolactone<br/>(PCL)</b> | 24 | 143  | 1.43  | 102 | 0.4  | 0.004  | 0    | <1 | 82.35 | 0.8 | 1.7 | 0.2 | 0.002 |
|                                   | 48 | 25   | 0.25  | 25  | <0.5 | <0.005 | 0.19 | <1 | 38.92 | 0.4 | 0.6 | 0.3 | 0.003 |
|                                   | 72 | <25  | <0.25 | <10 | <0.5 | <0.005 | 0.02 | <1 | 26.39 | 0.3 | 0.9 | 0.3 | 0.003 |
|                                   | 96 | <25  | <0.25 | <10 | <0.5 | <0.005 | <0.2 | <1 | 24.87 | 0.2 | 1.0 | 0.1 | 0.001 |
| <b>Softwood</b>                   | 24 | 1171 | 11.71 | 234 | <0.5 | <0.005 | 0.05 | <1 | 250.6 | 2.5 | 4.7 | 0.3 | 0.003 |
|                                   | 48 | 273  | 2.73  | 53  | 2.6  | <0.005 | 0.05 | <1 | 67.76 | 0.7 | 4.0 | 0.4 | 0.004 |
|                                   | 72 | 43   | 0.43  | 27  | 1.9  | 0.019  | 0.02 | <1 | 36.99 | 0.4 | 1.2 | 0.3 | 0.003 |

|  |    |     |       |     |      |        |      |    |       |        |            |     |       |
|--|----|-----|-------|-----|------|--------|------|----|-------|--------|------------|-----|-------|
|  | 96 | <25 | <0.25 | <10 | <0.5 | <0.005 | <0.2 | <1 | 23.54 | 0.2354 | 1.06202209 | 0.1 | 0.001 |
|--|----|-----|-------|-----|------|--------|------|----|-------|--------|------------|-----|-------|

**Table 3-2 Metal concentrations over 96 hours.**

| Sample                        | Time | Na       | Mg        | Al     | K        | Ca     | Fe     | Ni       | Cu      | Zn      |
|-------------------------------|------|----------|-----------|--------|----------|--------|--------|----------|---------|---------|
|                               | (h)  | (mg/g)   | (mg/g)    | (µg/g) | (mg/g)   | (µg/g) | (µg/g) | (µg/g)   | (µg/g)  | (µg/g)  |
| <b>Polycaprolactone (PCL)</b> | 24   | n/a      | 0.0000138 | 0.0513 | 0.000366 | 1.79   | 0      | n/a      | 0.133   | 0.0828  |
|                               | 48   | 0.00426  | 0.0000722 | n/a    | 0.00146  | 1.99   | n/a    | 0.000307 | 0.0233  | 0.00306 |
|                               | 72   | 0.000329 | n/a       | n/a    | 0.000502 | 0.465  | n/a    | n/a      | 0.00283 | 0.00479 |
|                               | 96   | 0.00376  | 0.000134  | n/a    | 0.0013   | 1.87   | n/a    | n/a      | 0.00137 | 0.0105  |
| <b>Softwood</b>               | 24   | 0.00631  | 0.0121    | 0.841  | 0.227    | 2.96   | 0.776  | 0.00832  | 0.106   | 0.41    |
|                               | 48   | 0.00568  | 0.00316   | 0.228  | 0.0758   | 13     | 0.302  | 0.0117   | 0.0334  | 0.57    |
|                               | 72   | 0.00238  | 0.00187   | 0.0926 | 0.038    | 8.36   | 0.0309 | 0.000361 | 0.011   | 0.0467  |
|                               | 96   | 0.00298  | 0.00143   | 0.0543 | 0.0229   | 6.29   | n/a    | n/a      | 0.00852 | 0.0289  |
| <b>Beech Sawdust (BSw)</b>    | 24   | 0.00386  | 0.0366    | 0.679  | 0.593    | 79.2   | 0.648  | 0.297    | 0.0491  | 0.629   |
|                               | 48   | 0.00269  | 0.0142    | 0.364  | 0.306    | 29.5   | 0.313  | 0.113    | 0.0315  | 0.215   |
|                               | 72   | 0.00168  | 0.00642   | 0.296  | 0.169    | 15.9   | 0.248  | 0.0416   | 0.0158  | 0.0875  |

|                               |  |    |         |          |        |        |      |        |          |         |         |
|-------------------------------|--|----|---------|----------|--------|--------|------|--------|----------|---------|---------|
|                               |  | 96 | 0.003   | 0.0029   | 0.0589 | 0.105  | 5.98 | 0.0568 | 0.0144   | 0.0144  | 0.0305  |
| <b>Hardwood</b>               |  | 24 | 0.0417  | 0.0216   | 0.506  | 0.465  | 54.6 | 0.597  | 0.0238   | 0.0688  | 0.328   |
|                               |  | 48 | 0.0155  | 0.00562  | 0.0962 | 0.163  | 16.3 | 0.0647 | 0.00526  | 0.0188  | 0.0861  |
|                               |  | 72 | 0.0101  | 0.0038   | 0.0538 | 0.103  | 12   | 0.0298 | 0.00434  | 0.0124  | 0.0743  |
|                               |  | 96 | 0.00704 | 0.00243  | 0.0239 | 0.0652 | 7.43 | n/a    | 0.0011   | 0.00763 | 0.0362  |
| <b>Spruce Wood Bark (SWB)</b> |  | 24 | 0.0459  | 0.0101   | 0.428  | 0.43   | 53.2 | 0.514  | 0.0235   | 0.0323  | 0.656   |
|                               |  | 48 | 0.0108  | 0.00168  | 0.114  | 0.147  | 9.4  | 0.0754 | 0.00308  | 0.00851 | 0.103   |
|                               |  | 72 | 0.00643 | 0.00105  | 0.0762 | 0.0985 | 5.35 | 0.0124 | 0.000484 | 0.00161 | 0.0488  |
|                               |  | 96 | 0.00553 | 0.000575 | 0.062  | 0.0741 | 2.75 | n/a    | n/a      | 0.00285 | 0.00885 |
| <b>Barley (BSt) Straw</b>     |  | 24 | 0.45    | 0.236    | 0.1882 | 14.5   | 2620 | 0.958  | 0.0434   | 0.66    | 1.254   |
|                               |  | 48 | 0.1336  | 0.0894   | 0.0664 | 4.26   | 1198 | 0.1788 | 0.00582  | 0.284   | 0.258   |
|                               |  | 72 | 0.0542  | 0.0422   | 0.0358 | 1.692  | 600  | 0.348  | 0.00346  | 0.1758  | 0.1986  |
|                               |  | 96 | 0.0302  | 0.0234   | 0.0107 | 0.72   | 346  | 0.274  | 0.000522 | 0.1264  | 0.101   |

### 3.3.5 Statistical Test

The statistical analyses conducted on the various organics yielded individual p-values that were all less than the significance level of 95% (Table 3-3). As a result, the null hypotheses for the different parameters were rejected, indicating significant differences in at least two of the medians. The multiple comparison tests, with PCL as the control, further revealed significant differences between barley straw and the control for all the organics measured. These findings provide evidence to support the conclusion that there are substantial variations in the organics under leached from the different materials under investigation.

**Table 3-3 Results of Kruskal-Wallis test on data**

| Parameter                 | Adjusted P-value ( $\alpha= 0.05$ ) |
|---------------------------|-------------------------------------|
| COD (mg/g)                | 0.045                               |
| NO <sub>3</sub> -N (mg/g) | 0.007                               |
| TP (mg/g)                 | 0.025                               |
| DOC (mg/g)                | 0.024                               |

### 3.4 Conclusions

In this study, six of the most common materials found in the UK were tested for their potential to be used in BNR, with attention to the organics likely to be leached, the rates of leaching, and how this affects their suitability as external carbon sources for denitrification. To the author's knowledge, some of these materials (e.g. wood bark) have been tested in this study for the first time and so there was no direct comparison to made with existing data.

It was noted that both soft and hard woodchips, barley straw, spruce wood bark, and beech sawdust released quite high levels of COD, which may not necessarily be available to microbes at the start of the systems, and so will result in high

effluent COD concentrations in the initial stages Biodegradable polymer (PCL), on the other hand indicated significantly less COD concentrations throughout the study but has been reported to be a good carbon source for nitrate removal. This suggests that easily leached COD may not necessarily be readily available to microorganisms and there may be activity directly on the media. The results of the leaching tests showed that PCL released minimal nitrate or ammonium-nitrogen over the course of the trial. The studies also demonstrated that total phosphorous (TP) was released from all media. Barley straw leached much higher quantities of total phosphorous than softwood, which leached at a relatively faster rate. The release of phosphorous from the media should not be overlooked as this can result in effluent phosphorous exceeding the allowable consents. Barley straw generally demonstrated the highest leaching amongst all the tested media and poses the highest risk for secondary pollution when used as carbon source for BNR. Straw generally is easily hydrolyzed, leading to high concentrations of organics and the potential to quickly run out of carbon. By the fourth day of this study, most of the leachable organics had been depleted. Depending on the volume of media used in SPD systems and the scale of operations, this initial leaching phase is likely to vary from a few days to weeks and needs to be monitored. The results from the metal analysis suggests low concentrations which would not necessarily inhibit denitrification or pose a significant health risk to aquatic organisms and humans. The concentrations demonstrated may even be helpful in improving enzymatic activities. Pre-treatment techniques such as washing may minimize the quantities of organics leached.

Overall, all the media tested in this study have the potential to be used as supporting carbon sources for SPD in terms of carbon content. However, it should be noted that barley straw has the highest potential to cause secondary pollution/pollution swapping. Also, straw generally has the ability to be easily hydrolysed, causing a fast rate of organics depletion. Sawdust has good potential but may however, be easily washed into the effluent due to the particle sizes. Woodchips and wood bark exhibited similar properties however, care has to be taken when

using wood bark as this is the first study to the knowledge of the author and more work needs to be done. The woodchips seemed to exhibit a good balance between the needed carbon content and the risk of secondary pollution. It should also be noted that, different results might be seen depending on the type of lignocellulosic material and plant species, as well as the effluent type or bioreactor systems used.

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## 4 Pilot Scale Evaluation of Woodchip as Media for Tertiary Solid-phase Denitrification in Municipal Wastewater

Affo Souleymane, A., Alibardi, L., and Pidou, M.

Cranfield University, Bedfordshire, MK43 0AL, UK

**Abstract:** The performance of four columns for solid-phase denitrification using woodchip media was studied for a period of 168 days. Average nitrate removal efficiency was observed to be between 62-75% with the best performance seen in the hardwood seeded with sludge column. All columns displayed limited nitrate removal in the first 10 days of operation, followed by a significant decrease in the effluent nitrate concentrations, indicating the denitrification process was triggered in all systems. The addition of sludge from a wastewater treatment plant helped to speed up the start-up process by about 6 - 8 days compared to the system without seed sludge. The results of the study were found to be in line with typical range of nitrate removal reported in mg/L for woodchip-based solid-phase denitrification systems, with the current study performing better in terms of daily removal rates (mg/L/day) compared to many studies in the literature. The results suggest that higher removal rates can be expected with higher influent nitrate concentrations. Over time, significant variability in the performance was observed which could not be clearly explained.

**Keywords:** solid-phase denitrification, woodchip media, start-up process, nitrate removal

## 4.1 Introduction

New total nitrogen (TN) consents are being enforced by the Environment Agency in England on some sewage treatment works (STW) requiring residual total nitrogen (TN) as low as 8 – 10 mg/L (European Environment Agency, 2020). TN removal is typically achieved biologically through the nitrification and denitrification processes (Equation 4-1). The majority of nitrogen present in raw municipal wastewater is in the form of ammonium ( $\text{NH}_4^+$ ) and can be converted by micro-organisms to nitrate ( $\text{NO}_3^-$ ) in the presence of oxygen (aerobic conditions). The nitrate can then be reduced to nitrogen gas by micro-organisms under anoxic conditions (low oxygen levels) as organic matter is also oxidized in the process (Metcalf & Eddy, 2003). Most STWs are set up to achieve nitrification, but it is not always possible to retrofit existing facilities to implement the denitrification step. In this case, additional systems will be required and from a practical point of view, implementation of denitrification at the tertiary treatment stage is seen as more feasible. It is important to note that the denitrification process requires the presence of organic matter in the water, but the level of organics is generally too low at the tertiary stage as they have been removed in the previous steps of the STW. In order to achieve denitrification in these conditions, the use of external carbon sources is then necessary.

### Equation 4-1 Nitrification (1,2)/Denitrification reactions (3)

1.  $\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+$
2.  $\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^-$
3.  $4\text{NO}_3^- + 5\text{C} + 2\text{H}_2\text{O} \rightarrow 2\text{N}_2 + 4\text{HCO}_3^- + \text{CO}_2$

Different studies have focused on a range of water sources such as for example stormwater (Lopez-Ponnada et al., 2017), agriculture run-offs (Manca et al., 2020), aquaculture (Von Ahnen et al., 2016), municipal wastewater (Yang et al., 2020) as well as numerous cases with synthetic waters (Zhao et al., 2020) in which nitrate is spiked. Most of the systems reported rely on attached growth, in which the micro-organisms grow in a biofilm attached to a media. There are essentially four main configurations with either a fixed or fluidized bed of the media and then with the water to be treated flowing either horizontally or vertically

in the reactor. The more common configuration applied to municipal wastewater is the moving bed bioreactor (MBBR) in which, typically a plastic carrier is maintained in suspension in the water and the water flows horizontally through the reactor (McQuarrie & Boltz, 2011). The MBBRs can have aerobic and anoxic zones to provide both nitrification and denitrification. Fluidised biofilm bioreactors (FBBR) are based on a similar approach with plastic carriers (Burghate & Ingole, 2013), which in this case, are fluidized as the water flows upwards (Water Research foundation, 2019). Systems using sand as a media have also been reported (Debarbadillo et al., 2006; Wei et al., 2014), relying on the deep bed filter configuration with up or down flow. Various types of organic media have been investigated and evaluated by numerous works at laboratory, pilot, and full scale in a bid to understand the factors affecting denitrification and the efficiency of these materials as external carbon sources for microbes. The media used in existing works can be classified into solid, liquid and gas based on their phases (Fu et al., 2022). As mentioned above, at the tertiary stage where the levels of organics are depleted, these systems will require the dosing of an external carbon source to ensure good denitrification. Methanol, ethanol, and acetate have been widely used as a traditional external carbon source in biological denitrification (Timmermans & Van Haute, 1983; Lee et al., 2013; Pelaz et al., 2018). Despite the high removal efficiency, these carbons have the ability to inhibit microbe growth due to the accumulation of nitrites (Glass & Silverstein, 1998; Lee et al., 2013). Moreover, due to the large quantities needed to successfully reduce high nitrate influent loads and the associated expenses, many companies are being forced to invest in finding alternative carbon sources which will provide the benefits of cost-efficiency, stability, and homogeneity of operations, along with a reasonable level of removal efficiency (Kaplan et al., 1987; Lee et al., 2013). A number of studies have investigated the use of carbon sources other than methanol with varying results reported.

Leverenz et al. (2010) highlighted the potential of solid-phase denitrification in which a solid media is used as both a support for the micro-organisms to grow on and the carbon source for the process. Due to the dual-purpose served by the

solid media and its potential affordability, this alternative is gaining more popularity. To illustrate this, products such as woodchip (Damaraju et al., 2015; Von Ahnen et al., 2016 and 2018) also combined to non-organic materials such as sand or gravel, sawdust (Hu et al., 2019; Yang et al., 2020) corn cobs (He et al., 2016; Feng et al., 2017) and rice straw and hull (Lu et al., 2017; Luo et al., 2018) have been trialed. Also, biodegradable plastics (Yamada et al., 2013; Li et al., 2016) including the reuse of waste biodegradable snack ware (Wang & Wang, 2013) have been investigated. These are expected to provide a more reliable and controllable source of carbon but will be expensive and to date are not readily available on the market. Solid-phase denitrification (SPD) has generally been applied in fixed bed reactor, either in columns with the water flowing upwards or in horizontal bed filter as seen for the large-scale implementation of woodchip systems (Von Ahnen et al., 2018b).

Evaluation of the performance reported in the literature have generally shown that the different types of systems with the different types of carbon sources can all provide significant removals of nitrate with the majority achieving more than 80% removal of the nitrate (Chapter 2). Interestingly, as a cheap and readily available material, woodchip has been extensively tested with water sources such as aquaculture up to full scale treatment but there is limited understanding of its application to municipal wastewater.

Von Ahnen et al. (2016) conducted a study to examine the use of woodchips as an external carbon source for nitrate removal in a recirculating aquaculture system. They used freshly chopped willow woodchips and reported nitrate removal rates ranging from 5.20 to 8.98 g/m<sup>3</sup>/day with an average efficiency of 98%. Another study by (Díaz-García et al., 2021) used citrus woodchips to treat brine from groundwater desalination plants and reported nitrate removal rates between 15-25 g/m<sup>3</sup>/day and 5-10 g/m<sup>3</sup>/day, depending on temperature changes. (Lopez-Ponnada et al., 2017) tested the use of softwood and hardwood to treat stormwater and achieved removal efficiencies of 75.2% and 63%, respectively. Manca et al. (2020) studied the use of softwood and a hardwood mix to treat groundwater, with average removal rates of 2.0 and 1.6 g/m<sup>3</sup>/day. Maxwell et al.

(2022) used a mixture of hardwood and softwood to treat surface drainage and reported a removal efficiency of over 80% for an average influent nitrate concentration of 6.4 mg/L. Finally, Von Ahnen et al. (2018) evaluated the performance of full-scale hardwood bioreactors in treating aquaculture effluent and reported average removal rates of 4.8, 4.5, and 7.8 g/m<sup>3</sup>/day, with corresponding nitrate removal values of 84%, 84%, and 92%, respectively. Aside from using woodchips alone, some studies have utilized a combination of woodchips with non-organic materials. This combination, typically with sand or gravel, is believed to increase the stability of bioreactor systems while preserving the performance reported for systems that use woodchips only. For example, Šereš et al. (2019) tested the use of birch and spruce woodchips mixed with gravels to treat synthetic wastewater and reported removal rates of 1.4 and 1.2 mg/L/day with average removal efficiencies of 96% and 85%, respectively. Zhao et al. (2018) used a mixture of woodchips and quartz sand to treat synthetic water and achieved a nitrate removal success rate of approximately 95%. The use of woodchips and non-organic materials together in solid-phase denitrification systems is gaining popularity as it offers several benefits. Non-organic materials, such as sand or gravels, help to add stability to the bioreactor systems, which is important for maintaining the long-term performance of the systems. The presence of these non-organic materials also helps to reduce greenhouse gas emissions and mitigate the risk of pollution (Šereš et al., 2019). Additionally, the combination of woodchips and non-organic materials can help to mitigate some of the limitations associated with using woodchips alone, such as clogging or compaction. Studies have shown that the use of woodchips mixed with non-organic materials can maintain similar nitrate removal efficiencies as those reported for woodchip-only systems (Zhao et al., 2018; Šereš et al., 2019). This has however not been extensively explored as there is limited information available.

An approach for improving denitrification efficiency is through the inoculation of active denitrifying bacteria. One common method of inoculation is sludge seeding which refers to the addition of a source of active denitrifying bacteria to the

system, typically in the form of pre-existing sludge from a similar treatment system (Cao et al., 2013). The objective of sludge seeding is to inoculate the system with a well-established population of nitrate-reducing bacteria, which can help to speed up the establishment of a stable denitrification process. Several studies have reported the benefits of sludge seeding in denitrification. For example, Chi et al. (2018) found that sludge seeding improved the performance and stability of an Anammox denitrification system. Sludge seeding has been shown to be an effective method for enhancing denitrification in solid-phase systems, however, conflicting findings have also been reported in the literature. For example, in a study by Wang & Wang, (2013), it was reported that sludge seeding is not necessary for acclimation of denitrifying bacteria in a solid-denitrification system. On the other hand, a study by Chi et al. (2018) suggests that the quality and composition of the seed sludge are important factors to consider when implementing sludge seeding. In conclusion, while sludge seeding has been shown to be effective in enhancing denitrification in solid-phase systems, conflicting findings have also been reported. The success of sludge seeding may depend on the quality and composition of the seed sludge, as well as the specific conditions of the system. Further research is necessary to fully understand the effects of sludge seeding and to determine its optimal application. There are also other methods of inoculation such as bioaugmentation, the introduction of immobilized bacteria, use of synthetic inoculum and natural acclimatization. This study factors sludge seeding and natural acclimatization techniques.

Overall, the diversity in system configuration and options for carbon sources combined to the high performance reported highlight the potential for tertiary denitrification but further evaluations are required to determine the applicability in real conditions for tertiary municipal wastewater treatment. This current study then aimed to evaluate current SPD systems for tertiary denitrification and in particular assess the potential of woodchips as alternative carbon sources and examine the use of seed sludge in the start-up process. Another objective is to

look at how the mix of woodchips and non-organic materials such as gravels affect the performance of the technology.

## **4.2 Materials and Methods**

A pilot plant comprising of four columns was installed at Cranfield University's National Research Facility for Water and wastewater and ran for 168 days (Figure 4.1). Each Perspex column was 50 cm in diameter and 1.5 m tall and fitted with a diffuser at the bottom for the distribution of the feed and an outlet port at 1.3 m height (6.2Appendix A). The columns were then filled with 1 m of media. The system was connected to a Watson Marlow 520S peristaltic pump (0.1 – 220 rpm rotor speed range, flow rate of 4  $\mu$ L to 3.5 L/min) with four pump heads connecting to the four columns for effluent feed. A storage tank was installed at the front of the columns to allow for homogenization of the feed prior to entering the columns, and to store water for short-term disruptions to the effluent supply. During operation, the secondary treated effluent from the University's sewage works, following treatment through screens, primary settling, trickling filters and a secondary clarifier, was pumped from the holding tank to the bottom of the columns and was flowing upward through the media bed to overflow at the outlet port by gravity. The configuration allowed to maintain a 30 cm head of water above the media to ensure anoxic conditions. For these trials, 100% natural, chemical-free, hardwood, and softwood woodchips sourced from environmentally friendly sources, in compliance with BSEN 1177:1998 standards (CPA Horticulture, Llanelli, UK, <https://www.cpa-horticulture.co.uk>) with sizes between 10 and 30 mm were used. Different conditions were tested in each of the columns to evaluate impact on start-up and general performance. The four columns contained respectively, hardwood and sludge (HS), hardwood only (H), hardwood, sludge, and washed gravel (HGS), and softwood and sludge (SS). The sludge was collected from a suspended growth biological system with a denitrification zone from a large Sewage Treatment Works (STW) located in the Midlands and used as seed biomass in the columns. Also, washed gravels (20 mm) were added to one of the columns to evaluate its impact on the structure of the bed (Šereš et al., 2019). The columns were operated to achieve an empty

bed contact time (EBCT) of 12 hours, and an average theoretical HRT of 6 hours at 50% porosity, but due to a range of challenges with operation, especially in the initial weeks of the trials the average EBCTs were recorded as  $11.5 \pm 2.7$ ,  $12.7 \pm 2.1$ ,  $10.9 \pm 3.3$  and  $15.0 \pm 2.7$  hours for the columns with hardwood, hardwood and sludge, hardwood, sludge and gravel and softwood and sludge, respectively.



**Figure 4.1 Initial pilot plant setup**

#### **4.2.1 Sampling and Laboratory analysis**

Samples were collected from the influent holding tank and the effluent pipes of each of the columns and analysed for their content in nitrate, ammonium, phosphate, and chemical oxygen demand (COD) with cell tests (Merck Life Science UK Limited, Watford, UK) and measured in a Merck Spectroquant NOVA 60. The samples were also analysed for pH with a Jenway 3510 pH meter (Cole-Parmer, St Neots, UK) and for dissolved oxygen (DO) with a LE621-IP67 DO meter (Mettler Toledo, Leicester, UK) and dissolved organic carbon (DOC) using a Shimadzu TNM-1 TOC Analyzer (Shimadzu 3201, Japan).

### **4.2.2 Statistical Analysis**

Statistical analyses were performed using the Minitab software for statistical analysis. The assumptions used for the statistical tests were that the observations within each group are not influenced by values in other groups and the data follows a normal distribution. Homogeneity of variances was conducted using Levene's test. One-way ANOVA tests were applied, and all statistical differences based on 95% confidence level ( $\alpha = 0.05$ ).

## **4.3 Results and Discussion**

### **4.3.1 Nitrate Removal**

Over the duration of the study, the secondary effluent fed to the columns had nitrate concentrations ranging between 26 and 62 mg/L with an average at 44 mg/L (Figure 4.2a). Similar nitrate removal behaviours were observed for all four columns, with limited removal over the first 10 days of operation, followed by a significant decrease in the effluent nitrate concentrations up to day 18, indicating that the denitrification process was triggered in all systems (See 6.2Appendix C for evidence of microbial activity). To illustrate, at 18 days, the feed nitrate concentration was 52 mg/L and the residual nitrate concentrations in the hardwood, hardwood and sludge, hardwood, sludge and gravel and the softwood and sludge columns were 1.0, 10.2, 15 and 1.7 mg/L, representing removals of 98.1, 80.4, 71.2 and 96.7%, respectively. Generally good performances were then maintained up to about 46 days of operation as the H column achieved between 48 and 99% nitrate removal, 70 to 98% with HS, 43 to 94% with the HGS, and 56 and 98% with the SS. All the columns reached levels of below 8 /L at some point in the operation, the expected new consent to come in place but none could maintain these very low levels in the effluent as some variability was observed for all the columns. The feed water nitrate levels decreased significantly from 62 to 30 mg/L between days 32 and 46 of operation, gradually increased back to 51 mg/L at day 69 and then decreased again to reach a concentration of 30 mg/L at day 101. The effluent concentrations were found to follow a similar trend with an increase (days 46 to 69) and a subsequent decrease. Further

analysis of other parameters did not allow to explain these variations. Overall, all the columns achieved good removal as the hardwood and sludge delivered the best average removal with 74.8% while the other columns achieving 62 to 65% on average (Figure 4.2a). The softwood and sludge system displayed the most variability (Figure 4.2b)

When comparing to existing data from the literature, the systems tested in this study were found to be within the typical range of performance reported for woodchip based solid-phase denitrification system (Figure 4.3b). Interestingly, when looking at the removal rates (mg/L/d), the systems from the current study were found to perform better than the majority of those reported in the literature. Indeed, rates obtained from the literature were generally below 40 mg/L/d while rates of 60, 68, 83 and 48 mg/L/d were obtained for the hardwood (H), hardwood and sludge (HS), hardwood, sludge, and gravel (HGS) and softwood and sludge systems (SS). As stated above, the different columns displayed relatively similar average percentage removal of nitrates but due to the variations in the EBCT effectively applied, the actual nitrate removal rates were found to be different. With an average EBCT of just under 11 hours, the system with the hardwood, sludge and gravel was in fact found to perform the best. It is also important to note that the system with the gravel had less woodchip overall than the others yet performed better clearly demonstrating an advantage over the other configurations. With an average mass removal of 107.4 mg/kg (dry mass media), the system with the hardwood, sludge and gravel configuration still provided the best removal compared to 91.7, 92.9, and 72.8 mg/kg (dry mass media) for HS, H, and SS, respectively (Figure 4.2). For the statistical analyses, the null hypothesis stated that all means are equal, while the alternative hypothesis proposed that not all means are equal. The results of the ANOVA indicated a significant difference in the means of the nitrate removed (mg/kg) ( $p$ -value = 0.020). Further analysis using the Fisher Least Significant Difference (LSD) method with a 95% confidence level revealed distinct groupings among the levels of the factor (Table 4-1 Statistical Analyses on nitrate removed (mg/kg) from all the four columns.). Specifically,  $\text{NO}_3\text{-N}$  Removed (mg/kg) in HGS exhibited a

significantly higher mean compared to the other groups. Meanwhile, NO<sub>3</sub>-N Removed (mg/kg) in columns SS and HS showed similar means but were significantly different from NO<sub>3</sub>-N Removed (mg/kg) in column H. These findings provide evidence to reject the null hypothesis and support the alternative hypothesis that there is a significant difference in the performances of the different columns.

**Table 4-1 Statistical Analyses on nitrate removed (mg/kg) from all the four columns.**

### Analysis of Variance

| Source | DF  | Adj SS | Adj MS | F-Value | P-Value |
|--------|-----|--------|--------|---------|---------|
| Factor | 3   | 17787  | 5929   | 3.40    | 0.020   |
| Error  | 114 | 198622 | 1742   |         |         |
| Total  | 117 | 216409 |        |         |         |

### Means

| Factor                           | N  | Mean   | StDev | 95% CI          |
|----------------------------------|----|--------|-------|-----------------|
| NO3-N Removed (mg/kg) <b>HS</b>  | 29 | 90.52  | 38.38 | (75.17, 105.88) |
| NO3-N Removed (mg/kg) <b>H</b>   | 31 | 72.12  | 36.30 | (57.27, 86.98)  |
| NO3-N Removed (mg/kg) <b>HGS</b> | 29 | 106.43 | 37.95 | (91.08, 121.79) |
| NO3-N Removed (mg/kg) <b>SS</b>  | 29 | 91.28  | 52.61 | (75.92, 106.63) |

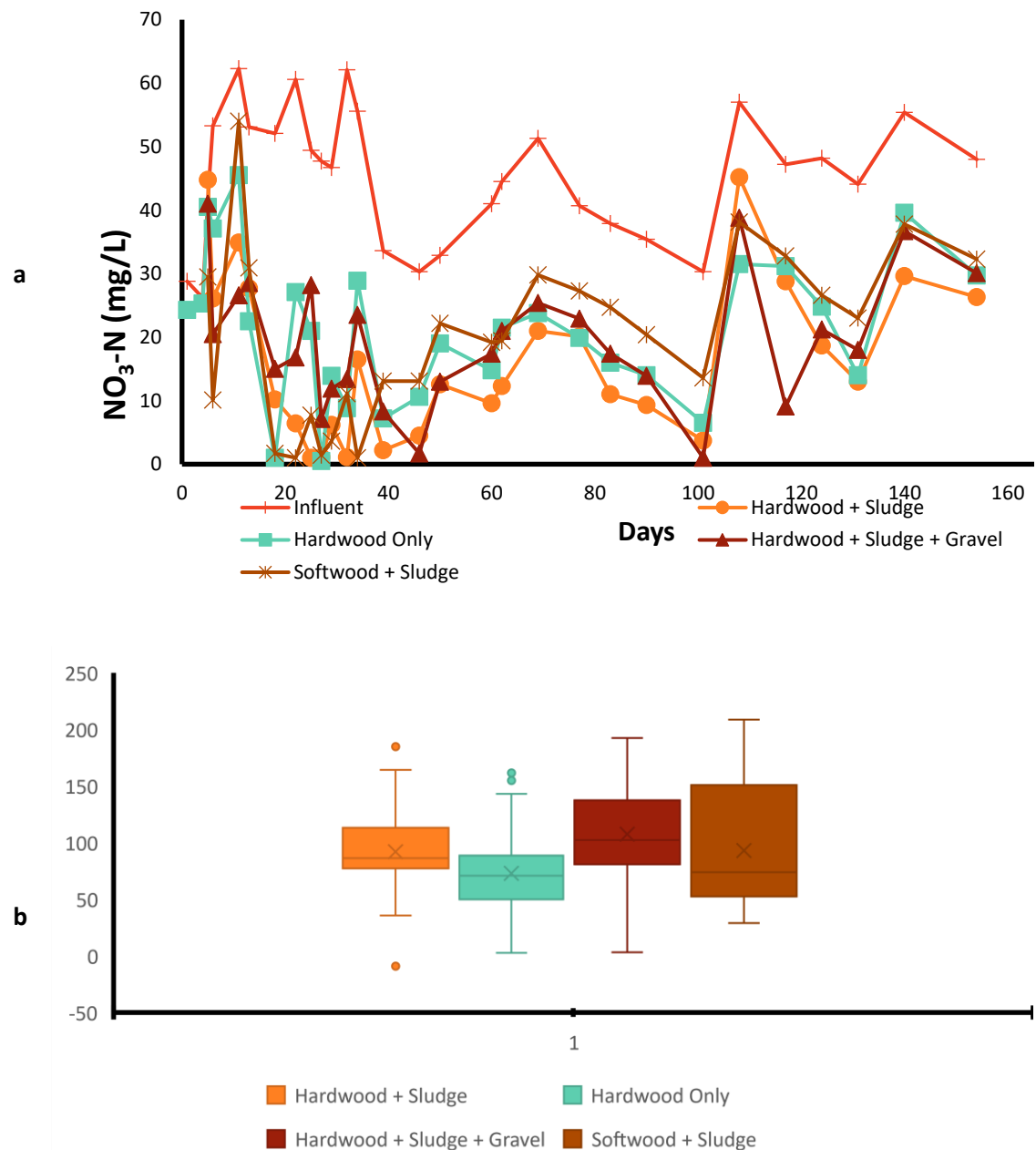
*Pooled StDev = 41.7408*

## Fisher Pairwise Comparisons

### Grouping Information Using the Fisher LSD Method and 95% Confidence

| Factor                                           | N  | Mean   | Grouping |   |
|--------------------------------------------------|----|--------|----------|---|
| NO <sub>3</sub> -N Removed<br>(mg/kg) <b>HGS</b> | 29 | 106.43 | A        |   |
| NO <sub>3</sub> -N Removed<br>(mg/kg) <b>SS</b>  | 29 | 91.28  | A        | B |
| NO <sub>3</sub> -N Removed<br>(mg/kg) <b>HS</b>  | 29 | 90.52  | A        | B |
| NO <sub>3</sub> -N Removed<br>(mg/kg) <b>H</b>   | 31 | 72.12  |          | B |

Also, the difference in performance when comparing to the literature can be explained by the types of water used in the trials. To illustrate, the studies reported in the literature on the application of woodchip based solid-phase denitrification used a range of sources including agriculture run-offs, aquaculture water, storm water and synthetic waters, most with influent nitrate concentration below 30 mg/L. These results highlight that with a higher influent concentration, higher removal rates can be expected. Since the effluent trends seem to follow that of the influent, the poor performance observed on some of the days may be due to a limitation in influent nitrate load. However, there is no clear explanation for the variability in the results observed in the current study. Parameters such as temperature have been reported to affect the denitrification process (Hoover et al., 2016), but no trend could be found. It should however be noted that the data reported in this study was mostly obtained over the summer (June to October 2022). The temperature and nitrate removal data over the colder months are not enough to draw conclusions on the effect of the temperature change on the nitrate removal rates.



**Figure 4.2 Nitrate removal over time (a) and variability in the performance (b) for the four configurations tested.**

In the box plot, the lower and higher levels of the box represent the first and third quartile of the data set, the horizontal line inside the box represents the median

and the extremities of the error bars represent the minimum and maximum values within range. The dots represent the outlier values. The x is the average.

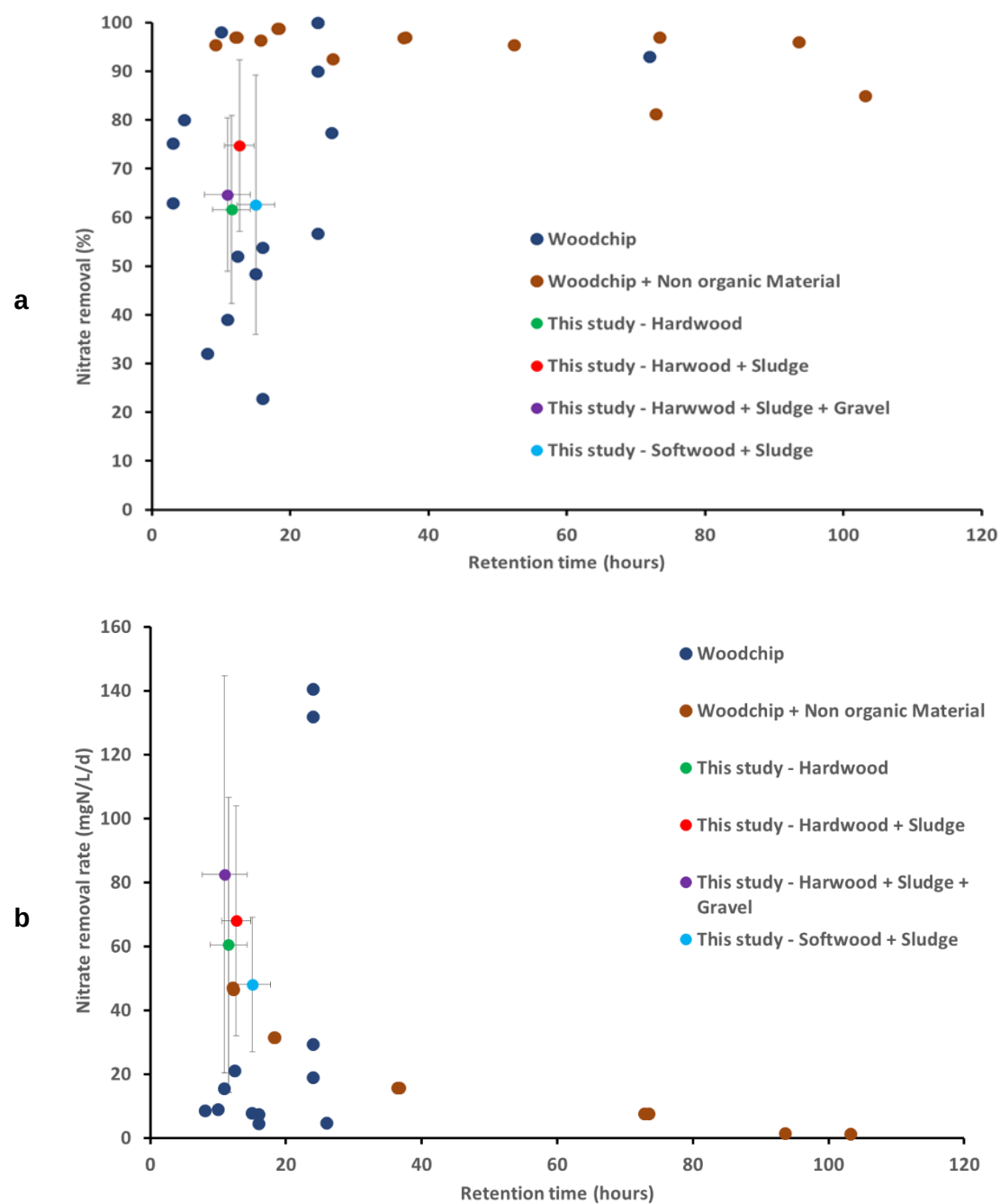


Figure 4.3 Nitrate removal percentage (a) and rate (b) compared to existing data from the literature.

#### 4.3.2 Start-up Period and Bed Structure

As mentioned above, sludge from a STW with a denitrification stage was added to 3 of the columns in view of speeding up the start-up of the denitrification process. All four columns displayed poor removal over the first 10 to 12 days of operation. After this, the nitrate effluent concentration decreased to low levels (<20 mg/L) (Figure 4.4). A similar start-up period of 11 days to reach stable conditions was reported for a large scale (12.5 m<sup>3</sup>) woodchip system treating fish farm water (Von Ahnen et al., 2016b). The hardwood and sludge and softwood and sludge systems maintained low levels of nitrate at below 10 mg/L between days 14 and 28 of operation. The system with hardwood, sludge and gravel delivered higher effluent concentrations between 7 and 28 mg/L. Finally, the system with only the hardwood woodchip only reached a low value (<20 mg/L) after 18 days suggesting a slight delay in the process but generally delivered effluent qualities in a similar range to the other system over the first month of operation.

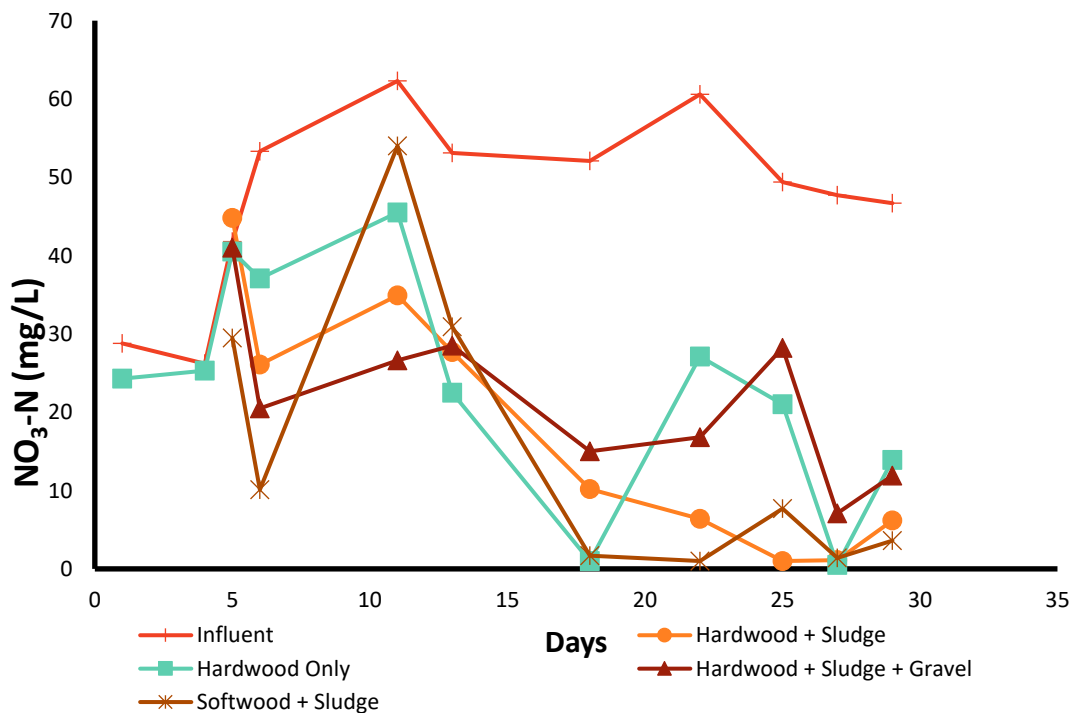
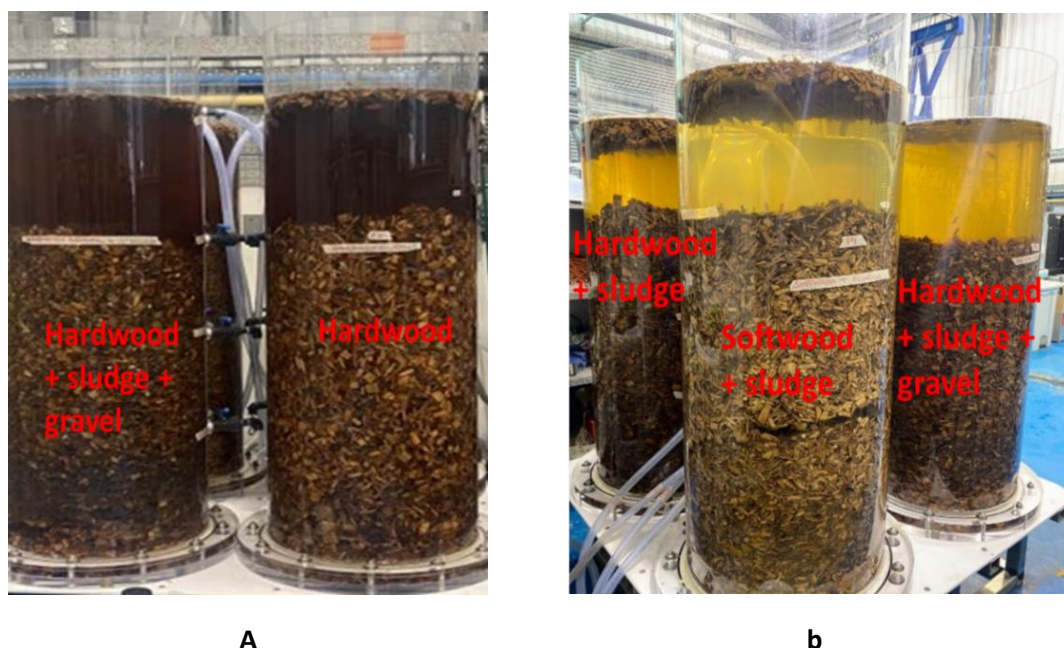


Figure 4.4 Nitrate removal over the start-up period.

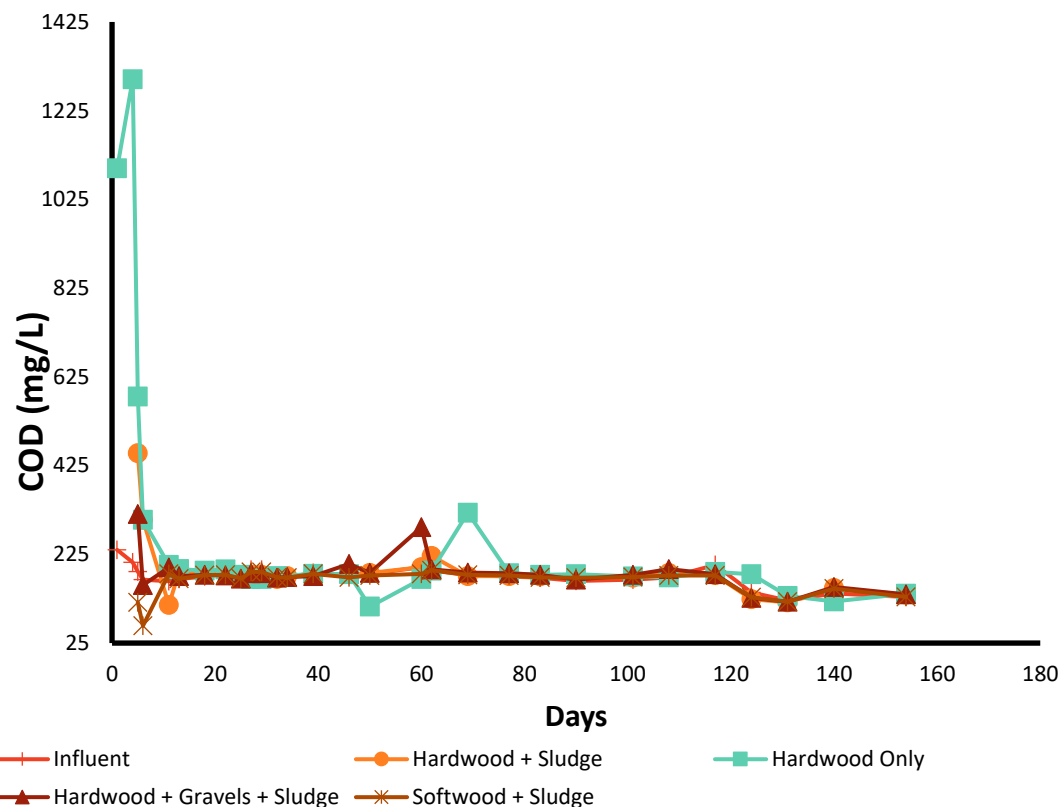
Observation of the columns during the start-up period demonstrated that in the first few days of operation, some leaching of material from the media in the effluents occurred, as highlighted by the dark colour of the column of water on top of the media beds in Figure 4.5a. The colour of the effluent was found to get lighter over time (Figure 4.5b), and no distinction of colour between the influent and effluent could be seen after two weeks of operation. This demonstrates that the poorer treatment performance observed during the start-up period may partially be due to the fact that the denitrification process is not yet established but also due to the release of material in the effluents from the media. It was also observed that at the start of the trial, a significant fraction of the woodchip media was floating in the hardwood only, hardwood and sludge and softwood and sludge systems. Most sank back down at the bottom of the columns within a few days, but a small fraction remained at the top for the rest of the trial. However, the gravel was found to help maintain the structure of the bed as very little woodchip was observed to float. This can be a very important factor when scaling up the technology. This phenomenon was observed by others and often systems were implemented with a mesh, plate, or gravel at the top to avoid movement of the bed (Hu et al., 2019). Finally, it should be noted that small bubbles were observed in all columns after the start-up period demonstrating the presence of gas. It can be assumed that the gas is in fact nitrogen formed as part of the denitrification process, again confirming that the process took place as expected.



**Figure 4.5 Columns after (a) 2 days and (b) 8 days of operation.**

#### **4.3.3 pH, Organics and Other Nutrients**

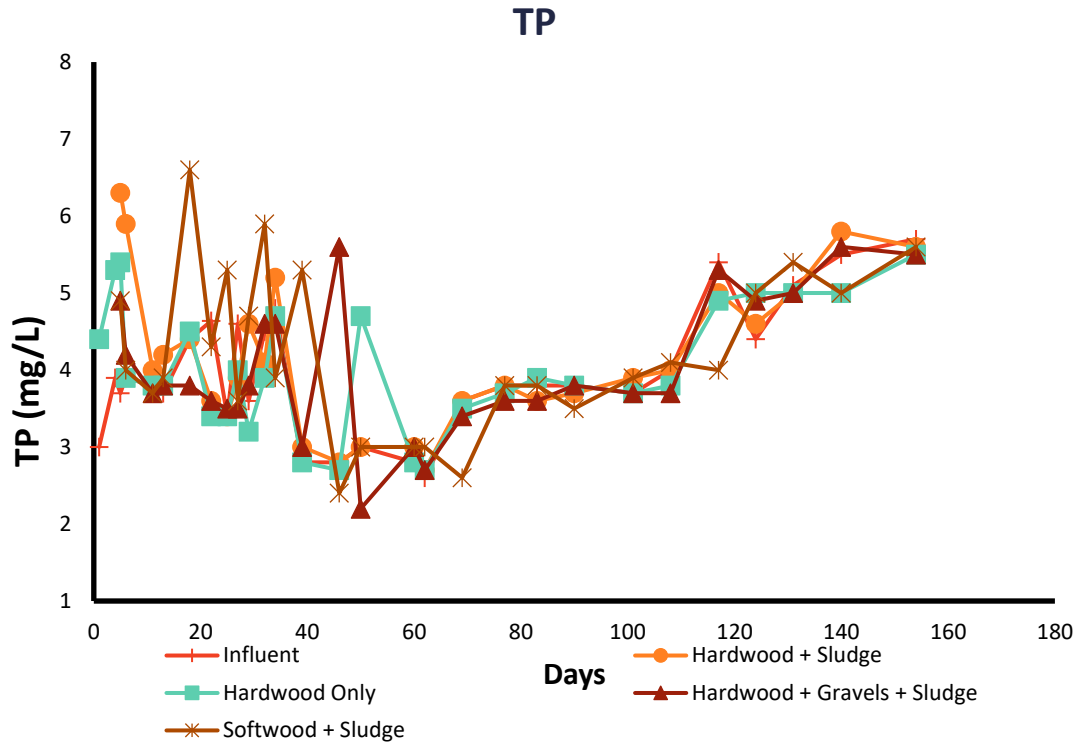
The effluents from the different systems were also analysed for a range of other parameters that may help understand their behaviours. As stated before, a suitable level of organics is required for the denitrification process and the media used, here woodchip, is expected to provide some of this organic matter. As highlighted by the dark colour of the effluents during the start period (Figure 4.5), high levels of organics, above that of the influent, were found in the effluents (Figure 4.6) confirming the leaching of materials from the media. This is also confirmed by the COD data from the pilot plants (Figure 4.6) as after the start-up period the COD in the influent and effluents were found to be very similar (~ 124 – 180 mg/L) hence suggesting very little leaching occurred and/or what is leached is consumed within the system. As mentioned by di Capua et al. (2019), anoxic conditions are needed for biological denitrification to take place. Hence the systems were all kept at always DO concentrations between 0.1 – 0.5 mg/L to ensure the systems were consistently kept anoxic.



**Figure 4.6 COD measurements over time in the effluents from all four columns.**

Phosphorus levels were also found to be higher than the influent concentration especially at the start of the trial, with also some significant variability over the first two months of operation (Figure 4.7). The leaching tests again confirmed that phosphorus is released from the media. In this case, high levels were found with the hardwood with a concentration of 1.4 mg/L after 24 hours. In a similar way to the COD, the release then decreased over time. For the softwood, the release was relatively stable for the four days of the leaching tests at a concentration of about 0.3 mg/L. Phosphorus leaching from woodchip was also observed in another study (Sharrer et al., 2016b). This is non negligible, especially if on a site with a very tight consent for total phosphorus and would need to be consider when scaling up the technology. Based on this, it would be expected that the levels of phosphorus become similar to the influent concentration after the start-up period. The variability observed in the pilot trial can then possibly also be

explained by some of the variation in flow observed in some of the columns. Indeed, the value stabilized in the later stage of the trial when the flow rates were more closely controlled.



**Figure 4.7 Total Phosphorous measurements over time from all four columns.**

The ammonia levels were found to vary significantly for the first half of the trial, including in the feed (Figure 4.8). They were then found to stabilize towards the end, in the same way as for the phosphorus. The stabilization of the flow within the pilot plants may have helped but it should also be noted that this period also coincides with the commissioning of a new submerged aerated filter on the site to help with the nitrification (only treats a quarter of the flow but it has been performing very well). Interestingly, the leaching tests did not show any release of ammonium and also nitrate in the water suggesting that these should not be changed by the media in the system. A spike in ammonium levels was detected on day 117, but this was consistent with an increase in ammonium concentrations from the influent wastewater. Finally, the pH was found to remain very stable as

no significant changes were observed in the pilot trials (Figure 4.9) and leaching tests (Chapter 3).

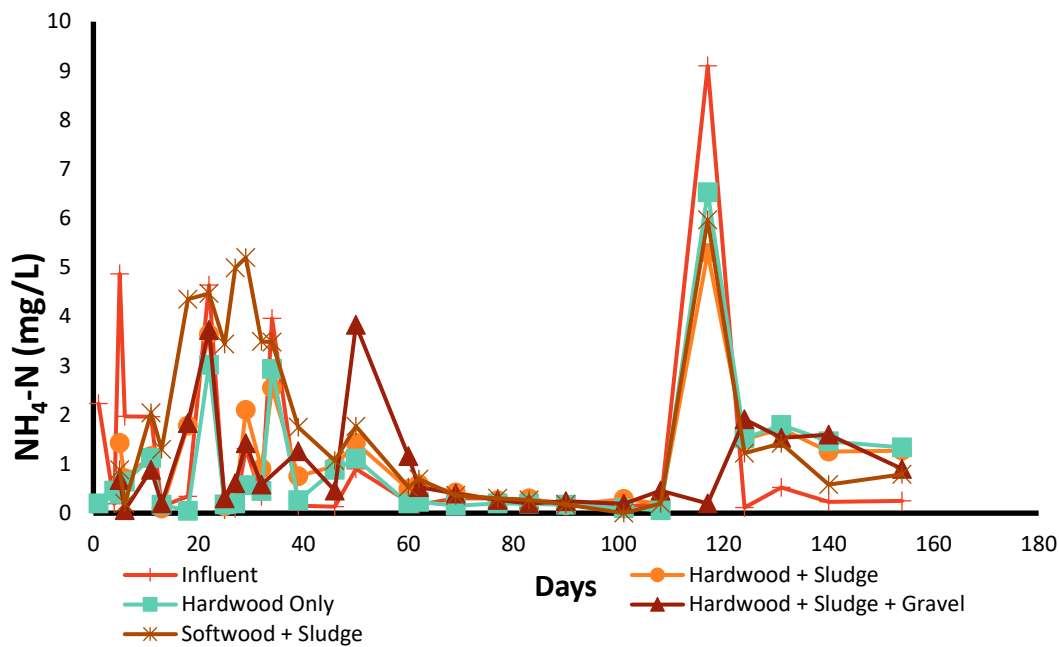


Figure 4.8 Ammonium-Nitrogen measurements from all columns over time.

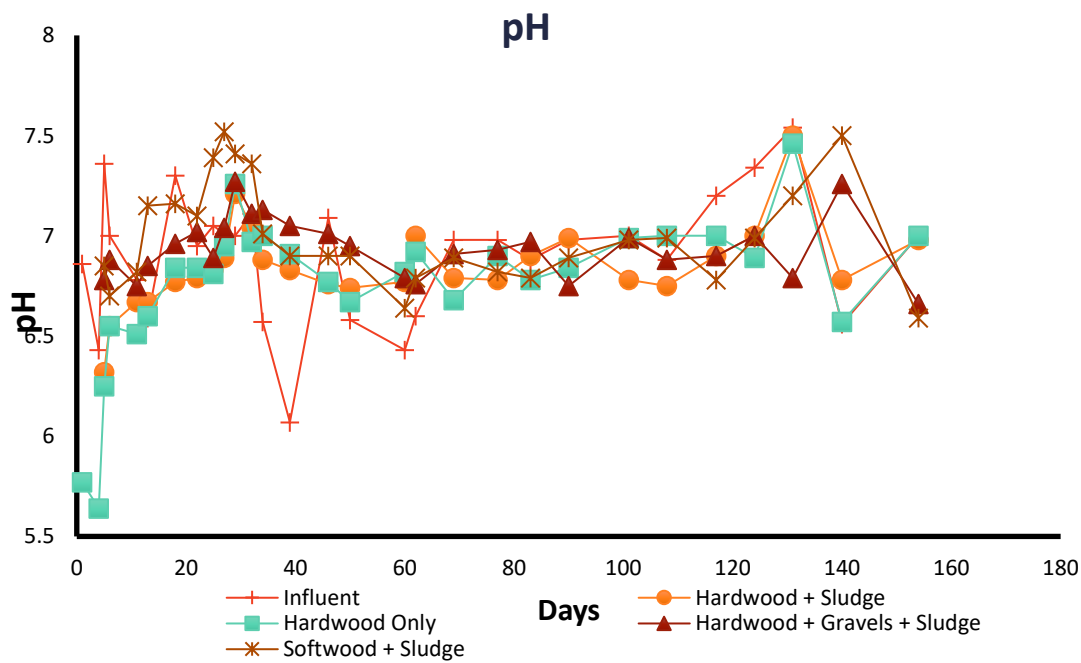


Figure 4.9 pH measurements from all colums over time.

## 4.4 Conclusions

In conclusion, the performance of solid-phase denitrification using woodchip media in four columns was evaluated in this study. The results showed that the nitrate removal efficiency was between 62 - 75%, with the hardwood and sludge column having the best performance with. It was observed that all columns displayed limited nitrate removal in the first 10 days of operation, which was then followed by a significant decrease in the effluent nitrate concentrations, indicating that the denitrification process was triggered in all systems. The addition of sludge from a wastewater treatment plant was found to be helpful in speeding up the start-up process a little. The results of this study were found to be in line with the typical range of performance reported for woodchip-based solid-phase denitrification systems, with the current study performing better in terms of removal rates compared to most studies in the literature most likely due to the nature of water being treated in this study. These findings suggest that higher removal rates can be expected with higher influent nitrate concentrations, although the variability in the results remains unexplained. In light of these results, it is recommended that further studies be conducted to understand the factors affecting the denitrification process and the removal rates in order to improve the performance of solid-phase denitrification systems using woodchip media.

## 4.5 References

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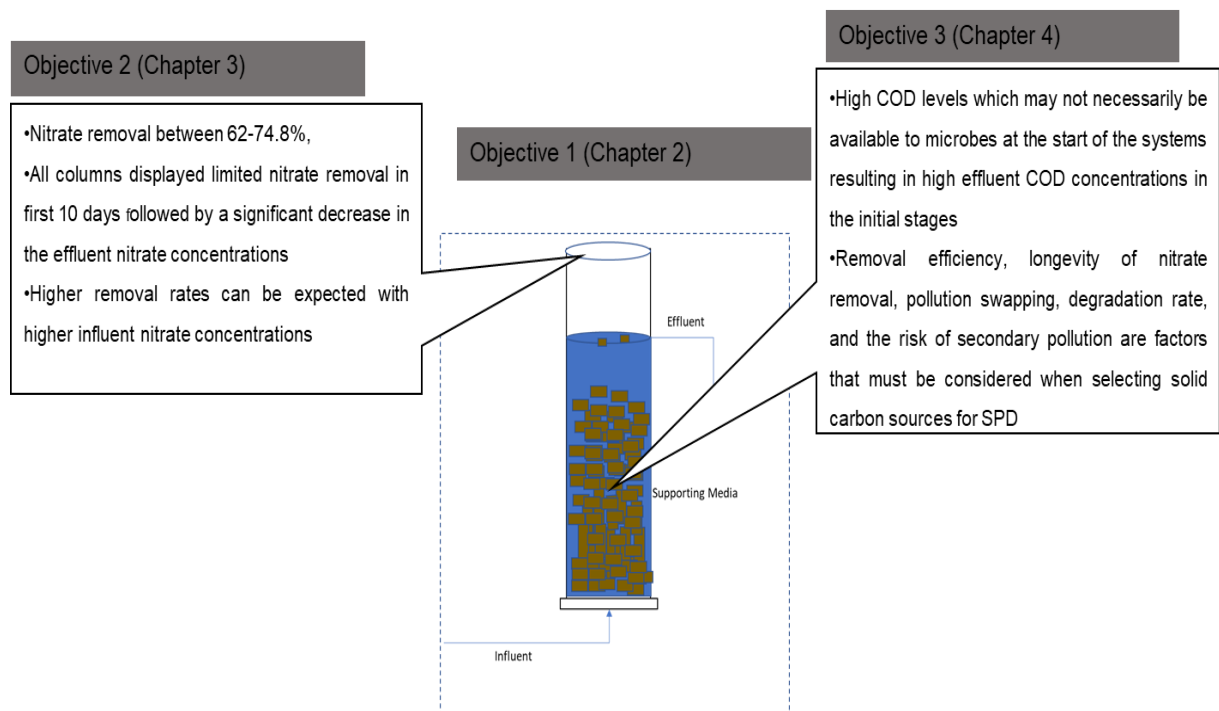
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## 5 Overall Discussion and Practical Implications of The Work

The overall aim of the study was to “establish the efficacy of the novel solid-phase denitrification technology using low-cost solid media for tertiary removal of nitrates from municipal wastewater” through experimental work divided into two sections (Figure 5.1). One part of the work explored at lab scale, the potential of a range of low-cost solid media to be used as a supporting carbon source in solid-phase denitrification. The media explored were selected based on their availability in the UK and their use in previous works or lack thereof, including woodchips, straw, sawdust, biodegradable polymer, and wood bark. The second part of the work was a pilot trial of the solid-phase denitrification technology using woodchips as the external carbon substrate.



**Figure 5.1 Summary of the experimental work.**

## 5.1 Potential for the release of compounds in SPD

It was shown that in the start-up phase, all the materials, except the polymer tested in this study are likely to release high concentrations of COD, usually indicated by the dark-coloured leachate in the first few days. The polymer released the least COD but has been proven in different studies to provide good nitrate removal efficiency despite the low COD concentrations. This suggests that the easily leachable COD, may not necessarily be an indicator of carbon substrate availability to microbes. The barley straw used in this study poses the highest risk of secondary pollution as it released the highest concentrations of organics amongst all the media. The straw is a softer substrate that is easily hydrolysed, leading to high organics concentration and the risk of running out of a carbon after a short period. For all the media, the initial leaching phase is of concern and requires monitoring. Indeed, for both the hardwood and softwood media, COD levels of over 1000 mg/L were measured over the first 24 hours. Interestingly, the COD levels were then found to decrease significantly over the subsequent 3 days of the leaching tests suggesting that the media contained some easily leachable materials that is released initially but, over time, the leaching stops (Chapter 3). This was also observed with the pilot trials plants operated with the same media, as after the start-up period the COD in the influent and effluents were found to be very similar hence suggesting the occurrence of almost no leaching (120 – 180 mg/L COD). When leachable organics appeared to run out, this allowed for microbial activity to stabilize, and the denitrification process kickstarted (Chapter 4). The onset and continuation of denitrification despite the low values measured for leached organics suggests that these materials may contain carbon that is more readily available to microbes for nitrate removal, and that microbes may not be using leachable carbon in the denitrification process but rather localised activities on the media itself (Wang & Chu, 2016). This raises the possibility that leaching tests may have limitations in accurately assessing the full potential of a media, indicating the need for further research at the micro-scale to better understand the processes occurring within the media.

Furthermore, the results of the pilot study indicated significant variation in ammonia levels during the first half of the trial, including in the feed, which then stabilized towards the end, likely due to the commissioning of a new submerged aerated filter to assist with nitrification (Chapter 4). Interestingly, leaching tests did not show any release of ammonium or nitrate in the water, suggesting that the media used in the system did not negatively impact water quality (Chapter 3). The pH remained stable throughout the trial and leaching tests, implying that the SPD process can effectively remove nitrate from water sources without significant negative impacts on water quality when maintained and monitored properly.

This study also found that the elemental metal concentrations in the supporting carbon materials used in SPD were generally within safe limits, with only a few metals such as K showing significant values leaching from barley straw. The presence of elements such as K, Na, and Mg in lignocellulosic materials like barley straw may promote microbial activities in the denitrification reactor. Additionally, elements such as Al, Ca, Fe, Ni, Cu, and Zn, observed in lower concentrations than the inhibitory level, may stimulate bacterial activity and provide additional energy sources for denitrification. These findings suggest that SPD using the selected materials has the potential to be an effective and environmentally sustainable solution for removing nitrate from water sources. However, careful consideration and monitoring are necessary to prevent the release of harmful compounds into the environment during the denitrification process. To reduce the risk of leaching too high level of compounds, some studies such as Schipper et al. (2010) have suggested pre-treatment measures, among which the most popular are pre-washing of the carbon substrate to strip it off the easily leachable organics, and treatment with acid or alkali.

These findings highlight the potential for SPD as a promising technology for water treatment.

## **5.2 Performance of woodchip-based SPD technology**

In the second part of the experimental work, a pilot-scale solid-phase denitrification reactor was trialed, using woodchips as the supporting carbon substrate. Different configurations of the bioreactor were tested including inoculation with sludge from an existing wastewater facility, and a mixture of woodchips with washed gravels.

Overall, all the columns exhibited good removal. The system made up of softwood and sludge showed the highest variation. The systems examined in this work were found to be within the typical range of performance reported for woodchip-based solid-phase denitrification systems when compared to data already available from the literature. Interestingly, the systems from the current investigation performed better than the bulk systems reported in the literature when looking at the removal rates (mg/L/d). Despite the system with the gravel having less woodchip than the others, it still worked better, proving that it may be superior to the other arrangements. The findings from this study also demonstrated that higher removal rates are likely to occur at higher influent concentrations. However, the variation in the outcomes observed in the current investigation had no obvious cause. For example, temperature has been claimed to have an impact on the denitrification process (Hoover et al., 2016); however, no trends were discovered. It should be noted that most of the data included in this study were collected throughout the summer (June to October 2022). Data on temperature and nitrate removal during the colder months were insufficient to make inferences about how temperature variation affects nitrate removal rates. The variance in flow seen in some of the columns can thus possibly also account for some of the variability observed in the pilot study. In fact, as the study progressed and the flow rates were tightly controlled, the value stabilized.

Overall, the results of this study provide valuable insights into the potential of solid-phase denitrification systems and underscore the need for continued research and development to optimize their effectiveness for water treatment purposes. The use of woodchips as a supporting carbon substrate, as

demonstrated in this study, can provide an affordable and sustainable solution for nitrate removal and these findings have important practical implications for the design and operation of denitrification systems for the treatment of nitrate-contaminated water sources.

### **5.3 Implementation of SPD**

Solid-phase denitrification, which refers to the use of a solid substrate to support the growth of denitrifying microorganisms, is a relatively new development in wastewater treatment. This technique has drawn much interest as a potential improvement over current biological denitrification techniques in terms of efficiency and cost. Solid-phase denitrification for wastewater treatment has been a subject of further research in recent years. As a result, there is now a greater understanding of the critical elements that influence the effectiveness of the process, such as the type of solid substrate used, type of denitrifying bacteria used, and ambient environmental conditions. Additionally, higher influent nitrate concentrations can lead to increased removal rates due to more available substrate for denitrification processes.

The significance of the type of solid substrate chosen is one of the important results of a recent study. The effectiveness of denitrification can be affected by the diverse qualities of different substrates, such as porosity, water-holding capacity, and surface area, found in sawdust, wood chips, and straw, amongst others. According to previous research, substrates with a large surface area and a decent carbon content work best.

The costs of purchasing carbon sources and sludge management should be considered when selecting solid carbon sources for SPD systems. It is also important to consider the longevity of nitrate removal, pollution swapping (increase in one pollutant to remove another), risk of secondary pollution, slow-release rate from the decomposition process, low concentrations of released compounds, and high denitrification rates. This study concludes that there is a range of low-cost media options available for tertiary solid-phase denitrification in municipal wastewater, and each has its own advantages and disadvantages. It

also highlights the importance of evaluating these materials carefully before making any decisions about which materials to use to ensure optimal performance at an affordable cost.

Biodegradable plastics and polymers have been found to be good carbon sources for nitrate removal, with lower concentrations of released compounds compared to other solid carbon sources. They also tend to exhibit slower degradation rates than other materials, which can help ensure longevity in terms of the nitrate removal efficiency. Additionally, biodegradable plastics and polymers are associated with a lower risk of secondary pollution when used as external carbon sources in denitrification processes. However, there are limitations in terms of the cost. Barley straw generally has the highest leaching rate among all tested media, which can lead to high concentrations of organics in wastewater and the potential for quickly running out of carbon. This initial leaching phase is likely to vary from days to weeks depending on the volume used and the scale of operations; therefore, so it needs careful monitoring is required. High levels of COD released by barley straw may not be immediately available for microbes, leading to higher effluent COD concentrations during the start-up phases. There is also an increased risk of secondary pollution due to its easily hydrolyzed nature when used as an external carbon source in BNR systems. Pre-washing of carbon media helps reduce the leaching rates of organics by removing any impurities or contaminants that may be present on the surface. This can help prevent the release of these substances into wastewater, which can lead to secondary pollution and other environmental issues. Additionally, pre-washing also reduces organic matter in the water itself, allowing for more efficient nitrate removal processes, as well as reducing potential risks associated with high concentrations of organics in a system.

The main advantage of using woodchip media for solid-phase denitrification is that it can be more efficient in terms of removal rates than other materials. Additionally, the use of woodchips as a medium allows for better control over the process because of their physical properties, such as porosity and surface area, which facilitate microbial activity (Lopez-Ponnada et al., 2017). Woodchips also

provide an external carbon source needed by microbes during denitrification, making them ideal for this type of application. The addition of sludge from a wastewater treatment plant can help speed up the start-up process of SPD systems. This is because the sludge contains active denitrifying bacteria that can quickly establish themselves and begin reducing nitrate concentrations, leading to faster results than if no seeding was performed. Different configurations of woodchip systems for SPD applications can affect both performance and cost-effectiveness. Columns are generally more efficient in terms of nitrate removal, but they require a larger space for installation compared to horizontal bed filters, which may be better suited for large-scale implementation because of their smaller footprint. Additionally, columns tend to have higher capital costs associated with them than other types of reactors, such as horizontal filter beds, because they need additional components such as pumps or valves that increase installation complexity and cost.

The effectiveness of solid-phase denitrification can also be affected by environmental factors such as temperature, pH, and dissolved oxygen (Zhong et al., 2020). According to previous research, denitrification occurs best at temperatures between 15 and 30 °C, pH values between 5.5 – 8 with values ranging from 6.5 – 7.5 recorded for this study, and low concentrations of dissolved oxygen (Yang et al., 2015; Díaz-García et al., 2021). Solid-phase denitrification can be significantly influenced by hydraulic retention time as variability in nitrate removal was observed in the pilot trials with varying retention times.

A longer HRT improves the amount of time available for denitrification, resulting in more effective nitrogen component elimination. However, an excessive HRT might result in the build-up of organic materials, which lowers denitrification efficiency and raises the possibility of reactor failure (Zhong et al., 2020). Also, a shorter HRT may lead to inadequate denitrification and insufficient nitrogen compound elimination. In conclusion, HRT is a critical component of the SPD process and should be regulated to strike a compromise between the nitrogen removal efficiency and system stability. Laboratory testing and SPD process observations can be used to determine the appropriate HRT.

## 5.4 Conclusions

In conclusion, Solid-Phase Denitrification has been shown to be a successful approach for nitrate removal from wastewater. This method uses a solid substrate, such as sawdust or woodchips, to provide denitrifying bacteria with a surface to live on and turn nitrate into nitrogen gas. However, it is crucial to consider the expense of implementing this technology because it can necessitate significant investments in infrastructure and equipment. The process is also controlled by pH, which should be maintained within a specified range to guarantee maximum nitrate removal effectiveness and temperature, with optimal performance observed within a limited temperature range. It is possible to obtain high levels of nitrate removal and improve water quality by carefully considering these parameters and by correctly constructing and operating SPD systems. The effectiveness of nitrate removal by SPD is significantly influenced by the hydraulic retention time or the time that the wastewater is in contact with the solid substrate. While a longer HRT often leads to higher nitrate removal rates, it also increases the volume of the SPD system that is necessary as well as the cost of operation. To maintain the optimum performance and efficiency of the SPD system, it is crucial to balance the HRT with other parameters, such as the desired level of nitrate removal, available area and resources, and economic viability of the SPD system.

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## 6 Conclusions and Further Works

### 6.1 Conclusions

The following conclusions can be drawn for each of the objectives.

**Objective 1:** To evaluate current knowledge through the review of existing literature on the application of liquid and solid external carbon sources for denitrification in municipal wastewater treatment and understand their application at the tertiary treatment stage.

- Different organic liquids can be used as carbon sources to optimize biological denitrification in tertiary treatment steps by providing an additional energy source for the bacteria involved. This helps increase their activity and efficiency, leading to more effective nitrate removal from wastewater. Additionally, different types of media, such as sawdust, straw, and woodchips, are compatible with various wastewaters, making them suitable for many applications.
- The current understanding of the mechanisms and processes involved in solid-phase systems for nitrate removal from wastewater is that these involve a combination of physical, chemical, and biological processes. Physical processes include adsorption onto surfaces or particles as well as filtration through media such as woodchips. Chemical reactions can also occur between organic compounds present in the water and nitrogenous species to form new products, which are then removed by denitrifying bacteria.
- Finally, microbial activity plays an important role in breaking down complex molecules into simpler forms, which can be easily assimilated by other organisms or released back into the environment.

Limitations include:

- There has not been much research on woodchip-based solid-phase denitrification in municipal wastewater treatment systems, so there may still be room for improvement in this area.

**Objective 2:** To determine the potential of a range of low-cost solid media as carbon sources for denitrification.

- There have been reports on a variety of materials, including woodchips, straw, sawdust, organic cotton, biodegradable plastics, and corn cob. Most research typically discuss the effectiveness and rates of nitrate removal. However, there are other criteria that should be considered when establishing these media's applicability in addition to their functionality as effective for BNR. The possibility of secondary contamination, pollutant swapping, cost, availability, and material lifespan are a few of these considerations.
- It was noted that both soft and hard woodchips, barley straw, spruce wood bark, and beech sawdust released quite high levels of COD, which may not necessarily be available to microbes at the start of the systems, and so will result in high effluent COD concentrations in the initial stages. Biodegradable polymer (PCL), on the other hand indicated significantly less COD concentrations throughout the study but has been reported to be a good carbon source for nitrate removal.
- When utilised as a carbon source for BNR, barley straw typically showed the highest leaching of all the investigated media and presents the greatest potential for secondary pollution. Straw is easily hydrolysed, resulting in large levels of organics and the possibility of rapidly running out of carbon. Most of the leachable organics had been used up by the fourth day of our investigation. This first leaching phase needs to be watched carefully because it could last anywhere from a few days to weeks, depending on the quantity of media used in SPD systems and the size of the activities.
- Mitigation measures such as pre-washing of carbon media may be necessary to reduce the high leaching rates of organics measured in the initial phase of bioreactors.

**Objective 3:** To assess the effects of different operating and start-up conditions on the efficacy of woodchip based solid-phase denitrification.

- The results of this study suggest that woodchip-based solid-phase denitrification systems can be used to effectively remove nitrate from wastewater. This study found higher removal rates than many studies in the literature, indicating that these types of systems may offer a more efficient way to remove nitrates than other methods. Additionally, adding sludge from a wastewater treatment plant was seen to accelerate the start-up process and further increase efficiency.
- These findings have practical implications as they provide an effective method for treating water with high levels of nitrogen pollution, which is becoming increasingly common because of human activities, such as agriculture and industry. The results of this study showed that the nitrate removal efficiency was between 62-75%, with the best performance observed in columns containing hardwood and sludge media. All columns displayed limited nitrate removal during the first 10 d, followed by a significant decrease in effluent concentrations, indicating that denitrification had been successfully triggered.
- These findings are consistent with the typical performance range reported for woodchip-based solid-phase denitrification systems, suggesting that higher removal can be expected when influent nitrogen levels are high. This study found higher removal rates than many studies in the literature, indicating that these types of systems may offer a more efficient way to remove nitrates than other methods.

The key takeaways of this study are that woodchip-based solid-phase denitrification systems can be used to effectively remove nitrate from wastewater. Additionally, adding sludge from a wastewater treatment plant was seen to speed up the start-up process and increase efficiency even further. The advantages of using woodchip media for solid-phase denitrification compared to other materials include woodchips, which are readily available and inexpensive, making them an attractive option. They also provide a large surface area that can be colonized by nitrate-reducing bacteria, allowing for efficient removal of nitrates from wastewater.

Additionally, the organic nature of woodchips provides additional carbon sources that may help speed up the start-up process and increase efficiency even further when combined with sludge seeding.

## **6.2 Future Works**

Some key areas where further work will prove beneficial have been identified in this study:

1. More long-term trials are required with the solid-phase denitrification to properly ascertain the effect of different weather conditions on the efficiency of the technology. Because the trials in this study were mostly done in the summer with warmer temperatures, it was difficult to determine how the systems' behaviour would change in colder temperatures. Long-term trials can also help to study the long-term stability and sustainability of solid-phase denitrification.
2. Trials on different pre-treatment techniques can be tested on different media to understand how this affects the leaching of organics and its further impact on potential secondary pollution or pollution swapping.
3. The SPD technology can be integrated with other wastewater treatment processes such as activated sludge to investigate the potential to use these technologies side by side in a treatment chain.
4. Future research may focus on investigating the mechanisms and pathways through which organic matter is utilized in the process. Specifically, there is a need to better understand the types and quantities of organic matter that are most effective in promoting denitrification, as well as the specific microorganisms that are involved in this process.

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## APPENDICES

### Appendix A Images and data of pilot scale experiment



Figure A-1 New pilot plant setup



**Figure A-2 Column media before and after effluent flow**

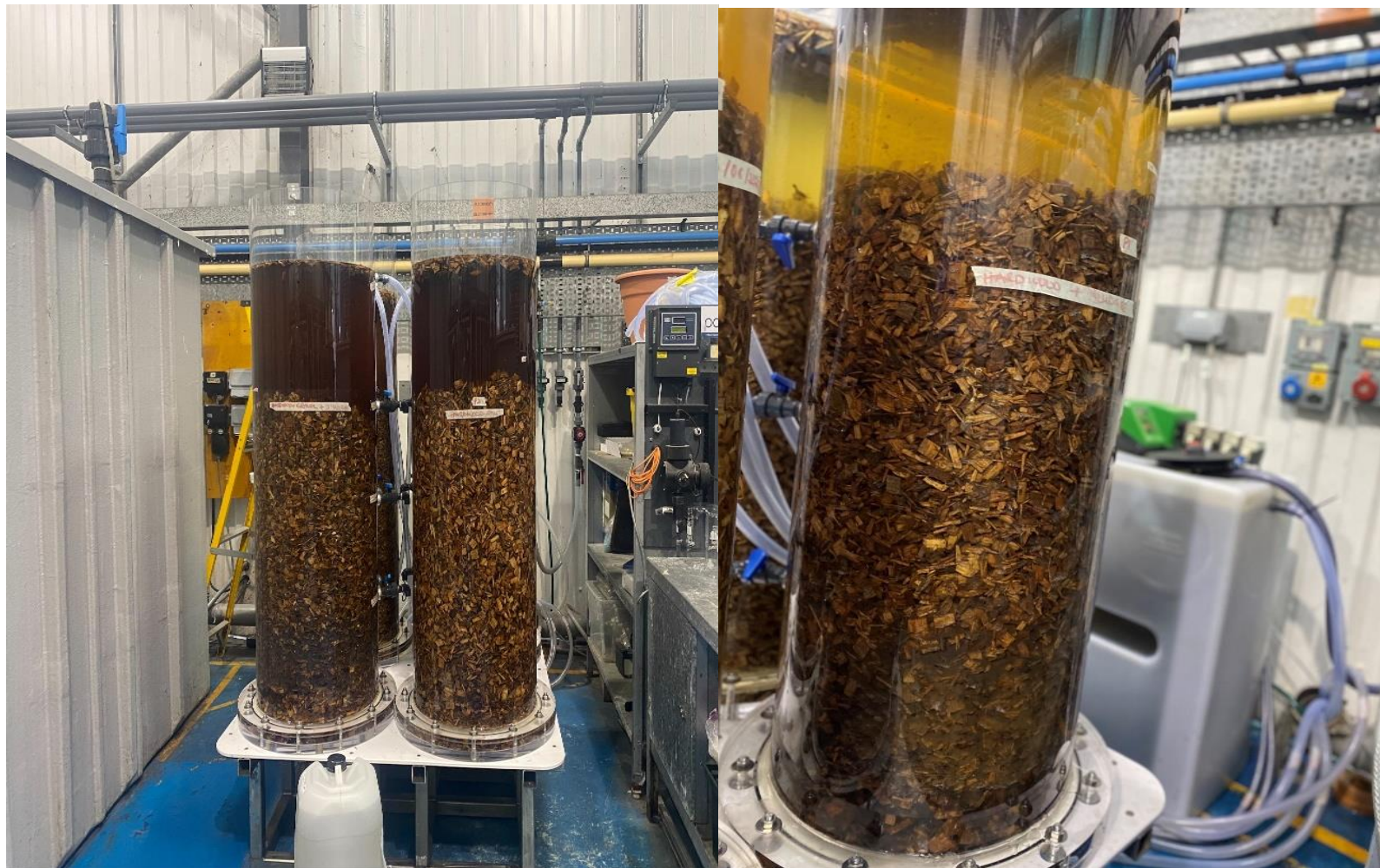


Figure A-3 Column effluent on day 1 vs day 10

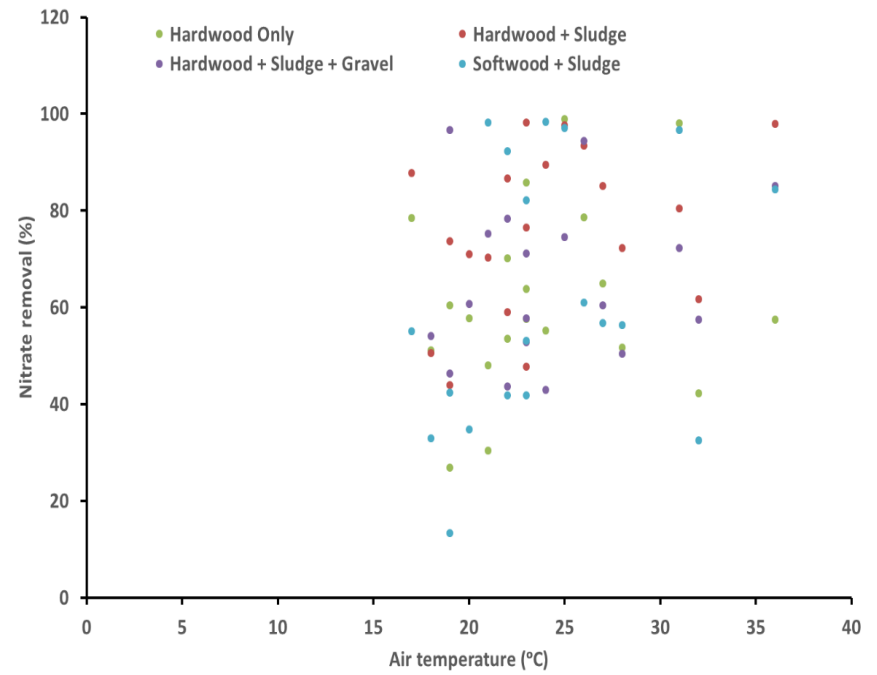
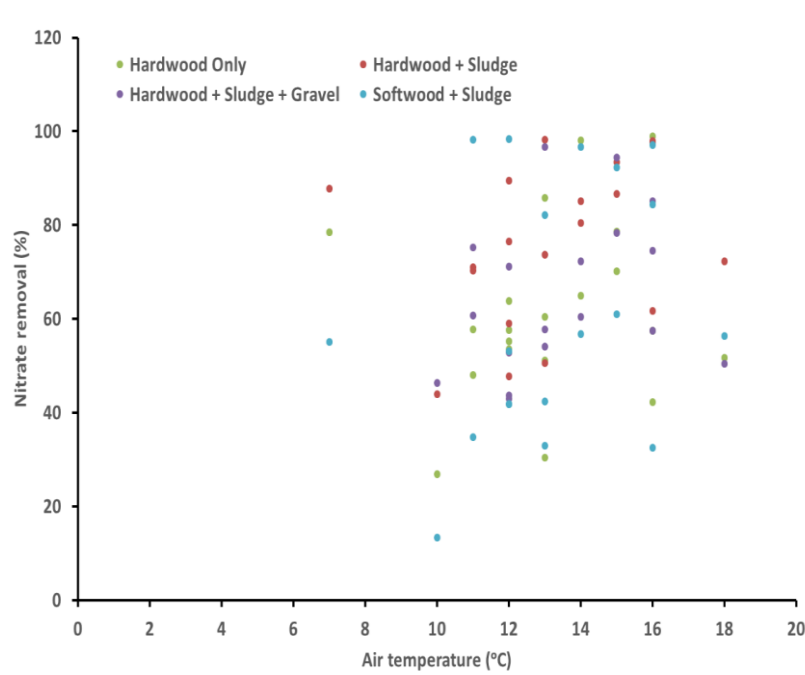


Figure A-4 Nitrate removal against air temperature (1) high (2) low

## Appendix B Data from literature review

Table B-1 Literature review table

| Carbon Source             | C/N | EBCT/<br>HRT | Tempe               | pH                  | %<br>Removal | Nitrate<br>In | Nitrate<br>Out | Removal<br>Rate<br>(mg/L/d) | Type of<br>Water        | System<br>Type | Reference                     |
|---------------------------|-----|--------------|---------------------|---------------------|--------------|---------------|----------------|-----------------------------|-------------------------|----------------|-------------------------------|
| Cotton wool               |     |              |                     |                     |              | 137.16        | 2.08           |                             | Synthetic<br>wastewater | Column         | (Aloni & Brenner,<br>2017)    |
| Wheat straw               |     |              | 31                  | 7.5                 |              | 100           | 9              |                             | Synthetic<br>wastewater | Continuous     | (Aslan & Türkman,<br>2005)    |
| Pine bark                 |     |              | 21                  |                     | 59.7         | 60            | 24.2           | 0.09                        | Synthetic<br>wastewater | Batch          | (Capodici et al.,<br>2014)    |
| Sawdust                   |     |              | 21                  |                     | 77.7         | 60            | 13.4           | 0.37                        | Synthetic<br>wastewater | Batch          | (Capodici et al.,<br>2014)    |
| Cork                      |     |              | 21                  |                     | 80.8         | 60            | 11.5           | 0.28                        | Synthetic<br>wastewater | Batch          | (Capodici et al.,<br>2014)    |
| Olive nut                 |     | 11.78        |                     |                     | 99.66        | 30            |                | 0.023                       | Synthetic<br>wastewater | Column         | (Capodici et al.,<br>2018)    |
| Olive nut                 |     | 12.5         |                     |                     | 99.47        | 50            |                | 0.034                       | Synthetic<br>wastewater | Column         | (Capodici et al.,<br>2018)    |
| Olive nut                 |     | 12.63        |                     |                     | 76.3         | 75            |                | 0.039                       | Synthetic<br>wastewater | Column         | (Capodici et al.,<br>2018)    |
| Wine industry by-products | 7   |              | 15                  | 6.2                 |              | 12.04         |                |                             | Synthetic<br>wastewater | Column         | (Carrey et al.,<br>2018)      |
| Cotton                    |     |              | 23                  | 9.5                 |              | 110           |                | 190                         | Synthetic<br>wastewater | Column         | (Della Rocca et al.,<br>2006) |
| Cotton + Steel wool       |     |              | 23<br>18.3<br>±0.54 | 9.5                 |              | 110           |                | 236                         | Synthetic<br>wastewater | Column         | (Della Rocca et al.,<br>2006) |
| Citrus Woodchip           |     | 24           | °C                  | 7.6<br>6.9 -<br>7.8 | 80 -95       | 40 -50        | 10             | 18.9                        | Brine<br>Synthetic      | Batch          | (Díaz-García et al.,<br>2021) |
| Maple/birch               |     | 6            |                     |                     | 88           | 7.6           | 6.68           |                             | Stormwater              | Pilot          | (Ergas et al., 2010)          |

|                                 |    |          |              |      |                 |       |       |                           |                       |
|---------------------------------|----|----------|--------------|------|-----------------|-------|-------|---------------------------|-----------------------|
| Corn cob                        |    |          |              | 92.5 | 3.88 -<br>4.79  | 4     |       |                           | (Feng et al., 2017)   |
| Acid pre-treated corn cob       |    |          |              | 94.1 | 3.88 -<br>4.79  | 4.2   |       |                           | (Feng et al., 2017)   |
| Alkaline pre-treated corn cob   |    |          |              | 93.3 | 3.88 -<br>4.79  | 4.2   |       |                           | (Feng et al., 2017)   |
| Rice straw                      | 24 | 25 +/- 2 |              | 91.2 | 3.88 -<br>4.879 | 4     |       | Synthetic Wastewater      | (Feng et al., 2017)   |
| Acid pre-treated rice straw     | 24 |          |              | 95   | 3.88 -<br>4.79  | 4.2   |       |                           | (Feng et al., 2017)   |
| Alkaline pre-treated rice straw | 24 |          |              | 85.1 | 3.88 -<br>4.79  | 4.3   |       |                           | (Feng et al., 2017)   |
| Rice hull                       | 24 |          |              | 56.4 | 3.88 -<br>4.79  | 2.2   |       |                           | (Feng et al., 2017)   |
| Acid pre-treated rice hull      | 24 |          |              | 77.3 | 3.88 -<br>4.79  | 3.2   |       |                           | (Feng et al., 2017)   |
| Alkaline pre-treated rice hull  | 24 |          |              | 81.3 | 3.88 -<br>4.79  | 4.1   |       |                           | (Feng et al., 2017)   |
| Mulch                           |    | 22 - 24  | 6.2 -<br>7.4 | 89.7 | 32.2            | 0.066 |       | Spiked groundwater Batch  | (Gibert et al., 2008) |
| Softwood                        |    | 22 - 24  | 6.2 -<br>7.4 | 98.7 | 32.2            | 0.067 | 0.067 | Spiked groundwater Batch  | (Gibert et al., 2008) |
| Softwood                        |    | 22 - 24  | 6.6 -<br>7.7 | 96   | 50              | 0.022 | 0.034 | Spiked groundwater Column | (Gibert et al., 2008) |
| Hardwood                        |    | 22 - 24  | 6.2 -<br>7.4 | 98.7 | 32.2            | 0.035 | 0.035 | Spiked groundwater Batch  | (Gibert et al., 2008) |
| Coniferous                      |    | 22 - 24  | 6.2 -<br>7.4 | 95.1 | 32.2            | 0.048 | 0.048 | Spiked groundwater Batch  | (Gibert et al., 2008) |
| Willow                          |    | 22 - 24  | 5.5 -<br>6   | 86.3 | 32.2            | 0.056 | 0.026 | Spiked groundwater Batch  | (Gibert et al., 2008) |
| Leaves                          |    | 22 - 24  | 6.2 -<br>7.4 | 93.9 | 32.2            |       | 0.217 | Spiked groundwater Batch  | (Gibert et al., 2008) |
| Compost                         |    | 22 - 24  | 6.2 -<br>7.4 | 92.7 | 32.2            |       | 0.026 | Spiked groundwater Batch  | (Gibert et al., 2008) |

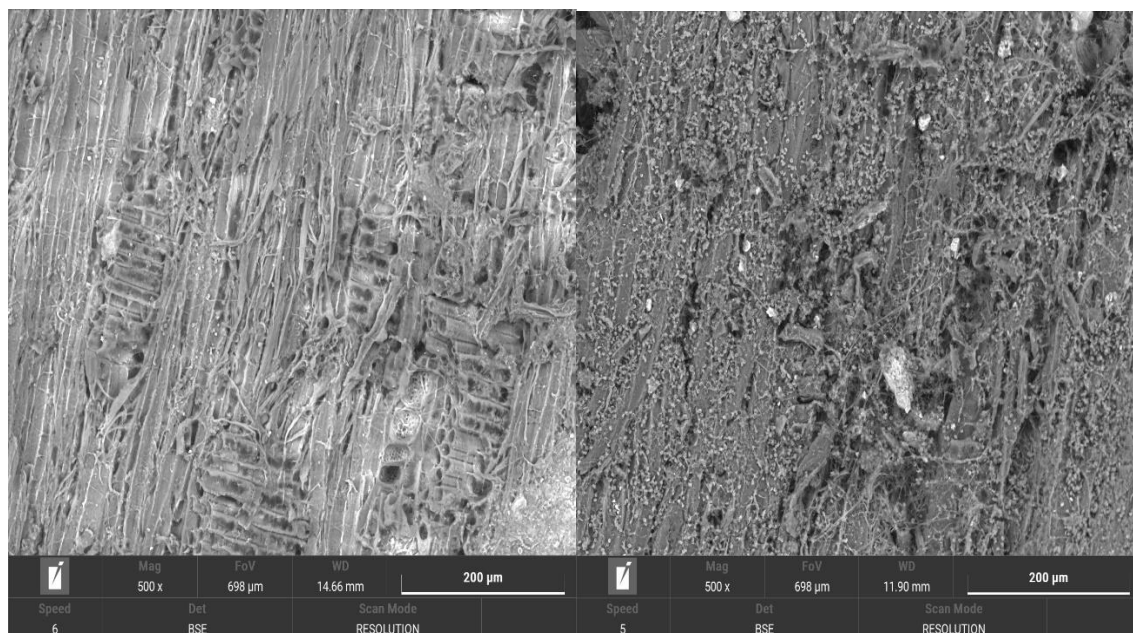
|                                |       |         |         |   |       |       |       |                      |                    |                              |
|--------------------------------|-------|---------|---------|---|-------|-------|-------|----------------------|--------------------|------------------------------|
| Cornstalks                     | 42.6  |         |         |   | 405   | 39.4  | 2.88  | Spiked groundwater   | Batch              | (Greenan et al., 2006)       |
| Cardboard                      | 280.3 |         |         |   | 150   | 10.1  | 1.05  | Spiked groundwater   | Batch              | (Greenan et al., 2006)       |
| Woodchips                      | 448.9 |         |         |   | 75    | 16    |       | Spiked groundwater   | Batch              | (Greenan et al., 2006)       |
| Fresh hardwood                 |       | 10.9    | 21.5    |   | 39    | 30    | 15.6  | Synthetic Wastewater | Up-flow packed bed | (Hoover et al., 2016)        |
| Weathered hardwood             |       | 12.4    | 20      |   | 52    |       | 21    |                      |                    | (Hoover et al., 2016)        |
| Untreated sawdust              |       |         |         |   | 56.64 |       | 29.3  |                      | Packed Column      | (Hu et al., 2019)            |
| Lime treated sawdust           |       | 24 (12) | 25 - 35 | 7 | 100   |       | 131.9 | Synthetic wastewater |                    | (Hu et al., 2019)            |
| Peracetic-acid treated sawdust |       |         |         |   | 100   |       | 140.6 |                      | Batch              | (Hu et al., 2019)            |
| Sand coated oil                |       |         |         |   | 20    | 21    |       |                      | Open top barrier   | (Hunter, 2001)               |
| Wheat straw                    |       |         |         |   | 22.6  |       | 53    |                      | Column             | (Ine et al., 1998)           |
| Wheat straw                    |       |         |         |   | 22.6  |       | 0.31  |                      | Column             | (Ine et al., 1998)           |
| Biodegradable plastic          |       |         |         |   | 50    | 40.27 |       |                      | Column             | (Zhang et al., 2012)         |
| Brewery spent grain            | 2.3   |         |         |   | 95    |       |       |                      |                    | (Kaplan et al., 1987)        |
| Hydrated soluble potato solids | 1.7   | 96      |         |   | 95    |       |       |                      |                    | (Kaplan et al., 1987)        |
| Softwood                       |       |         |         |   | 75.2  | 33.4  | 5.84  | Stormwater           | Batch/Column       | (Lopez-Ponnada et al., 2017) |
| Hardwood                       |       |         |         |   | 63    | 11.6  | 3.45  | Stormwater           | Batch/Column       | (Lopez-Ponnada et al., 2017) |
| Hardwood                       |       |         |         |   |       | 2.3   | 2.29  |                      | Column             | (Lynn et al., 2015)          |
| Hardwood Mix                   |       | 201.6   |         |   |       |       | 1.6   |                      | Groundwater Trench | (Manca et al., 2020)         |

|                                       |     |           |              |         |            |                       |                      |                             |
|---------------------------------------|-----|-----------|--------------|---------|------------|-----------------------|----------------------|-----------------------------|
| Hardwood                              | 2   |           | 9.0 ± 3.69   |         | 9.0 ± 4.01 | Agriculture Drainage  |                      | (Martin et al., 2019)       |
| Hardwood                              | 8   |           | 32.1 ± 5.18  |         | 8.5 ± 2.42 | Agriculture Drainage  |                      | (Martin et al., 2019)       |
| Hardwood                              | 16  |           | 53.8 ± 17.55 |         | 7.4 ± 2.52 |                       |                      | (Martin et al., 2019)       |
| a ~70/30 mixture of hardwood/softwood | 4.7 |           | >80          | 6.4     | 0.3        | Surface drainage      | Paired Bioreactor    | (Maxwell et al., 2022)      |
| PHBV                                  |     | 25        | 7 - 7.6      | 80      |            |                       | Fixed bed            | (Mergaert et al., 2001)     |
| softwood                              |     |           |              | 3       | 1.44       | Synthetic stormwater  | Column               | (Peterson et al., 2015)     |
| Maple                                 |     |           |              | 3       | 1.86       | Synthetic stormwater  | Column               | (Peterson et al., 2015)     |
| Wild cherry                           |     |           |              | 3       | 1.08       | Synthetic stormwater  | Column               | (Peterson et al., 2015)     |
| Oak                                   |     |           |              | 3       | 1.86       | Synthetic stormwater  | Column               | (Peterson et al., 2015)     |
| Beech                                 |     |           |              | 3       | 0.96       |                       | Column               | (Peterson et al., 2015)     |
| Polycaprolactone                      |     | 30        |              | 31.66   |            |                       |                      |                             |
| Polycaprolactone                      | 2.5 | 28+/- 0.5 | 94           | +/- 3.3 | 0.94       | 29.7                  | Synthetic Wastewater | (Shen et al., 2020)         |
| Hydrolysed molasses                   |     |           |              |         |            |                       |                      | (Zhang et al., 2016)        |
| Woodchips                             |     |           |              | 22.6    | 155.52     |                       | SBR                  | (Quan et al., 2005)         |
| Waste cellulose solids                |     |           | 7.3          | 4.8     | 1.04       | Agricultural drainage | Bed                  | (Robertson & Merkley, 2009) |
| Waste cellulose solids                |     |           |              | 74      | 1.2        | 0.2                   | 0.7                  | (Robertson et al., 2000)    |
| Waste cellulose solids                |     |           |              | 58      | 4.8        | 2                     |                      | (Robertson et al., 2000)    |
| Waste cellulose solids                |     |           |              | 80      | 57         | 11.6                  | 2.6                  | (Robertson et al., 2000)    |

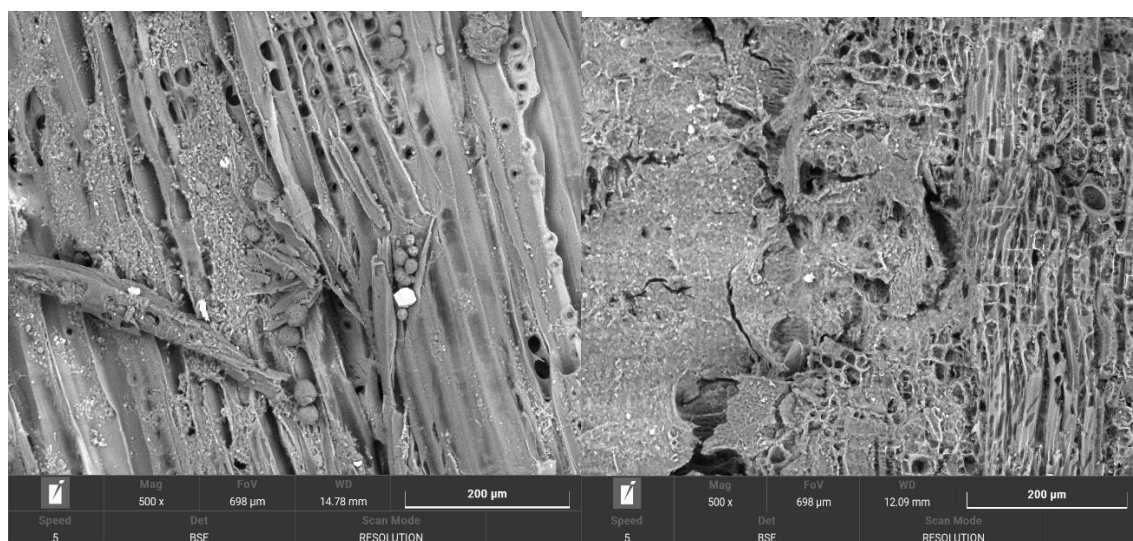
|                                  |       |         |           |           |            |           |                            |                       |                      |                                 |
|----------------------------------|-------|---------|-----------|-----------|------------|-----------|----------------------------|-----------------------|----------------------|---------------------------------|
| Crab shell chitin                |       |         | 8.5       |           | 22.7       |           | 2.4                        | Synthetic groundwater | Batch                | (Robinson-Lora & Brennan, 2009) |
| Crab shell chitin                | 16.2  |         | 6.5       |           | 22.7       |           | 24.5                       | Synthetic groundwater | Column               | (Robinson-Lora & Brennan, 2009) |
| Woodchips                        |       | 15 - 26 |           |           | 53         |           | 1.4                        | Dairy effluent        | Bed                  | (Schipper et al., 2010)         |
| Sawdust                          |       |         |           |           | 50         |           | 1.4                        | Dairy effluent        | Walls                | (Schipper et al., 2005)         |
| Washed gravels + Birch woodchips | 93.6  |         |           | 96 +/- 6  |            |           | 1.4 +/- 0.7                |                       |                      | (Šereš et al., 2019)            |
| Washed gravel + Spruce woodchips | 103.2 |         |           | 85 +/- 20 |            |           | 1.2 +/- 0.7                |                       |                      | (Šereš et al., 2019)            |
| Activated sludge                 | 6     | 18 - 23 |           |           | 15         |           |                            | WWTP effluent         | UASB                 | (Sun et al., 2017)              |
| Wood particles                   |       |         |           |           | 11.8       | 3.9       |                            | Agricultural drainage | Beds                 | (Van Driel et al., 2006)        |
|                                  |       |         |           |           |            |           | 5.20 ± 0.02 to 8.96 ± 0.19 |                       |                      |                                 |
| Fresh willow woodchips           | 10    |         |           | 98        |            |           |                            | Aquaculture           | Horizontal Flow      | (Von Ahnen et al., 2016)        |
| Hardwood                         | 26    |         |           |           | 5.3 ± 0.2  | 1.0 ± 0.5 | 4.8 ± 2.3                  | Aquaculture           | horizontal down-flow | (Von Ahnen et al., 2018)        |
| Hardwood                         | 16    |         |           |           | 10.5 ± 3.5 | 7.5 ± 2.6 | 4.5 ± 1.9                  | Aquaculture           | vertical down-flow   | (Von Ahnen et al., 2018)        |
| Hardwood                         | 15    |         |           |           | 9.5 ± 2.8  | 4.9 ± 2.5 | 7.8 ± 2.1                  | Aquaculture           | vertical down-flow   | (Von Ahnen et al., 2018)        |
| Biodegradable snack ware         | 2     | 25      | 7.4 - 7.9 | 100       | 50         | 0.01      |                            | Synthetic groundwater | Continuous flow      | (Wang & Wang, 2011)             |
| Corncoobs                        |       | 29      | 8.5       | 87        | 25.3       | ~3.5      | 203                        | Synthetic water       | Column               | (Xu et al., 2009)               |
| Polycaprolactone                 |       | 30      |           | 87        | 50         |           |                            | Synthetic wastewater  | Batch                | (Shen et al., 2015)             |
| Starch/PCL                       |       | 30      |           | 97        | 50         |           |                            | Synthetic wastewater  | Batch                | (Shen et al., 2015)             |
| Starch/PCL                       | 0.5   | 25      |           |           | 15         |           | 640                        | Synthetic wastewater  | Packed bed           | (Shen et al., 2013)             |

|                                             |      |    |      |       |         |       |                      |                 |                     |
|---------------------------------------------|------|----|------|-------|---------|-------|----------------------|-----------------|---------------------|
| Starch/Polylactic acid                      |      |    |      | 50    |         | 607   | Synthetic wastewater | Packed bed      | (Shen et al., 2013) |
| Poly (butanediol succinate)                 | 2    | 25 | 7    | 50    | 1.92    | 600   | Synthetic wastewater | Packed bed      | (Shen et al., 2016) |
| poly(3-hydroxybutyrateco-3-hydroxyvalerate) |      |    | 5.68 |       | 0.73    |       |                      |                 |                     |
| (PHBV)                                      | 3    |    | -    |       | +/_     |       | WWTP Wastewater      | Continuous flow | (Yang et al., 2020) |
|                                             |      |    | 6.95 | 92.36 | 9.53    | 0.86  |                      |                 |                     |
|                                             |      |    |      |       |         | 0.99  |                      |                 |                     |
|                                             |      |    |      |       |         | +/-   | WWTP Wastewater      |                 | (Yang et al., 2020) |
| PHBV- Rice Hull                             | 3    |    |      | >95   |         | 0.95  |                      |                 |                     |
|                                             |      |    |      |       |         | 0.32  |                      |                 |                     |
|                                             |      |    |      |       |         | +/-   | WWTP Wastewater      |                 | (Yang et al., 2020) |
| PHBV-Sawdust                                | 3    |    |      | 96.64 |         | 0.38  |                      |                 |                     |
|                                             |      |    |      |       | 50.57 ± |       | Synthetic Wastewater |                 | (Zhao et al., 2020) |
| Woodchip and quartz sand                    | 9.16 |    |      | 95.4  | 1.32    | <5.00 |                      |                 |                     |

## Appendix C Images from Microscopy



**Figure C-1 500x SEM images of hardwood (a)before reactor (b)after 168 days**



**Figure C-2 500x SEM images of softwood (a) before reactor (b)after 168 days**



**Figure C-3 SEM image of microbial activity on softwood surface**