Evaluation of Fenton Pretreatment and MBBR Performance for Leachate Biodegradability Improvement

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Abstract:

The Master's thesis investigated the impact of Fenton oxidation, as a pretreatment method to enhance the biodegradability of landfill leachate, hence improve subsequent treatment performance in a Moving Bed Biofilm Reactor (MBBR). Leachates with varying COD levels were screened for optimal Fenton conditions (acidic pH, Fe^2+:H_2O_2 ratio of 1:5, 30-minute reaction time), and subsequently applied prior to 24-hour MBBR experiments. Fenton treatment led to significant COD reductions in the Denitrification reactors—up to 73% for Odense and 68% for Aarhus leachates. The results from OUR measurements, TOC, and OX_C suggested that the treated leachates were already highly biodegradable. Moreover inhibition analysis showed no detectable inhibitory elements. These results implied characteristics of young leachates. This indicates that Fenton primarily removed readily degradable organics, rather than converting refractory compounds into more bioavailable forms. In general the MBBR showed good performance, due ammonium oxidization and COD reductions.

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Appendix: 13
Hand in: 4th of June 2025

Preface

This Master's Thesis is produced by Reena Bausram Mosebo for the 4th semester of the Masters program of Water and Environmental Engineering at Aalborg University. The focus of the project was to investigate the effectiveness of Fenton pretreatment and MBBR performance in improving the biodegradability of landfill leachate. Most of the experiments were conducted in *Miljøteknik* department involved in wastewater treatment projects in the Danish Technological Institute (DTI), Aarhus. Additional analyses were conducted at the AAU Water Laboratory facilities in both Esbjerg and Aalborg. The project spanned from 3rd February 2025 to 4th June 2025.

I would like to express my gratitude to Caroline Kragelund Rickers and Jesper Gemke along my colleagues at the *Miljøteknik* department at DTI, for their valuable guidance and feedback throughout the project. In addition, special thanks are extended to Flemming Husum of the Marselisborg Wastewater Treatment Plant, Aarhus Vand, for promptly providing activated sludge when needed.

My sincere appreciation goes to my supervisors AAU: Jens Muff and Asbjørn Haaning Nielsen for their constructive feedback, and expert guidance throughout this master's thesis. A special appreciation to Peter Roslev, from the Department of Chemistry and Bioscience, AAU, for his time, and valuable feedback, which made it possible to carry out the inhibition analysis.

Reading Guide

The project report is divided into chapters representing the different assignments that the student has worked on. All material that is not produced by the student, is cited by [Surname, Year]. The citation is referred to the bibliography found at the end of the report, where a full list of all references can be found.

All figures, tables, and equations are numbered according to the chapter where the figure, table, or equation is found. Thereby, the first figure in the first chapter is named 1.1, the second figure in the first chapter is 1.2, and so on.

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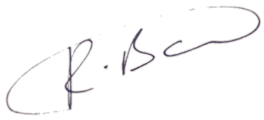

Reena Bausram Mosebo

Table of Contents

1 Introduction

In Denmark, landfill leachate management is distributed as follows: 49% is treated at municipal treatment facilities, 33% is treated on-site, 10% is recirculated within the landfill, and 8% is handled through alternative methods [Dakofa, 2024]. The EU Landfill Directive 1999/31/EC mandates strict operational standards for landfill sites, including effective sealing systems, leachate collection, and treatment. While landfill waste across the EU has declined from 23% to 16% [EEA, 2024], leachate management remains a significant challenge. Treating leachate on-site is often challenging, so it is commonly sent to municipal wastewater treatment plants. However, this approach is not the most effective, as leachate often contains a complex mix of hazardous substances and an increasing concentration of micropollutants over time. Therefore, wastewater treatment plants face the added challenge of managing contaminated sludge while ensuring consistent water quality.

Micropollutants (MP) such as Per- and polyfluoroalkyl substances (PFAS), heavy metals and Polycyclic Aromatic Compounds (PAHs) are persistent contaminants that pose serious risks to both the environment and human health. They are frequently found in landfill leachate due to their widespread use in industrial and consumer products. These substances pose significant challenges for wastewater treatment systems as they resist degradation and tend to accumulate in lakes, coast and streams [DTI3, 2024]. Leachate is a black or brown liquid formed when rainwater filters landfill waste, carrying harmful substances such as organic matter, heavy metals, nitrogen compounds, and toxic chlorinated organics, which pose environmental risks [Saxena et al., 2022]. Additionally, leachate requires nutrient treatment and removal of nitrogen and phosphorus.

A major challenge in leachate treatment is there is low concentration of biodegradable organic content combined with high concentrations of refractory hazardous substances. The reduced level of biodegradable organic material in leachate limits the efficiency of biological treatment processes that rely on microorganisms to degrade organic matter as well as the subsequent adsorption on activated carbon or resins required for PFAS removal. Additionally, the presence of toxic or refractory substances can inhibit microbial activity [Remmas et al., 2023]. Heavy metals can poison microbial cells, while PFAS compounds, known for their persistence, are harmful to humans, resist degradation by conventional biological methods [Grgas et al., 2023].

When treating leachate with regards to PFAS removal, simple and robust techniques such as flocculation, precipitation, and adsorption using activated carbon or other adsorbents (like resins) are often preferred. However, to enhance the efficiency of these methods, it is crucial to reduce chemical oxygen demand (COD). High levels of organic matter, which is measured as COD in mg/L can compete with micropollutants, which are measured in µg/L or ng/l, for adsorption sites and precipitation reagents [Goukeh et al., 2025]. Therefore, the hypothesis is that micropollutant removal techniques will be more effective if the leachate is pre-treated using the Fenton process. This treatment can also make recalcitrant organic matter more biodegradable, allowing it to be processed in a moving bed biofilm reactor. A treatment technology that supports the development of a healthy biofilm capable of removing nitrogen through nitrification/denitrification.

1.1 Problem Statement

Therefore, this project aims to evaluate the impact of Fenton oxidation as a pretreatment method to enhance biodegradability, enabling the effective use of a Moving Bed Biofilm Reactor (MBBR) through assessment of potential for nitrification and denitrification processes. This study also look into any inhibitory effects of the leachate before and after both Fenton oxidation and MBBR treatment. Hence, the problem statement is:

What is the effect of using Fenton oxidation as a pretreatment to improve biodegradability in MBBR technology for leachate treatment?

In the process, the following questions will be investigated:

- Can Fenton help reduce inert COD and hence improve the biodegradability of leachate?
- What is the suitable dosage of hydrogen peroxide (H_2O_2) and iron (II) sulfate ($FeSO_4$) for Fenton oxidation in the screening of the different leachates?
- What is the nitrate removal rate in MBBR (aerobic and anaerobic treatment)?
- Are there any inhibitory elements before and after Fenton oxidation and MBBR for leachate treatment?
- Is there a difference in using a leachate with high COD compared to one with low COD?

1.2 Project Strategy

Addressing the problem statement requires testing different combinations of the H_2O_2 and $FeSO_4$ dosage as pretreatment, and running the pre-treated through a lab-scale MBBR reactor. The MBBR is used to show nitrification and denitrification processes. The following steps are taken to analyze of the four leachates from the Norrecco landfill:

1. Water quality of the different leachates
2. Testing reagents dosage for different COD target reductions (30%, 40%, 60%, 80%)
3. Testing $FeSO_4$ dosage with regards to H_2O_2 dosage (1:5 and 1:4 molar ratios)
4. Measurement of Total Organic Carbon (TOC) and COD before and after Fenton oxidation
5. Measurement of nitrates and ammonium before and after MBBR
6. Inhibition analysis before and after the chemical and biological treatment

1.3 MFS Eliminator

This master's thesis project is in collaboration with Danish Technological Institute (DTI), Aarhus within the environment technology division under the water technology center. In DTI, the MFS Eliminator lighthouse project is a Danish Environmental Protection-approved project under the MUDP (*Miljøteknologisk Udviklings- og Demonstrationsprogram*) to build a full-scale plant for reduction of micropollutants directly at the source point in leachate treatment. This data-driven project has also as goals to minimize the amount of water needed for leachate treatment and implement a cleaning solution at the source [DTI1, 2024]. Figure 1.1 demonstrates the different phases of an MUDP project at DTI, and the master's project is part of work package 2-3 (AP2-3), which focuses on optimization in the LAB and pilot scale development [DTI2, 2023].

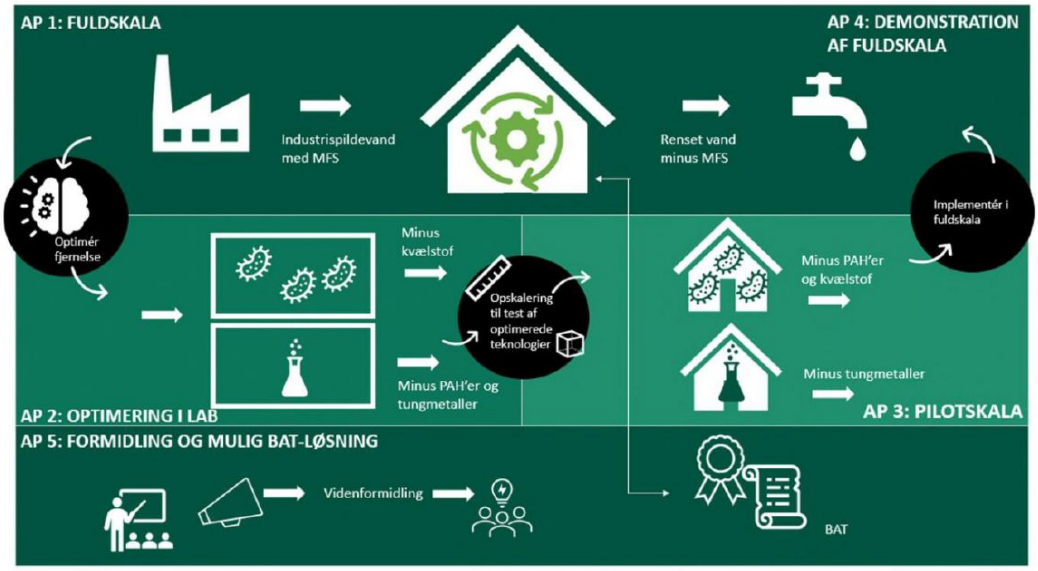

Figure 1.1: An MUDP project schema[DTI2, 2023]. (AP 1)Fullscale, (AP 2)Optimisation in lab, (AP 3) Pilot scale, (AP 4) Demonstration at fullscale and (AP 5) Knowledge sharing and possible BAT solution

This project aims to apply Fenton oxidation as a pretreatment for various leachates to reduce COD and improve their biodegradability. The effectiveness of this approach will be evaluated through the integration of MBBR system. The results will contribute to the MUDP lighthouse project, and help with the design and development of a full-scale treatment plant.

The key partners in the Lighthouse MFS-Eliminator project include DTI, Norrecco, MUTAG, and AAU (Department of Chemistry and Biosciences). MUTAG, with over 20 years of expertise in biological wastewater treatment, introduced its MUTAG BioChip in 2008. This biochip, or carrier, is utilized in the bioreactor to support microbial growth, which degrades the organic and inorganic materials in the leachate.

Norrecco, a landfill company handles soil and construction waste, focusing on resource recycling [Norrecco, 2024]. The waste primarily includes wood, plastics, soil, and building demolition debris. These materials are sorted, cleaned, and then sent to various locations for further use or processing. The central treatment plant is located in Copenhagen at Prøvestenen

and Nordhavn, with additional treatment facilities spread across Denmark, including in Greve, Lynge, Odense, Kolding, Aarhus, and Agerskov. The leachates to be tested are from Copenhagen, Odense and Aarhus sites.

1.4 Project Roadmap and Timeline

Figure 1.2 serves as a roadmap for processes involved in the thesis project.

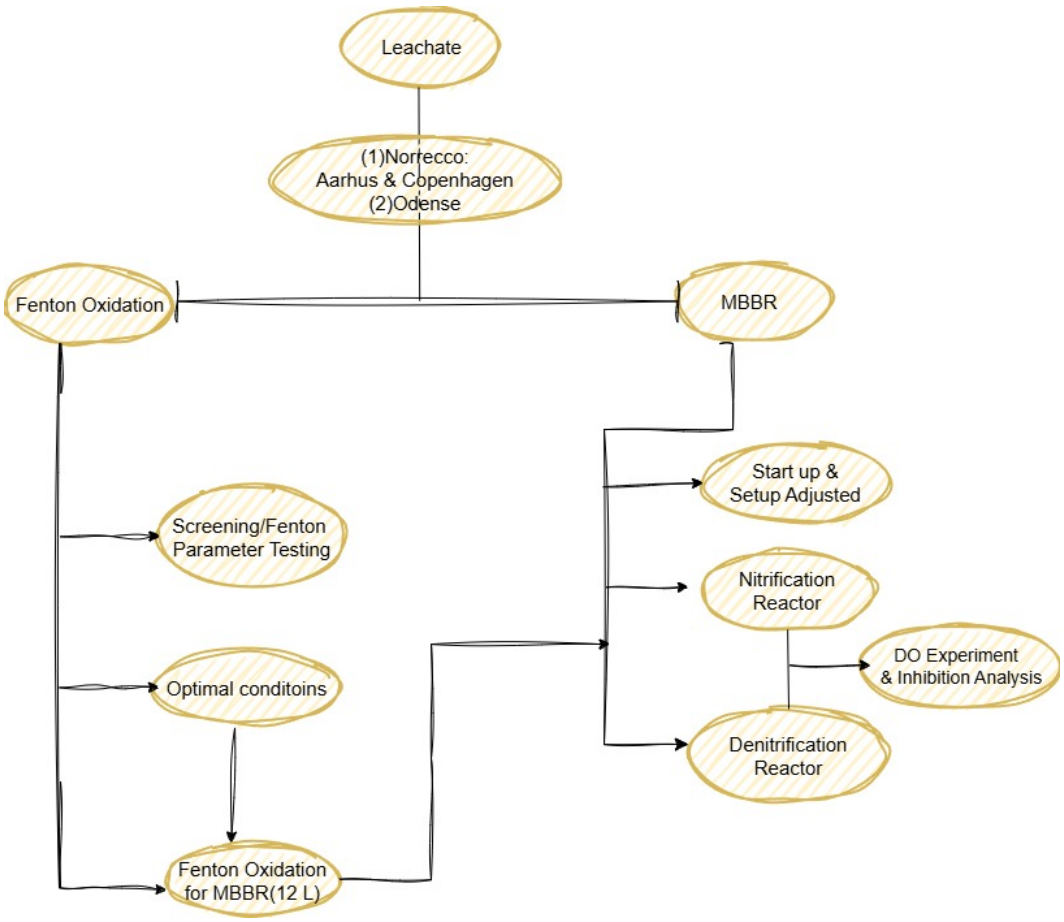

Figure 1.2: Main Tasks: Fenton Oxidation and MBBR lab setup

The schematic diagram outlines the main tasks and progress of the project workflow, along with the purpose of each step, as detailed in Chapter 3.

This study is conducted between the 2nd of February and 4th June 2025 for approximately 16 weeks. The Ghantt chart below shows the schedule and main activities for finding the Fenton optimal conditions, testing the MBBR setup, applying the optimal condition as leachate pretreatment, and treating the leachate through the MBBR systems (see Appendix A).

2 | Literature Review

2.1 Fenton Oxidation

2.1.1 Application of Fenton Oxidation

Fenton oxidation is a powerful advanced oxidation process widely applied for the treatment of industrial and municipal wastewater, especially for effluents containing recalcitrant organic contaminants. In the textile industry, Fenton oxidation has synthetic color removal rates that can exceed to 90% and COD reductions between 61% and 85% [Bury et al., 2024; Mahtab et al., 2021]. Heterogeneous Fenton applications, that is the iron catalyst in solid form can further achieve up to 94% color and 81% COD removal [Mahtab et al., 2021].

Homogeneous Fenton oxidation, where the iron catalyst is in soluble form, effectively targets phenolic compounds and high-strength organic loads that account for up to 75% COD removal in the olive oil sector [Aramyan & Moussavi, 2017]. The pulp and paper industry benefits from solar photo-Fenton processes, which achieve rapid COD reductions of 85 to 92%, demonstrating robustness against highly colored and lignin-rich wastewater [Aramyan & Moussavi, 2017; Ziembowicz & Kida, 2022]. Pharmaceutical wastewater, often loaded with micro-pollutants such as antibiotics, also responds well to Fenton-based treatments. These systems can remove 80–97% of total organic carbon (TOC) with complete decontamination of antibiotics and micro-pollutants [Aramyan & Moussavi, 2017; Ziembowicz & Kida, 2022].

Numerous experimental studies have demonstrated that toxic and recalcitrant compounds can be broken down by the Fenton process into low-molecular-weight organic compounds, which typically exhibit enhanced biodegradability [Almudena Vilar & Veiga, 2013; Chen et al., 2007; Lopez et al., 2004; Mahtab et al., 2021]. As a result, the Fenton process is frequently applied to improve the biodegradability of landfill leachate. Using Fenton's reagent in different forms, the removal of COD from landfill leachate has been reported to be 55– 81% and TOC removal to 71%, with a better BOD / COD ratio, making further biological treatment more feasible [Mahtab et al., 2021]. Deng and Zhao [2015] also found out that Fenton processes have COD removal efficiencies between 31% and 95% for 71% for landfill leachate. However, Fenton oxidation is less effective at ammonia-nitrogen removal therefore, is is often used as pretreatment step with 90% removal of UV-absorbing organic matter, which helps downstream disinfection in wastewater plants [Deng & Zhao, 2015].

2.1.2 Fenton Oxidation Reactions

Fenton oxidation is a radical chain reaction that uses reagents such as hydrogen peroxide (H_2O_2) and iron salts (commonly ferrous sulfate, $FeSO_4$), as catalyst to generate hydroxyl radicals (•OH) [Walling et al., 2021]. These radicals are highly reactive and the second strongest oxidant, that can degrade a variety of organic pollutants [Tagga et al., 2017; Walling et al., 2021]. The initial Fenton reactions, that is hydroxyl generation [Bury et al., 2024] can be described as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^- \tag{2.1}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \tag{2.2}$$

The generated hydroxyl radicals, (•OH) can break down complex, recalcitrant contaminants into smaller, non-toxic byproducts, such as water and carbon dioxide. The Equation (2.2) is a much slower process, where ferric ions is reduced to ferrous ions by reaction with (H_2O_2) [Bricker et al., 2014]. Other reactions represent propagation and determination stages for the chain reaction. The key parameters such as pH, reagent concentrations, and reaction time are factors that influence the effectiveness of the process [Bricker et al., 2014; Tagga et al., 2017; Walling et al., 2021].

2.1.3 Experimental Parameters

The optimal conditions for the key parameters are found experimentally to ensure effective pollutant degradation. Table 2.1 presents the optimal condition ranges for the specific characteristics of wastewater.

Table 2.1: Experimental parameters and recommended values

Parameters	Values	Recommended	Authors
Mass ratio COD:H_2O_2	0.5:1 to 1:4	1:1	[Bury et al., 2024]
Ratio H_2O_2:Fe^{2+}	1:1 to 1:10	1:4	[Bury et al., 2024]
pH	3.0 and 2.5-3.5	3.0	[Bury et al., 2024; Kumari & Kumar, 2023; Walling et al., 2021]
Reaction Time	30-60 minutes	–	[Bury et al., 2024; Ziembowicz & Kida, 2022]

The dosage of H_2O_2 and Fe^{2+} can be determined theoretically based on pollutant indicators such as Total Organic Carbon (TOC), Chemical Oxygen Demand (COD) or Biological Oxygen Demand (BOD) [Bury et al., 2024]. In order to maximize the decomposition of organic compounds, it is essential to select the appropriate ratios of reactants, and organic compounds present in the sample wastewater [Payandeh et al., 2017].

2.1.4 Fenton Process

The 2.1 below illustrates the Fenton oxidation process, which is designed to achieve a 40% reduction in COD.

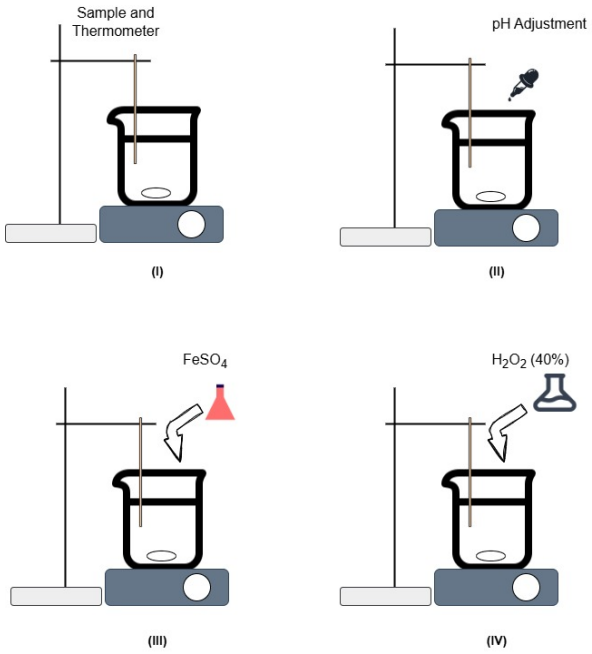

Figure 2.1: The Fenton process at 40% dosage

After the theoretical estimation of the required hydrogen peroxide and iron sulfate dosages, the Fenton oxidation process proceeds in two main steps. First, the pH is adjusted, followed by the addition of the reagents: iron sulfate is added first, followed by hydrogen peroxide, as illustrated in Figure 2.1.

The scavenging effect is undesirable consumption of hydroxyl radicals, •OH, by excess hydrogen peroxide, H_2O_2, or Fe^{2+}, and this can diminish the overall efficiency of the Fenton process [Bury et al., 2024; Mahtab et al., 2021]. Therefore, careful optimization of Fe^{2+} and hydrogen peroxide dosage is essential to enhance pollutant removal while minimizing waste generation and operational costs. In addition, chlorides or carbonates can react with hydroxyl radicals, leading to radical scavenging, which can decrease Fenton oxidation efficiency [Deng & Zhao, 2015].

Fenton oxidation process is cost-effective and practical due to inexpensive, moderately reactive, and easily manageable reagents (Fe^{2+} and H_2O_2) [Aramyan & Moussavi, 2017]. In addition, its simplicity and flexibility can allow for a standalone or hybrid system with integration into existing water treatment methods such as coagulation, filtration, and biological oxidation [Aramyan & Moussavi, 2017]. However, a major drawback is the generation of iron sludge, which needs disposal, and increases risks of cross contaminations, thereby can lead to additional costs [Deng & Zhao, 2015; Mahtab et al., 2021]. Moreover, the process can produce toxic byproducts if not carefully controlled, with low pH and high chemical dosages raise operational costs and limit scalability [Mahtab et al., 2021; Ziembowicz & Kida, 2022].

2.1.5 Oxidation Level of Carbon (OX_C) in Wastewater Treatment

Hvitved-jacobsen [2002] describes the oxidation level of carbon (OX_C) as a quantitative measure for assessing the redox state of organic matter in wastewater. This parameter is derived from the stoichiometric relationship between chemical oxygen demand (COD) and total organic carbon (TOC), and is given by:

$$OX_C = 4 - 1.5 \cdot \frac{COD}{TOC} \tag{2.3}$$

The redox level serves as an indicator of substrate quality, but on its own, it does not fully determine biodegradability. A reduced substrate, characterized by a negative OX_C value—for example, fatty acids (around –1 to –2), ethanol (–1.5), and glucose or acetate (approximately 0)—tends to offer more energy [Hvitved-jacobsen, 2002]. In contrast, more oxidized substrates, with a positive OX_C, provide less energy and are often less favorable for microbial degradation.

2.2 Moving Bed Biofilm Reactor (MBBR)

2.2.1 MBBR

The nitrification and denitrification processes play a vital role in nitrogen cycling by removing excess ammonium and nitrate from ecosystems. In a Moving Bed Biofilm Reactor (MBBR), these processes are effectively carried out, preventing the accumulation of these compounds and thus reducing the risk of pollution in natural water systems.

The MBBR, as a biological treatment has become widely adopted primarily due to its cost-effective and eco-friendly approach to nitrogen treatment [M.Safwat, 2018; Pan et al., 2022]. Microorganisms play an essential role in the biodegradation of organic contaminants. Landfill leachate contains a a high concentration of organic matter and NH_3-N, and its treatment produces carbon dioxide (CO_2) and sludge as the end product. In principle, MBBR operates similarly to activated sludge, however biofilm are formed on the carriers, that move freely in water, which are contained within the reactor by sieves positioned at the outlet [R.Vieira et al., 2022]. A possible design representing this versatile biological wastewater treatment method is shown in Figure 2.2.

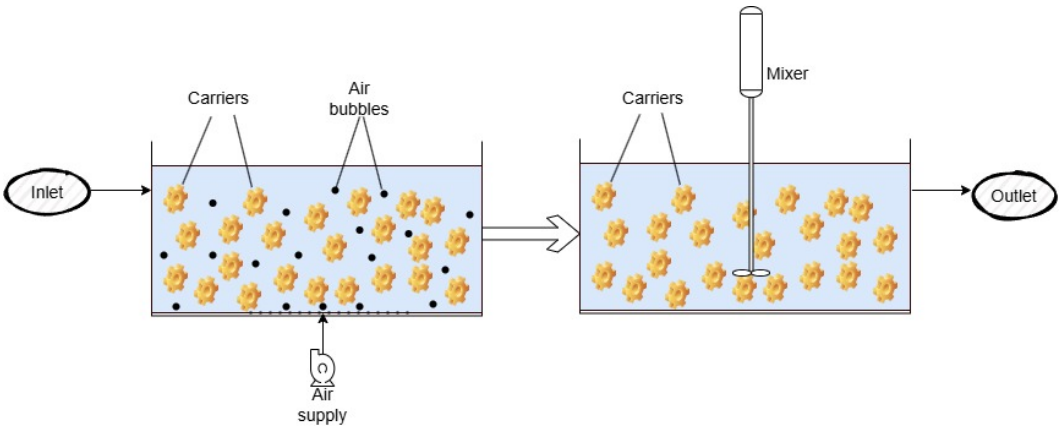

Figure 2.2: An MBBR setup illustration [Bausram, 2024]

The process is initiated by inoculation with sludge from wastewater treatment plants, which supply the microorganisms that form the biofilm on the carriers [M.Safwat, 2018]. MBBR is used for aerobic, anoxic, and anaerobic applications. Nitrifying bacteria enable the nitrification process as illustrated in the first tank then to the denitrification tank, where nitrates are removed under anoxic conditions. The biofilm's higher active biomass concentration enhances efficiency. According to R.Vieira et al. [2022], there is 85% to 95% biological oxygen demand (BOD) removal with 25% to 30% nutrient reduction using MBBR technology, which effectively treat urban wastewater, industrial effluents, and micropollutants. MBBR includes no sludge recycling, full reactor utilization for biomass growth, low head losses, and no need for periodic backwashing [Gupta et al., 2022; M.Safwat, 2018].

In biological wastewater treatment, a nitrification buffer is applied to maintain optimal conditions for ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) [Rossi et al., 2015]. These bacteria require stable pH and alkalinity levels to operate efficiently, as the process of converting ammonium to nitrate consumes alkalinity. The buffer stabilizes these conditions, preventing significant pH drops that can hinder bacterial activity during

nitrification. Meanwhile, an acetate buffer serves as an external carbon source and as an electron donor [Rossi et al., 2015], primarily in denitrification processes. Denitrifying bacteria rely on an easily accessible carbon source to convert nitrate into nitrogen gas under anoxic conditions.

2.2.2 Nitrification/Denitrification

Nitrification is the first step in the biological treatment of nitrogen under aerobic conditions, and is carried out by Ammonia Oxidizing Bacteria (AOB), which convert ammonium (NH_4^+) into nitrite (NO_2^-) [Gupta et al., 2022]. The microorganism group responsible for this reaction is *Nitrosomonas*, which facilitates the oxidation of ammonia, a toxic pollutant harmful to aquatic environments and plants [1H2O3, 2024; Henze et al., 2006].

This process [Henze et al., 2006] occurs slowly and can be represented as follows:

$$NH_4^+(aq) + \frac{3}{2}O_2(aq) \rightarrow NO_2^-(aq) + H_2O(l) + 2H^+(aq) \tag{2.4}$$

The second step of nitrification involves the oxidation of nitrite (NO_2^-) to nitrate (NO_3^-) a process facilitated by a group of Nitrite Oxidizing Bacteria (NOB), specifically *Nitrobacter*. This conversion is crucial because nitrate is less harmful and more easily absorbed by plants. The reaction [Henze et al., 2006] can be represented as follows:

$$NO_2^-(aq) + \frac{1}{2}O_2(g) \rightarrow NO_3^-(aq) \tag{2.5}$$

AOBs and NOBs are referred as nitrifiers, and are autotrophic by nature, meaning they obtain energy by using carbon dioxide as their carbon source. There is also a third type: Comammox, represented by the Genus *Nitrospira*, that performs complete nitrification and is found in wastewater treatment plants (WWTP), which can even dominate some municipal wastewater treatment systems [Spasov et al., 2020].

Denitrification is the second stage in the biological treatment of nitrogen, during which nitrates (NO_3^-) are converted to nitrogen gas (N_2), a harmless and inert component of the atmosphere. This process [Henze et al., 2006] is performed by denitrifying bacteria in oxygen-limited environments as shown below:

$$NO_3^-(aq) \rightarrow NO_2^-(aq) \rightarrow NO(g) \rightarrow N_2O(aq) \rightarrow N_2(g) \tag{2.6}$$

Denitrifying bacteria use nitrates as an electron acceptor, reducing them to nitrogen gas in an oxygen-limiting environment. This process can produce intermediates such as nitrite (NO_2^-), nitric oxide (NO), and dinitrogen oxide (N_2O) [Henze et al., 2006]. Denitrifiers are heterotrophs that obtain energy and carbon from organic compounds instead of carbon dioxide. While nitrous oxide is less abundant than nitrogen gas in the air, it is harmless. However, nitrate is highly toxic and has significant health risks, such as blue baby syndrome (methemoglobinemia), harmful effects on the digestive system, and potential carcinogenic impacts on living organisms [Gupta et al., 2022].

2.2.3 Carriers and Microbial Biofilm

The carriers, usually made of polyethylene with a density of 0.94 to 0.98 g/cm^3, are designed to remain buoyant and suspended in water [Gupta et al., 2022]. Typically featuring circular and longitudinal sections, they act as the interface between attached and suspended growth systems, offering a large surface area for biomass or biofilm development [Gupta et al., 2022; R.Vieira et al., 2022]. According to [R.Vieira et al., 2022], aerators provide oxygen to the MBBR system and can also ensure uniform distribution of the carriers due to coarse and fine bubble air distribution systems. Figure 2.3 shows a new carrier compared to a fully developed carrier.

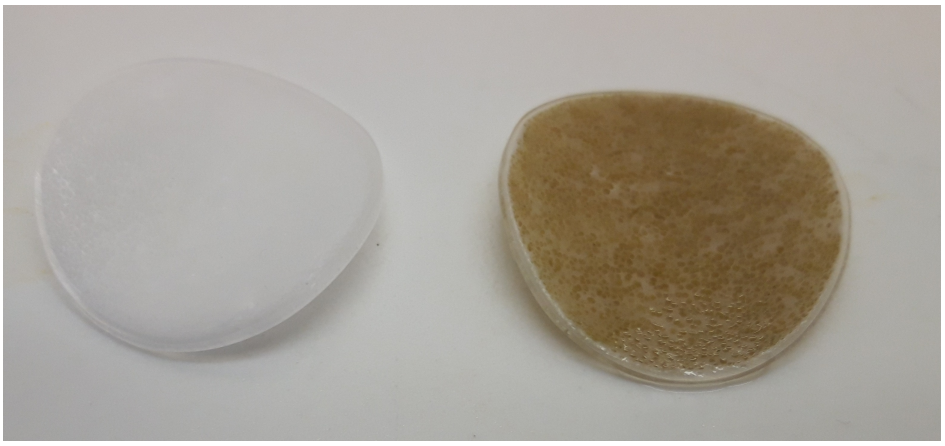

Figure 2.3: Left: New carrier, Right: carrier with biomass, almost a year old

Biofilm formation occurs on surfaces with access to water and nutrients. These complex structures of cells are embedded in a protective extracellular polymeric substance (EPS) matrix [Ancion et al., 2014; Gupta et al., 2022]. Biofilm development depends on factors like nutrient availability, pH, temperature, and shear forces [Gupta et al., 2022]. Growth on carriers is beneficial, supporting diverse microbial groups such as bacteria, fungi, viruses, protozoa, and algae [Lear, 2016], helping to counteract the destructive effects of toxic industrial compounds often present in traditional biomass formation [Gupta et al., 2022].

2.2.4 MBBR Lab Setup: System check and startup

The MBBR reactors used for the project were reactors already in use for earlier projects and they are repurposed for this project. The nitrification and denitrification reactors were emptied, tap water and buffer added and the nitrification and denitrification process were assessed over two weeks to let biological activity.

2.3 Leachate

Leachate is a complex mixture of recalcitrant organic compounds, heavy metals, and nutrients such as ammonium and nitrates. Moreover, the treatment of leachate is challenging due to high variability in the composition in terms of quality and quantity [Bove et al., 2015]. For determining the appropriate leachate treatment, factors such as leachate age, COD concentration, and the BOD/COD ratio can be considered and a classification of the characteristics of leachate based on age are listed in Table 2.2.

Table 2.2: Leachate characteristics [Saxena et al., 2022]

Parameters	**Young**	**Intermediate**	**Old**
Age (years)	<5	5–10	>10
COD (mg/L)	>10000	4000–10000	<4000
NH_3-N (mg/L)	<400	–	>400
BOD/COD	>0.3	0.1–0.3	<0.1
pH	<6.5	6.5–7.5	>7.5
Organic Compounds	80% VFA	5-30% VFA, humic/fulvic acids	Humic/fulvic acids
Biodegradability	High	Medium	Low
Heavy metals	Low-medium	Low	Low

VFA: Volatile fatty acid.

The biodegradability of leachate is reduced, whereas the organic load is enriched in recalcitrant organic compounds, heavy metals, and toxic organic pollutants as leachate ages [Morais & Zamora, 2005; Naumczyk et al., 2012]. Additionally, microorganisms cannot propagate and consequently reduce nitrogen removal due to reduced levels of biodegradable organic contaminants in mature leachate [Lopez et al., 2004].

MP can be toxic, endocrine-disrupting, mutagenic, or carcinogenic, even at low concentrations, making their removal a critical environmental challenging [Mahtab et al., 2021]. Consequently, effective treatment and removal these pollutants from wastewater while meeting stringent discharge regulations is critical. However, Mahtab et al. [2021] argue that conventional biological treatment methods have been ineffective, highlighting the need for more advanced treatment solution. This process is especially effective for treating industrial effluents contaminated with organic pollutants, and those that are recalcitrant and difficult to degrade through conventional means [Mahtab et al., 2021]. In recent years, advanced oxidation processes (AOPs) have gained recognition as one of the most promising alternatives due to their high efficiency and broad applicability. Fenton oxidation as an AOP operates under near-ambient temperature and pressure, AOPs generate large quantities of highly reactive hydroxyl radicals (•OH), which can effectively degrade and fully mineralize a wide variety of contaminants [Mahtab et al., 2021].

3 | Materials and Methods

3.1 Process Flow and Experimental Setup

The process diagram (Figure 3.1) illustrates the overall experimental workflow, including Fenton oxidation, MBBR treatment, Dissolved Oxygen (DO) experiment and inhibition analysis.

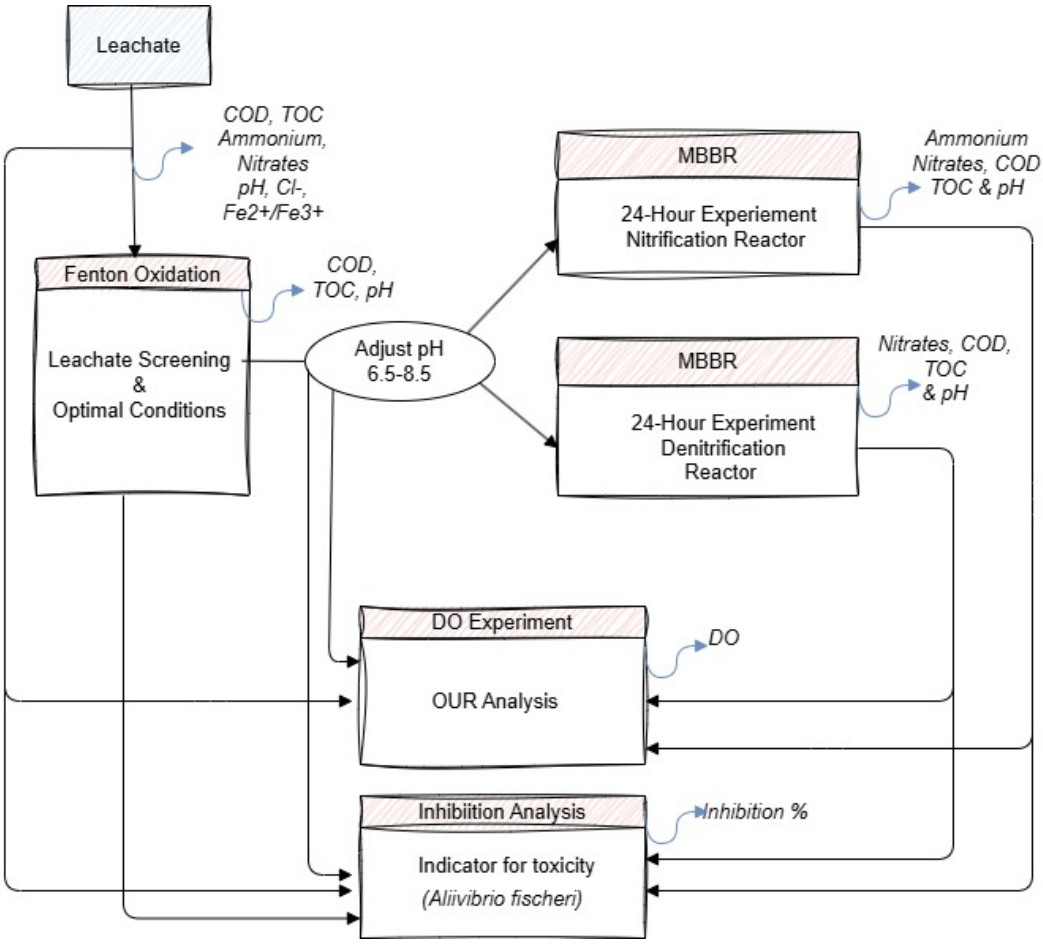

Figure 3.1: Experimental Workflow for Leachate Treatment, DO Experiment, and Inhibition Analysis with corresponding measurements at each stage

This flow diagram provides an overview of the experimental process, illustrating each stage and the sequence of steps used to evaluate COD reduction efficiency through Fenton oxidation applied to four different leachates: (1) Hvej, (2) Bvej, (3) ODE, and (4) AAR. It also outlines the biodegradability assessment performed only on ODE and AAR leachates using Moving Bed Biofilm Reactor (MBBR) treatment and Oxygen Uptake Rate (OUR) analysis through the DO experiments. Finally, the diagram includes inhibition analysis conducted to evaluate the toxicity and inhibition potential of the treated ODE and AAR leachates.

3.2 Leachate from Norrecco

The current leachate treatment process at the Norrecco site in Aarhus begins with mixing a polymer with tap water to reach the desired concentration. This mixture is then added to the leachate along with a flocculant, which is directed to a settling tank. A series of treatment stages are followed: it first passes through a sand filter, then through two activated carbon columns, and finally through a resin column. After treatment, part of the water is sent to the sewer system, another portion is mixed with tap water in a tank before being discharged into the rainwater outlet, and the remaining water is used for washing. This process is outlined in the treatment flow diagram (see Appendix B).

Leachate samples were named according to their respective landfill sites: Bvej and Hvej for the Copenhagen sites, and AAR for the Aarhus site.

The Odense leachate (ODE) was from a landfill in Odense, but was collected from Noreccco Aarhus. This leachate, rich in microorganisms, is from their nitrification/denitrification onsite treatment tank. It is being used to inculate carriers in the pilot MBBR located in Norrecco Aarhus.

All leachate samples were included in the initial screening, but only AAR and ODE leachates were selected for the Fenton and MBBR treatment processes in this project.

3.2.1 Sampling

The sampling of the different leachates were conducted at different Norrecco sites and stored in DTI basement in sealed containers at a temperature of 4°C to prevent any chemical degradation or biological activity before analysis. The leachate samples were filtered using a 0.45 μm membrane filter to remove suspended solids and prevent interference in the analytical measurements before the various experimental processes.

3.3 Fenton Reagents

In this study, the Fenton process was applied to treat leachate with *in situ* generation of hydroxyl radicals (•OH) through the catalytic decomposition of hydrogen peroxide (H_2O_2) by ferrous ions (Fe^{2+}). This allowed the oxidative breakdown of complex molecules into less harmful compounds. All solutions were prepared with demineralized water. Hydrogen peroxide (30% w/w) served as the oxidant, stored at 4°C in a dark container and diluted to a final concentration of 1M prior to use. Ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$, CAS No. 7782-63-0) was used as the Fe^{2+} source. A 1M stock solution was prepared in demineralised water and used to achieve Fe^{2+} to H_2O_2 molar ratios of 1:5 and 1:10 in the different experimental setups. The leachate pH in the acidic condition was maintained in the optimal range of 2.95 to 3.10 using 0.5 M diluted sulfuric acid. Sodium hydroxide (0.5M) was used to neutralize the pH after treatment when necessary.

3.4 Fenton Process

The Fenton process is based on careful dosage of the reagents and balance of different parameters. H_2O_2 is dosed based on stoichiometric relationships to achieve the desired COD reduction, ensuring sufficient hydroxyl radical generation, •OH, while avoiding excess H_2O_2, which could lead to inefficient reactions and decreased pollutant degradation efficiency.

The screening part of the project is to determine the optimal parameters (See Appendix G) for the dosage of H_2O_2 and $FeSO_4$ in Fenton oxidation to reduce COD and hence, improve biodegradability. This is achieved by examining the effects of operating parameters:

- Natural and acidic pH
- COD desired reduction: 30 %, 40 %, 60 % and 80 %
- Ratio Fe^{2+} to H_2O_2, 1:5 and 1:4

A constant COD to •OH radicals ratio was assumed to be 1:4 . The optimal parameter conditions then are applied as a pre-treatment on the selected leachates (ODE and AAR) to be used in the MBBR lab setup for biologically treatment. The concentration of Fe^{2+} and H_2O_2 is determined by the initial COD value and the targeted COD removal.

3.4.1 Hydrogen Peroxide Dosage

H_2O_2 is dosed based on stoichiometric relationships to achieve the desired COD reduction, ensuring sufficient hydroxyl radical generation, •OH, while avoiding excess H_2O_2, which could lead to inefficient reactions, decreased pollutant degradation efficiency and damage the biofilm in the carriers.

The following calculations demonstrate an example to determine the dosage of H_2O_2 and Fe^{2+} for a targeted reduction of COD of 40% for ODE.

Initial COD and H_2O_2 stock

Given the initial COD:

$$\begin{aligned}\text{Initial COD of leachate} &= 249\,[\text{mg/L}] \\ &= 249/1000/32 = 7.78 * 10^{-4}\,[\text{mol/L}]\end{aligned}$$

The stock of H_2O_2 is 30 % weight by volume (300 g/L), and the molar weight of H_2O_2 is 34 [g/mol], therefore concentration is as follows:

$$\text{Stock concentration} = 300/34 = 8.82\,[\text{mol/L}]$$

With a dilution factor of 20 for example, the applied stock solution is given by:

$$\text{Applied stock concentration} = 8.82/20 = 0.44\,[\text{mol/L}]$$

COD Target Reduction and H_2O_2 Dosage

For 40% COD reduction and taking COD to •OH stoichiometric ratio (1:4) into account, the concentration of H_2O_2 required are as follows:

$$\begin{aligned}\text{Applied Stock concentration} &= 40\% * \text{ COD in reactor} * 4 \\ &= 40\% * (7.78 * 10^{-4}) * 4 \\ &= 12.45 * 10^{-3} \ \text{[mol/L]}\end{aligned}$$

The volume required of the solution stock to be used to oxidize the initial COD is calculated as follows:

$$\begin{aligned}\text{V1M1} &= \text{V2M2} \\ \text{V}_1 &= \frac{12.45 \times 10^{-3}\,\text{[mol/L]} \times 0.1\,\text{[L]}}{0.44\,\text{[mol/L]}} \\ &= 2.82\,\text{[ml]} \\ &= 2822\,[\mu\text{L}]\end{aligned}$$

3.4.2 Iron(II) Sulphate Dosage

For the concentration of Fe^{2+}, different ratios of Fe^{2+}: H_2O_2 is tested for example 1:5. The calculation below shows the ratio of 1:5:

Given: Fe^{2+} stock solution of 1 M $Fe_2(SO_4)_3 \cdot 7H_2O$

$$\begin{aligned}\text{Fe}^{2+} \text{ in solution} &= 1/5 * \text{ Amount of } \text{H}_2\text{O}_2 \text{ in total volume} \\ &= 1/5 * 12.45 * 10^{-3} \\ &= 2.49 * 10^{-4} \ \text{[mol/L]}\end{aligned}$$

Since the stock of Fe^{2+} is 1 M ([mol/L]), the volume required is determined as follows:

$$\begin{aligned}\text{V1M1} &= \text{V2M2} \\ \text{V}_1 &= \frac{2.49 \times 10^{-4}\,\text{[mol/L]} \times 0.1\,\text{[L]}}{1\,\text{[mol/L]}} \\ &= 0.249 \ \text{[ml]} \\ &= 249\,[\mu\text{L}]\end{aligned}$$

Calculations on the different ratios and reduction percentage can be found in Appendix C.

3.4.3 Materials and Equipment

The following is a list of materials and equipments used to set up the fenton oxidation process:

- Hydrogen peroxide (H_2O_2) - 1 M
- Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) - 1 M
- Sulphuric acid and NAOH (to adjust pH) - 0.5 M
- Leachate (Norrecco: Aarhus and Copenhagen sites and Odense)
- Filter paper (Coffee filter)
- Beakers, pipettes, and pipette tips
- Magnets and magnetic stirrer
- Spectrophotometer (Hach DR3900) and Digester (Hach HT200S)
- pH meter (Hach HQ Series) and ORP meter

3.4.4 Experimental Procedure

The experimental setup (see Figure 3.2) is based on the Fenton process (see Section 2.1.4), used reagent dosages calculated from initial COD measurements and the desired COD reduction, as detailed in Section 3.4.

Figure 3.2: Fenton Oxidation setup: 40% COD reduction with acidic pH

The leachate, filtered using coffee filters, was allowed to reach room temperature and prepared by measuring 100 ml in a 250 ml beaker with a magnetic stirrer (Figure 3.2). After pH adjustment, Fe^{2+} was added, followed by H_2O_2 to initiate the Fenton reaction. The reaction proceeded for 20 minutes, after which samples were collected for COD, TOC, and pH analysis. Most measurements were conducted in duplicates, where possible.

3.4.5 MBBR: Nitrification/Denitrification Reactors

The Fenton-treated leachate was then used in the MBBR to evaluate the nitrification and denitrification processes. The MBBR lab setup at DTI (see Figure 3.3), previously used in earlier projects, was adapted and reused to assess the biological treatment processes of nitrification and denitrification.

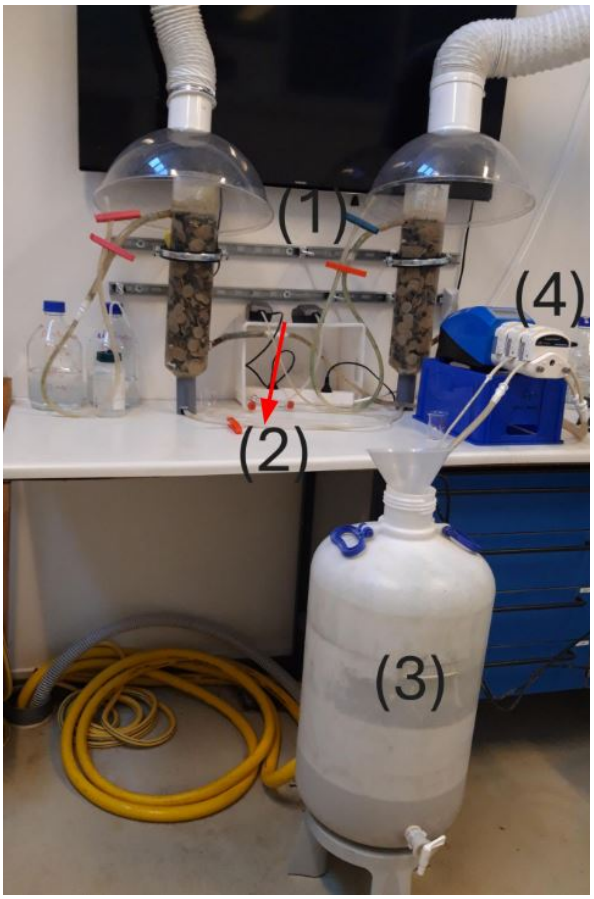

Figure 3.3: MBBR Lab setup: (1) Two nitrification reactors, (2) two air pumps, (3) denitrification reactor and (4) peristaltic pump

For this project, the peristaltic pump was not used; instead, water and buffer additions were carried out manually. The system was configured to perform batch leachate treatment. The volume of carriers to 10-30% of the total used in the reactors were assessed to be between 10-30% water volume of the reactors [DTI3, 2024]. The two aerobic reactors consisted of 3 L glass columns with 0.75 L of carriers and 2.25 L. The anaerobic reactor, can accomodate a total 10-30 L of water with the carriers. A total 10 L water volume with 1 L volume of carriers were tested in the system [DTI3, 2024].

3.4.6 MBBR Start up and Stabilisation period

On the 11th March 2025, the MBBR reactors were emptied and the carriers were weighed (See Appendix G). All the equipment and setup were tested to ensure it was fully operational, that is the connections were leak-proof, and was ready for nitrification and denitrification processes. The reactors were filled with tap water and the required nitrification/denitrification buffers were added in the nitrification and denitrification reactors. The measurements of ammonium, nitrates and COD were regular taken and buffers added when necessary.

The system was operated from 11th March to 28th March 2025 as stabilization period to allow microbial communities to adapt before starting the main experiments.

A buffer stock solution was prepared for both the aerobic and anaerobic reactors of the MBBR setup, following specifications provided by DTI as detailed in [DTI3, 2024].

- A nitrification buffer containing 561.8 mg NH_4^+-N/L was prepared by dissolving 5.04 g of $NaHCO_3$ and 2.65 g of $(NH_4)_2SO_4$ in 1 L of demineralized water in a 1 L blue-cap bottle. The solution was stirred on a magnetic stirrer for approximately 5 minutes until fully dissolved.
- The denitrification (acetate) buffer, which contained 400 mg $NO_3 - N$/L and 4000 mg/L COD, the following compounds were dissolved in 1 L of demineralized water in a blue-cap bottle: 2.42 g $NaNO_3$, 8.48 g CH_3COONa, 7.1 g Na_2HPO_4, and 1.2 g KH_2PO_4. A magnetic stir bar was added, and the solution was stirred for approximately 5 minutes until fully dissolved.

Each buffer solution was applied at a 1:9 proportion relative to the total water volume in the MBBR.

A modified buffer solution was used for leachate samples treated with Fenton oxidation. This solution was based on the standard denitrification buffer but was prepared without the addition of nitrate and COD sources. The modified buffer was applied in both the aerobic nitrification reactor and the denitrification reactor to support the respective biological treatment processes.

3.4.7 24-hour Nitrification/Denitrification Experiment

A 24-hour experiment was conducted to assess nitrification and denitrification performance, to confirm the biological activity supported by the MBBR carriers.

Following the stabilization phase, the MBBR system was initially filled with tap water and buffer, and a 24-hour nitrification and denitrification test was conducted. For the main experiments, 12 L of untreated leachate was first introduced into one of the nitrification columns and the denitrification tank for the 24-hour experiment to establish a baseline. Then, the MBBR was emptied and refilled with the Fenton-pretreated leachate, which then underwent the same nitrification and denitrification process for comparison. The leachate were pretreated using the Fenton process, applying optimal conditions.

Table 3.1 presents the specifications for the 24-hour experiment comparing leachate with and without Fenton pretreatment.

Table 3.1: Specifications for 24-hour Nitrification/Denitrification Test

Test	**Sample Type**	**Buffer**	**Note**
Nitrification 1	Tap water	Nitrification	-
Denitrification 1	Tap water	Denitrification	-
Nitrification 2*	Leachate	Nitrification	Without ammonium
Denitrification 2*	Leachate	Denitrification	Without ammonium
Nitrification 3*	Pretreated Leachate	Modified Nitrification	Without ammonium
Denitrification 3*	Pretreated Leachate	Modified Denitrification	Without COD and nitrates

** Performed with twice with different leachates*

The nitrification reactor contained 2.7 L of water and 0.3 L of nitrification buffer. In the denitrification reactor, 9 L of water was combined with 1 L of denitrification buffer. A modified buffer was used for the 24-hour experiment using leachate.

Water samples were collected were chosen time interval, and were filtered using a 0.45 µm filter and a syringe. The samples were then analyzed for ammonium, nitrates and COD in duplicates. The pH was also recorded throughout the process.

3.5 Analytical Procedure

Several reagent kits from Hach Lange were used to analyze the water samples during the study, as summarized in Table 3.2.

Table 3.2: Hach Measurement Kits and Range

Cuvette	**Test Kit**	**Range**
Cl^-	LCK 311	1–1000 mg/L
Fe^{2+}/Fe^{3+}	LCK 320	0.2–6.0 mg/L
NH_4-N	LCK 303	2–47 mg/L
NH_4-N	LCK 304	0.015–2 mg/L
NO_3-N	LCK 339	0.23–13.50 mg/L
NO_3-N	LCK 340	5–35 mg/L
COD	LCK 1814	7–70 mg/L

The water samples were diluted when necessary to fit the required ranges and analyzed according to the manufacturer's recommended procedures [Hach, 2022].

The concentration chloride (Cl^-) was measured using the Hach LCK 311 kit. First, 0.1 mL of the water sample was added to the cuvette and close it securely. It was shaken gently for mixing, then allowed to stand for 3 minutes. The cuvette was cleaned and placed it in a DR1900 spectrophotometer.

Total iron (Fe^{2+}/Fe^{3+}) was measured using the Hach LCK 320 kit. For Fe^{2+}, 2 mL of sample was added to the reagent cuvette, gently mixed, and after 5 minutes, the reddish-colored solution was measured in a spectrophotometer. To determine Fe^{2+} , Dosicap A was added after the initial 5-minute reaction, mixed again, and allowed to stand for another 5 minutes before measurement.

The LCK 303 test kit determined the concentration of ammonium. A volume of 0.2 mL of the water sample was pipetted into a cuvette, sealed with a DosiCap Zip, and shaken vigorously which ensured proper mixing. After a reaction time of 15 minutes, the cuvette was cleaned, and the absorbance was measured using a DR1900 spectrophotometer, showing the ammonium concentration in mg/L of NH_4-N.

For nitrate analysis, the LCK 340 test kit was used where, a 0.2 mL of the water sample was pipetted into a cuvette. A nitrate-specific reagent was then carefully added, followed by gentle shaking to mix the contents. After the cuvette was left to rest for 15 minutes, similar procedure to ammonium was applied to measure the nitrate concentrations.

COD was measured using LCK 1814 cuvettes. After thoroughly mixing any settled sediments, 1.8 mL of the water sample was added to each cuvette, which was then sealed, gently inverted, and digested at 150°C for 15 minutes using the HS method. After cooling to room temperature and cleaning the exterior, the cuvettes were analyzed using a spectrophotometer.

Selected samples were frozen at DTI and sent to AAU Esbjerg for TOC analysis.

3.6 DO Experiment: Oxygen Uptake Rate

The DO experiment (see Figure 3.4), a modified respiratory test, was conducted by measuring oxygen consumption over time in a sealed conical flask containing activated sludge and the sample leachate under un-aerated conditions. The decrease in dissolved oxygen over time is used to determine the slope, represents the oxygen uptake rate (OUR) [Al-Ahmady & Fakhri, 2012; Hagman & l. C. Jansen, 2007]. The OUR measures the rate at which microorganisms in activated sludge consume dissolved oxygen during the biological oxidation of both organic and inorganic compounds [Hagman & l. C. Jansen, 2007]. It serves as an indicator of microbial activity and the biodegradability of constituents present in the leachate.
Activated sludge was collected from the Marselisborg wastewater treatment plant several times during the study. It was stored under aerated conditions for 3–4 days and used as needed for the DO experiments. A high oxygen uptake rate (OUR) indicates high biodegradability, while a low OUR suggests lower biodegradability or potential microbial inhibition of the leachate.

Equipment

- Erlenmeyr flask flask: 100 mL
- Magnetic Stirrer and Magnet: 3-4 cm long
- Dissolved Oxygen (DO) Meter: Hach Lange HQ40D
- Stand: To hold the oxygen meter.
- Computer: With HQ40D PC Software
- Parafilm and Timer

Sample Preparation

- Test Samples: Mix the sample water with activated sludge at a ratio of 1:1 (v/v).
- Blank Control: Use only activated sludge without any added organic sample.

The Figure 3.4 shows the setup for the DO experiment.

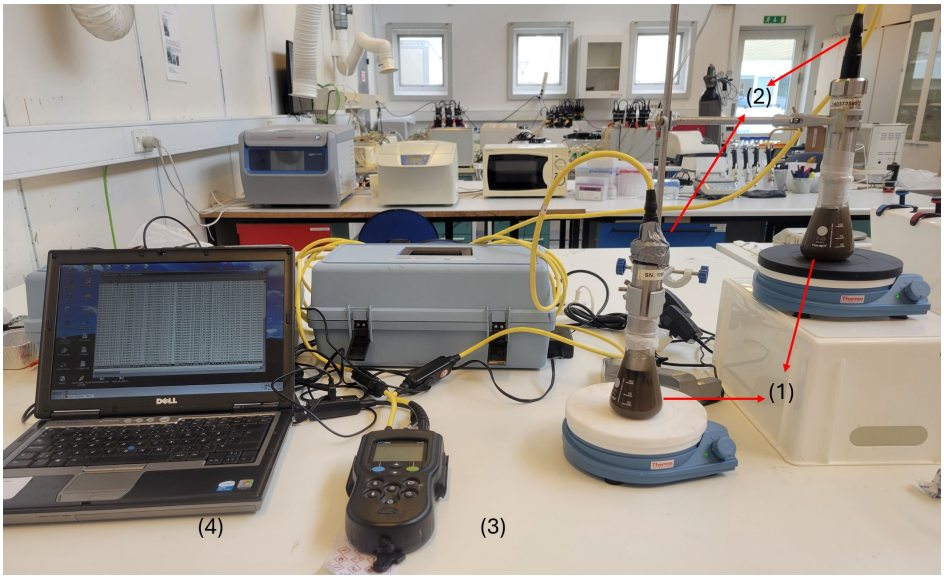

Figure 3.4: Biodegradability Test: (1) Sample mixture of leachate and activated sludge with magnetic stirrer, (2) DO meter sensor probes, (3) DO meter and (4) PC with DO software connected to DO meter and sensor

Two sets of sample mixtures connected to two DO probes can be tested simultaneously, as shown in the Figure 3.4. The probes were calibrated prior to the experiment to ensure accuracy, and readings were recorded at regular intervals.

Procedure

The steps to set up the experiment are as follows:

1. Connect the "PC for Hach DO sensor" to the DO meter and open the software for DO measurement.
2. Fill a flask with the test mixture, leaving minimal headspace.
3. Attach the oxygen meter (DO meter) to the stand and lower the oxygen meter into the testing sample.
4. Tightly seal the flask with parafilm to ensure no external oxygen enters.
5. Start the magnetic stirrer, set it to about 200 rpm.
6. Press START on the measuring unit and press PLAY on the PC software.
7. Run the experiment for the required time period of 30 minutes to 1 hour.
8. Stop the measurement after the set time intervals and save the data, which is analysed using the Hach DO software.

Due to malfunctioning of the DO monitoring software, the collected data were exported as CSV files and analyzed using Microsoft Excel. Linear regression functions were applied to determine the slopes, from which the OUR was subsequently calculated.

3.7 Inhibition Analysis

A modified version of the standard protocol from ISO 11348-1 (2007) was used and provided Aalborg University and produced by Roslev [2024]. The test works on the principle that a decrease in light emission from the luminescent bacterium *Aliivibrio fischeri* (*A. fischeri*) serves as an indicator of the acute toxicity of chemical substances present in the sample. The inhibition analysis was performed on raw leachate, leachate without Fenton after MBBR treatment, and samples of leachate before and after Fenton and MBBR treatment.

3.7.1 Preparation of *A. fischeri* Reagent

The cultivation of *A.fischeri* before toxicity testing is a multi-step process designed to ensure that the bacterial culture is healthy, active, and exhibits strong luminescence at the time of the assay. The procedure starts by thawing a frozen stock culture of *A. fischeri*. Next, 10–20 mL of autoclaved Mar+ medium is added to a sterile 100 mL blue-cap bottle, and the thawed culture is inoculated (20–50 µL). This inoculated bottle is then incubated for 24 to 72 hours at 20°C with shaking at approximately 100 rpm; visible turbidity and luminescence indicate successful growth. Three new blue-cap bottles containing 10–20 mL Mar+ medium are prepared and inoculated with different volumes (0.01 mL, 0.1 mL, and 1.0 mL) of the initial culture in order to optimize the test. They are then incubated for another 16–24 hours at 20°C with shaking. After incubation, the luminescence of each culture is measured using a plate reader. The culture exhibiting the highest light emission is selected for testing since this will offer greater sensitivity and consistency. For the experiment a diluted *A. fischeri* culture is estimated from the amount needed (about 10 mL per 96-well plate), with VF-Tox medium

to achieve 10^5 to 10^6 Relative Light Units (RLU). Then shake and incubate the dilution for 30–60 minutes in the dark at 20°C.

3.7.2 Sample Preparation

9 grams of sodium chloride (NaCl) were weighed and dissolved in demineralized water to obtain 450 ml of NaCl 2% w/v. The solution was refrigerated for each use and the pH was measured and adjusted to 7.0 ± 0.2 for each use. This solution was provided by Aalborg University lab.

All the samples were filtered and the chloride content was measured. Using the chloride content and the sample volume, the amount of NaCl salt to be added was estimated, which set the salinity of the samples to 2% w/v. The samples were then stirred and the pH adjusted to 7.0 ± 0.2. It is to be noted that the samples can be prepared in advanced and stored for up to 48 hours before use.

The VF Tox Medium was made using: 8 g/L D(+)-Glucose monohydrat, 20 g/L NaCl, 2.035 g/L $MgCl_2 \cdot 6H_2O$, 0.3 g/L KCl, 11.9 g/L HEPES (N-2-Hydroxyethyl)piperazine-N-(2-ethanesulfonic acid) and distilled water.
pH is adjusted to pH 7 using NaOH or HCl. The solution is autoclaved.

The VF-Mar+ Medium was made using: 37 g/L Marine Broth, 5 g/L Peptone and distilled water. The solution is autoclaved.

The Medium for freezing A. fischeri culture was made using: VF-Mar+ Medium, 40% Glycerol (mL/mL). The solution is autoclaved.

3.7.3 Sample Analysis

For 2-fold dilutions, a 96-well plate was used where column 11 holds undiluted sample, columns 2–10 contained serial 2-fold dilutions, column 1 is control, and column 12 is blank. Column 12, Blank contains : 100 μl 2% NaCl and 100 μ VF Tox Medium. Column 1, control contains 100 μl 2% NaCl and 100 μl *A. fischeri.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
B	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
C	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
D	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
E	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
F	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
G	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank
H	Control	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2	Blank

Figure 3.5: Plate setup

Details regarding equipment requirements are provided in the Appendix F.

3.7.4 Data Processing

The data from plate reader was collected in Relative Light Units (RLU) and transferred to an Excel spreadsheet. All recorded data were analyzed statistically, with the mean and standard deviation calculated. Relative inhibition, denoted as q, was calculated using the following equations:

$$q = \left(1 - \frac{A_{DX} - A_{blank}}{A_0 - A_{blank}}\right) \tag{3.1}$$

A_{DX} refers to the bioluminescence measured at each specific toxicant concentration. A_0 is the bioluminescence recorded for control samples without any added toxicant.

The q represents the relative inhibition, which is expressed as a value between 0 and 1. These q values were visualized on a dilution-series graph.

Data analysis and graphical visualization of the measured results were performed using both Excel and Python.

3.8 Data Management

Data collection was organized using an Excel spreadsheet, with a separate sheet maintained for each experiment. For every experiment conducted, relevant data and associated units were recorded.

For probe procedures, the pH of the sample and the multimeter readings were noted. For the Fenton testing, the volume of the sample, the volumes of the reagents, and the specific conditions used to evaluate different Fenton parameters were documented.

In analytical tests, information recorded included the dilutions used, raw results, and the calculated concentrations.

Data analysis and graphical visualization of the measured results were performed using both Excel and Python.

4 | Results and Discussion

4.1 Leachate Charaterization

The characterization of Hvej, Bvej, ODE, and AAR leachates was performed to assess starting concentrations so that it enabled informed selection of analytical techniques and sample preparations before conducting experiments. The starting concentrations of COD, chloride ion concentrations (Cl^-),iron (Fe^{2+}/Fe^{3+}) and pH were measured on filtered samples of the raw leachate are shown in Table 4.1.

Table 4.1: Water Quality Parameters for different Leachate Samples: Initial Concentrations

Parameter	**Hvej**	**Bvej**	**ODE**	**AAR**
COD [mg/L]	33.6	43.6	249	72.6
Cl^- [mg/L]	471	955	671	513
Fe^{2+} [mg/L]	-	N/A	N/A	0.26
Fe^{3+} [mg/L]	-	N/A	0.026	N/A
pH	7.3	7.9	7.5	8.0

N/A: Not applicable as measurements were negative or below minimum limit

The COD were measured from a range of 33.6 mg/L to highest being 249 mg/L for ODE. Hvej had the highest Cl^- measurement of nearly 1000 mg/L, while the concentrations of Fe^{2+} and Fe^{3+} are below 1 mg/L for Hvej, ODE and AAR. The pH ranges from 7.3 to 8.0 initially, which is near neutral pH. The COD and pH levels decreased over time, while the concentrations of Cl- and Fe^{2+} and Fe^{3+} remained consistent throughout the duration of the study.

Based on the characterisation of leachate from Table 2.2, all the four leachate samples (Bvej, Hvej, ODE, and AAR) can be classified as old leachates if only considering their low COD concentrations (<4000 mg/L) and elevated pH values (>7.5). Older leachate show low biodegradability and a predominance of refractory organics like humic and fulvic acids [Saxena et al., 2022]. Consequently, treating old leachates with biological methods alone is challenging, highlighting the need for advanced oxidation processes such as Fenton as a pretreatment step.

The Cl^- measurements for the all samples were below 1000 mg/L. Excessive Cl^- concentrations can impact Fenton process negative by acting as scavengers [Bury et al., 2024]. Additionally, according to Hach [2024], chloride concentrations above this threshold can potentially cause elevated COD readings. When Cl^- is too high, diluting the sample to reduce chloride concentration or using a COD method tolerant to high chloride levels can be a solution.

The initial concentrations of Fe^{2+}/ Fe^{3+} were very low or below the minimum limit of the Hach kit, thereby not expected to impact the Fenton process. Excess Fe^{3+} can lead to rapid precipitation as ferric hydroxide, reducing available catalyst and hindering hydroxyl radical generation [Bury et al., 2024; Walling et al., 2021]. Moreover, Fe^{3+} also promotes undesired H_2O_2 decomposition and side reactions that scavenge hydroxyl radicals, further lowering efficiency [Babuponnusami & Muthukumar, 2014; Ziembowicz & Kida, 2022]. If iron levels are

too high, pre-treatment methods such as pH adjustment, coagulation, or filtration can be used to remove excess iron before the Fenton process.

Over time, the pH of the samples decreased, which required pH adjustments following the requirements of each experiment. Since pH is a critical parameter for the Fenton process to function effectively, it was also carefully regulated in experiments involving microorganisms [Bury et al., 2024; Gupta et al., 2022]. The COD and pH values were remeasured before each experiment to account for their variability over time, ensuring accurate assessment of the Fenton process and sample preparation for analytical assessment, such as diluting samples.

4.2 Leachate Screening and Optimal Operating Condition

The leachate screening and optimal operating condition assessment aimed to determine effective and ineffective Fenton treatment conditions, demonstrating the sensitivity of the process and the response of leachate to varying operating parameters.

4.2.1 pH

The results of Fenton process screening with pH as parameter shown in Figure 4.1.

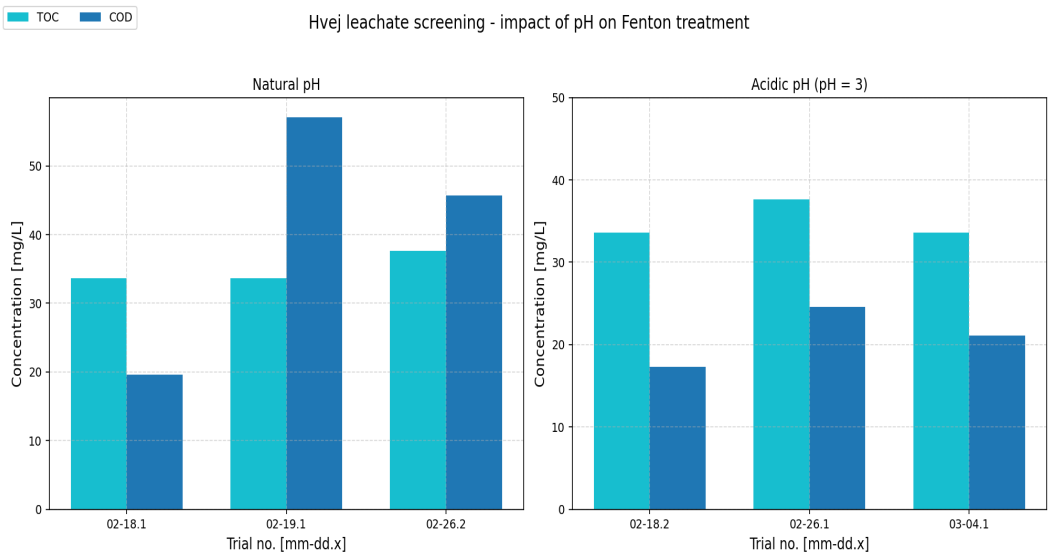

Figure 4.1: Leachate screening - Impact of pH on Fenton treatment: Hvej

Testing both natural and acidic pH aims to assess whether the Fenton process can be effective without acidification, potentially reducing costs especially when considering scale-up. Natural pH conditions only worked for the Hvej leachate, whereas acidic pH around 3, consistently yielded results in both Hvej and Bvej samples. Therefore, an acidic pH (2.95–3.10) was maintained for further experiments during the study. The acidic conditions proved effective for all four different leachates, which is consistent with other studies where the COD removal was between 31-95% [Bury et al., 2024] and the measure for biodegradability increased from a BOD/COD of 0.2 to 0.5 [Lopez et al., 2004].

4.2.2 Fe^{2+}:H_2O_2 molar ratio

The results of Fenton process screening with molar ratio as parameter shown in Figure 4.2.

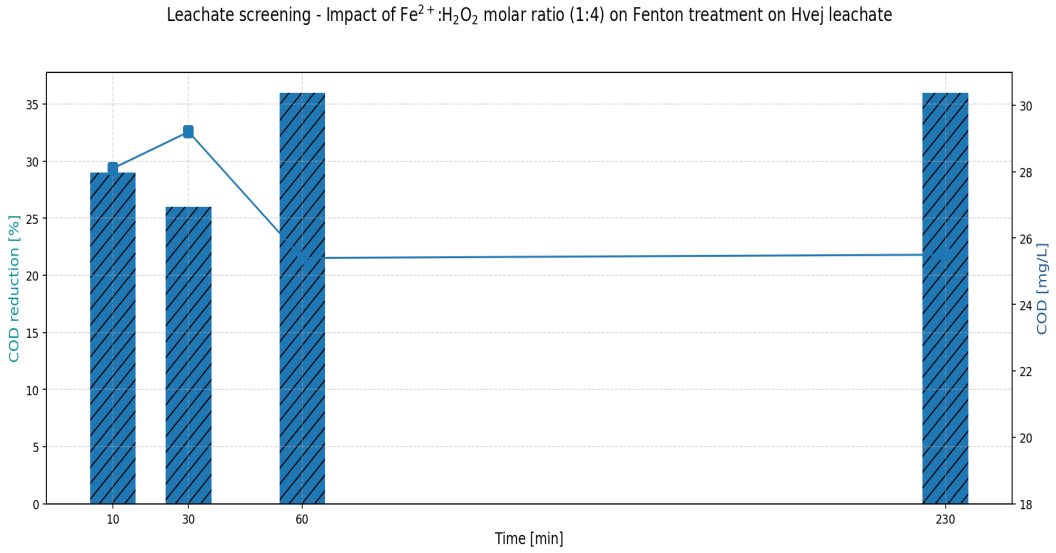

Figure 4.2: Leachate screening - Impact of molar ratio on Fenton treatment: Hvej

When the Fe^{2+}:H_2O_2 molar ratio was altered for Hvej to 1:4 as recommended by Bury et al. [2024], The measured decrease in COD was lower than the targeted reduction over a period exceeding two hours, indicating that while the dosage was effective, the process was slow. In contrast, the 1:5 ratio proved more efficient, delivering the required dosage more quickly and making the treatment process faster—ultimately saving time. This is beneficial from an economic perspective, as it supports cost-effective treatment if the process is adopted and scaled up for full-scale operation in Norrecco for instance. As a result, the 1:5 ratio was adopted for all further Fenton oxidation experiments.

4.2.3 Target COD reduction

Two additional leachate sources, ODE and AAR, were selected for further experiments based on leachate availability, and to enable a comparison between two distinct COD levels—ODE with a higher initial COD and AAR with a lower one. For both ODE and AAR leachates, screening tests were conducted to evaluate the effects of reaction time and target COD reduction. The pH was maintained at acidic conditions, with fixed molar ratios of COD:H_2O_2 (1:4) and Fe^{2+}:H_2O_2 (1:5).

The results of Fenton process screening with target reduction as parameter for Hvej and Bvej leachates shown in Figure 4.3, and for AAR and ODE leachates in Figure 4.4.

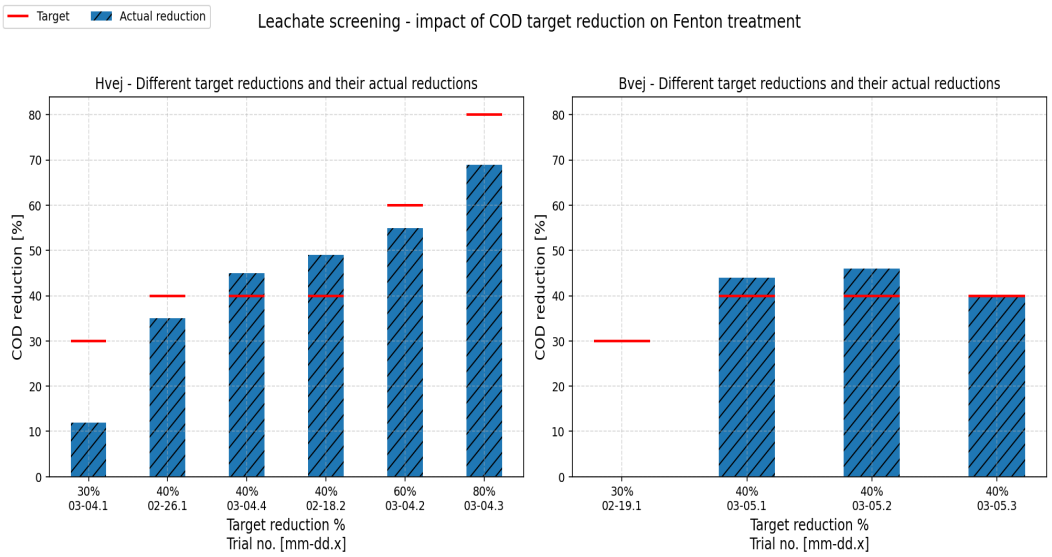

Figure 4.3: Leachate screening - Impact of target reduction on Fenton treatment: Hvej and Bvej

For Hvej, the estimated COD reductions of 30%, 40%, 60%, and 80% resulted in 12%, 35-49%, 55%, and 69% COD reduction, respectively. In comparison, Bvej at 40% target reduction showed reductions ranging from 40% to 46% for the same target values. The estimated 30% COD reduction suggests that the reagent dosages were insufficient for effective Fenton oxidation through radical generation, likely resulting in scavenging effects due to other compounds such as carbonate [Bury et al., 2024]. In contrast, the target 40% and 60% COD reduction indicated adequate dosages of Fe^{2+} and H_2O_2.

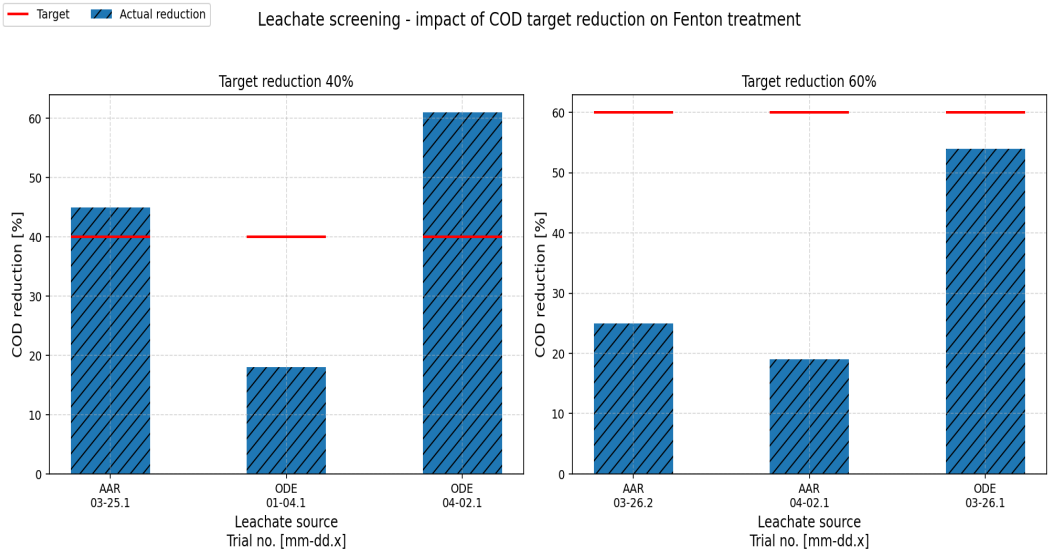

Figure 4.4: Leachate screening - Impact of target reduction on Fenton treatment: AAR and ODE

In the case of ODE, the target reductions of 40%, 40 % and 60% produced COD reductions of 18%, 61% and 54%, respectively. For AAR, the target COD reductions of 40%, 60% and 60% resulted in reductions of approximately 45%, 25%, and 19%, respectively. Initial COD for ODE being higher than AAR that is 210 mg/L produced higher COD reduction.
This indicates that the organic content in ODE leachate is more readily oxidizable under Fenton conditions. A greater proportion of the COD in ODE is possibly made up of reactive or non-refractory organics, such as volatile fatty acids or lower molecular weight compounds, which are more susceptible to hydroxyl radical attack [Bury et al., 2024].
For AAR, a higher starting COD of 72.6 mg/L achieved a greater reduction (25%) than the sample starting at 48.6 mg/L, which showed only an 19% reduction. This suggests that more organic pollutants are present, giving hydroxyl radicals more to react with, which can lead to higher removal efficiency.

The results also highlighted that the complex composition of leachate [Ates & Argun, 2021; Payandeh et al., 2017] can make it difficult to consistently reproduce effective Fenton oxidation. Therefore, the results were expected to vary, so a target with large margins was desired. Both targets of 40% and 60% show this property, but 40% was somewhat arbitrarily adopted for all further Fenton oxidation experiments.

4.2.4 Running time

Figure 4.5 illustrates the COD concentration over a 30-minute period of Fenton oxidation for both Hvej leachats under acidic conditions, molar ratio of iron to hydrogen peroxide of 1:5 and with a target of 40% COD reduction, while Figure 4.6 show the same but for AAR and ODE leachates.

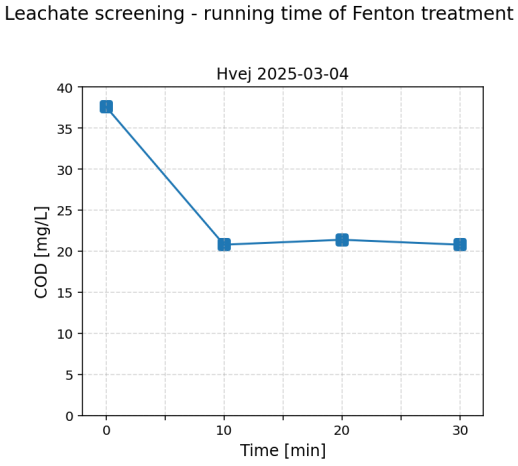

Figure 4.5: Leachate Screening- Impact of Time COD after Fenton oxidation: Hvej

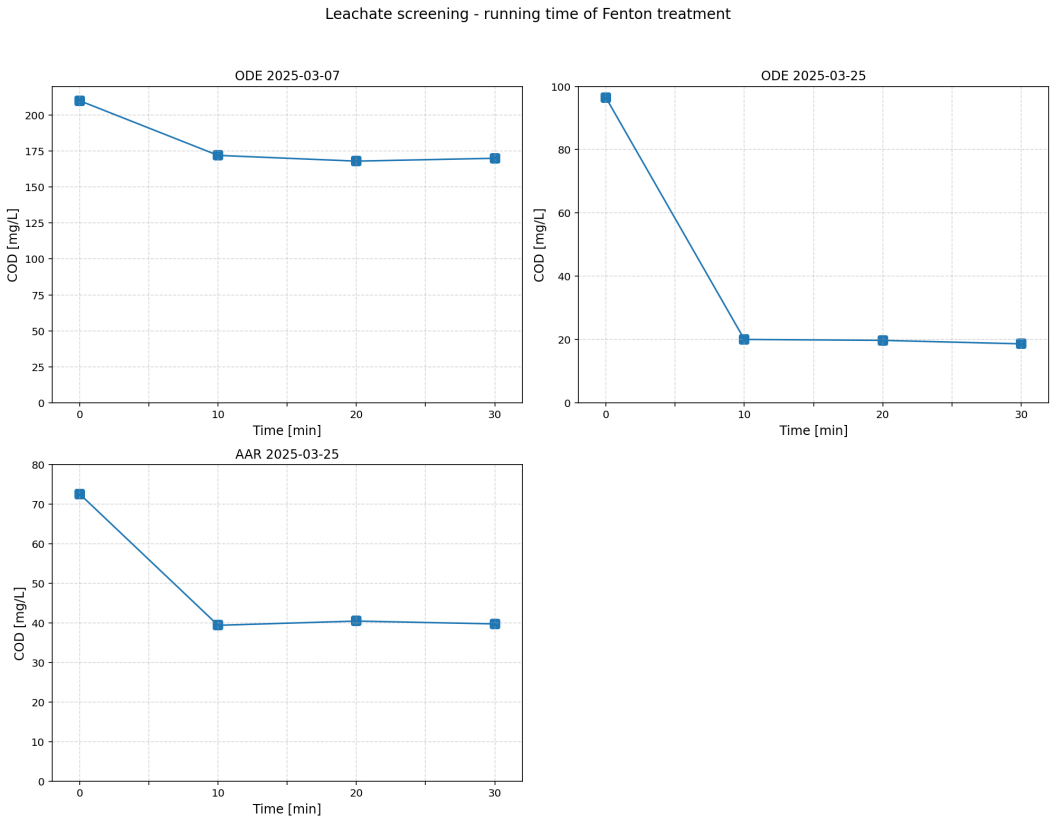

Figure 4.6: COD after Fenton oxidation: ODE and AAR

In all cases, a rapid reduction in COD was observed within the first 10 minutes. For ODE, COD dropped sharply from approximately 96 mg/L to around 20 mg/L, after which the curve plateaued with minimal further reduction. A similar trend was noted for all other trials, although the initial COD and the reduction differed. The results suggest that most of the Fenton reaction occurred within the first 10 minutes. This trend indicates that the reagents, Fe^{2+} and H_2O_2 were likely consumed early in the process, and no significant oxidation took place afterwards. This aligns with studies by Bury et al. [2024] and Bricker et al. [2014] in particular where rapid Fenton oxidation for mature leachate increased biodegradability within the reaction time of 1-5 minutes. An inital decision of a reaction time of 20-30 for subsequent experiments was chosen based on Hvej data alone, but later corroborated with ODE and AAR data.

4.2.5 Optimal Operating Conditions

After the screening stage, the optimal operating conditions applied on ODE and AAR as pretreatment before the MBBR experiment is summarised in Table 4.2.

Table 4.2: Fenton Process Operating Conditions

Conditions	**Experimental Values**
pH	2.95–3.10
Fe^{2+}:H_2O_2 molar ratio	1:5
Target COD Reduction	40%
Reaction Time	30 minutes

The optimal conditions for the Fenton oxidation experiments were determined based on consistent performance across different leachate types that align with literature recommendations (see Table 4.2). It is to be noted that the COD:H_2O_2 molar ratio was assumed to be 1:4 and the experiments were carried out at ambient temperature.

The screening matrix and raw data can be found in Appendix C.

4.3 MBBR: 24-hour Experiment

Leachates from the Norrecco Aarhus (AAR) and Odense (ODE) landfills were used in 24-hour MBBR experiments (nitrification and denitrification reactor), both with and without Fenton treatment for each leachate. Water samples were collected at regular intervals during the tests and analyzed for ammonium, nitrates, and COD. The pH was also measured at each interval. The results can be found in Appendix D.1.

4.3.1 MBBR:ODE without Fenton

The graph below illustrates the results of the nitrification experiments carried out over 24 hours for ODE without Fenton pretreatment (see Figure 4.7).

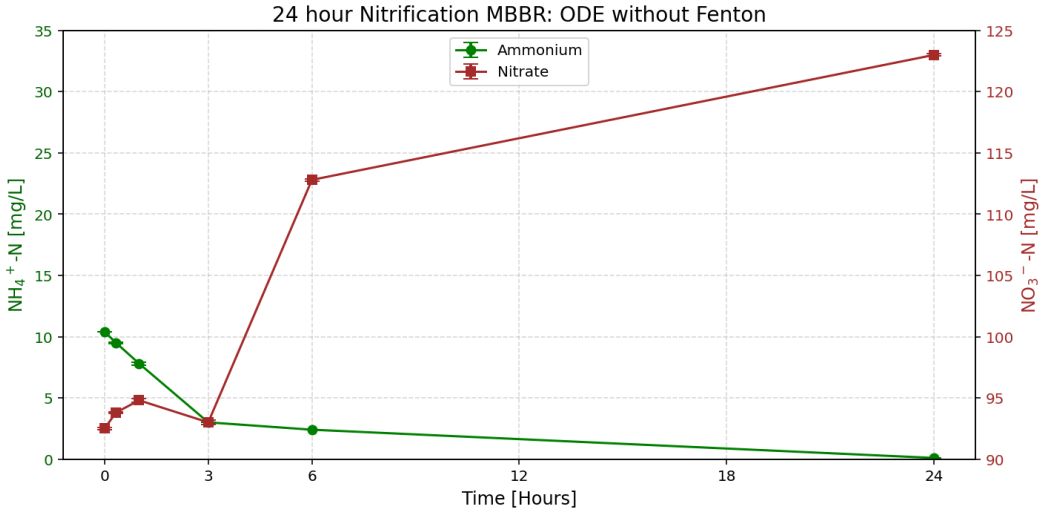

Figure 4.7: Ammonium and Nitrate concentrations over 24 hours in MBBR, nitrification reactor without Fenton pretreatment (ODE leachate) conducted on 08-04-2025

The ammonium concentration decreased from approximately 10 mg/L to approximately 2 mg/L within the first 3 hours, indicating active nitrification. A complete nitrification was observed after 6 hours, with near-zero levels at the 24-hour mark. Meanwhile, nitrate levels increased from 113 mg/L to a final value of 123 mg/L, suggesting effective conversion of ammonium through biological oxidation by autotrophs. In addition, the COD decreased from from 69 mg/L to 21 mg/L, pointing reduction of organic load of 70%. The pH ranged from 6.9 to 7.0 during the 24 hour period, which indicates a good environmental condition for microorganisms performance. A important factor for microbial biofilm growth is pH [Gupta et al., 2022] and most microorganisms have good performance around pH neutral conditions with minimum pH fluctuations [Madan et al., 2022].

The nitrification reactor successfully achieved complete nitrification of the ODE leachate within 6 hours without the need for Fenton pretreatment. This is indicated by the sharp decrease in ammonium and corresponding increase in nitrate, suggesting active nitrifying bacteria. The good performance of the MBBR aerobic reactor, indicates well-established population of nitrifying microorganisms on the biofilm carriers [Chen et al., 2007]. Furthermore, the observed COD reduction suggests that other microbial communities were also active, confirming the

presence of readily biodegradable organic matter—characteristic of a young, biodegradable leachate [Lopez et al., 2004]. However, the main purpose of nitrification process is not COD removal.

Figure 4.8 shows the results from the denitrification experiment carried out over 24 hours with ODE without Fenton pretreatment.

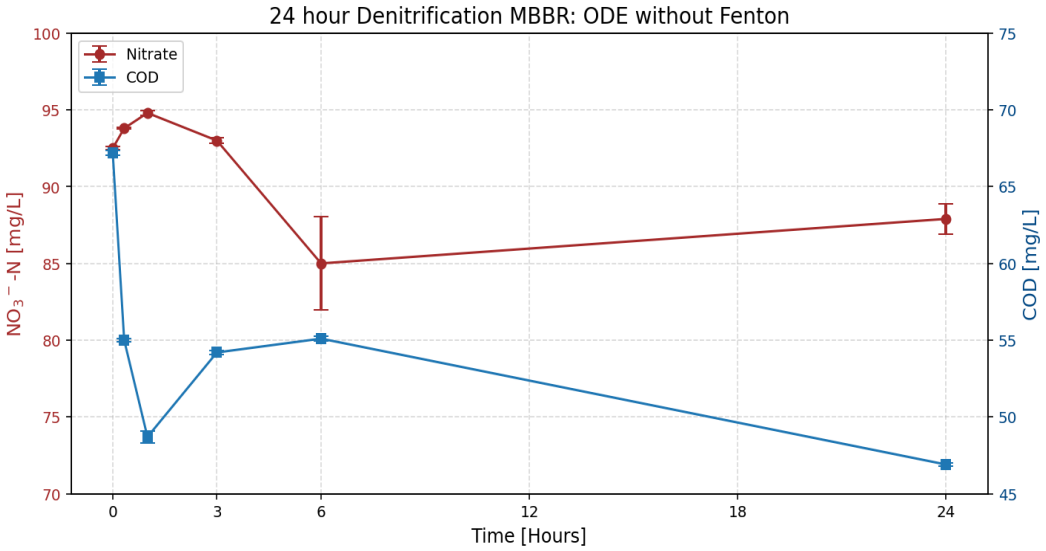

Figure 4.8: Nitrate and COD concentrations over 24 hours in the denitrification MBBR without Fenton pretreatment (ODE leachate) conducted on 08-04-2025

In the denitrification reactor, nitrate levels fluctuated in the early hours, initially increasing before decreasing with an overall 5% reduction. The COD levels decreased from 67 mg/L to 46.9 mg/L over the same period. The third measurement at the 1-hour mark can be considered to be a measurement error, as it deviates from the overall decreasing COD trend. The pH remained relatively stable, ranging from 6.9 to 7.1, a favourable condition for biofilm [Gupta et al., 2022].

The modest decrease in nitrate concentration, alongside a more notable reduction in COD, suggests limited denitrification activity, but an indication of ongoing microbial degradation of organic materials. The denitrification process may have been limited, potentially due to non-optimal conditions such as insufficient carbon availability or a low C/N ratio. This is supported by the theoretical C/N ratio required for effective denitrification is approximately 3.74 [Chiu & Chung, 2003], a condition not achieved in this experiment.

4.3.2 MBBR:ODE with Fenton

The outcome for the nitrification MBBR experiment carried out over 24 hours for ODE with Fenton pretreatment is shown in Figure 4.9.

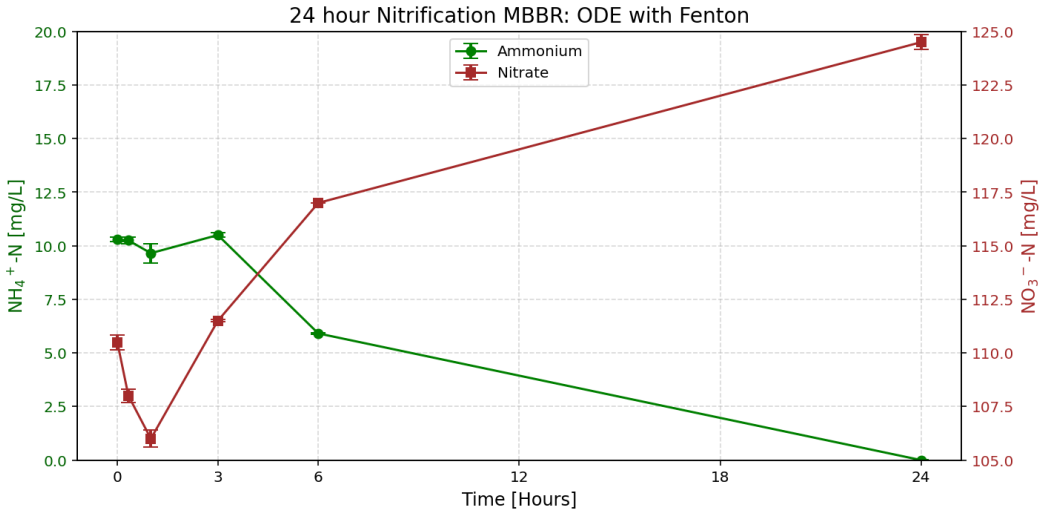

Figure 4.9: Ammonium and Nitrate concentrations over 24 hours in the nitrification MBBR with Fenton pretreatment (ODE leachate) conducted on 10-04-2025

The ammonium concentration showed a declining trend, decreasing from 10.3 mg/L to approximately 6.0 mg/L over the first 6 hours, which is slower compared to the nitrification experiment without Fenton pretreatment. Complete nitrification is observed after the 6-hour mark. Nitrate levels gradually increased, rising from an initial value of 111 mg/L to a final concentration of 125 mg/L, nearly reflecting the oxidation of ammonium. Firstly, the COD dropped from 67 mg/L to 36 mg/L with Fenton treatment, and further to 17.6 mg/L after 24 hours in the MBBR, while pH remained stable between 7.1 and 6.7, similar neutral environment as previous experiments.

The results indicate effective nitrification and biodegradation in the MBBR reactor following Fenton pretreatment, as shown by complete ammonium conversion to nitrate and a 75% COD reduction. However, this represents only a modest 5% COD removal efficiency improvement over the experiment without Fenton, suggesting that the overall performance was quite similar. The slight difference may be attributed to analytical uncertainty rather than a significant treatment effect. This further implies that Fenton treatment may have primarily acted on compounds that were already biodegradable, rather than targeting truly refractory substances. Older leachate has higher proportions of refractory compounds, such as humic and fulvic acids are difficult to treat [de Sousa et al., 2023]. The complete nitrification indicates strong microbial performance, unaffected by inhibitory compounds that might be present in leachate in general. Additionally, the observed COD reduction suggests the presence of easily degradable organic matter, further confirming that the leachate is highly biodegradable.

Figure 4.10 presents the results from the denitrification experiment carried out over 24 hours for ODE without Fenton pretreatment (see Figure 4.10).

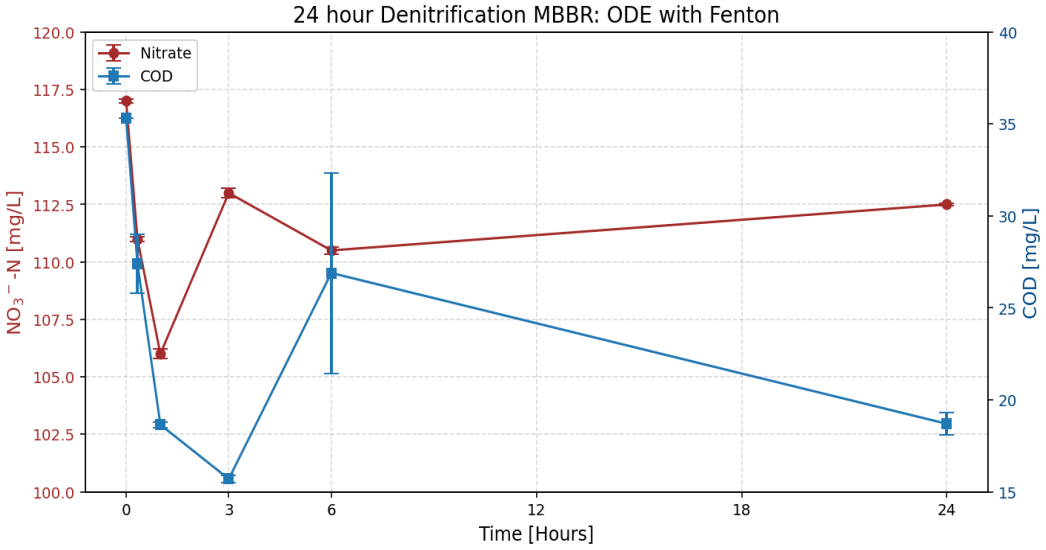

Figure 4.10: Nitrate and COD concentrations over 24 hours in the denitrification, MBBR with Fenton pretreatment (ODE leachate) conducted on 10-04-2025

Nitrate concentrations showed minor fluctuations, decreasing from around 117 mg/L to 106 mg/L at 3 hours, then gradually increasing to approximately 113 mg/L by the end of the experiment. In contrast, COD levels exhibit a declining trend with fluctuating measurements from about 35 mg/L to below 20 mg/L, indicating organic matter removal. The pH in the reactor ranged from 7.0 to 6.4, which is within a range favorable for microbial activity in the MBBR system. The overall trend suggests that while COD removal was effective, nitrate reduction was limited, suggesting that denitrification was not fully achieved, potentially due less optimal denitrification conditions such as an imbalanced C/N ratio [Chiu & Chung, 2003] similar to the experiment of ODE without Fenton. However, the observed COD reduction may result from anaerobic digestion or fermentation—a biological process in which microorganisms degrade organic matter without oxygen, producing biogas primarily composed of methane and carbon dioxide. Additives like iron (from Fenton treatment) can enhance microbial activity, improve process stability, and increase methane production [Pashaki et al., 2024], thereby boosting overall efficiency, the reduction in pH supports the production of CO_2.

Moreover, the interruption of stirring in the denitrification MBBR experiments likely caused non-uniform conditions, reducing nitrate availability to denitrifiers and disrupting anoxic conditions—both detrimental to nitrate removal. Adequate mixing and hydrodynamics are important for healthy biofilm and substrate diffusion [Madan et al., 2022]. Furthermore, this may explain the fluctuations in COD measurements, as unhomogeneous mixing can lead to uneven distribution of colloids, resulting in variability and measurement uncertainty. Therefore, operational malfunction can have some impact on the results of the 24-hour denitrification experiment.

4.3.3 MBBR: AAR without Fenton

Figure 4.11 shows AAR leachate that was treated in the MBBR without any Fenton pretreatment.

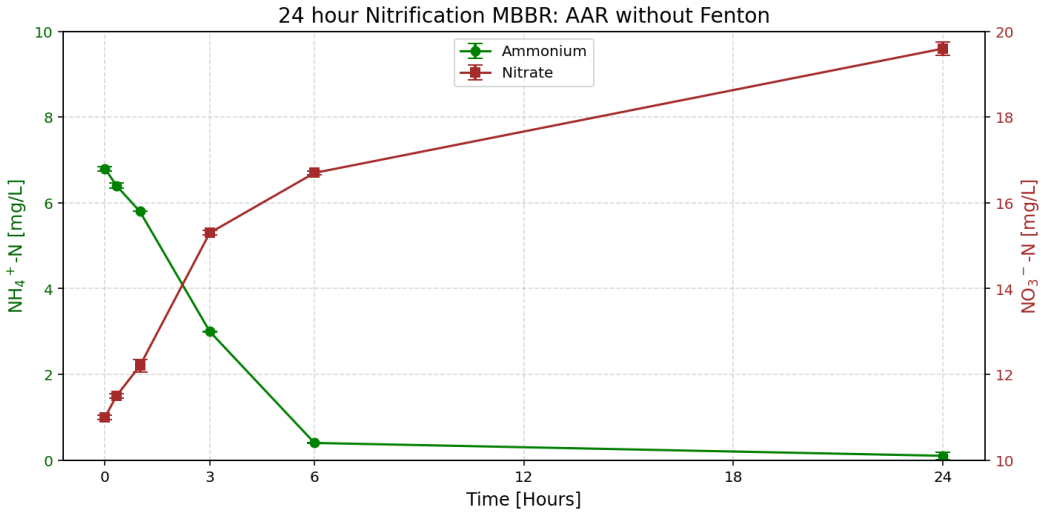

Figure 4.11: Ammonium and Nitrate concentrations over 24 hours in the nitrification MBBR without Fenton pretreatment (AAR leachate) conducted on 14-04-2025

The MBBR nitrification experiment with AAR leachate (without Fenton) showed that ammonium decreased from 6.8 mg/L to nearly zero within 6 hours, indicating rapid nitrification. While nitrate levels increased from 10 mg/L to 19.6 mg/L over 24 hours corresponding to the ammonium oxidation. The COD also decreased from 39.8 mg/L to 19.1 mg/L, while pH remained stable between 7.2 and 7.7, supporting microbial activity. These results show efficient nitrification and organic matter biodegradation within the MBBR. The ammonium oxidation and nitrate accumulation indicate active presence of nitrifiers on the carriers. Similar to ODE, the aerobic reactor can be considered to have a good performance by complete nitrification and reduction of 25% COD, suggesting a biodegradable leachate [Lopez et al., 2004]. AAR seems to exhibit characteristics of a young leachate with easily degradable organic matter, which is similar to ODE. Therefore, relying solely on COD and pH values is not sufficient to reliably characterize the leachate as old, nor to assume that a large portion of the organic matter is refractory, as discussed in Section 4.1.

Figure 4.12 presents the performance of the denitrification MBBR in treating AAR leachate without the application of Fenton pretreatment.

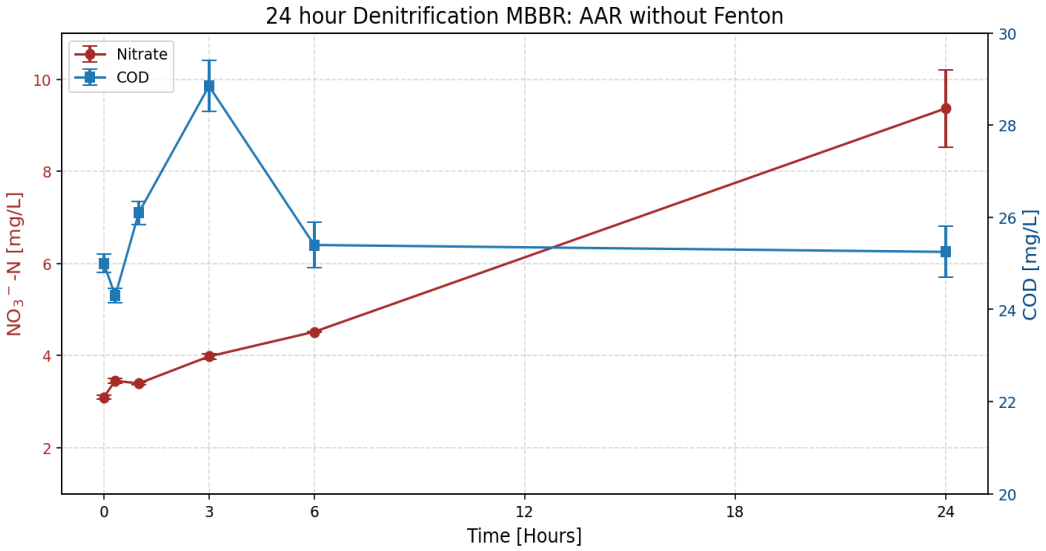

Figure 4.12: Nitrate and COD concentrations over 24 Hours in the denitrification MBBR without Fenton pretreatment (AAR leachate) conducted on 14-04-2025

The denitrification experiment using AAR leachate (without Fenton pretreatment) showed an increase in nitrate concentration from approximately 3 mg/L to over 9 mg/L over 24 hours, contrary to expectations for a denitrification process. COD concentrations fluctuated slightly, beginning at 25 mg/L and ending at 25.25 mg/L, showing no meaningful change. This indicates that neither nitrate reduction nor organic matter degradation occurred effectively in the denitrification reactor, suggesting that heterotrophic denitrifiers were either inactive or inhibited. The increase in nitrate concentration suggests that ammonium oxidation may have taken place, indicating a failure to maintain true anoxic conditions.
Additionally, the carbon-to-nitrogen (C/N) may not have been optimal [Chiu & Chung, 2003], restricting heterotrophic denitrifier activity, hence hindering the denitrification process.

The fluctuating COD measurements, including the increase observed at the 3-hour mark, may be attributed to a malfunctioning flow meter, which disrupted homogeneous mixing in the reactor and potentially affected the results, similar to the ODE denitrification experiment.

4.3.4 MBBR: AAR with Fenton

It should be noted that Fenton treated AAR leachate used in the MBBR 24-hour experiments did not produce the target reduction of 40%, instead it showed no reduction. The Figure 4.13 shows the performance of the nitrification MBBR in treating AAR leachate pretreated with Fenton oxidation.

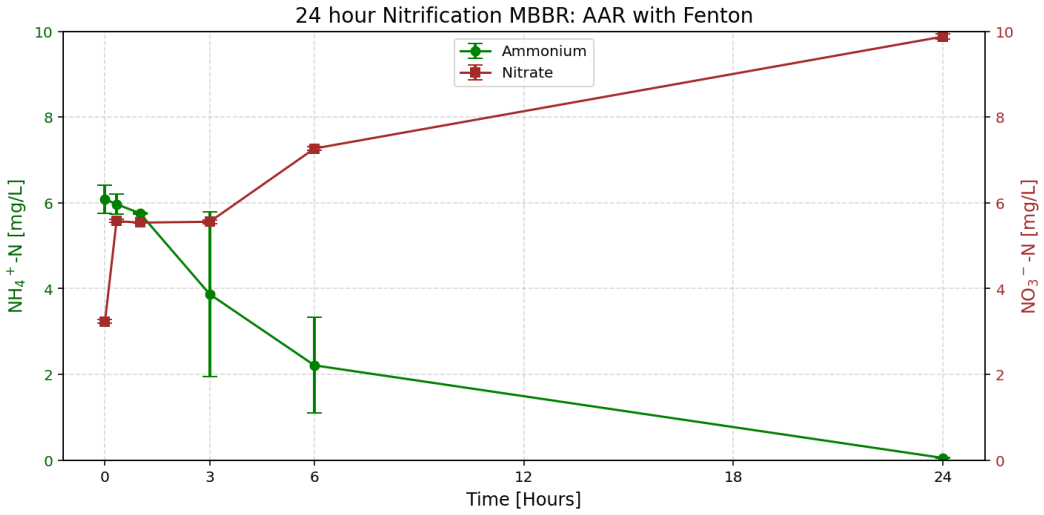

Figure 4.13: Ammonium and Nitrate concentrations over 24 Hours in the nitrification MBBR with Fenton pretreatment (AAR leachate) conducted on 15-04-2025

In the nitrification experiment with the AAR leachate, the ammonium concentration decreased from 6 mg/L to approximately 4 mg/L within the first 3 hours, and further declines to around 2 mg/L by the 6-hour mark. A complete nitrification was observed after 6 hours. Simultaneously, the nitrate levels increase over time from 3 mg/L to about 10 mg/L, which reflects the oxidation of ammonium. The COD decreased from an initial value of 38.6 mg/L to 15 mg/L after 24 hours, while the pH declined slightly from 7.6 to 7.0. This is a stable condition for a healthy biofilm.

In comparison to the AAR leachate without Fenton and ODE with Fenton, the oxidation of ammonium occurred at a slower rate. The Fenton pretreatment temporarily affect nitrifiers, similar to the experiment carried out with ODE leachate. Moreover, the slower rate than ODE with Fenton can be attributed to residual H_2O_2, potentially resulting from an ineffective Fenton dosage. However, MBBR was effective in showing complete nitrification after the 6-hour mark. There is also a COD reduction after 24 hours, even at a lower starting COD of 38.6 mg/L, suggesting a variety of microbial communities. The COD measurement taken immediately after Fenton treatment showed only 3% reduction, indicating that the process did not significantly mineralize the organic matter. This may be attributed to the initial COD measurement not accurately reflecting the actual COD used for reagent dosage calculations. The second sampling of AAR revealed the presence of numerous small living organisms, indicating that the leachate was highly biodegradable and rich in organic matter. This could have led to rapid COD degradation, independent of the Fenton treatment. Moreover, after 24 hours in the aerobic reactor, COD decreased to 15 mg/L, compared to 19 mg/L in the AAR sample without Fenton. This minimal difference suggests that Fenton, if complete, had little to no impact on overall COD removal.

The results of the denitrification MBBR in treating AAR leachate with Fenton pretreatment is shown in Figure 4.14.

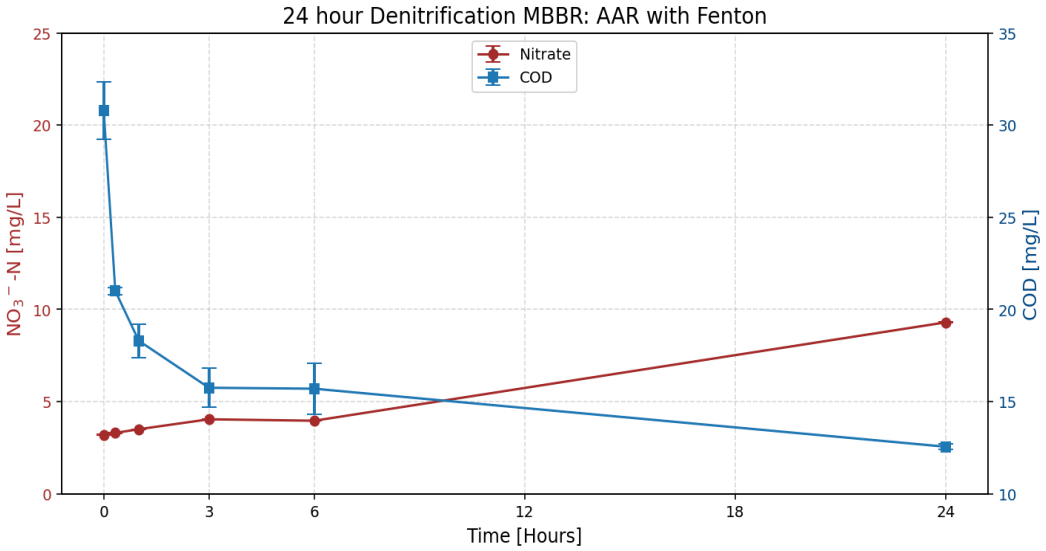

Figure 4.14: Nitrate and COD concentrations over 24 hours in denitrification MBBR with Fenton pretreatment (AAR leachate) conducted on 15-04-2025

The denitrification experiment showed that the nitrate concentrations increased during the 24-hour MBBR experiment in the denitrification reactor, rising from just over 3 mg/L to approximately 10 mg/L. The increasing nitrate concentration in the denitrification reactor suggests that ammonium is being oxidised, which is unexpected in an anoxic environment. The COD is declining, and the pH showed a slight drop, decreasing from 7.4 to 6.8. This could suggest fermentation, but an increase in nitrate indicates aerobic conditions. The COD shows that microbial activity, implying that the leachate is highly biodegradable, similar to ODE.

It is important to note that during the denitrification reactor experiments, the flow meter used to mix the water sample was found to have stopped at some point during the 24 hours even with addition of a new meter. This may have affected the denitrification results, since homogenous mixing is an important factor for substrate diffusion in biofilm [Madan et al., 2022]. In this case, the COD is very low, and the C/N ratio of 4 is not maintained. This also affects the denitrification process.

Detailed measurements for all 24-hour experiments are provided in the Appendix D.

4.3.5 Summary of results: 24-hour MBBR Experiment

Table 4.3 show the balance of nitrate and ammonium in the Nitrification experiments.

Table 4.3: N-balance in MBBR experiments

Sample	ΔNO_3^--N [mg/L]	ΔNH_4^+-N [mg/L]	**Net-N [mg/L]**
AAR Nitrification	8.6	-6.7	1.9
AAR Fenton + Nitrification	6.7	-6.0	0.7
ODE Nitrification	19.5	-10.4	9.1
ODE Fenton + Nitrification	14.0	-10.3	3.7

The AAR Nitrification MBBR experiment are nearly balanced, showing a complete nitrification of ammonium. The small discrepancy in AAR nitrification could be associated to analytical measurement error. There is complete nitrification in ODE experiments, however, the net nitrogen is positive, suggesting a other nitrogen source. This could be due to the presence of nitrite in the raw leachate and the Fenton process that partially oxidizes it before nitrification.

Figure 4.15 presents the COD concentrations and their respective removal efficiencies for ODE and AAR leachates with and without Fenton treatment in MBBR nitrification compared to Raw leachate.

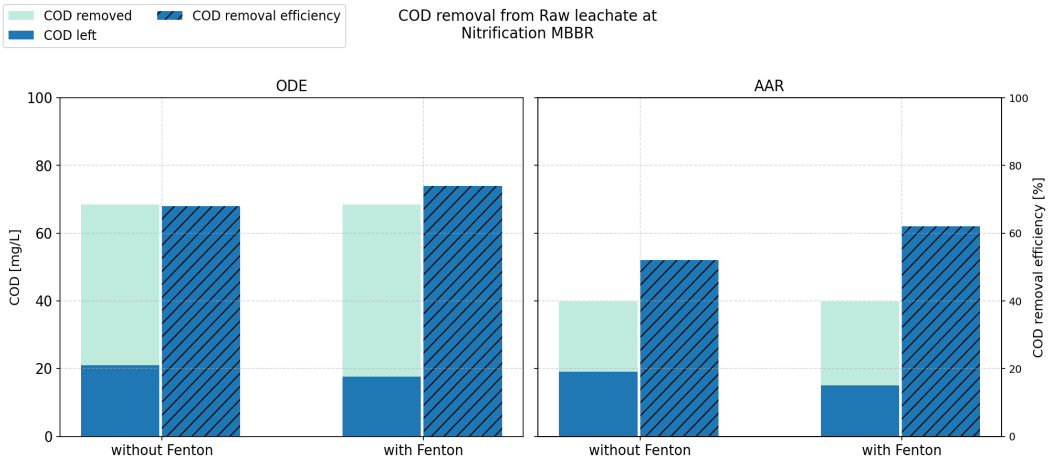

Figure 4.15: COD Removal in Nitrification MBBR with/without Fenton pretreatment compared to Raw: ODE and AAR

Both setups exhibited effective nitrification and biodegradation, with the Fenton-pretreated leachate showing a higher overall COD removal. This slight improvement may be attributed to Fenton pretreatment, enhancing the biodegradability of more complex organic compounds, making them more accessible to microbial degradation in the MBBR. However, the small difference suggests that the ODE leachate likely already contained a substantial fraction of readily biodegradable compounds, and therefore, the effect of pretreatment was negligible.

Figure 4.16 presents the COD concentrations and their respective removal efficiencies for ODE and AAR leachates with and without Fenton treatment in MBBR denitrification.

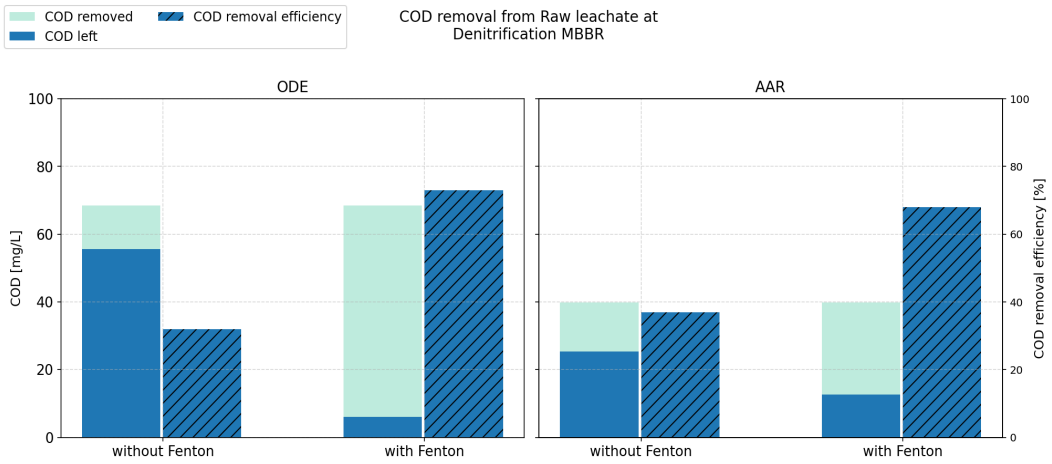

Figure 4.16: COD Removal in Denitrification MBBR with/without Fenton pretreatment compared to Raw: ODE and AAR

For ODE, COD removal efficiency increased from 32% to 73% after Fenton pretreatment, and for AAR 37% to 68%. This shows that Fenton pretreatment significantly improved biodegradability of leachate for the microorganisms present in the Denitrification reactor.

4.4 Oxygen Uptake Rate (OUR) - DO Experiment

The DO experiment is an adapted respiratory assessment designed to evaluate the biodegradability of leachate at different stages of experiments with and without Fenton oxidation and MBBR. In this procedure, samples—including untreated leachate (Raw), leachate treated with Fenton reagent (Fenton), and leachate subjected to both Fenton treatment and subsequent MBBR process (Nitrification, Denitrification)—are mixed with activated sludge. Dissolved oxygen levels in these mixtures were monitored over time, and the resulting changes were used to calculate the oxygen uptake rate. The results are based on single-run experiments conducted after each treatment stage, which can introduce a level of uncertainty. The data and an example calculation can be found in Appendix E.1.

The resulting OUR values for ODE, which indicate the oxygen consumption rate of each sample, are shown in Figure 4.17.

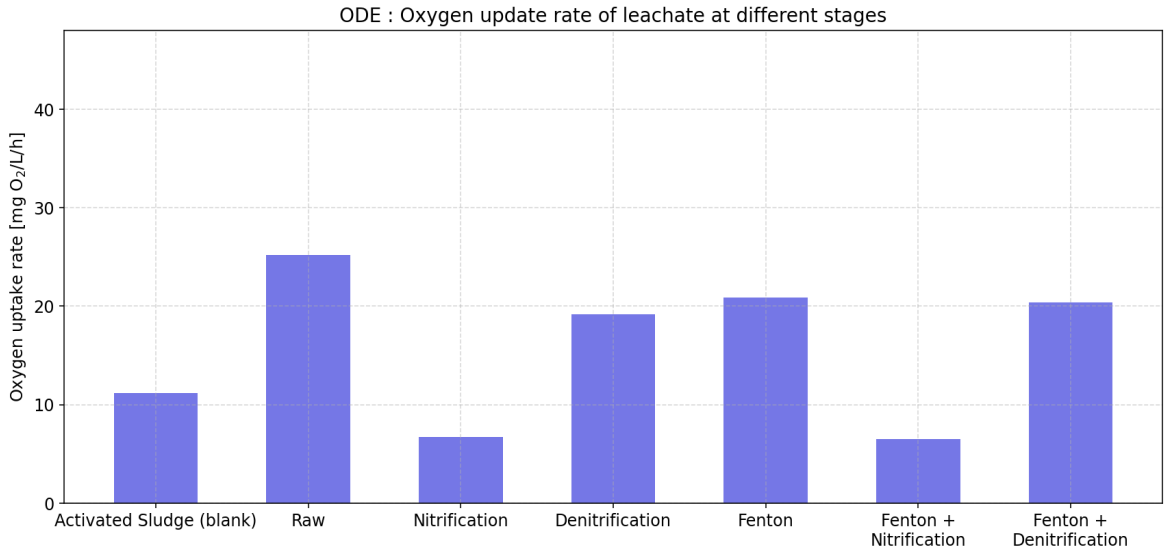

Figure 4.17: OUR for Activate sludge, raw ODE, and the different stages of treatment

The activated sludge alone exhibited a baseline OUR of 11.2 mg O_2/L/h. The raw leachate, ODE showed an OUR of 25.2 mg O_2/L/h and 20.9 mg O_2/L /h after Fenton. The lower OUR after Fenton suggests that the treatment reduced the availability of substrates for microorganisms in the DO experiments. It can therefore, be deduced that ODE leachate was naturally highly biodegradable in its raw state, as also indicated by the decrease in OUR to 6.8 mg O_2/L following the MBBR nitrification reactor. This decrease suggests that the microorganisms efficiently utilized the available organic matter, leading to a reduced demand for oxygen. In contrast, the denitrification reactor recorded a much higher OUR of 19.2 mg O_2/L/h, implying that the microorganisms in this reactor were less effective. This aligns with the results from the 24 hour MBBR experiments, which also indicated poor performance in the denitrification stage.

The Fenton-treated leachate showed an OUR of 6.5 mg O_2/L/h after the nitrification reactor, nearly identical to the value observed without Fenton. This suggests that the Fenton process did not enhance the ODE's biodegradability.
The results for Denitrification after Fenton pretreatment is nearly identical with Denitrification without Fenton, in contrast with highly improved COD removal rates (Figure 4.16), suggesting that the additional COD removed was not substrate for the microorganisms in the activated sludge.

The resulting OUR values for AAR samples at different treatment stages are shown in Figure 4.18.

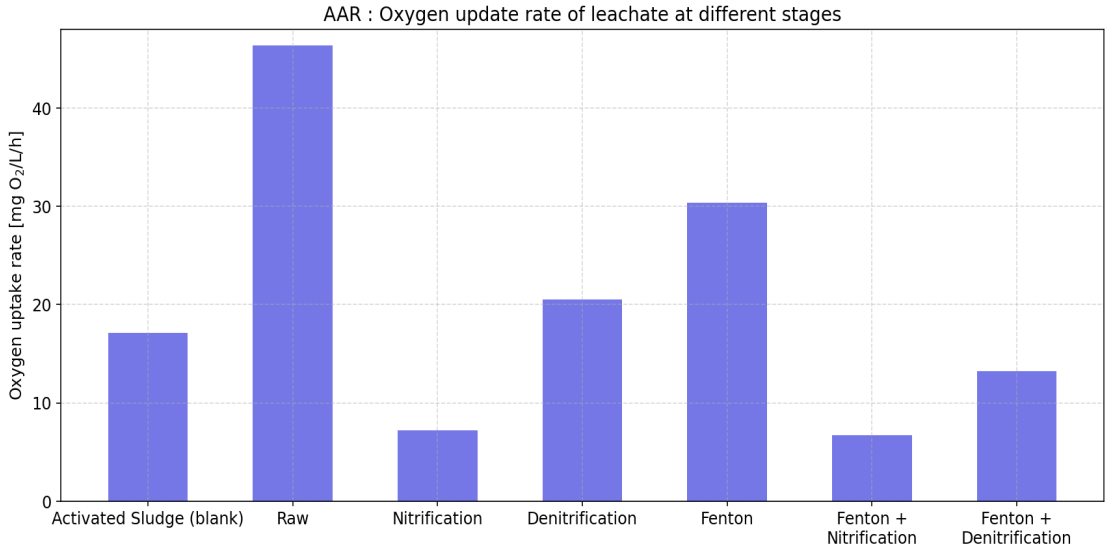

Figure 4.18: OUR for Activated Sludge and AAR leachate at different treatment stages

For the AAR samples, the activated sludge showed an OUR of 17.1 mg O_2/L/h. The raw AAR shows an OUR of 46.4 mg O_2/L/h and a lower OUR of 30.9 mg O_2/L/h after Fenton. Higher respiratory activity in the untreated leachate reflects a higher availability of substrates, allowing for greater microbial consumption [Hagman & l. C. Jansen, 2007]. The OUR value for AAR was significantly higher compared to ODE. During a second sampling of AAR, numerous small living organisms were observed in the sample, suggesting that the leachate contained an abundance of readily available organic matter. This supports the indication that the AAR leachate was highly biodegradable.
After the nitrification reactor, the OUR value without and with Fenton was estimated to be 7.2 mg O_2/L/h and 6.7 mg O_2/L/h respectively, which is unchanged consumption. This indicates that Fenton did not enhance the biodegradability of the leachate, or that the biodegradable organic matter present was already readily available for microbial consumption, regardless of pretreatment.
The denitrification reactor showed an OUR of 20.5 mg O_2/L/h and 13.2 mg O_2/L/h without and with Fenton, respectively. Alternatively, the significant decrease in OUR in the Fenton-treated leachate suggests improved performance of the reactor with the Fenton-treated sample in the denitrification experiment. However, this does not necessarily confirm that Fenton treatment had a meaningful impact on the AAR leachate, as the high OUR observed in the raw leachate indicates the presence of biodegradable substrates.

The DO experiments conducted on both ODE and AAR leachates showed similar patterns, with the highest OUR values observed in the raw leachates, indicating the presence of a readily biodegradable carbon source. As noted Hagman and l. C. Jansen [2007], a higher OUR reflects a more easily degradable carbon source. Following Fenton treatment, a decrease in OUR was observed, indicating that microorganisms in the MBBR carriers consumed the available organic matter. However, the total oxygen consumption with and without Fenton was very similar, showing that this experiment cannnot confirm earlier findings of increased COD removal in Denitrification. This indicates that it was anaerobic microorganisms responsible for the

increased COD removal.

4.5 COD, TOC Measurements and Oxidation level of Carbon, OX_C

The COD, TOC and OX_C measurements are analysed to give an overview of the quality and treatability of leachate substrates, particularly about their biodegradability, energy content, and degree of oxidation or reduction.

Figure 4.19 illustrates the variation in TOC, IC and COD concentrations across ODE leachate samples subjected to different treatment stages, both with and without Fenton oxidation.

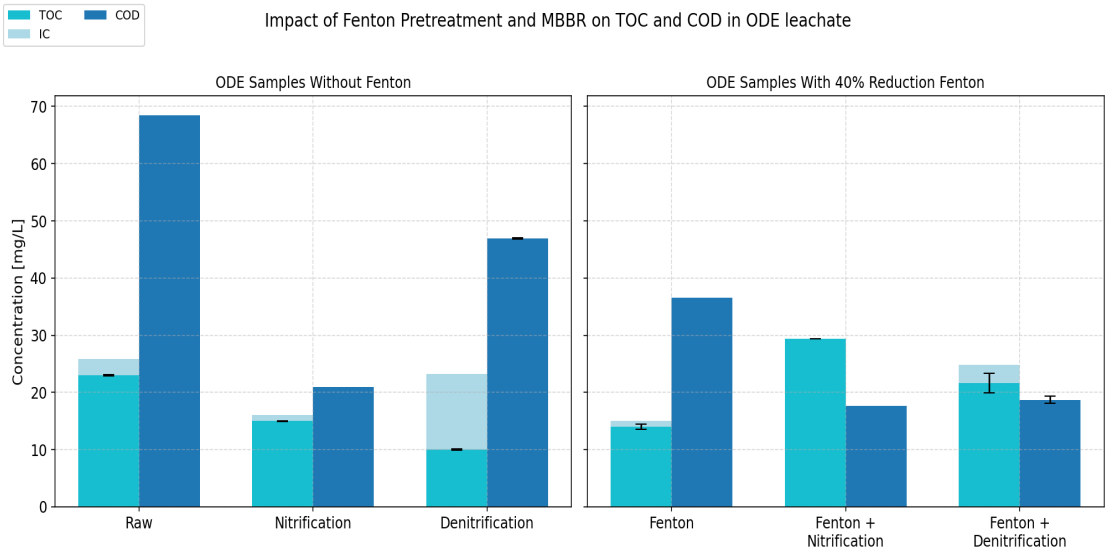

Figure 4.19: TOC and COD concentration measured without Fenton/MBBR treatment, with only MBBR treatment and with Fenton /MBBR treatment for ODE

The reduction of TOC and COD after the ODE nitrification MBBR indicates the presence of effective and heterogeneous community of microorganisms, since COD removal is not the main purpose of Nitrification MBBR. However, the high COD level after the denitrification stage suggest that it is not performing as intended. The IC value implies production of CO_2 dissovled as bicarbonate (HCO_3^-), it supports possible fermentation.
The decrease in TOC following Fenton treatment indicates that hydroxyl radicals targeted and oxidized the available organic carbon in the leachate and mineralizing a significant part. This behaviour is supported by beaker experiments in the screening process, showing CO_2 release—likely due to outgassing caused by stirring and low pH conditions (see appendix C.1). Moreover, this reduction is also supported by TOC reduction performed by [Aramyan & Moussavi, 2017; Mahtab et al., 2021; Ziembowicz & Kida, 2022]

The higher TOC values observed after Fenton treatment and MBBR exceeded the corresponding COD values. This may be attributed to biofilm detachment, contributing additional carbon to the samples or potential experimental errors. Denitrification with Fenton treated leachate shows some IC hinting at fermentation.

Figure 4.20 illustrates the variation in TOC, IC and COD concentrations across AAR leachate samples subjected to different treatment stages, both with and without Fenton oxidation.

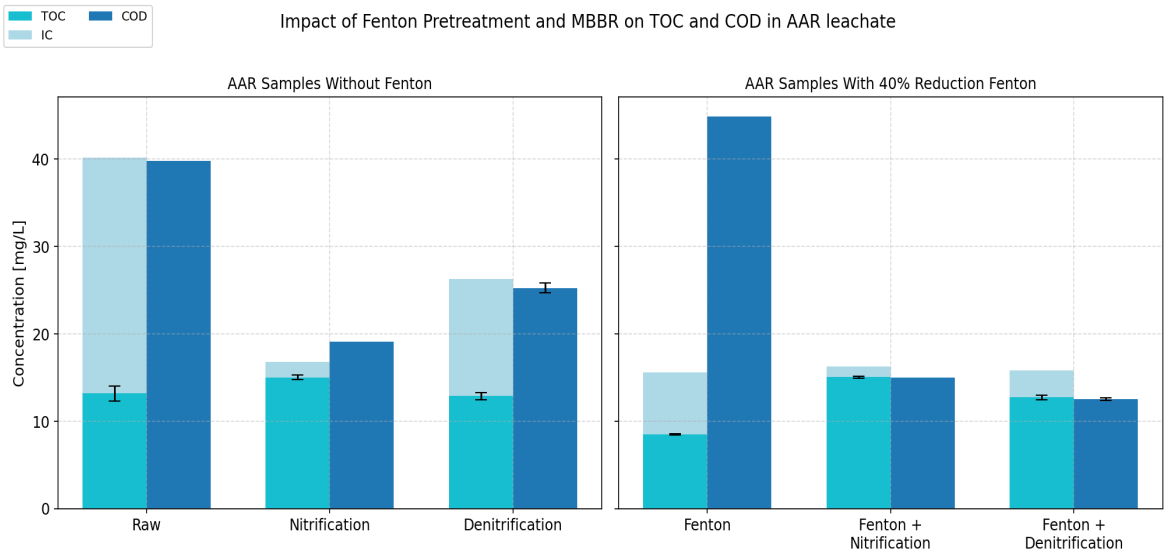

Figure 4.20: TOC and COD concentration measured on Raw AAR, without Fenton/after MBBR treatment, and with Fenton/after MBBR.

In samples without Fenton treatment, COD decreased from 39.8 mg/L to 19.1 mg/L after nitrification and to 25.25 mg/L after the denitrification reactor. While TOC remained relatively stable overall.

After Fenton, slightly higher COD was measured, which is an indication of residual H_2O_2 affecting the HACH measurements. This could be due suboptimal reagent dosages. This also supported by the IC, where none was expected. It is to noted that the Fenton process may have completed at a later stage.

TOC increases after Fenton, both in Nitrification and Denitrification reactors, could again be attributed to biofilm from the reactors. The Fenton stage left bicarbonate in the leachate which nitrifiers could convert back to TOC, and combined with a malfunctioning Denitrification reactor (see section 4.3.4).

After Fenton both the Nitrification and Denitrification reactors behaved similarly, in both COD and TOC, suggesting that while the Fenton treatment did not work as expected, it did have an effect.

Table 4.4 and 4.5 present the TOC and COD concentrations, removal efficiencies, and oxidation level of carbon (OX_C) for ODE and AAR leachates subjected to MBBR treatment—with and without 40% Fenton pretreatment.

Table 4.4: TOC, COD, Removal Efficiencies with their corresponding OX_C for ODE Samples

Sample	TOC [mg/L]	COD [mg/L]	TOC Removal %	COD Removal %	OX_C
Raw	23.0 ± 0.12	68.5	–	–	-0.47
Nitrification	15.1 ± 0.07	21	34	69	1.91
Denitrification	10.2 ± 0.15	46.9 ± 0.10	56	32	-2.89
Fenton	14.0 ± 0.48	36.6	39	47	0.08
Fenton + Nitrification	29.4 ± 0.01*	17.6	-28	74	3.10*
Fenton + Denitrification	21.6 ± 1.73*	18.7 ± 0.60	6	73	2.70*

* TOC values may not represent true values as discussed previously.

Table 4.5: TOC, COD, Removal Efficiencies with their corresponding OX_C for AAR Samples

Sample	TOC [mg/L]	COD [mg/L]	TOC Removal %	COD Removal %	OX_C
Raw	13.2 ± 0.86	39.8	–	–	-0.53
Nitrification	15.0 ± 0.27	19.1	-14	52	2.09
Denitrification	12.9 ± 0.43	25.3 ± 0.55	2	37	1.06
Fenton	8.6 ± 0.07	44.9	35	-12	-3.83
Fenton + Nitrification	15.1 ± 0.11*	15.0	-14	62	2.51*
Fenton + Denitrification	12.7 ± 0.27*	12.6 ± 0.15	3	68	2.52*

* TOC values may not represent true values as discussed previously.

Both ODE and AAR have an OX_C value, calculated by Equation 2.3, of -0.47 and -0.53, respectively, indicating that the leachates are in a reduced form and a favorable substrate quality for microbial activity [Hvitved-jacobsen, 2002]. In contrast, the positive OX_C values in nitrification/denitrification reactors for both ODE and AAR suggest more oxidized substrates. This indicates that the leachate has been oxidised by the microorganism in the MBBR, with the exception of the negative value of in denitrification reactor for ODE. This negative value may be due to malfunctioning of the denitrification reactor, therefore this value can be discarded. After ODE Fenton the OX_C value rises to near zero, leaving a more oxidized substrate by lowering both COD and TOC, meaning that Fenton acted upon biodegradable organics in the leachate and mineralizing a significant part. The value of -3.83 after AAR Fenton again reflects the malfunctioning Fenton treatment discussed earlier.
Due to inflated TOC results after Fenton treatment for both AAR and ODE, the OX_C values are in turn also inflated and should discarded.

The OX_C values relates to COD removal rates, but instead gives an indication of how depleted the source material is, thereby could be of help in decision making when stop COD reduction.

4.6 Inhibition Analysis

The inhibition analysis is performed using the bacteria *A. Fischeri* to assess the relative inhibition, q (see Equation 3.1) of the leachate. The more inhibitory compounds the samples contains, the slower the bacteria grow, up to complete inhibition with a reported value of 1. Figure 4.21 shows an example of the inhibition result for only the raw leachates at different dilutions.

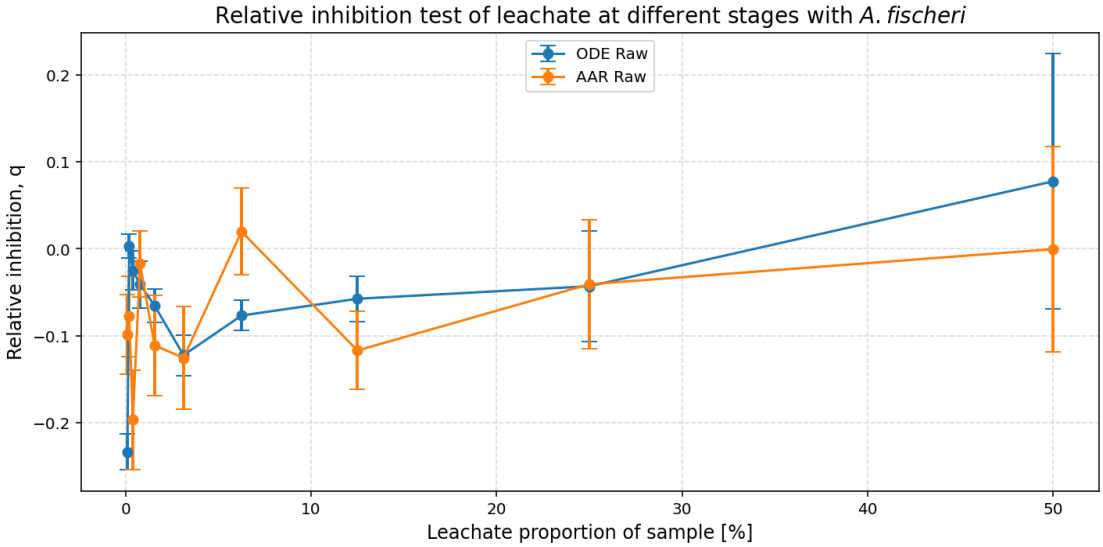

Figure 4.21: Inhibition test - q values for ODE and AAR at different dilutions with *A. Fischeri*

At 50% concentration, the q is estimated around zero. Across the various dilutions, the values showed a general declining trend with some fluctuations at higher dilution levels. This is likely due to pipetting variability. Data for the other stages shows similar results. Data and graphs for all stages are available in Appendix F.
Since data did not conform to the expected sigmoidal fit of the Hill equation, making it impossible to determine an EC_{50} which is usually reported with this analysis [Roslev, 2024]. This further supports the conclusion that the samples did not exhibit any concentration-dependent inhibition.

The results of the inhibition analysis in ODE and AAR with leachate at 50% volume of the sample at different stages is shown in Figure 4.22.

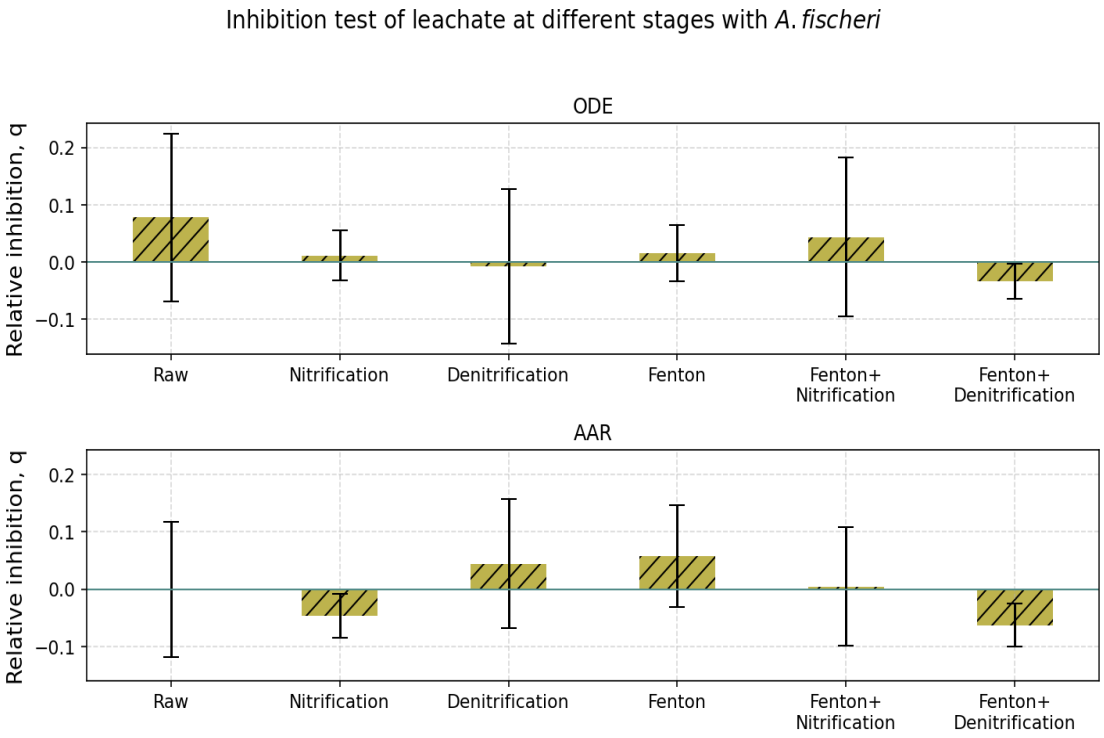

Figure 4.22: Inhibition analysis - Relative inhibition to reference culture

High values (1) shows complete inhibition, while 0 shows none. Negative values may indicate promotion of growth.

This assay indicated no detectable inhibition in both ODE and AAR at the different dilutions. This is seen by the q values being near zero or negative even at 50% leachate volume, and zero within the standard deviation or close. As a consequence, the data did not align with the Hill curve model, which prevented calculation of EC_{50} which is usually reported with this analysis [Roslev, 2024].

Remmas et al. [2023] assert that metals, xenobiotics, and pharmaceuticals—including CHCs, PFCs, phenols, PAHs, and plasticizers—contribute to the recalcitrant nature of landfill leachate and can pose significant risks to living organisms even at low concentrations.
ODE was sampled from a nitrification/denitrification tank, meaning it had already received some level of biological treatment, which likely explains the lack detection of inhibitory effects observed. This likely explains the absence of toxic effect, since previous treatments would reduce the concentration of inhibitory compounds. Additionally, its use as inoculum for a pilot MBBR at Norrecco further suggests that this leachate supports active microbial communities.
AAR was collected after a settlement tank and is likely a relatively young leachate. Regular waste rotation at the Aarhus landfill and prior experiences requiring rainfall for leachate collection support this assessment. These factors may explain the absence of detectable inhibitory effects in the AAR leachate sample.
Since previous experiments also showed that the leachates were highly biodegradable, thereby implying less refractory content, this supports no detectable inhibitory characteristics.

It is possible that a more targeted and sensitive analytical approach could yield more definitive results, particularly if the specific contaminants present in the leachate were identified. A way to improve detection sensitivity and provide a more comprehensive understanding of potential toxicity could be by extending the duration of exposure, such as increasing it to more than one hour instead of 30 minutes exposure time in the plate reader. There are potential uncertainties that may also arise from sample preparation methods and prolonged storage.

5 | Conclusion

The primary objective of the Master's thesis was to assess whether Fenton pretreatment could improve leachate biodegradability by breaking down complex refractory organic compounds into simpler and more bioavailable forms, thus improving the effectiveness of subsequent treatment in a Moving Bed Biofilm Reactor. The Denitrification MBBR 24-hour experiments showed positive results in this regard. Moreover, the lowering of the COD levels also reduces competition with micropollutants for adsorption or reaction sites, potentially enabling more effective and efficient micropollutant removal.

In the screening of different leachate samples was conducted to determine optimal operating conditions and evaluate the impact of key parameters on reagent dosing efficiency (H_2O_2 and Fe^{2+}). The identified optimal conditions included an acidic pH, a Fe^{2+} to H_2O_2 molar ratio of 1:5, and a target COD reduction of 40%. Moreover, the COD:H_2O_2 molar ratio was assumed to be 1:4. These conditions were applied with a reaction time of 30 minutes to treat the ODE and AAR leachates before the 24 hour MBBR experiments.

In the denitrification reactor, Fenton pretreatment led to significantly higher COD removal—ODE increased from 32% to 73% and AAR from 37% to 68%. These results from the 24-hour MBBR denitrification experiments indicate that Fenton treatment enhanced the biodegradability of the leachates for the denitrifiers. However, subsequent experiments could only show that ODE and AAR, were highly biodegradable from the beginning of the project.

Initially, based on COD and pH measurements, the Hvej, Bvej, ODE, and AAR leachates were classified as mature. However, subsequent experiments with ODE and AAR indicated otherwise. The AAR and ODE leachate behaved similar in most experiments, even though COD was higher in ODE. Both leachates demonstrated complete nitrification and moderate COD reduction in the MBBR, indicating good nitrification performance and the presence of readily biodegradable compounds, both with and without Fenton treatment. A small 5% improvement in COD reduction for ODE following Fenton in the nitrification reactor suggests that Fenton primarily removed already biodegradable organics. Additionally, ODE leachate had already received biological treatment, therefore the lack of detectable inhibitory effects.

The DO experiments revealed higher OUR values for ODE and AAR raw leachates, which decreased after Fenton treatment. This suggests that Fenton reduced the availability of biodegradable substrates for microorganisms, indicating that the raw leachates were already highly biodegradable—characteristic of younger leachates.

Furthermore, the negative OX_C values for ODE and AAR indicate a reduced substrate, a favorable quality for microbial activity. After Fenton OX_C increases, showing a more oxidised form of the substrates, indicating the Fenton reaction occurred on biodegradable organics and not the refractory part. The TOC increased after nitrification and denitrification experiments, and it was attributed to biofilm addition from the reactor. Therefore, the OX_C values for these experiments cannot be considered. The relationship with OX_C values and COD removal can be used to assess COD reduction.

An inhibition analysis was performed and the results showed no detectable inhibitory compounds present in the leachates. This indicated a younger leachate, since the recalcitrant nature of mature leachate shows some toxicity even at low concentrations. A specific inhibition analysis or the extension of the exposure duration could potentially yield different results.

The study demonstrated that Fenton oxidation was generally effective in reducing COD across the various leachate samples. An improvement in biodegradability is shown by the signification COD reduction in both ODE and AAR after Fenton treatment in the MBBR denitrification reactor. However, results from DO measurements, TOC, and OX_C analyses indicated characteristics typical of younger leachates. This implies that the COD reduction was likely due to the removal of already biodegradable compounds, rather than the transformation of refractory organics. Therefore, it cannot be conclusively stated that Fenton oxidation enhanced the biodegradability of the ODE and AAR leachates based on these experiments. The project could benefit from the inclusion of BOD measurements, as it would have provided a more direct assessment of biodegradability and better supported the findings from the 24-hour denitrification experiments.

Nonetheless, the reduction in COD achieved through Fenton treatment could still be beneficial in PFAS removal processes, for instance, particularly where high COD levels may compete for adsorption sites in resin-based treatments. The conclusions drawn from the results support the ongoing MFS Lighthouse Eliminator project at DTI and offer valuable insights for Norrecco, helping with more informed decisions in future research and development. As part of a broader laboratory optimisation effort, this project makes a modest yet meaningful contribution to advancing leachate treatment and, ultimately, enhancing PFAS remediation strategies, promoting more effective and sustainable environmental practices.

6 | Perspective

For future studies, it is recommended to use older leachates to evaluate the hypothesis that refractory compounds can be converted into more biodegradable, bioavailable forms, which can subsequently be treated in the MBBR system. Leachates containing PFAS or other toxic and refractory compounds would be especially valuable, as these characteristics would enable a more meaningful evaluation of the Fenton treatment and the underlying hypothesis.

Moreover, conducting multiple 24-hour experiments under consistent parameters would allow for a clearer distinction between signal and noise, offering a more robust understanding of the behavior of Fenton-treated leachate. This would offer insights into the progression of nitrification and denitrification processes as well as COD degradation across different treatment stages over time. It would also be beneficial to include BOD measurements, along with a defined protocol for their implementation, to assess biodegradability more accurately and compare results with already published materials.

For process optimisation, having at least two people involved in the experimental workflow would significantly improve efficiency. The logistical demands of transferring leachate from the Fenton oxidation stage to the MBBR system, conducting sample analyses, and performing DO measurements require well-organised, timely, and carefully sequenced execution. The current design leaves no room for error by providing no redundancy. Moreover, a sufficient volume of leachate must be available, especially if the same denitrification reactor is to be used, that is minimum 10 L.

A redesign of the denitrification reactor similar to the 3 L nitrification reactor would reduce headspace, thereby minimising the risk of oxygen intrusion and making it easier to maintain an anoxic environment. The current reactor's large headspace may have contributed to suboptimal anoxic conditions. Using nitrogen purging could also help establish and maintain anoxic conditions more effectively. From a data quality perspective, monitoring nitrite levels, a critical intermediate in the nitrification process, would give a better overview of system performance. Moreover, this would support more reliable mass balance calculations and analysis regarding nitrogen transformation.

A more targeted and sensitive analytical approach, combined with extended exposure durations—beyond the current 30 minutes—could improve detection sensitivity and offer a clearer understanding of potential toxicity, especially if specific contaminants in the leachate are identified.

These adjustments would help optimise reactor performance, minimise variability and errors, and enable the collection of more reliable data—ultimately improving the effectiveness and sustainability of landfill leachate treatment. There is potential for further research, particularly given the growing relevance of PFAS and other persistent emerging contaminants to public health, environmental protection, and regulatory strategies.

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A | Gantt chart - Project timeline

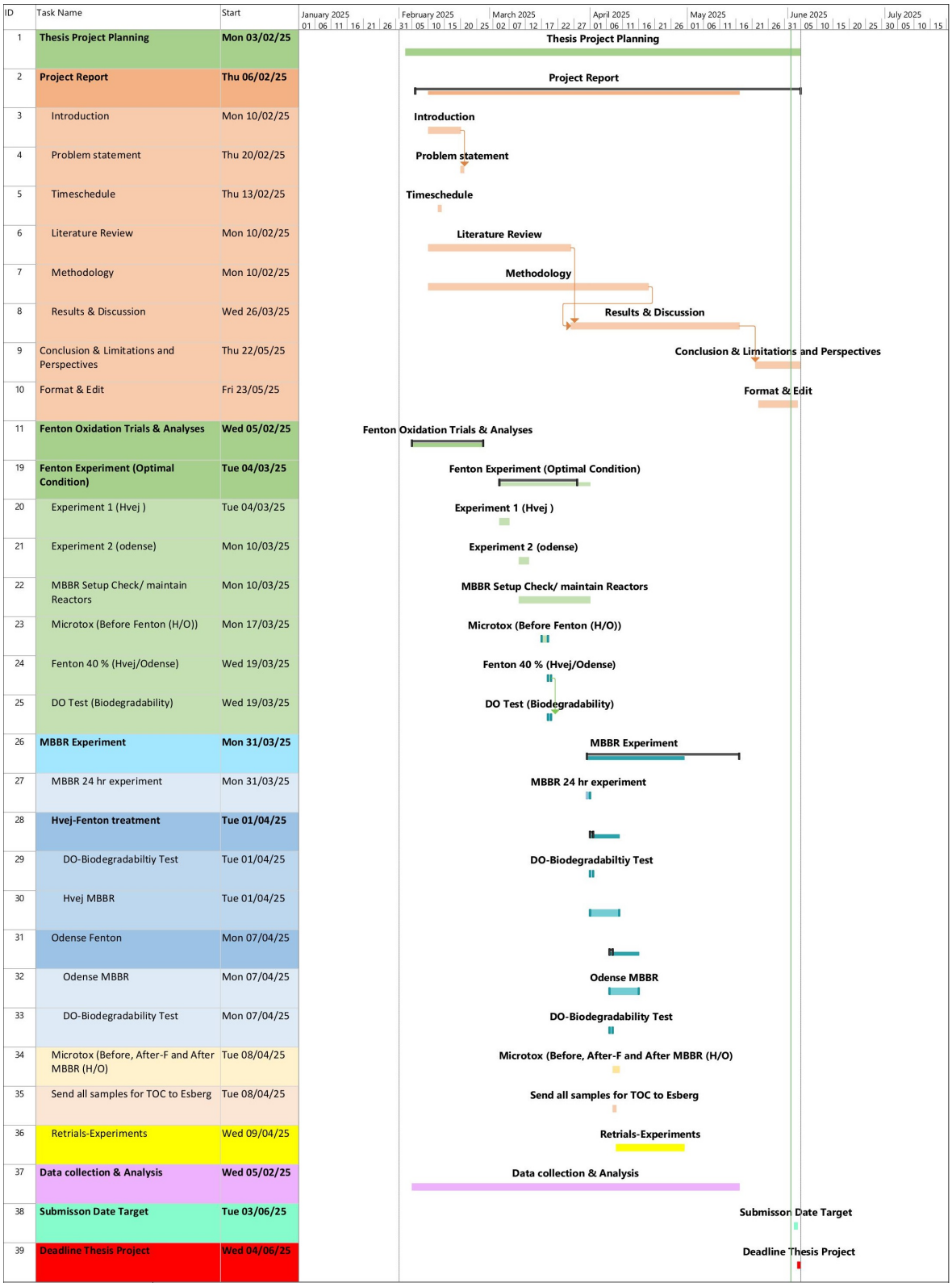

Figure A.1: Gantt chart, the Project timeline

B | Norrecco Leachate Treatment: Flow Diagram

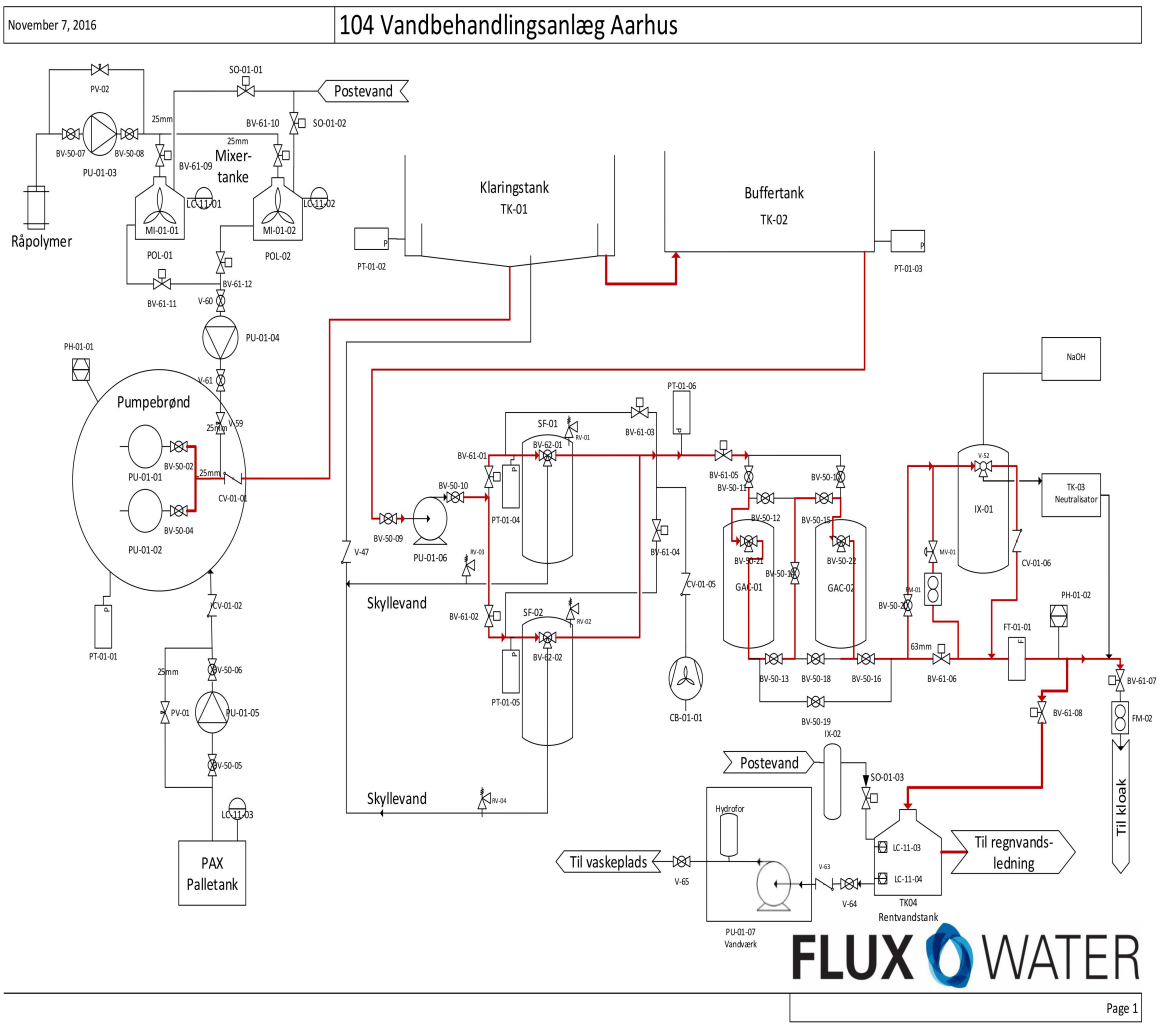

Figure B.1: Overview of Leachate treatment in Norrecco, Aarhus obtained from DTI (2024)

C | Leachate screening data tables

The following link directs to an folder containing results of the experiments (please note that Google sheets do not render the files properly, they are intended to be downloaded and opened in Excel): *[Data]*
The file "Screening and TOC.xlsx" contains results from the MBBR experiments.

C.1 Summary tables

The Table C.1 and C.2 present the results of a Fenton process screening conducted on Hvej and Bvej.

Table C.1: Impact of Fenton parameter changes on treatment efficiency: Hvej

Parameter	Trial	Fe^{2+}:H_2O_2	pH	Time (min)	Target Red. %	Initial COD	Final COD	% Red.	Fenton
pH	02-18.1	1:5	Natural	10	40	33.6	19.6	43.35	✓
pH & Target Reduction	02-19.1	1:5	Natural	10–30	30	33.6	57.1*	–	✗
	02-18.2	1:5	Acidic	10	40	33.6	17.3	48.51	✓
	03-04.1	1:5	Acidic	30	30	33.6	21.13	11.97	✓
	02-26.1	1:5	Acidic	40	40	37.6	24.6	34.57	✓
	02-26.2	1:5	Natural	40	40	37.6	45.7	–	✗
Target Reduction	03-04.2	1:5	Acidic	10	60	37.6	17.0	54.79	✓
	03-04.3	1:5	Acidic	10	80	37.6	11.5	69.41	✓
Running Time	03-04.4	1:5	Acidic	10	40	37.6	20.8	44.68	✓
	03-04.5	1:5	Acidic	20			21.4	43.09	✓
	03-04.6	1:5	Acidic	30			20.8	44.68	✓
Fe^{2+}:H_2O_2	03-10.1	1:4	Acidic	10	40	39.7	28.1	29.22	✓
	03-10.2			30	40	–	29.2	26.45	✓
	03-10.3			60	40	–	25.4	36.02	✓
	03-10.4			230	40	–	25.5	35.77	✓

Note:✓indicates Fenton process effective and ✗indicates Fenton was uneffective. Initial and Final COD measured in [mg/L] and Time measured in [minutes]. *Average over all trials.

Table C.2: Impact of Fenton parameter changes on treatment efficiency: Bvej

Parameter	Trial	H_2O_2:Fe^{2+}	pH	Time (min)	Target Red. %	Initial COD	Final COD	% Red.	Fenton
pH & Target Reduction	02-19.1	1:5	Acidic	10	30	43.6	43.6	0.00	✗
	03-05.1	1:5	Acidic	20	40	43.6	24.4	44.04	✓
	03-05.2	1:5	Acidic	60	40	43.6	23.6	45.87	✓
	03-05.3	1:5	Acidic	80	40	43.6	25.9	40.60	✓

Note:✓indicates Fenton process effective and ✗indicates Fenton was uneffective. Initial and Final COD measured in [mg/L] and Time measured in [minutes]

The Table C.1 and C.2 present the results of a Fenton process screening conducted on ODE and AAR.

Table C.3: Impact of Fenton parameter changes on treatment efficiency: ODE

Parameter	Trial	H_2O_2:Fe^{2+}	pH	Time	Target Red. %	Initial COD	Final COD	% Red.	Fenton
Running Time	03-07.1 - 6	1:5	Acidic	10-240**	40	210	106	49.52	✓
	03-25.1 - 3	1:5	Acidic	10-30**	40	96.40	19.42	79.86	✓
Target Reduction	03-26.1	1:5	Acidic	20	60	91	42	53.85	✓
	04-01.1	1:5	Acidic	20	40	91	74.34	18.35	✓
	04-02.1	1:5	Acidic	20	40	111.40	60.50	60.50	✓

Note:✓indicates Fenton process effective and ✗indicates Fenton was uneffective. Initial and Final COD measured in [mg/L] and Time measured in [minutes]

Table C.4: Impact of Fenton parameter changes on treatment efficiency: AAR

Parameter	Trial	H_2O_2:Fe^{2+}	pH	Time (min)	Target Red. %	Initial COD	Final COD	% Red.	Fenton
Running Time	03-25.1 to 3	1:5	Acidic	10–30**	40	72.6	39.87	45.08	✓
Target Reduction	03-26.1	1:5	Acidic	20	60	72.6	54.15	25.41	✓
Target Reduction	04-02.1	1:5	Acidic	20	60	48.6	39.40	18.93	✓

Note:✓indicates Fenton process was considered effective. Initial and Final COD: [mg/L] and Time [minutes]

D | MBBR

D.1 MBBR results

The following link directs to an folder containing results of the experiments (please note that Google sheets do not render the files properly, they are intended to be downloaded and opened in Excel): *[Data]*
The file "MBBR.xlsx" contains results from the MBBR experiments.

E | DO Experiments-OUR

E.1 OUR results

The following link points to a folder containing results of DO/OUR experiments:
Folder: DO.

The folder contains the following summary of results: "DO - Summary.xlsx"

Individual results and calculations from the experiments, such as in Figure E.1, are provided in the files listed below (please note that Google sheets do not render the files properly, they are intended to be downloaded and opened in Excel):

		Line est. Probe 1		
	a/slope	-172.1183095	7.324600819	b/constant
	stnd err of a	0.402905345	0.002433854	stnd err of b
	R2	0.999545397	0.010549209	stnd err of y est
	F-stat	182493.7879	83	df
	regression of sum of sqs	20.30896798	0.009236722	residual sum of sq
		Line est. Probe 2		
	a/slope	-492.3469018	6.648499751	
		5.468097855	0.021319368	
	R2	0.993018283	0.082800998	
		8107.180884	57	
		55.58287549	0.390792305	
		Probe 1 O2 rate	7.17159623	mg/(L*h)
		Probe 2 O2 rate	20.51445424	mg/(L*h)
		ratio 1/2	0.349587474	
		2 x more than 1	1.860514394	
		delta 1-2	-13.34285801	

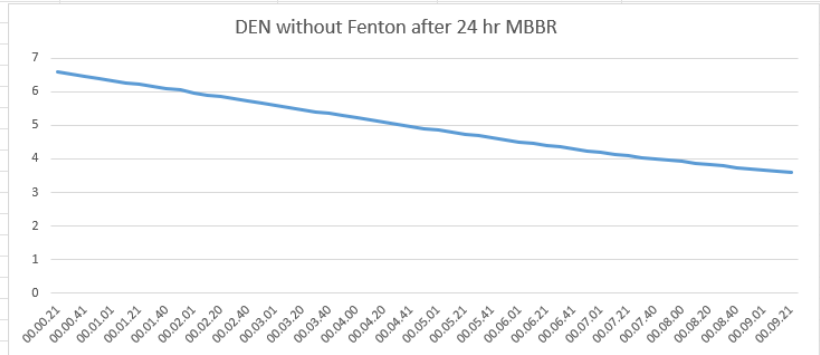

Figure E.1: DO calculation in Excel - example

DO - AAR Fenton Nitri - AAR Fenton Denitri.xlsx
DO - AAR Fenton.xlsx
DO - AAR Nitri - AAR Denitri.xlsx
DO - AAR Raw - AAR Act sludge.xlsx

DO - ODE Act sludge - ODE Fenton.xlsx
DO - ODE Act sludge - ODE Raw.xlsx
DO - ODE Fenton - ODE Denitri.xlsx

DO - ODE Fenton Nitri - ODE Fenton Denitri.xlsx
DO - ODE Nitri.xlsx

F | Inhibition

F.1 Inhibition results

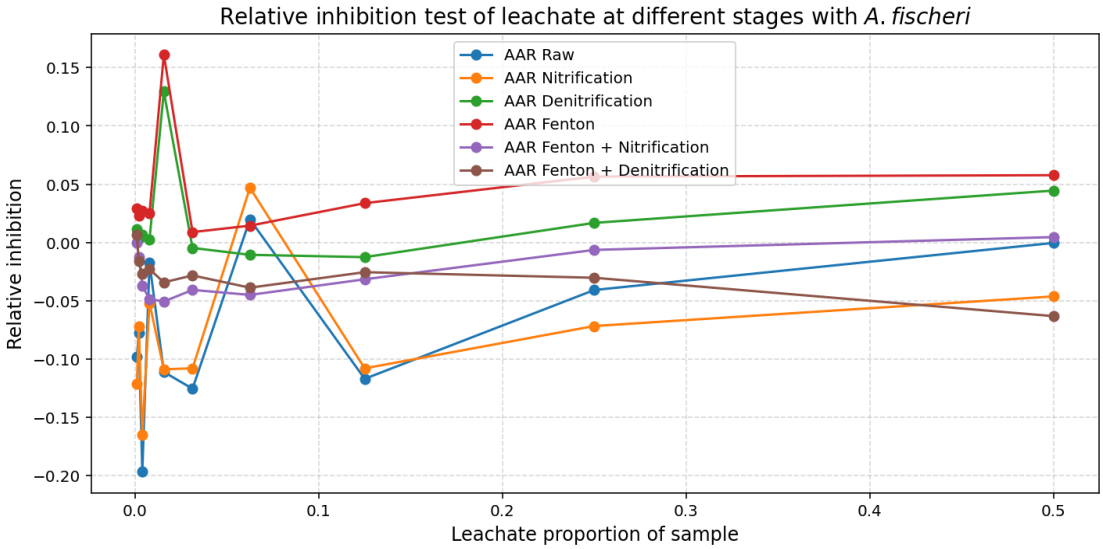

Figure F.1: Inhibition test - q values for AAR

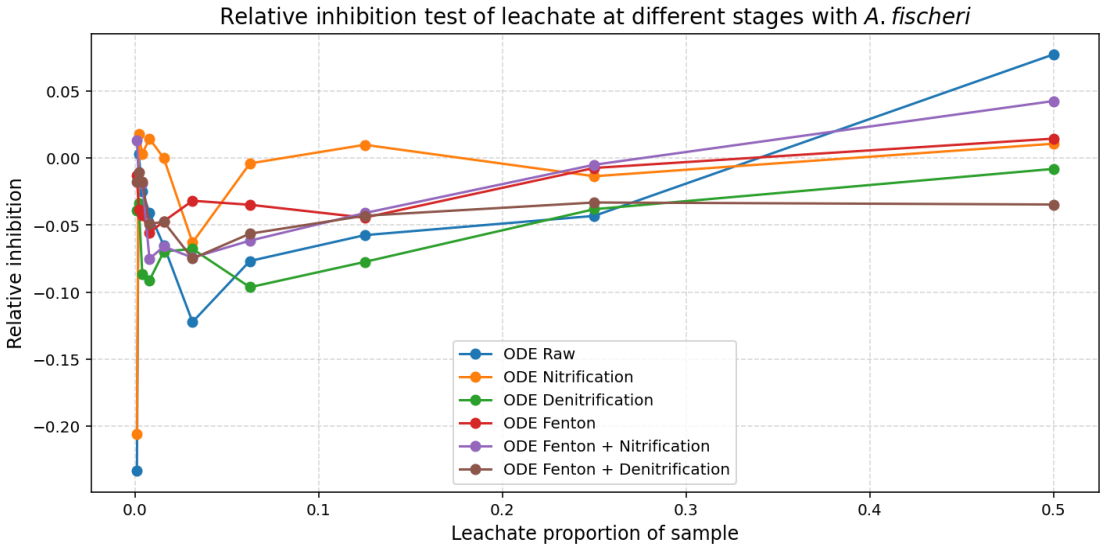

Figure F.2: Inhibition test - q values for ODE

The following link directs to an folder containing results of the experiments (please note that Google sheets do not render the files properly, they are intended to be downloaded and opened in Excel): *[Data]*
The file "Inhibition.xlsx" contains results from the inhibition test.

F.2 Materials List

- Vibrio fischeri DSM 7151 (=*Aliivibrio fischeri* DSM 7151)
- Autoclaved 100 mL Blue cap bottles
- Autoclaved Mar+ medium for cultivation of A. fischeri
- Autoclaved Tox medium for incubation of A. fischeri during test fischeri
- Autoclaved 2% NaCl solution (20 g/L NaCL in distilled water)
- VICTOR X Multilabel Reader
- SDI Luminescence analyzer
- White 96-well microplates
- Multichannel pipette and standard pipettes + tips
- Sterile Petri dishes
- Shaker at 20 °C

G | Lab setup

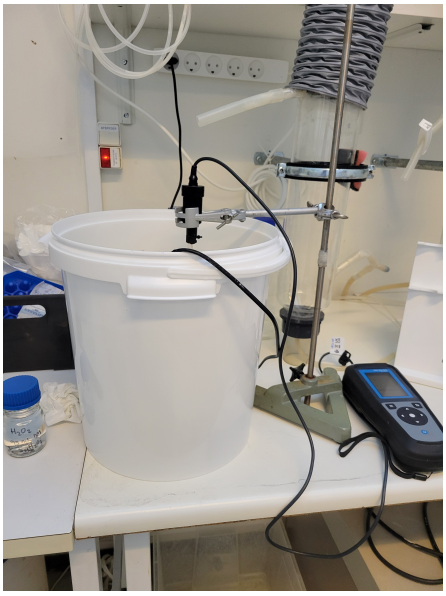

Figure G.1: Fenton setup - before MBBR ODE

Figure G.2: Aften Fenton treatment and pH adjustment to 7

Figure G.3: Filtering ODE

Figure G.4: Filtering ODE - before MBBR

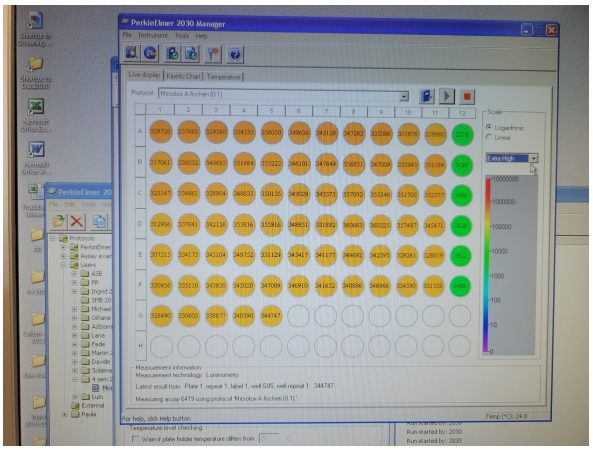

Figure G.5: Plate reader example

Figure G.6: Sample for DO - after fenton MBBR ODE

Figure G.7: Startup carriers from Denitrification reactor

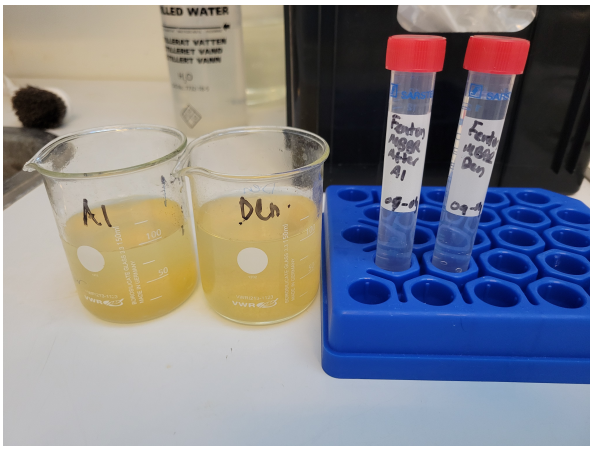

Figure G.8: Unfiltered samples - After MBBR Nitrification

Figure G.9: Screening trial