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Nitrogen removal in MBBRs

The effect of low temperatures

Master's thesis in Infrastructure and Environmental Engineering

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Master's thesis ACEX30

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Cover:
Plastic carriers floating and moving in the water.

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Abstract

Nitrogen removal from wastewater in regions with low temperatures and long snow-melting periods represents a challenge for the performance of biological treatment processes and information on the efficiency of nitrification and denitrification processes under these conditions is lacking. This study evaluated the implementation of the MBBR treatment process in a pilot plant for wastewater treatment at Fillan WWTP in Sundsvall, Sweden, between January and May 2023. Each line had 6 different reactors, referred to as zones, including nitrification and denitrification (pre- and post-). They were used to evaluate the nitrogen removal efficiency during continuous plant operation. Furthermore, batch activity tests were performed to follow up the biomass activities depending on temperature changes, and laboratory scale activity tests were used to analyze the influence of DO on the denitrification process. From the results, it was concluded that high nitrogen removal efficiencies can be achieved at low temperatures. Nevertheless, the denitrification process was highly affected during the snowmelt period, especially in April, when the nitrogen removal efficiency dropped below 50% in both lines. From the batch activity tests, the Arrhenius temperature dependence coefficient was calculated for the denitrification and nitrification processes. Although θ values were close to the values reported in the literature, no clear difference was observed between them. From the laboratory-scale activity test, the negative effect of high DO concentrations was evidenced not only in the denitrification process but also in $\text{NO}_2\text{-N}$ production, which is also related to the excess available carbon source.

Keywords: *wastewater, low temperature, nitrogen removal, MBBR.*

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List of Acronyms

- AS	Activated Sludge
- AOB	Ammonia Oxidizing Bacteria
- BOD	Biochemical Oxygen Demand
- COD	Chemical Oxygen Demand
- DO	Dissolved Oxygen
- EPA	Environmental Protection Agency
- EU	European Union
- HRT	Hydraulic Retention Time
- MBBR	Moving Bed Biofilm Reactor
- NH ₄ -N	Ammonium – nitrogen
- NOB	Nitrite Oxidizing Bacteria
- NO ₂ -N	Nitrite – nitrogen
- NO ₃ -N	Nitrate – nitrogen
- OLR	Organic Loading Rate
- pe	population equivalents
- TS	Total Solids
- UWWTD	Urban Waste Water Treatment Directive
- WWTP	Wastewater Treatment Plant

1 Introduction

Inorganic nitrogen compounds such as ammonium (NH_4^+) and nitrate (NO_3^-), coming from anthropic activities mainly related to food production and the use of fertilizers, can be found among the components of wastewater (Menció et al., 2023). Along with other nutrients such as phosphorus, these compounds represent a risk to the environment since, in high concentrations, they can unbalance aquatic ecosystems, contributing to algal blooms and eutrophication (Adolph et al., 2023; Soro et al., 2023), impacting water quality and aquatic biodiversity (U.S. EPA, 2011).

Therefore, the European Union (EU) has been regulating discharges of treated wastewater from Wastewater Treatment Plants (WWTPs) through the Urban Waste Water Treatment Directive (UWWTD) since 1991 (European Parliament, 1991). By incorporating the directive, the Swedish Environmental Protection Agency (EPA) determined that all waters in Sweden have been classified as being sensitive to the discharge of phosphorus, while the receiving water bodies along the Baltic Sea, north of Norrtälje, are designated as Less Sensitive Areas to nitrogen discharges, thus, WWTPs in the area are designed to reduce the concentration of organic matter and phosphorus but not nitrogen.

Recent studies suggest a decrease in oxygen levels in the Bothnian Sea and an increase in the risk of eutrophication (Atangana Njock et al., 2023; Rolff & Elfving, 2015; Teutschbein et al., 2017; Vigouroux et al., 2021), which could lead to a modification of the current regulations by the EU and Swedish authorities. Demanding WWTPs to implement treatments for the removal of both nutrients (nitrogen and phosphorus) represents a challenge in the region as the efficiency of the biological processes involved is affected by low temperatures (Fillos et al., 1996; Wild et al., 1971; Wong-Chong & Loehr, 1975).

For that reason, in the city of Sundsvall, Sweden, led by the company MittSverige Vatten och Avfall and with the assistance of IVL Swedish Environmental Research Institute, a project was developed in a pilot plant involving the Moving Bed Biofilm Reactor (MBBR) process for the treatment of a portion of the city's wastewater, and to support the decision-making process related to the future of the wastewater treatment in the region. Even though the MBBR treatment process was created in the late 80s, there is still a lack of information on its performance during the cold months and in regions with long snow-melting periods.

Thus, this master's thesis aims to i) review the existing parameters and design values for the implementation of an MBBR-based nitrogen removal with emphasis on low-temperature

conditions; ii) investigate the influence of temperature on the nitrification and denitrification processes (pre- and post-) in the pilot plant; iii) analyze the other factors influencing the nitrogen removal processes in treatment plants located in regions with a long period of snowmelt; and finally iv) discuss the possibilities to achieve nitrogen removal in northern Sweden and additional measures to be taken compared to the other WWTPs in southern Sweden.

The scope of this master's thesis includes the analysis of nitrogen removal in a pilot plant utilizing the MBBR process to treat wastewater from a specific area of Sundsvall, Sweden. The pilot plant was designed to represent a biological treatment step of a WWTP, although phosphorus removal is not incorporated.

2 Background

This section explains the context of this master's thesis by reviewing the current regulations related to wastewater discharges in Sweden and the European Union, the nitrogen removal process including its temperature dependence, the characteristics of the MBBR treatment process, and different case studies around the world.

2.1 Regulations related to wastewater discharges

Currently the EU, according to the UWWTD, classifies receiving water bodies from urban WWTPs into Sensitive Areas and Less Sensitive Areas based on the risk and potential for eutrophication (European Parliament, 1991). Based on this directive, Sweden's coastal areas are classified as shown in Figure 1.

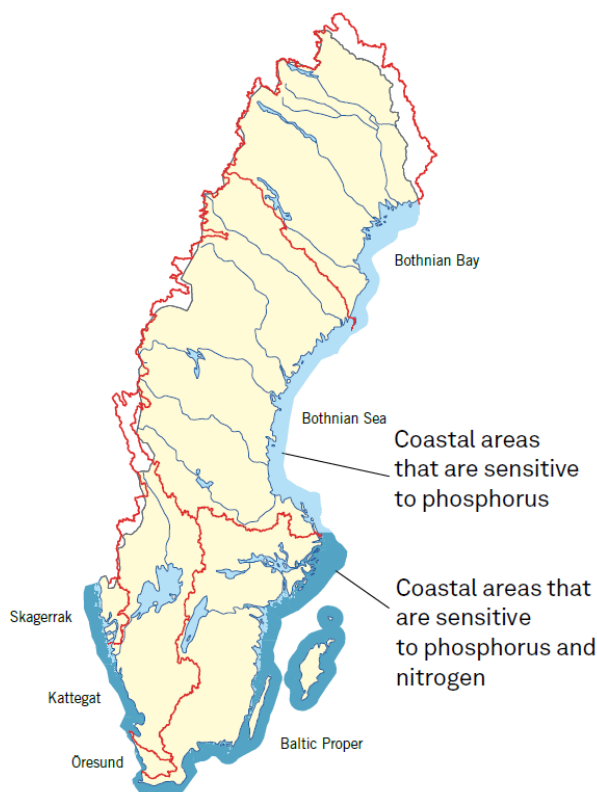


Figure 1 Classification of coastal areas in Sweden according to the UWWTD. Source: Naturvårdsverket (2020).

In the case of Sensitive Areas, specific guidelines have been established, which include the maximum nutrient concentrations (as an annual mean value) in the discharges or the minimum percentage of reduction required. These guidelines are presented in Table 1.

Table 1 Discharge requirements from urban WWTPs to Sensitive Areas according to the UWWTP

Parameters	Concentration	Minimum percentage of reduction
Total Phosphorus	2 mg/l (10 000 – 100 000 pe)	80
	1 mg/l (more than 100 000 pe)	
Total Nitrogen	15 mg/l (10 000 – 100 000 pe)	70 - 80
	10 mg/l (more than 100 000 pe)	

Adapted from European Parliament (1998). pe: population equivalents.

The percentage of reduction is calculated based on the influent loading, and Total Nitrogen (N-tot) refers to the sum of organic nitrogen (N_{org}), ammonium – nitrogen (NH_4-N), nitrate – nitrogen (NO_3-N), and nitrite – nitrogen (NO_2-N):

$$N-tot = N_{org} + NH_4-N + NO_3-N + NO_2-N \quad (1)$$

Within the national framework, discharges to the receiving water are regulated by the Swedish EPA's regulations NFS 2016:6 (Naturvårdsverket, 2016), the Swedish Environmental Assessment Ordinance SFS 2013:251 (Klimat- och näringslivsdepartementet, 2013), and the WWTPs' environmental permits, which serve as mechanisms for the implementation of the UWWTD (Naturvårdsverket, 2020). Additionally, in 2022 a proposal for a revised UWWTD was published. It is stipulated that nitrogen must be removed in all WWTPs with > 100 000 pe that discharge in sensitive areas. Although the limit value after removing nitrogen is not yet defined, the Baltic Sea is explicitly considered a sensitive area in the proposal (Directorate-General for Environment, 2022).

2.2 Nitrogen removal in traditional mainstream wastewater treatment

Nitrogen removal in traditional mainstream wastewater treatment can occur through two biological processes, carried out by specific bacteria, as shown in Figure 2. First, nitrification is the oxidation of ammonium to nitrate by two groups of autotrophic bacteria in aerobic conditions and includes two reaction steps (Holmes et al., 2019).

- Nitrification by Ammonia Oxidizing Bacteria (AOB), mainly *Nitrosomonas*:



- Nitrification by Nitrite Oxidizing Bacteria (NOB), mainly *Nitrospira* and *Nitrotoga*:



- The total nitrification process:

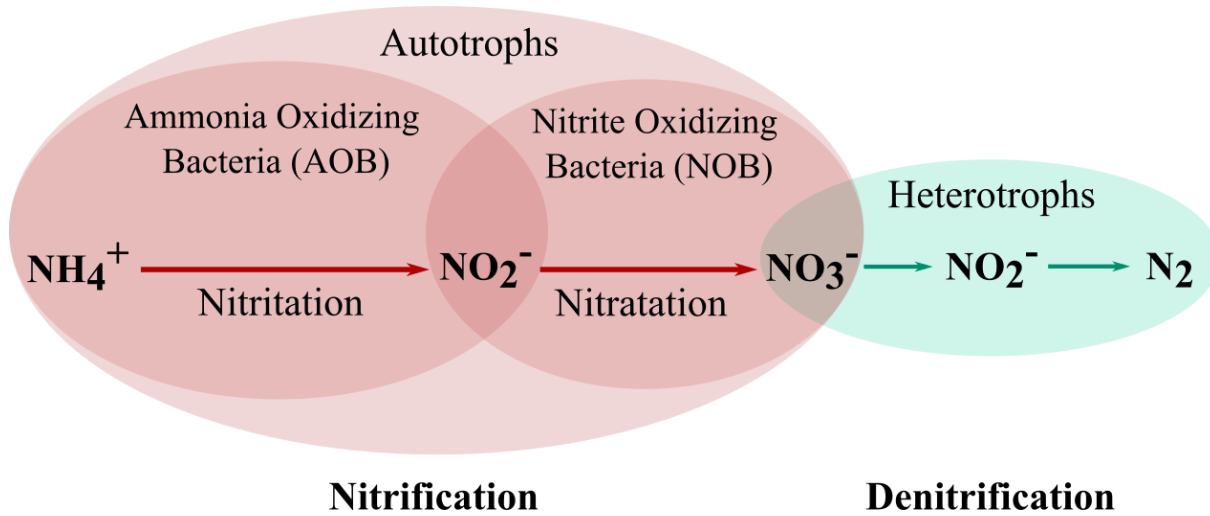


Figure 2 Nitrification and denitrification processes in the traditional mainstream wastewater treatment

The reaction rate of nitrataion in conventional wastewater treatment is higher than that of nitritation resulting in a low accumulation of nitrite in the aerobic process (Grady Jr. et al., 2011). In addition, the nitrification process reduces the alkalinity of the wastewater (Wilczak et al., 1996). The second step, denitrification, is the heterotrophic reduction of nitrate to nitrogen gas (Zumft, 1997). This process occurs only in anoxic conditions and organic matter must be available as a substrate.

Nitrification and denitrification processes are influenced by various environmental factors, including temperature, pH, oxygen availability, substrate availability, and bacterial abundance. Temperature plays a crucial role in enzymatic reaction rates, and thus growth rate, and metabolism (Metcalf & Eddy, 2013). Optimal growth for most nitrifiers occurs at temperatures ranging from 25 to 30 °C (Watson et al., 1981), and a pH between 7.2 and 8.0 is suggested (Wong-Chong & Loehr, 1975). Denitrifiers thrive at a pH of 7.0 to 7.5 (Thomas et al., 1994) and temperatures between 30 and 35 °C (Liao et al., 2018; Saleh-Lakha et al., 2009). Nevertheless, the processes still can be performed by bacteria in a much wider range of temperatures and pH.

2.3 Temperature dependence coefficient from Arrhenius equation

The Arrhenius equation is a widely used model to describe the relationship between biochemical reaction rates and temperature, particularly when an elevation in the temperature does not inhibit

the process (Sobotka et al., 2021). Equation 5 represents a simplification of the Arrhenius equation that incorporates a dimensionless temperature dependence correction coefficient (θ), enabling the estimation of a reaction rate k_{T_1} at a specific temperature T_1 , providing reference values from another temperature ($T_{ref}, k_{T_{ref}}$).

$$k_{T_1} = k_{T_{ref}} \cdot \theta^{(T_1 - T_{ref})} \quad (5)$$

The parameter θ is required for the design and modeling of biological treatment systems implemented in WWTPs, and according to the Norwegian design guidelines (Norsk Vann, 2020), the recommended θ value for the nitrification process using the MBBR treatment is 1.09, while 1.07 for denitrification, although different values have been described in the literature.

Hem et al. (1994) reported a value of θ equal to 1.044 between 8 and 18 °C, while Salvetti et al. (2006) obtained a θ average value of 1.058 by conducting nitrification of wastewater in a pilot-scale MBBR under oxygen-limiting conditions within a temperature range of 12.5 to 28.1 °C, and 1.098 under ammonium-limiting conditions within a temperature range of 13.4 to 22.0 °C. Almomani & Khraisheh (2016) used the MBBR process to treat septic tank effluents in both pilot plants and a laboratory-scale batch reactor, considering a range of temperatures of 8 - 25 °C and varying HRTs. The kinetics of ammonium removal was then correlated with an average θ value of 1.054, which was calculated using equation 6, where t represents the exposure time in days at a specific temperature.

$$\theta = 3.81 \times 10^{-2} \ln(t) + 9.83 \times 10^{-1} \quad (6)$$

In a separate study, Ahmed et al. (2019) investigated the nitrification performance of an MBBR treating wastewater with temperatures ranging from 10 to 4 °C. Their analysis revealed a θ value of 1.049, however, under cold shock conditions (down at 1°C) that value increased to 1.149. This finding aligns with the observations made by Hwang & Oleszkiewicz (2007), who reported an increase in the θ value when abruptly decreasing the wastewater temperature from 20 to 10 °C in a Sequencing Batch Reactor (SBR), yielding a θ value of 1.116.

Zhou et al. (2022) investigated the θ values in a series of MBBRs used for wastewater treatment in China over one year. The average temperatures observed during the summer, autumn, and winter seasons were 26.9, 21.5, and 15 °C, respectively. The experimental findings revealed θ values of 1.037 ± 0.010 and 1.045 ± 0.010 in two consecutive reactors operating under oxic conditions.

Furthermore, this investigation documented θ values observed in reactors subjected to anoxic conditions, specifically 1.023 ± 0.005 during the pre-denitrification process and 1.027 ± 0.007 during post-denitrification.

2.4 Biofilm systems

For over a century, the removal of pollutants in WWTPs has been done mainly by the Activated Sludge (AS) treatment process, maintaining in suspension a high concentration of microorganisms, by aeration and mechanical stirring (Rajasulochana & Preethy, 2016). However, there has been a notable surge in the adoption of biofilm systems since 1990-th, driven by their effectiveness (Asri et al., 2018).

Biofilm systems offer numerous advantages such as less space requirement in wastewater treatment, reduced Hydraulic Retention Time (HRT), low sludge production, and improved performance stability (Zhao et al., 2019). Some of these systems include the Rotating Biological Contactor (RBC), Trickling Filter (TF), Membrane Aerated Biofilm Reactor (MaBR), and MBBR, which rely on the ability of microorganisms to adhere to the surface of a supportive medium and interact with the wastewater to enhance the exchange of nutrients, substrate, and other products (Melo, 2003).

2.4.1 MBBR treatment process

Developed in Norway in the late 80s (European Patent No. 0,575,314; US Patent No. 5,458,779), the MBBR treatment process consists of a reactor containing free-moving plastic carriers as a supporting medium where most of the active biomass is present as attached biofilm (Hem et al., 1994) and as suspended flocs. The performance of the bioreactor is influenced by the characteristics of the plastic carriers (e.g. surface area, density, surface morphology, and microbial adhesion capacity) (Asri et al., 2018), and operational parameters such as pH, temperature, Organic Loading Rate (OLR), DO concentration, HRT, and the carriers filling ratio (Sonwani et al., 2022).

The MBBRs can be arranged in series and operated under either anoxic conditions, wherein a mechanical mixing system is incorporated to ensure continuous movement of the plastic carriers, or aerobic conditions, including a diffused aeration system (McQuarrie & Boltz, 2011). This versatile system has been implemented for the treatment of municipal wastewater (di Biase et al., 2019; Leyva-Díaz et al., 2020; Santos et al., 2020), as well as for advanced applications for the treatment of emerging contaminants (Abu Bakar et al., 2018; Wang et al., 2023).

2.5 WWTPs implementing the MBBR technology with low-temperatures conditions

Considering the adverse effect of temperature on the metabolic activity of microorganisms, influencing their biodegradation performance (Sonwani et al., 2022), as well as impacting the internal and external diffusion of substrates within the biofilm and the bioavailability and solubility of substances (El-Naas et al., 2014), it becomes crucial to evaluate the removal efficiency of the MBBR treatment process under low-temperature conditions and particularly, during the snow-melting period, when the temperature of the incoming wastewater drops, the concentration of contaminants is reduced, and the availability of a carbon source for the denitrification process decreases (Bjørn Rusten et al., 1995), hindering the capacity to achieve the required percentage of reduction specified by environmental regulations.

The implementation of MBBRs in sequence to treat municipal wastewater at full scale occurs worldwide and it is common in the Nordic countries under low-temperature conditions, e.g. in Norway, at the Lillehammer, Nedre Romerike, Nordre Follo, and Gardemoen WWTPs, in Sweden at the Ängholmen WWTP and the Ruka WWTP in Finland.

The findings from Ødegaard et al. (2023) provide a comprehensive overview of the performance of the aforementioned WWTPs and are compiled below. In 2018 and 2019, the Lillehammer WWTP had average wastewater temperatures of 10.8 and 10 °C, respectively, with minimum values of 3.9 °C and 5.3 °C. It consists of 9 MBBRs filled with K1 type carriers (surface area of 500 m²/m³) and 7 of them can operate under aerobic conditions, allowing combinations of phosphorus precipitation (pre- or post-), nitrification, and denitrification (pre- or post-) processes. During both years, the nitrogen removal rate surpassed 75%, with the nitrification rate being substrate-limited. Notably, the denitrification rate was significantly affected by dilution during snow melting.

The Nordre Follo WWTP, employing the same carriers and tank depth, incorporates pre-sedimentation, denitrification (pre- and post-), and nitrification reactors. Nitrogen removal in 2019 and 2020 reached approximately 80%, with average temperatures during winter of 9-10 °C and around 7-7.5 °C during snow-melting periods. Occasional methanol application at the beginning of the process was reported to enhance denitrification performance.

At the Nedre Romerike WWTP, the treatment process includes pre-sedimentation, pre-denitrification, nitrification, post-denitrification, and post-precipitation. Nitrification rates ranged from 0.31 to 0.41 g NH₄-N/m²-d during the years 2013, 2014, 2016, and 2018, with an average temperature of 8.6 °C and minimum temperatures between 5.2 and 7.4 °C. Pre-denitrification and

post-denitrification reaction rates ranged from 0.18 to 0.22 g NO_x-N/m² and 1.0 to 1.1 g NO_x-N/m²-d, respectively, with methanol typically applied during post-denitrification. However, the performance of the plant was significantly impacted by diluted water, resulting in removal rates being lowered by up to 50%.

The Gardemoen WWTP treats municipal wastewater, wastewater from an airport, and de-icing fluid. After pre-sedimentation, the plant incorporates pre-denitrification, nitrification, post-denitrification as well as post-precipitation. Analysis of data between 2019 and 2020 revealed a nitrogen removal of 85%. The average pre-denitrification reaction rate was 0.88 g NO_x-N/m²-d within a temperature range of 8.0-16.5 °C. However, at lower temperatures (5.4-8 °C), a reduction of up to 0.68 g NO_x-N/m²-d was observed. In contrast, the post-denitrification rate did not exhibit a clear correlation with temperature and had an average value of 1.2 g NO_x-N/m²-d.

3 Methods and materials

This section includes a detailed description of the pilot plant and the assessment of its performance through its continuous operation, as well as different activity tests, among others, to better understand the nitrification and denitrification processes by varying different parameters.

3.1 Case of study

In Västernorrland county, northeast of the city of Sundsvall, Fillan WWTP is located (Figure 3), which collects municipal sewage and stormwater from the Skön, Alnö, and Bosvedjan (including Sundsvall's hospital) areas, and then discharges the treated water into the Alnösundet strait. The plant has a maximum capacity of approximately 30 000 pe (750 m³/h) and includes mechanical, chemical, and biological treatment (AS process). Chemical precipitation of phosphate occurs before the pre-sedimentation by adding aluminum coagulant and subsequent mixing in flocculation tanks, to finally remove the precipitated phosphorus along with the primary sludge. Notwithstanding, the plant was not designed for nitrogen removal, following the UWWTD (European Parliament, 1991). In Appendix A the detailed process scheme of Fillan WWTP can be seen.

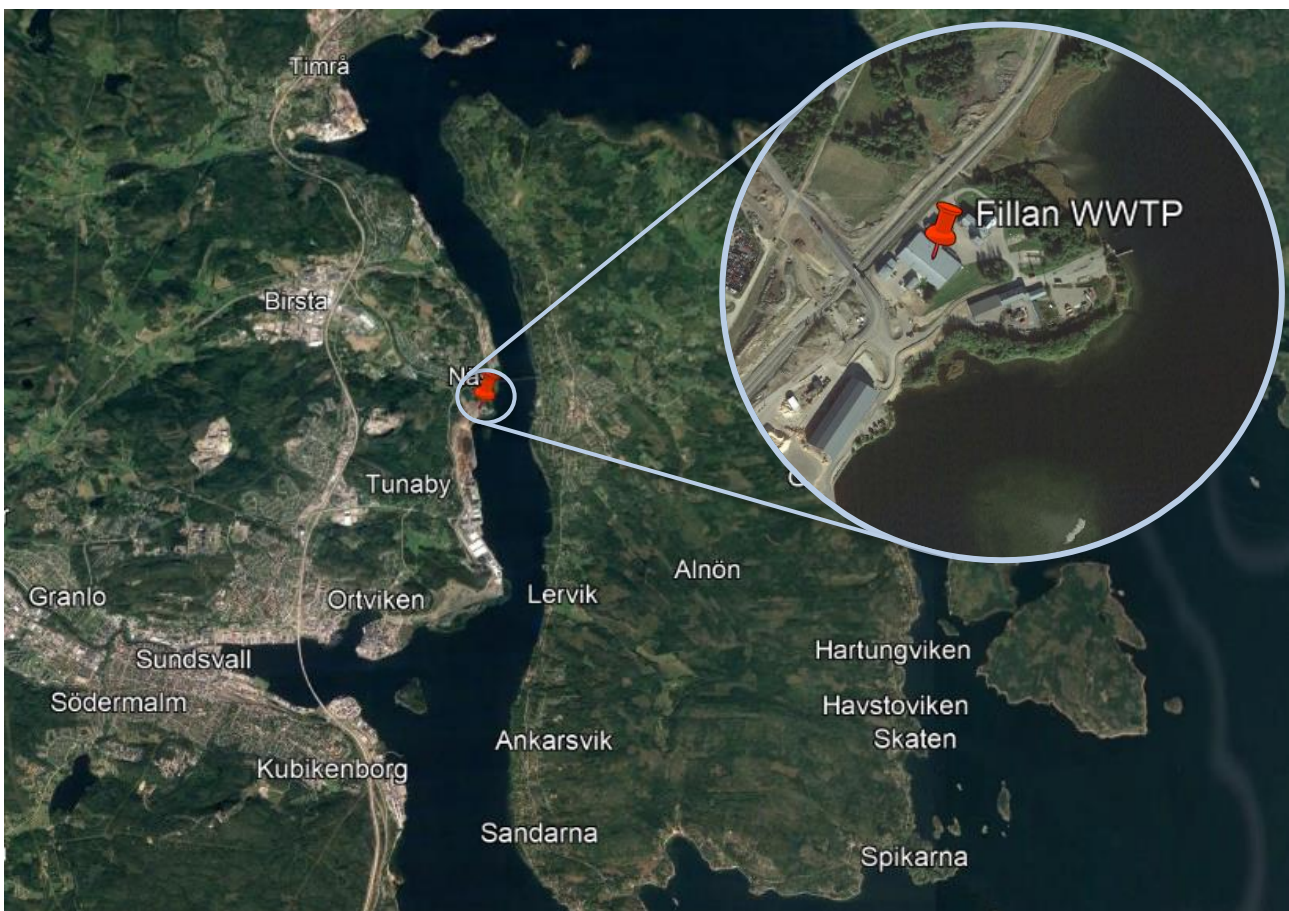


Figure 3 Location of Fillan WWTP, Västernorrland County, Sweden. Adapted from (Google Earth, n.d.)

Figure 4 represents the incoming flow to Fillan WWTP, the temperature of the wastewater in the flocculation step, and the concentration of the N-tot in the effluent throughout the years 2021 and 2022. According to the records, from the end of October, it is possible to observe temperatures of 11 °C, nevertheless, when the snow-melting period intensifies, between March and April, the flow can exceed 700 m³/h and during several weeks the temperature of the wastewater can be below 6 °C, and reach a minimum close to 4.8 °C, especially in April.

Although there is a correlation between high flow rates and low N-tot values in the effluent, in most cases N-tot values exceed 10 mg/l all year long. In December, January, and February, when the temperature of the wastewater ranges between 6 and 8 °C, it is possible to observe effluent values of N-tot higher than 15 mg/l. Moreover, if the incoming flow keeps its negative trend, the N-tot could exceed 20 mg/l in February due to the anticipated beginning of the snow-melting period (SMHI, n.d.-a).

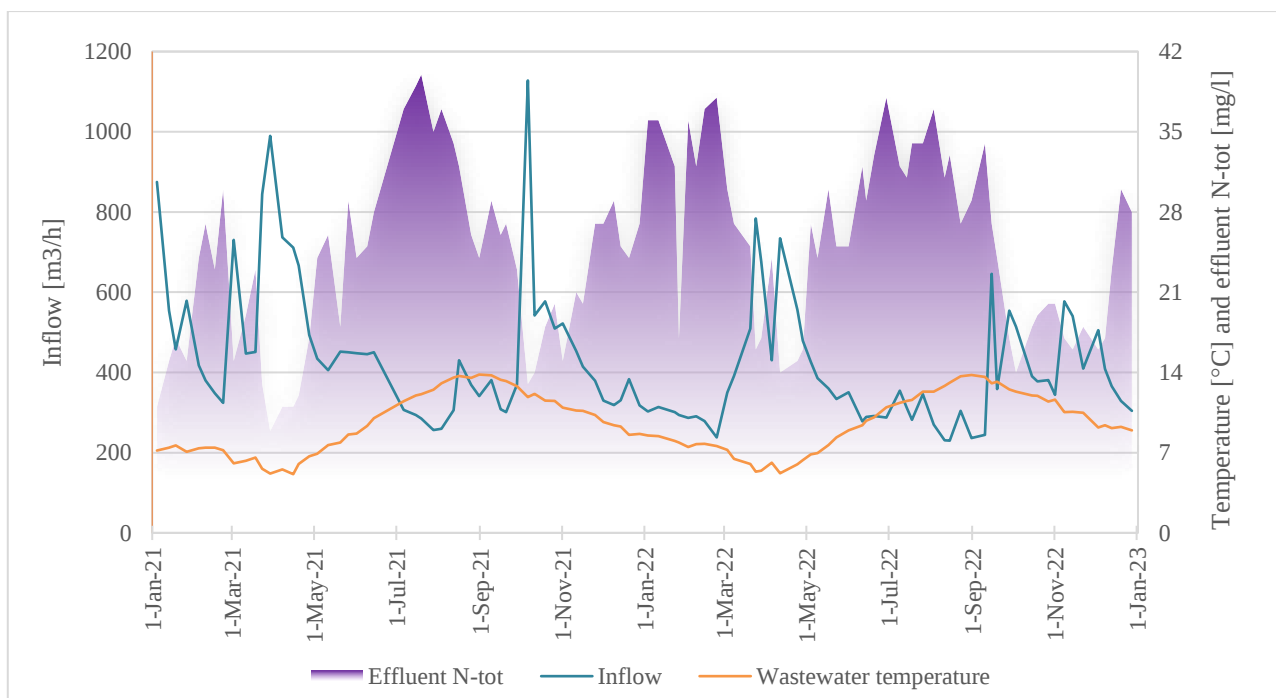


Figure 4 Inflow water flow and temperature at Fillan WWTP between 2021 and 2022

3.1.1 Pilot plan description

The pilot plant located at Fillan WWTP, inside a container (Figure 5), consists of two parallel lines with the MBBR process, which can be controlled independently. Each line has 6 zones where the nitrification and denitrification processes (pre and post) are carried out, as well as the removal of organic matter (Figure 6). The detailed flow scheme of the pilot plant can be seen in Appendix B. The width of all the zones is 0.6. The carriers chosen for the pilot plant are of the K3 type with a

surface area of $500 \text{ m}^2/\text{m}^3$, a nominal length of 12 mm, and a nominal diameter of 25 mm. Moreover, all the zones, except for the N2 zone, had agitators operating at the same frequency.



Figure 5 Pilot plant in March 2023 at Fillan WWTP

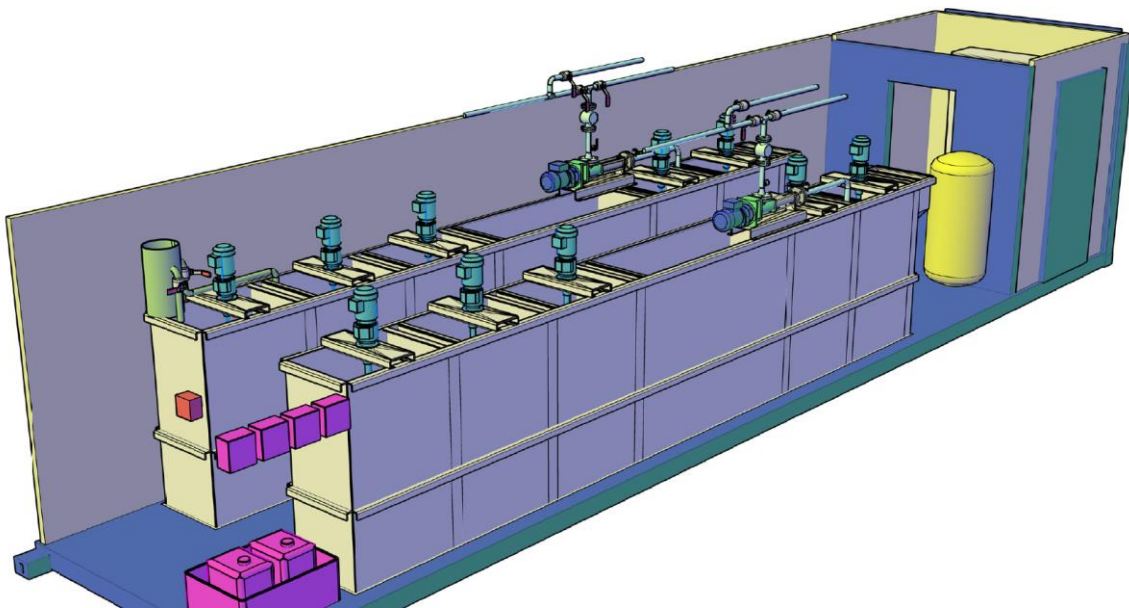


Figure 6 3D representation of the pilot plant. Adapted from PURAC (2021b)

Originally the pilot plant was designed with a filling ratio of carriers within the zones of 50%, however, due to strong agitation before the start of this master's thesis, some plastic carriers broke and/or changed their shape, reducing the available surface area and therefore, the amount of biofilm interacting with the nitrogen in the wastewater. During this master's thesis, the surface area per zone was recalculated taking into account a rough calculation of the loss of plastic particles (see Table 2)

since the filling ratio varied between 44% and 50% in the different zones. Additionally, these broken plastic particles caused operating problems by clogging the pipes and the heat exchanger.

Table 2 Water volume and surface area per zone in both lines

Zone	Water volume	Surface area [m ²]	
	[m ³]	Line 1	Line 2
FDN	0.65	155	156
FLEX	0.65	145	142
N1	0.65	146	146
N2	1.37	342	342
DE-OX	0.65	141	150
EDN	0.65	145	145
Total	4.6	1074	1081

In some of the zones, online meters were placed to monitor parameters such as temperature, pH, NH₄-N, NO₃-N, and DO concentrations (red squares in Figure 7). The online meters took measurements every minute, along with other devices that collected information about the inflow in each line, the recirculation flow, the dose of external carbon source supplied, and the opening of the valves, among others. However, when analyzing the measurements taken by the online meters in the pilot plant, the online meter located downstream of the N2 zone in line 1 displayed inconsistent NH₄-N concentration values (mostly 0) from March 17 to April 11. Subsequently, the online meter was replaced with a new device. This inconsistency had various implications for the performance of line 1, which will be elaborated upon in the subsequent sections.

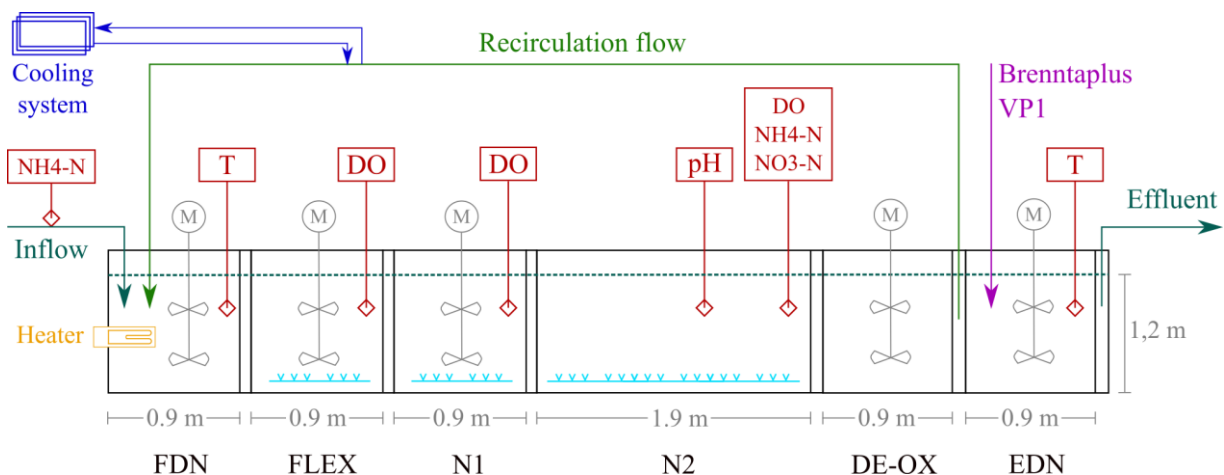


Figure 7 Scheme of one of the lines of the pilot plant. Note Permanent heater is only in line 1 and the cooling system is only in line 2

The wastewater to be treated came from Fillan WWTP, after treatment in drum sieves, a grit chamber, and primary sedimentation. A separate sedimentation tank without the preceding addition of aluminum-based flocculant was used to prepare the wastewater for further treatment in the pilot plant. Until 12/04/23, the inflow on line 1 was controlled to be 0.135% of the current inflow to Fillan WWTP, which represented a flow between 0.5 and 1.0 m³/h, while line 2 treated 0.1% of the current inflow to Fillan WWTP, which represented a flow between 0.4 and 0.7 m³/h. From 12/04/23, the flow was increased by 0.02% in both lines, to evaluate the efficiency of nitrogen removal under higher loading conditions.

3.1.2 Nitrogen removal per zone

Zone 1, FDN Nitrogen removal began in the first zone, where the pre-denitrification process occurred. This reactor received water from Fillan WWTP and mixed it with the recirculation flow from the fifth zone (DE-OX), after the nitrification process, which increased the NO₃-N concentration in the water. The zone did not have an aeration system to ensure anoxic conditions, but the agitator kept the water and the carriers in motion.

Zone 2, FLEX The second reactor was so named because it could be adjusted according to process performance. Since the zone had an aeration system, it could be used when a needed nitrification level could not be reached by aerating only zones N1 and N2. Otherwise, if it was used to carry out the pre-denitrification process, the air supply was stopped, and the agitator continued to operate. Nonetheless, the main function of this zone was the removal of organic matter from the wastewater.

Zones 3 and 4, N1 and N2 In these zones the nitrification process took place due to a high DO concentration maintained by the aeration system. In addition, the residual BOD that was not removed during pre-denitrification is removed in N1.

Zone 5, DE-OX Within this zone, the objective was to reduce the amount of residual DO coming from the nitrification process, to reach optimal conditions for the post-denitrification process. As a result of the nitrification process that occurred previously, the water from this zone contains a higher NO₃-N content and was therefore used as recirculation flow and sent to the FDN zone, to enable the pre-denitrification process. Over the study period, a recirculation flow of 100% of the inflow to the pilot plant at each line was maintained.

Zone 6, EDN In the last zone, the performance of the post-denitrification process was intended by adding an external source of organic carbon and keeping oxygen levels as low as possible. For this project, the product Brenntapplus VP1 was selected, with a COD content of 1.0 kg/l, and its dosing

was based on the $\text{NO}_3\text{-N}$ concentration after the N2 zone according to the online meters and the inflow rate (so-called Feedforward control). Nonetheless, due to the lack of stability in the results provided by the online meters, and the difficulty in estimating the required organic carbon and its consumption, a carbon source was dosed in excess at a relatively high dose of 9 g COD/g $\text{NO}_3\text{-N}$.

3.1.3 Temperature control

The temperature control consisted of heating/cooling systems based on incoming temperature readings. Through an external cooling system, the recirculation flow of line 2 was cooled to simulate low temperatures, while a fixed immersion heater heated the wastewater in line 2, 4 °C higher than in line 2. From 29/03/23, the temperature was controlled towards fixed set points set at 9 °C for line 1 and 5 °C for line 2.

Figure 8 shows the temperature of the wastewater after the sieves at Fillan WWTP (purple line), the temperature of the incoming wastewater to the pilot plant (green line), and the average temperature in both lines during the study period. The temperature after the sives at Fillan WWTP was collected only after 07/03/23, when a new sensor was installed, which showed that the temperature of the inflow to Fillan WWTP was lower than the inflow to the pilot plant.

The temperature reduction in line 1 between 12/02/23 – 15/02/23 was due to a sharp increase in temperature in the region (SMHI, n.d.-b), which led to a period of early snow-melting. On 10/03/23, and 13/04/23 there were technical problems in the operation of the fixed immersion heater which impeded the maintenance of the target temperature on line 1. In addition, it is possible to observe that the temperature control in line 2 was temporarily modified during some days when the batch tests were performed in the pilot plant (see Chapter 3.2.2).

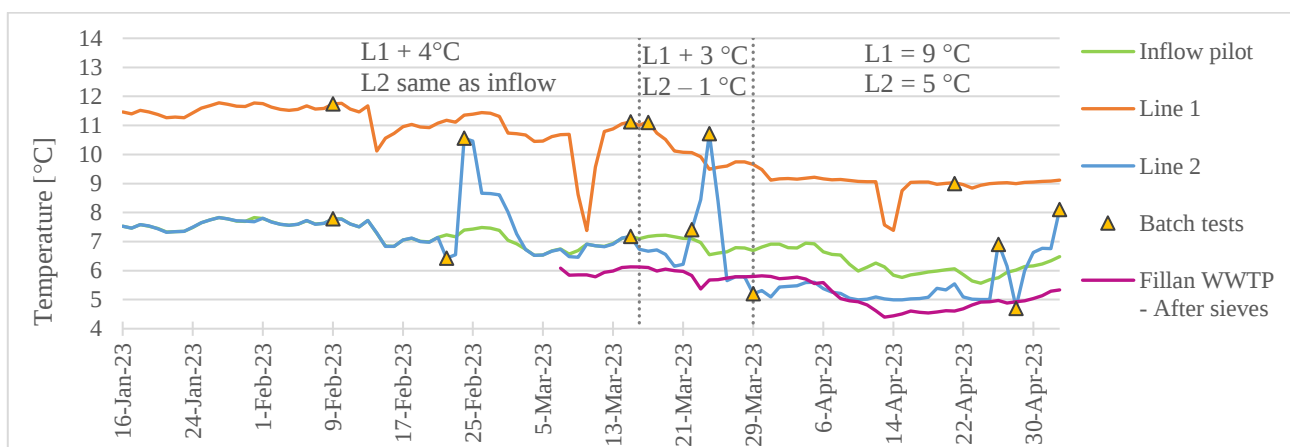


Figure 8 Temperature of the wastewater incoming to the pilot plant and the average temperature in both lines during the study period

3.1.4 Dissolved Oxygen control

To guarantee the nitrification process, even at low temperatures, the FLEX, N1, and N2 zones were equipped with an aeration system that automatically changes the air supply according to the $\text{NH}_4\text{-N}$ concentration monitored after the N2 zone, in order to i) maintain a high nitrification level with low effluent $\text{NH}_4\text{-N}$ concentrations; ii) allow a residual amount of $\text{NH}_4\text{-N}$ after the N2 zone, so it can be used in the DE-OX zone and help reduce the DO concentrations that will subsequently affect the denitrification processes in the FDN and EDN zones; and iii) optimize the air supply according to the performance of the nitrification process. Table 3 presents the different set points applied during the study period.

Table 3 DO supply per aerated zone during the study period

NH ₄ -N concentration after N2 zone [mg/l]		DO set-point [mg/l]		
16/01/23 – 01/02/23	01/02/23 – 03/05/23	FLEX	N1	N2
< 1.0	< 2.0	Off	2.0	4.0
1.0 - 2.0	2.0 - 3.0	Off	3.0	5.0
2.0 - 4.0	3.0 - 5.0	Off	5.0	5.0
> 4.0	> 5.0	2.0	5.0	5.0

Figure 9 represents a timeline with a record of the modifications made according to the most important parameters in the pilot plant during the study period. In addition, Appendix C compiles more detailed information in this regard.

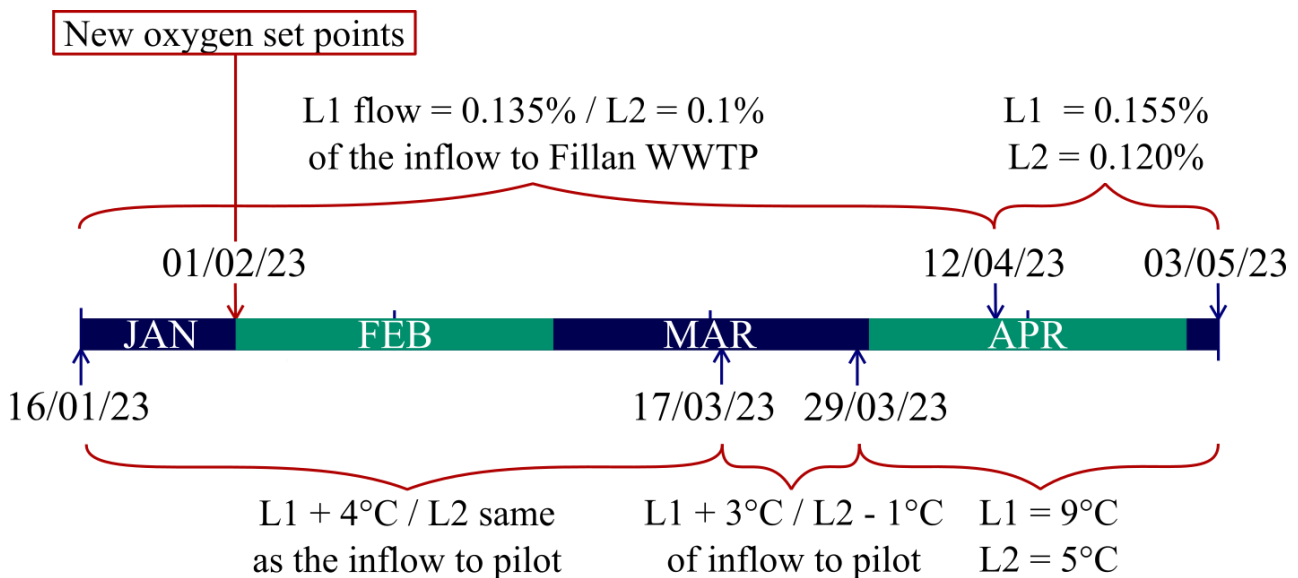


Figure 9 Timeline with representative modifications

3.2 Pilot plant performance

3.2.1 Continuous operation

The evaluation of the continuous operation of the pilot plant was accomplished through the analysis of the extended and the normal analysis packages by sampling the different zones (excluding the DE-OX zone) and then analyzing the concentrations of NH₄-N, NO₃-N, NO₂-N, N-tot, and COD in the laboratory through cuvette tests applying the DR6000 spectrophotometer from Hach Lange, Germany.

The extended analysis package was performed once per week and included samples of the different zones to track more accurately the processes that occur in each one. The normal analysis package was also performed once per week, usually two or three days after the extended package, and included only an analysis of the inflow and the outflow of each line. This allowed the calculation of the total removal efficiencies twice per week and a more detailed follow-up of the process once per week. For the N-tot and COD analyses of the inflow and effluent, the samples were not filtered, while a 0.45 µm filter was used for the rest. On 25/04/23 and 02/05/02, NO₂-N concentration was measured after the N2 zone as well. The sampling model is described in Table 4, and Appendix D goes into detail about the equations used to calculate nitrogen removal.

Table 4 Sampling plan per line during continuous operation of the pilot plant

Location	Sampling type	COD	N-tot	NH ₄ -N	NO ₃ -N	NO ₂ -N
Inflow Fillan WWTP	Daily sample proportional to flow	E/N ^a	E/N ^a	E/N	-	-
Inflow in pilot	Grab sample, filtered	E	-	-		-
After FDN zone	Grab sample, filtered	-	-	-	E	-
After FLEX zone	Grab sample, filtered	E	-	E	E	-
After N1 zone	Grab sample, filtered	-	-	E	E	-
After N2 zone	Grab sample, filtered	E	-	E	E	-
After EDN zone	Grab sample, filtered	E	-	-	E	E
Effluent	Daily sample proportional to time (sedimented before sampling)	-	E/N ^a	E/N	E/N	E/N
Regular number of analyses per week and line		6	4	7	7	3

Note. E – extended analysis package, N – normal analysis package. E/N samples were taken twice per week. E samples were taken once per week. ^a Samples were not filtered.

3.2.2 Activity tests

The activity tests included batch tests performed in the pilot itself in both lines and laboratory-scale activity tests performed in the laboratory using wastewater and carriers from line 2. Appendix E summarizes the conditions established in each activity test and the equations used to calculate nitrogen removal.

3.2.2.1 Batch activity tests

In order to follow up the actual biomass activities according to the changing temperature in the pilot, in line 1 a total of 3 batch activity tests were performed without temperature corrections (same temperature as the continuous operation of the pilot); whereas in line 2, batch activity tests at fixed temperatures of 4, 8 and 12 °C were performed close in time to each other assuming that the biomass did not change considerably between tests and aiming to see the influence of temperature in the short term. This set of 3 activity tests was performed three times during the study period, leading to a total of 9 batch activity tests on line 2. Due to various factors, it was not always possible to achieve the intended temperatures, so the actual test temperatures are shown in Appendix E.

The batch activity tests were performed in the pilot itself by separating the compartments and adding the substrates of the respective reaction. Adjustments in temperature and/or load were made from the afternoon of the previous day, to avoid a sharp change in operating conditions that could cause a load shock or insufficient nitrification. The normal sampling of the continuous operation was skipped during the weeks in which two batch tests were performed. The nitrification and the denitrification activity tests were performed in the whole line simultaneously according to the procedure below.

Nitrification activity. The inflow and the recirculation flow were stopped and the water level of the N1 and N2 zones was decreased. The oxygen supply was set at 5 mg DO/l for both zones and then, Ammonium Bicarbonate (NH_4HCO_3) and Sodium Bicarbonate (NaHCO_3) were added according to the incoming $\text{NH}_4\text{-N}$ concentration after the N2 zone (data from online meters) aiming to reach a concentration of $\text{NH}_4\text{-N}$ close to 22 mg/l at the beginning of the test and to control the pH and the alkalinity, respectively. The first sample was taken 5 minutes after the addition of the chemicals. A total of 5 samples were taken per zone every 25 minutes.

Denitrification activity. The inflow and the recirculation flow were stopped, the water level of the FDN, FLEX, and EDN zones was decreased and the oxygen supply on the FLEX zone was turned

off. Then, Sodium Nitrate (NaNO_3) and Brenntapplus VP1 were added according to the $\text{NO}_3\text{-N}$ concentration after the N2 zone (data from online meters) aiming to reach a concentration of $\text{NO}_3\text{-N}$ close to 20 mg/l and a biodegradable COD to nitrate ratio of 5 at the beginning of the test, respectively. 5 minutes after the addition of the chemicals, sampling began. The first sample was taken 5 minutes after the addition of the chemicals. A total of 5 samples were taken per zone every 25 minutes.

On 15/03/23, additional batch activity tests were performed in each of the FDN zones using the incoming water as the carbon source to compare the pre-denitrification capacity without the contribution of an external source of organic carbon but with the addition of NaNO_3 , and sampling as in the other tests.

3.2.2.2 Laboratory-scale activity tests

The influence of DO on the denitrification process was analyzed by simulating reactors using glass beakers, magnetic stirrers, and a water bath (see the setup in Appendix F). Three types of laboratory-scale tests were conducted as described bellow. In each set of tests, two reactors were run in parallel. Each reactor was filled with wastewater to 2 liters, with a carrier ratio equal to 50%, and both reactors were kept covered to limit oxygen diffusion at the water-air interface. While the first reactor was aerated intermittently to maintain a DO concentration of approximately 1 mg/l, the second reactor was not externally aerated and then, the DO concentration decreased close to 0.0 mg/l due to the high oxygen demand.

In the first two sets of tests wastewater and carriers from the FDN zone in line 2 were used but differed in that in the first test an external organic carbon source (Brenntapplus VP1) was added. During the third set of tests, wastewater and carriers from the EDN zone in line 2, and an organic carbon source were used. All these experiments were repeated in order to validate the results, for a total of 6 laboratory-scale tests during the study period.

After stabilizing the wastewater temperature in both reactors, the stirrers were switched on. After a few minutes, NaNO_3 and Brenntapplus VP1 were added according to the $\text{NO}_3\text{-N}$ concentration of the wastewater (data from online meters) aiming to reach a $\text{NO}_3\text{-N}$ concentration of about 22 mg/l and a COD/biodegradable nitrate ratio of 10 at beginning of the test, respectively. The first sample was taken 5 minutes after the addition of the chemicals. A total of 5 samples were taken per zone every 20 – 25 minutes.

3.2.3 *Biofilm analysis and additional tests*

The biofilm Total Solids (TS) were determined by analyzing 20 carriers from each of the zones for both lines on two occasions during the study period. The carriers were dried overnight at 100°C, weighed, and immersed first in 1M NaOH for at least 2 days and then in 32 vol% HCl for at least another 2 days, followed by washing, drying (overnight at 100 °C), and reweighing.

Complementary tests were carried out according to the results obtained weekly. Some of the tests performed include alkalinity, and PO₄-P tests, among others.

4 Results and discussion

This section includes a comprehensive compilation of the design parameters applied for nitrogen removal using MBBRs, with a specific focus on low-temperature conditions, an in-depth analysis, and a discussion of the results obtained from the continuous operation of the pilot plant situated at Fillan WWTP between January 16, 2023, and May 3, 2023. In addition, the results of the activity tests carried out during this period, among others, are reviewed.

4.1 Existing parameters and design values for the implementation of MBBR-based nitrogen removal with emphasis on low-temperature conditions

Currently, mainly Norwegian design parameters are used for the implementation of the MBBR technology (Norsk Vann, 2020). The design of WWTPs under low-temperature conditions is performed on a 10°C baseline, with the aim of achieving an effluent N-tot concentration of less than 10 mg/l and a percentage reduction of more than 70%. Although, additional measures are recommended for diluted cold wastewater, as a consequence of snow-melting periods. The existing parameters and design values recommended by the Norwegian Report No. 256 (Norsk Vann, 2020) for nitrogen removal using MBBRs under low-temperature conditions are described below.

4.1.1 Design of the MBBR

The reactor volume is determined based on the surface area loading rate in $\text{g}/\text{m}^2\text{-d}$, the proportion of filling of the carrier medium (%), and the effective specific surface area of the carrier medium (m^2/m^3). Table 5 describes the values for designing the surface area loading rate.

Table 5 Design values for MBBRs at 10 °C

Treatment target	Organic loading rate g BOD ₅ /m ² -d	Ammonium loading rate g NH ₄ -N/ m ² -d	NO ₃ -N _{equivalent} loading rate g NO ₃ -N/ m ² -d
A. The main part of the BOD ₅ should be removed: > 70% removal or < 25 mg BOD ₅ /l in the effluent			
- Without chemical additives	≤ 5 ¹		
- With polymer coagulation	≤ 8 ¹		
- With chemical post-precipitation	≤ 11.5 ¹		
B. In addition to A: The majority of NH ₄ -N should be nitrified: < 3 mg NH ₄ -N/l (getting < 15 mg BOD ₅ /l in the effluent)			
- Without pre-sedimentation or pre-denitrification.		≤ 0.50 ³	
- With pre-sedimentation or pre-denitrification.		≤ 0.60 ³	
- With pre-sedimentation and pre-denitrification.		≤ 0.65 ³	
- With pre-precipitation.		≤ 0.75 ³	
C. In addition to B: The majority of N-tot should be removed: > 70% (getting < 10 mg BOD ₅ /l in the effluent)			
			≤ 0.50 ^{4,5}
- With pre-denitrification.			≤ 1.50 ⁶
- With post-denitrification.			≤ 0.50 ^{4,5} in pre-DN reactor
- With a combination of pre- and post-denitrification.			≤ 1.50 ⁶ in post-DN reactor

Adapted from Norsk Vann, (2020)

¹ It is assumed that the reactor is divided into at least two separate chambers (specified value applies to total volume), and the residence time at $Q_{\max\dim}$ is ≥ 30 min.

² Dimensioning value for the part of the reactor that is expected to remove organic matter before nitrification.

³ Dimensioning value for the reactor section that is expected to remove ammonium by nitrification assuming that the residual concentration of ammonium is > 2 mg $\text{NH}_4\text{-N/l}$. If a lower residual concentration is required, the specified nitrification rates should be reduced linearly from the specified value at $\text{NH}_4\text{-N}$ concentration equal to 2 mg $\text{NH}_4\text{-N/l}$ to 0 at $\text{NH}_4\text{-N}$ concentration equal to 0 mg $\text{NH}_4\text{-N/l}$. No higher oxygen concentration than 5 mg $\text{O}_2\text{/l}$ should be assumed when dimensioning. The specified nitrification rates apply at oxygen concentration = 5 mg $\text{O}_2\text{/l}$.

⁴ Assuming that the C/N ratio in the incoming water to the pre-denitrification reactor is > 4 g $\text{BOD}_5\text{/g NO}_3\text{-N}_{\text{equiv, inflow}}$ and the residual concentration can be > 3 mg $\text{NO}_3\text{-N/l}$. If the C/N ratio is lower than this (which is normal), the rate should be reduced linearly from the specified value at $\text{C/N} = 4$ g $\text{BOD}_5\text{/g NO}_3\text{-N}_{\text{equiv, removed}}$ to 0 at $\text{C/N} = 2$ g $\text{BOD}_5\text{/g NO}_3\text{-N}_{\text{equiv, removed}}$. If there are two pre-denitrification reactors in series, the first should be dimensioned as specified and the last for < 0.30 g $\text{NO}_x\text{-N/ m}^2\text{-d}$.

⁵ Assuming that the recycled water volume from the nitrification to pre-nitrification reactor is $> 250\%$ $Q_{\text{mean, inflow}}$ in order to meet the requirement of 70% removal with pre-denitrification alone. With combined pre- and post-denitrification, the amount of recycling can be lower.

⁶ Assuming that an external carbon source is added, the C/N ratio in the incoming water to the post-denitrification reactor should be > 3 g $\text{BOD}_5\text{/g NO}_3\text{-N}_{\text{equiv}}$. (possibly 4.5 g $\text{COD/g NO}_3\text{-N}_{\text{equiv}}$) and the residual concentration of $\text{NO}_3\text{-N}_{\text{equiv}}$ can be > 3 mg $\text{NO}_3\text{-N/l}$. If a lower residual concentration is required, the loading rate for post-denitrification must be multiplied by a factor F . $F = \text{required concentration}/3$. These values apply when methanol or glycol is used as an external carbon source. If ethanol is used, the rates can be multiplied by 1.8.

At temperatures other than 10 °C, the loading rate must be corrected according to the following formula:

$$A_T = A_{10} \cdot \theta^{(T-10)} \quad (7)$$

Where A_T is the loading rate at the current temperature, A_{10} is the loading rate at 10 °C, and θ is the Arrhenius temperature dependence coefficient, which should be set to 1.07 for BOD_5 removal and denitrification, and 1.09 for nitrification.

WWTPs with nitrogen removal can be built with pre-denitrification, post-denitrification, or a combination of the two. The part of the plant that would provide organic matter removal and

nitrification is dimensioned according to treatment target B, while the denitrification reactors are dimensioned according to treatment target C, from Table 5.

In pre-denitrification plants, the volume required for the removal of organic matter, beyond the uptake (removal) in pre-denitrification, should be determined based on the remaining $BOD_{5,dissolved}$ after the pre-denitrification step. For the determination of the breakdown of organic matter by pre-denitrification, it should be assumed (unless data are available):

- a) The proportion of dissolved organic matter in wastewater as a percentage of the total ($BOD_{5,dissolved}/BOD_{5,total}$):
 - Without primary treatment stage: 25%
 - With primary treatment stage: 30 %
- b) The proportion of particulate organic matter that is converted to dissolved organic matter by hydrolysis: 25% of $BOD_{5, particulate}$ in the incoming wastewater.
- c) Breakdown of organic matter by pre-denitrification: $3.0 \text{ g } BOD_{5,dissolved} / \text{g } NO_3-N_{equiv, removed}$ where dissolved organic matter available for denitrification ($BOD_{5,dissolved}$) is the sum of the two fractions under points a) and b) above.

The load ($\text{kg } NO_3\text{-N/d}$) on the pre-denitrification reactor should be determined as the product of the recycled water volume and the concentration of nitrate in the outlet of the nitrification zone, with the addition of the converted oxygen volume (1 g oxygen corresponds to $0.35 \text{ g } NO_3\text{-N}_{equivalent}$).

In WWTPs where pre-denitrification is included, it will therefore be appropriate to reduce the oxygen content in a deoxygenation reactor before the nitrate-containing water is returned to the pre-denitrification reactor. This can be done by not aerating this reactor (using a stirrer) so that the biomass on the carriers consumes oxygen as a result of the decomposition of the remaining ammonium. The deoxygenation reactor can be dimensioned based on a decomposition rate of $0.225 \text{ g } NH_4\text{-N/m}^2\text{-d}$ (at 10°C temperature coefficient $\theta = 1.09$ - see equation 7). It can then be assumed that the oxygen concentration in the water to be recycled is $2 \text{ g } O_2/\text{m}^3$.

Similarly, in WWTPs where post-denitrification is included, it will be appropriate to use a deoxygenation reactor to reduce the oxygen load in the post-denitrification process. It will also be appropriate to use an aerated reoxygenation reactor after the post-denitrification reactor to ensure that any remaining organic matter from the external carbon source is decomposed. This

reoxxygenation reactor can be dimensioned for a residual organic matter concentration of 10 g COD_{dissolved}/m³ and a breakdown rate of 4 g COD_{dissolved}/m²-d at Q_{maxdim}. However, the retention time must not be set lower than 18 min at Q_{maxdim}.

4.2 Continuous operation

A summary of the average daily operational parameters for both lines, measured by the online meters during the study period, is presented in Table 6. It should be mentioned that the pH in both lines was lower than the recommended values to carry out the nitrification (pH between 7.2 – 8.0) and denitrification (7.0 – 7.5) processes.

Table 6 Summary of the operational parameters of both lines

Line	Parameter	Unit	Minimum	Maximum	Average
1	Flow	m ³ /h	0.5	1.7	0.8
	Temperature	°C	7.6	11.8	10.3
	pH in N2 zone	-	6.2	6.5	6.4
	DO in FLEX zone	mg/l	0.2	2.8	0.5
	DO in N1 zone	mg/l	2.0	4.0	2.3
	DO in N2 zone	mg/l	4.0	5.0	4.1
	Carbon source dose	ml/h	32.5	98.7	62.4
2	Flow	m ³ /h	0.4	1.3	0.6
	Temperature	°C	5.0	7.8	6.5
	pH in N2 zone	-	6.3	6.6	6.5
	DO in FLEX zone	mg/l	0.3	2.9	0.7
	DO in N1 zone	mg/l	2.1	4.8	3.1
	DO in N2 zone	mg/l	4.0	5.0	4.5
	Carbon source dose	ml/h	31.6	59.5	42.0

4.2.1 N-tot removal

Throughout the continuous operation of the pilot plant and according to the chemical analyses carried out twice a week (in most cases), a reduction of N-tot ranging from 40% to 86% was observed. Average values of 67% and 73% for lines 1 and 2, respectively, were achieved while comparing the N-tot concentrations in the pre-sedimented wastewater incoming to the pilot plant and the effluent of each line, as shown in Figure 10 and Figure 11, by including the post-denitrification process. An average contribution of the post-denitrification process of 24% in line 1 and 31% in line 2 can also be estimated during the N-tot removal.

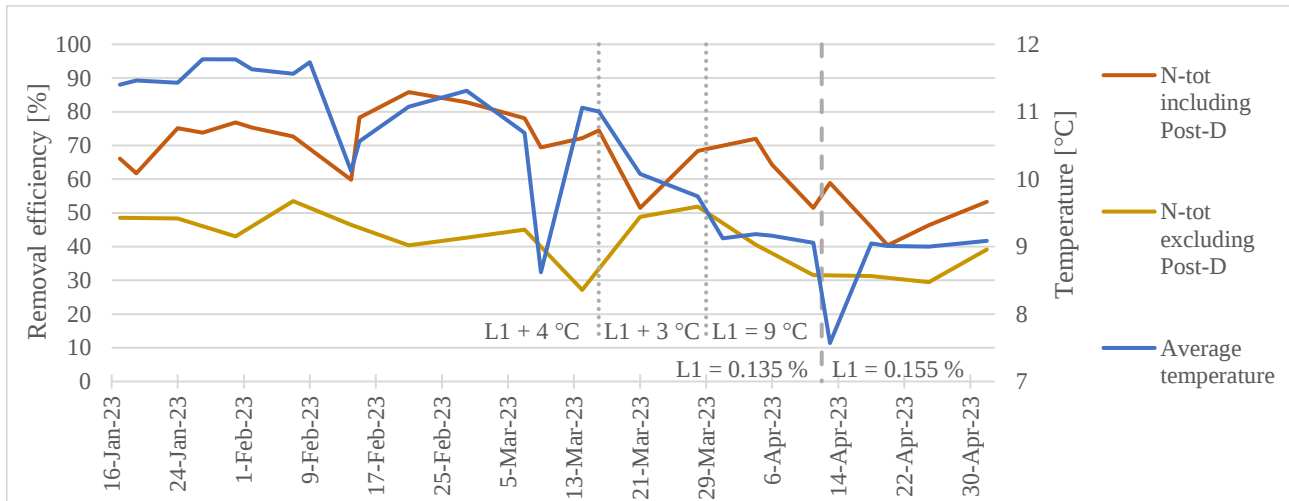


Figure 10 Nitrogen removal efficiency of line 1 during the continuous operation of the pilot plant

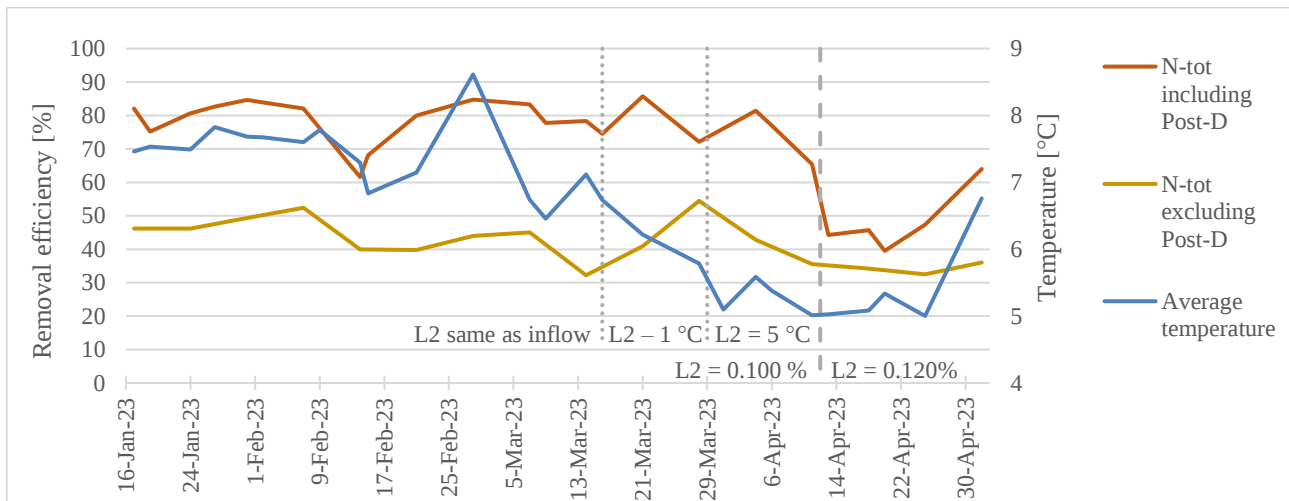


Figure 11 Nitrogen removal efficiency of line 2 during the continuous operation of the pilot plant

The lowest removal values, below 70%, were mainly associated with precipitation events and the snow-melting period, which occurred early between February 12 and 15 (SMHI, n.d.-b) and then resumed from April 4. However, the peak of the snow-melting period happened between April 12 and 29, with a notable surge in the incoming flow to Fillan WWTP on April 14, reaching up to 1355 m³/h, which is 4 times the lowest inflow observed during the study period.

The wastewater temperature reached a minimum temperature of 4.7 °C in line 2 on April 28 with an average inflow of 0.8 m³/h. On the day with the highest flow (April 14), line 1 had an average inflow of 2.05 m³/h and an average temperature of 7.4 °C, while line 2 had 1.63 m³/h and 5.0 °C. Table 7 describes the conditions in the pilot plant on the days that coincided with the snow-melting period and the water sampling and chemical analysis.

Table 7 Nitrogen removal during the snow-melting period

	Units	Date								
		14/02/23	15/02/23	06/04/23	11/04/23	13/04/23	18/04/23	20/04/23	25/04/23	02/05/23
Daily average precipitation – previous day ¹	mm	0.0	0.0	0.0	0.0	5.8	0.0	0.0	1.7	0.0
Daily average precipitation ¹	mm	0.0	0.0	4.9	0.4	0.6	0.0	0.0	7.0	0.0
Daily average temperature in Sundsvall – previous day ¹	°C	6.3	2.7	0.7	2.2	3.9	6.1	9.7	0.5	4.4
Daily average wastewater temperature – Line 1	°C	10.1	10.6	9.2	9.1	7.6	9.0	9.0	9.0	9.1
Daily average wastewater temperature – Line 2	°C	7.3	6.8	6.7	6.1	6.1	6.0	6.0	5.7	6.3
DO concentration at FDN – Line 1	mg/l	1.41	-	0.53	0.94	-	1.35	-	1.95	0.65
DO concentration at FDN – Line 2	mg/l	1.15	-	0.31	0.40	-	0.52	-	1.48	0.49
Influent COD concentration	mg/l	98.2	93.0	102.6	66.8	55.8	51.6	43.5	62.1	85.4
Influent N-tot concentration	mg/l	22.6	16.7	19.5	13.4	12.1	10.9	10.3	12.5	13.3
Effluent N-tot concentration – Line 1	mg/l	9.08	3.64	6.97	6.50	4.97	5.91	6.14	6.71	6.22
Effluent N-tot concentration – Line 2	mg/l	8.69	5.32	4.48	4.64	6.75	5.92	6.23	6.59	4.79
N-tot removal – Line 1	%	60	78	64	51	59	46	40	46	53
N-tot removal – Line 2	%	62	68	77	65	44	46	40	47	64

¹ Source SMHI, (n.d.-a)

As shown in Table 7, snow-melting periods are associated with dilute contaminant concentrations, low-temperature incoming wastewater, and high DO concentration. The elevated DO content accelerates the degradation of organic matter present in the network and additionally, the low temperatures limit hydrolysis, resulting in a higher presence of particulate organic matter, instead of easily biodegradable organic matter that can be used by the bacteria in the pre-denitrification process.

The negative impact of the snow-melting period on nitrogen removal has also been observed in WWTPs that implemented MBBR technology in Norway. It highlights the importance of including the post-denitrification process in WWTPs with low-temperatures conditions, as shown in Figure 10 and Figure 11 Table 7. The N-tot removal efficiency is higher while including the post-denitrification process, with an average contribution of 24% and 31% in lines 1 and 2, respectively.

Figure 12 and Figure 13 show the nitrogen compound concentrations in the inflow and effluent, along with the incoming COD concentration from Fillan WWTP. The average N-tot concentration in the effluent was recorded as 6.38 mg/l for line 1 and 5.03 mg/l for line 2. Inorganic nitrogen compounds (including $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$) accounted for an average contribution of 58%. It shows that, even though performed at lower temperatures, line 2 showed a more consistent performance compared to line 1.

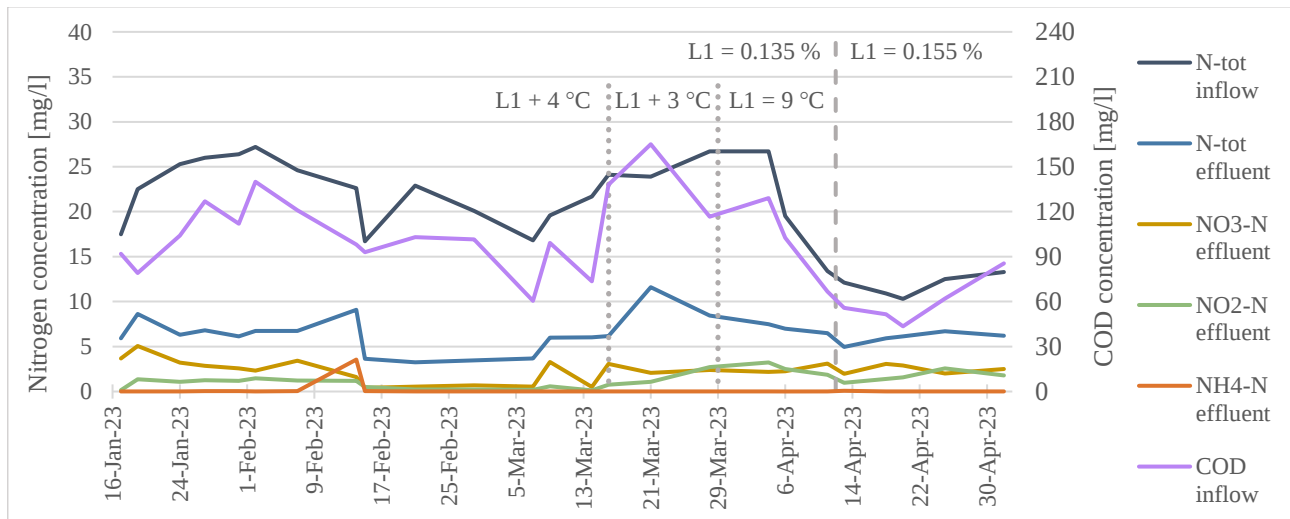


Figure 12 Inflow and effluent concentrations of nitrogen compounds and COD for line 1

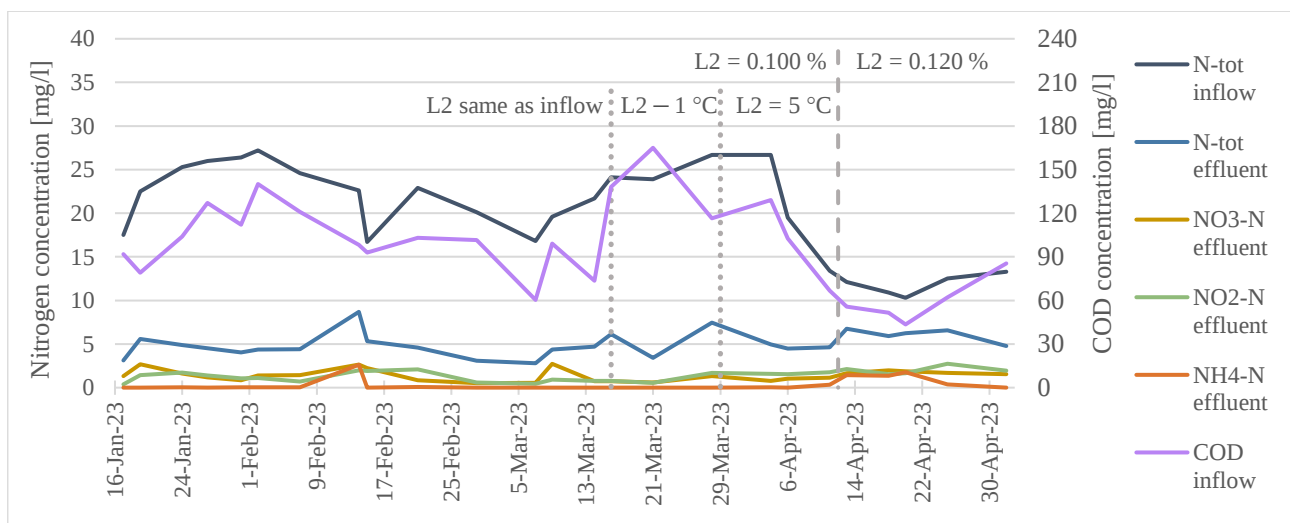


Figure 13 Inflow and effluent concentrations of nitrogen compounds and COD for line 2

Despite the equal stipulated dosage of the external carbon source in both lines (9 g COD/g NO₃-N) and a maximum difference of 2.9 mg/l between the NO₃-N concentrations after the N₂ zone of both lines, there is an average of 10 mg/l more biodegradable COD surplus after the post-denitrification process in line 1, which could promote the accumulation of NO₂-N.

The impact of wastewater dilution during snow-melting periods is evident, leading to a significant decrease in incoming concentrations of COD and N-tot. Throughout most of the study period, N-tot concentrations in the effluent were less than 10 mg /l, with NO₃-N and NO₂-N as the main contributing inorganic compounds. Although there was an increase in the effluent concentration of NH₄-N, especially in line 2, during the snow-melting period.

The only sampling that showed an effluent N-tot concentration of higher than 10 mg/l was on the 21st of March in line 1 with a value of 11.6 mg/l (see Figure 12). However, the inorganic nitrogen concentration in that sample was only 3 mg/l which suggests that the high effluent concentration was caused by unusually high sludge content in the sample or an analytic error.

4.2.2 Pre-denitrification

Figure 14 and Figure 15 compare the NO₃-N concentrations after pre-denitrification takes place in the FDN and FLEX zones, as expected, indicating a reduction in the NO₃-N concentration. After the snow-melting period began, NO₃-N concentrations in the diluted wastewater decreased drastically, however, after the pre-denitrification process, they did not change.

Manual measurements of DO in the FDN zone during the study period refer to concentrations between 0.22 – 1.95 mg/l, with an average of 0.77 mg/l in line 1 and 0.55 mg/l in line 2. Both values are above the 0.2 mg/l recommended in the reactors where the pre-denitrification process occurs (Seitzinger S et al., 2006). The FLEX zone of line 2 had higher DO concentrations, with manual measurements averaging 0.68 mg/l, compared to 0.44 mg/l in line 1.

The values close to 0 mg/l in the concentrations of NO₃-N after the pre-denitrification zones in line 2 on March 21, and May 2 (see Figure 15), can be attributed to the stop of the recirculation flow within the 24 hours of sampling. Moreover, the calculation of diluted wastewater is subject to limitations, as it relies on average daily concentrations that do not represent the continuous fluctuations. Additionally, the HRT in each tank is a few hours, and the recirculation flow has a smoother profile compared to the inflow to the pilot.

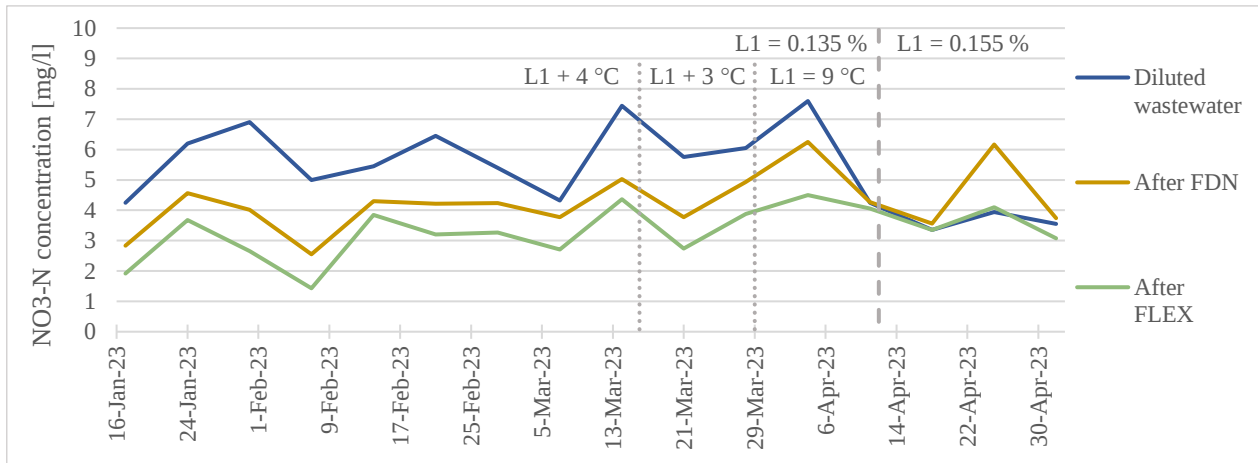


Figure 14 $\text{NO}_3\text{-N}$ concentration after pre-denitrification zones in line 1

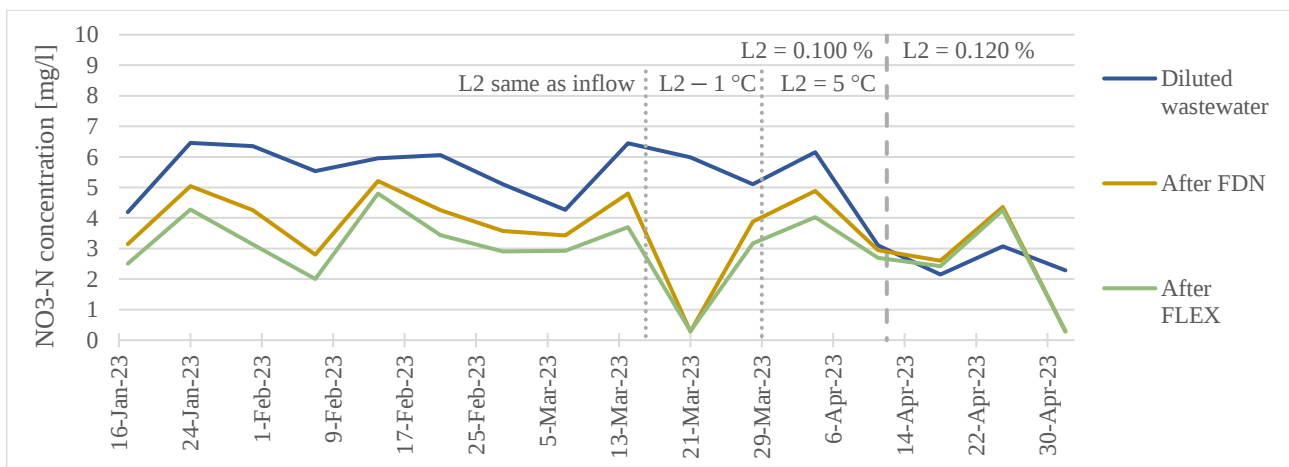


Figure 15 $\text{NO}_3\text{-N}$ concentration after pre-denitrification zones in line 2

BOD_7 measurements were conducted on six separate occasions for the incoming water to the pilot plant. The average BOD_7/COD ratio was 0.46, ranging between 0.40 and 0.48. These values suggest a high degree of biodegradability since a ratio of around 0.50 is considered optimal, indicating that a significant portion of the organic compounds present in the water can be readily degraded by microorganisms (Zhang et al., 2020). It should be noted that none of these measurements were taken during the snow-melting period, and therefore, the biodegradability characteristics of the wastewater during that specific period remain unknown.

4.2.3 Post-denitrification

The results demonstrate a notable reduction in the concentration of $\text{NO}_3\text{-N}$ following the post-denitrification process in both lines (see Figure 16 and Figure 17). Despite the dilution of pollutants during the snow-melting period, resulting in a decrease in the $\text{NO}_3\text{-N}$ concentration after N_2 , the concentrations in the effluent remain relatively unchanged. Manual measurements of DO concentrations in the EDN zone show a varying range of 0.12 to 0.45 mg/l, with an average of 0.26

mg/l in line 1 and 0.24 mg/l in line 2. The average COD concentrations after the EDN zone were 48 mg/l in line 1 and 39 mg/l in line 2.

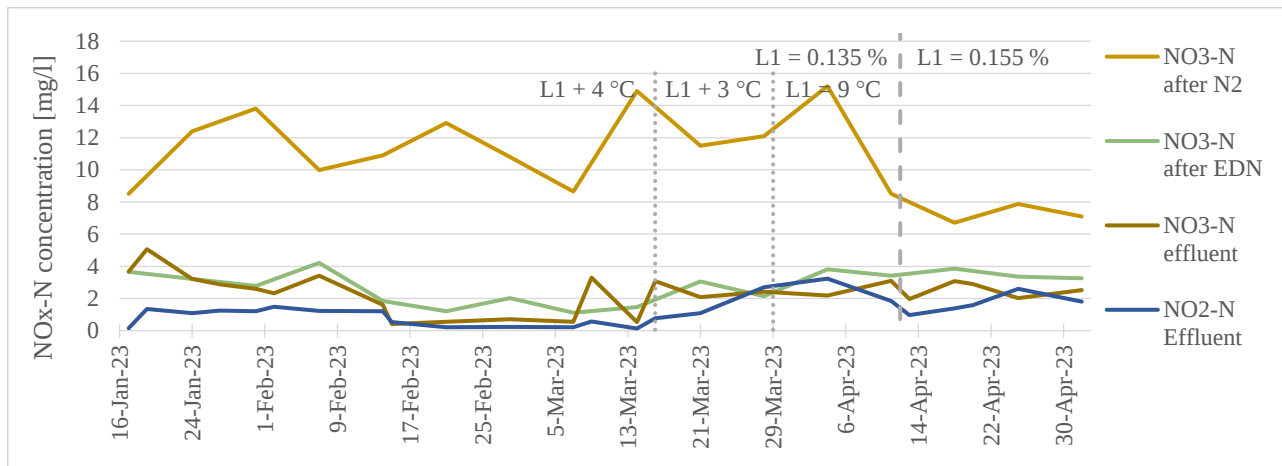


Figure 16 NO_x-N concentration before and after the post-denitrification in line 1

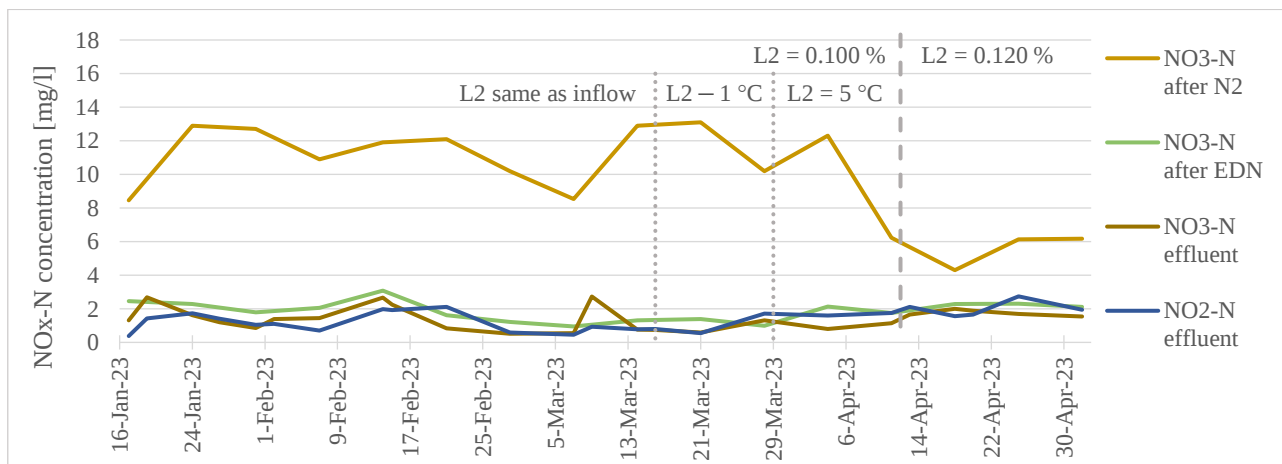


Figure 17 NO_x-N concentration before and after the post-denitrification in line 2

Additionally, the findings highlight the significant contribution of NO₂-N as an inorganic compound in the effluent, with an average of 1.20 mg/l on line 1, and 1.38 mg/l on line 2. This could be related to the excess organic matter available during the post-denitrification process since the external carbon source dose of 9 g COD/g NO₃-N is high compared to the 4 g COD/g NO_x-N recommended as optimal by Bjorn Rusten et al., (2006). Although, Brenntaplu VP1 was tested just after the completion of the master's thesis and determined to have a lower COD content than expected.

4.2.4 Nitrification

The nitrification process (ammonium removal) in both line 1 and line 2 exhibited a high removal efficiency of 100% for the majority of the study period. However, a decrease in removal efficiency

was observed during the snow-melting period. Despite the malfunctioning of the online meter located downstream of the N2, line 1 was only affected during the early snow-melting period, with the sudden temperature change, as previously noted by Hwang & Oleszkiewicz, 2007, by evidencing the impact of cold shocks on the rate of nitrification. Line 2 experienced the main impact during the peak of the snow-melting period, with a minimum reduction of 83% observed on April 20, as illustrated in Figure 18.

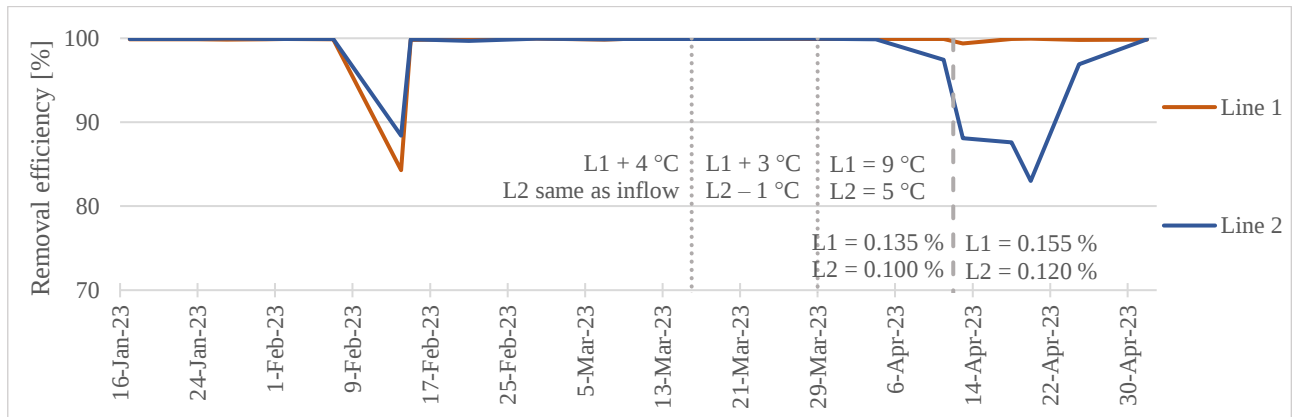


Figure 18 Nitrification removal efficiency in both lines

Based on the online meter readings, the average $\text{NH}_4\text{-N}$ concentrations after the N2 zone were 0.72 mg/l for line 1 and 1.95 mg/l for line 2, while the average $\text{NO}_3\text{-N}$ concentration was 9.51 mg/l for line 1 and 8.67 mg/l for line 2. However, the results for the $\text{NH}_4\text{-N}$ concentration on line 1 have a high uncertainty due to the previously mentioned inconsistencies (concentrations equal to 0 during long periods), which can be observed in Figure 19.

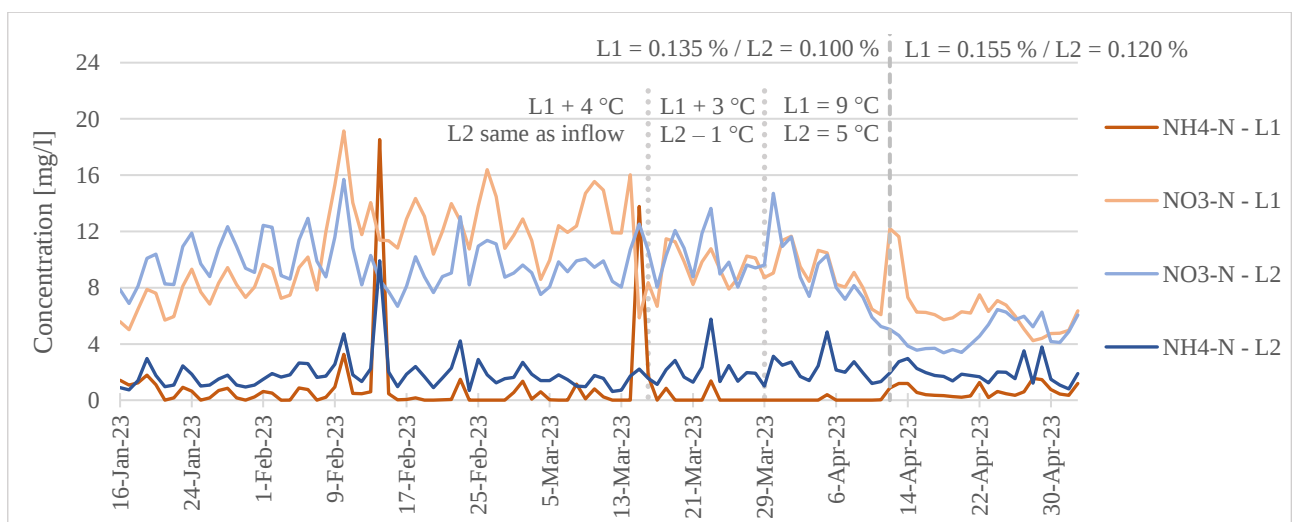


Figure 19 $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations after the N2 zone from the online meters on both lines

The high $\text{NH}_4\text{-N}$ concentration in line 1 on March 15 was due to a calibration in the online meter in the morning hours and a batch test in the afternoon hours followed by a 0.5 °C decrease in the wastewater temperature of the line. The high efficiencies of the nitrification process suggest the possibility of further reducing the DO supply, intending to reduce the DO concentration that is transferred to the zones where denitrification occurs. This also favors the occurrence of nitrification of residual $\text{NH}_4\text{-N}$ in the DE-OX zone, by taking advantage of the DO present there (manually measured average concentration of 1.72 mg/l in line 1 and 1.26 mg/l in line 2).

Notably, during the early snow-melting period, there was a significant increase in oxygen levels observed in the FLEX and N1 zones (see Figure 20). Although during the peak of the snow-melting period the FLEX zone was not being aerated, as the incoming $\text{NH}_4\text{-N}$ concentrations were low, it is possible to get an increase in DO concentrations in both lines due to the incoming water. On 21 April the online meters in the FLEX zones recorded the highest daily average DO concentrations at 1.08 mg/l and 1.38 mg/l in lines 1 and 2 respectively, which further suggests even more critical DO concentrations in the FDN zone.

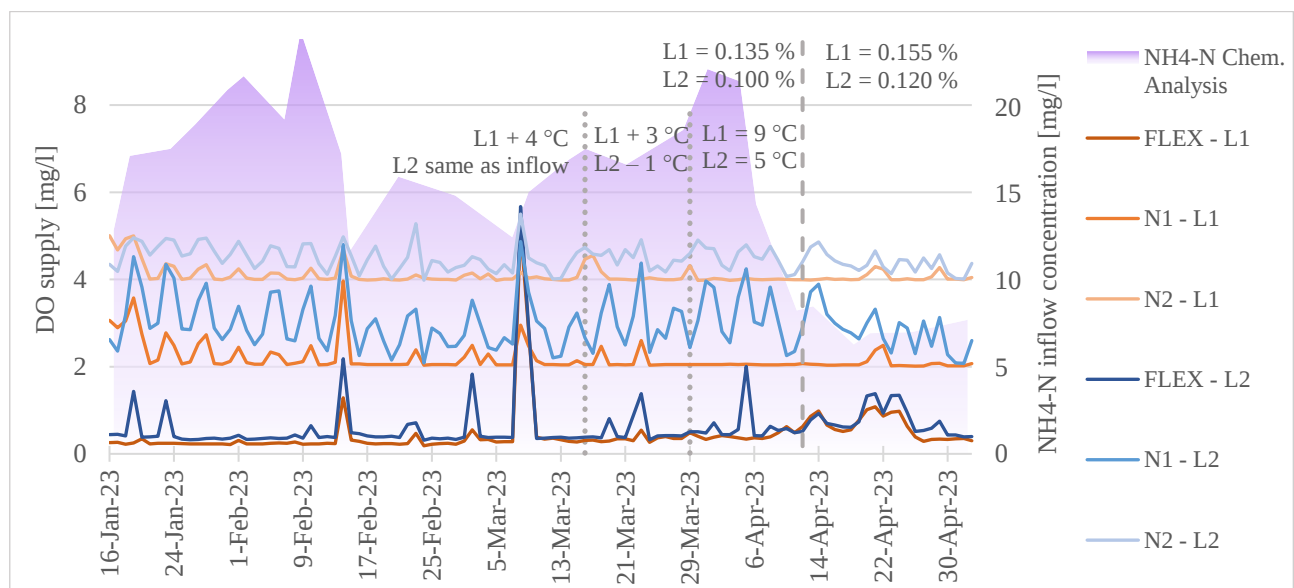


Figure 20 Daily average DO supply in the aerated zones of both lines and $\text{NH}_4\text{-N}$ inflow concentration

4.3 Activity tests

The results of the batch and laboratory-scale activity tests are presented below. Nevertheless, the results are mainly related to line 2, which had a cooling system, facilitating the analysis at low temperatures.

4.3.1 Batch activity tests

4.3.1.1 Typical profiles

Figure 21, Figure 22, and Figure 23 are an example of the results of the batch activity test carried out on 28/04/23 with an average temperature of 5 °C in line 2. The nitrification rate in the N2 zone was 0.40 g NH₄-N/m²-d and the denitrification rates in the FDN and EDN zones of 0.46 and 0.91 g NO_x-N/ m²-d, respectively. The average DO concentrations during the test were 5.1, 0.11, and 0.09 mg/l in the N2, FDN, and EDN zones, respectively.

As expected, in the denitrification process, NO₃-N and COD concentrations were reduced, while in the nitrification process, NO₃-N was produced by reducing NH₄-N. The EDN zone presented a non-linear behavior (Figure 23), limited by the COD concentration, and therefore, only the linear part of the curve was used to calculate the reaction rate. Additionally, an accumulation of NO₂-N in the denitrification process was observed. After 55 minutes, the NO₂-N concentration was 1.1 mg/l in the FDN zone and 4.7 mg/l in the EDN zone.

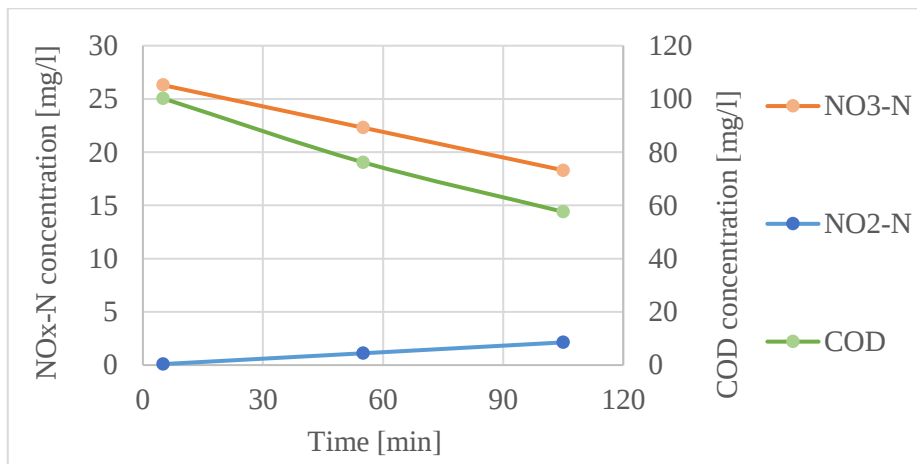


Figure 21 Denitrification batch activity tests on the FDN zone at 5 °C in line 2

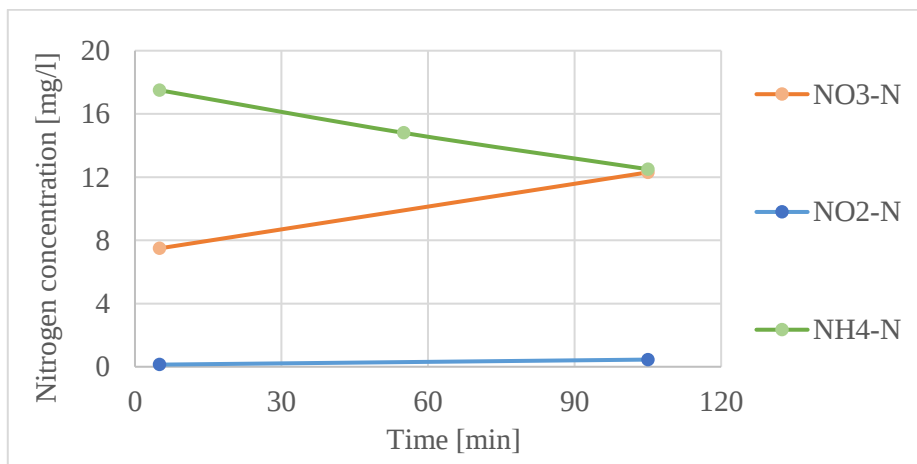


Figure 22 Nitrification batch activity tests on the N2 zone at 5 °C in line 2

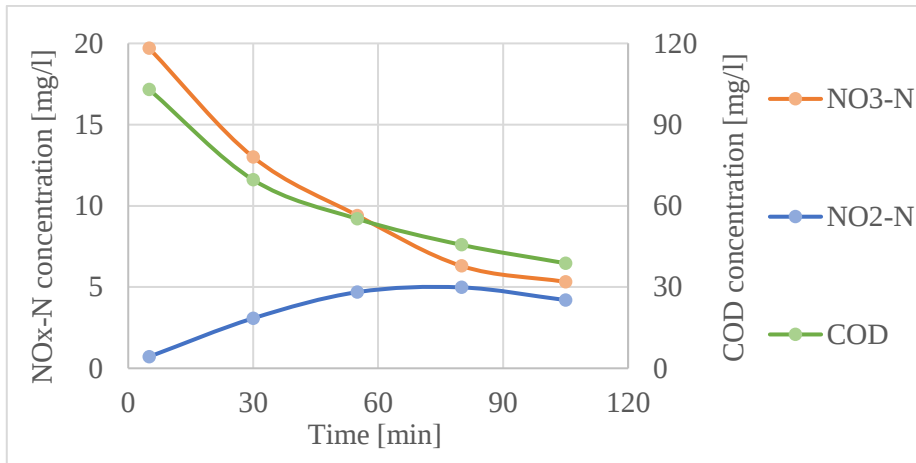


Figure 23 Denitrification batch activity tests on the EDN zone at 5 °C in line 2

4.3.1.2 Batch activity tests in FDN zones without external carbon source

The denitrification rate without the use of an external carbon source in line 1 was 0.32 g NO_x-N/m²-d, with an average temperature of 9.4 °C and a DO concentration of 0.1. In line 2, the denitrification rate was 0.22 g NO_x-N/m²-d with an average temperature of 7.0 °C and a DO concentration of 0.03.

Comparing these values with the regular batch activity tests where an external carbon source is used and at a similar temperature (9.1 °C and 7.5 °C), a reduction of the denitrification rate of more than 27%. Moreover, the low COD soluble/ N-tot removed ratios without using an external carbon source (1.4 in line 1 and 2.9 in line 2), compared to the ratios above 6.0 while using the external carbon source, suggests a contribution from the hydrolysis process during the denitrification, thereby increasing the concentration of available soluble COD.

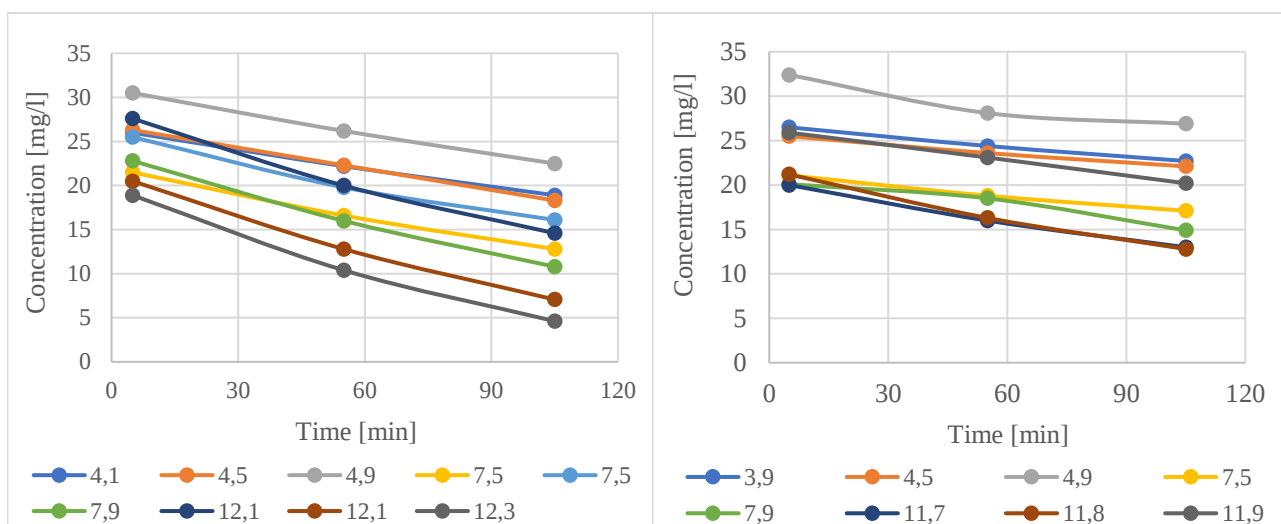
4.3.1.3 Comparison between lines 1 and 2

By maintaining a difference of 4 °C between both lines during the continuous operation of the pilot plant, it was possible to compare the results of the 3 batch activity tests performed on line 1 with line 2. It was observed that the greatest difference between activity rates occurred in the EDN and N₂ zones, with an average increase of 60% and 38%, respectively, when the wastewater was warmer. On average, the difference in the activity rates of the FDN and FLEX zones at 4 °C difference was less than 20%. In addition, when the range of temperatures was lower (line 2 at 5°C and line 1 at 9°C), the difference between the activity rates was 20% lower than when compared to the difference of the activity rates at higher temperature ranges (line 2 at 7.5°C and line 1 at 11.6°C).

4.3.1.4 Temperature dependence

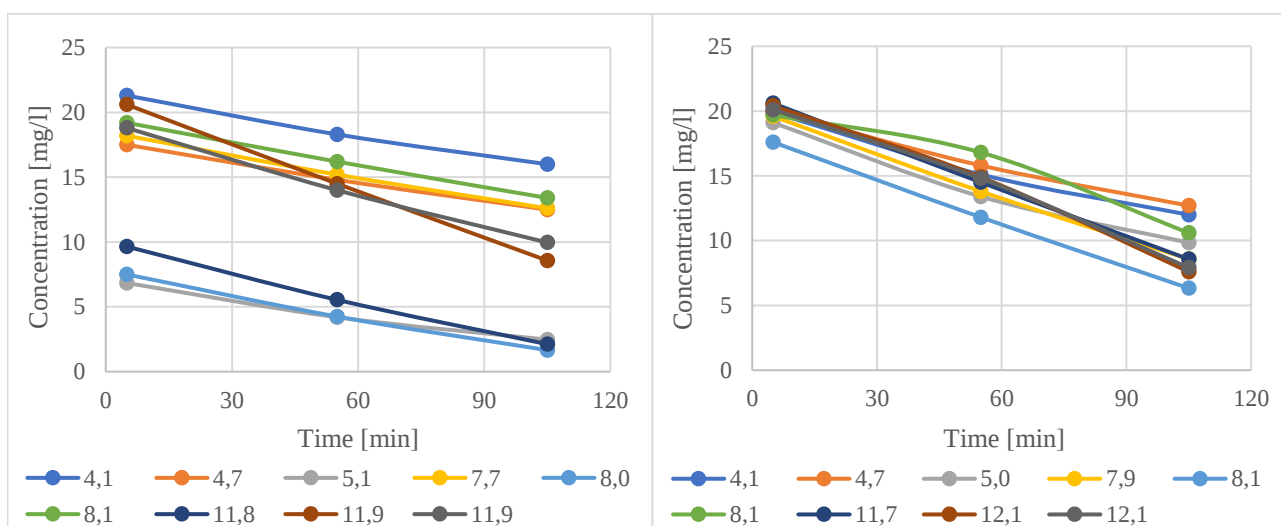
The reduction of inorganic compounds ($\text{NH}_4\text{-N}$ and $\text{NO}_x\text{-N}$) in the 3 sets of batch activity tests carried out on line 2 are presented in Figure 24. Some of the reduction patterns in the different nitrification and denitrification zones did not show the expected results, but this is because each test had different operational conditions that influenced the results. The initial test temperature, as well as the DO supply in the aerated zones, were some of the most difficult parameters to control.

The denitrification rates ranged from 0.42 - 0.84 g $\text{NO}_x\text{-N}/\text{m}^2\text{-d}$ in the FDN zone, 0.22 - 0.55 g $\text{NO}_x\text{-N}/\text{m}^2\text{-d}$ in the FLEX zone, and 0.99 - 1.84 g $\text{NO}_x\text{-N}/\text{m}^2\text{-d}$ in the EDN zone. The average DO concentrations in the FDN, FLEX, and EDN zones were 0.13, 0.13, and 0.10 mg/l respectively, which were low enough to not inhibit the denitrification. The nitrification rates ranged from 0.28 - 0.56 g $\text{NH}_4\text{-N}/\text{m}^2\text{-d}$ in the N1 zone and 0.41 - 0.71 g $\text{NH}_4\text{-N}/\text{m}^2\text{-d}$ in the N2 zone. The average DO concentrations in zones N1 and N2 were 5.42 and 4.90 mg/l, respectively, which is near the targeted 5 mg/l.



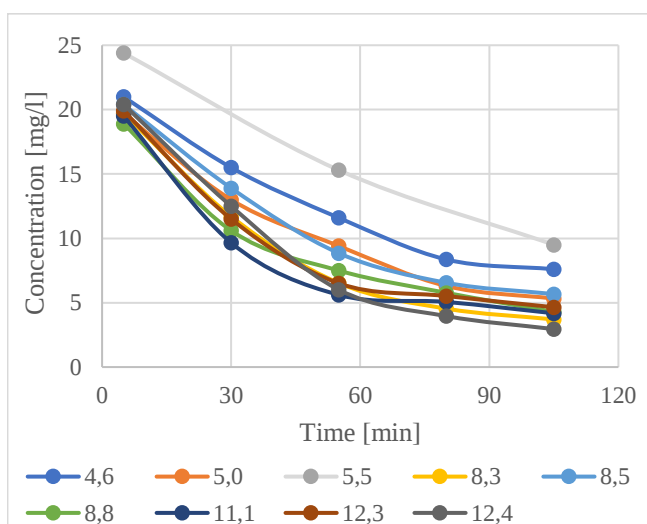
a) NO_x-N reduction in FDN

b) NO_x-N reduction in FLEX



c) NH₄-N reduction in N1

d) NH₄-N reduction in N2



e) NO_x-N reduction in EDN

Figure 24 NH₄-N and NO_x-N reduction during the batch activity tests in line 2. Note: The different colors represent the different temperatures.

From Figure 25 and Figure 26, it can be concluded, as expected, that both processes (nitrification and denitrification) are temperature-dependent. The lower nitrification rate of the N1 zone compared to N2 is related to the removal of organic matter that also occurs in N1. Compared to the EDN zone, FDN and FLEX zones presented lower denitrification rates. This can be related to the fact that the bacteria in these zones are not adapted to the use of that specific external carbon source. In addition, the structure and composition of the biofilm are important factors, since as observed in the results of the continuous plant operation, pre-denitrification occurs to a lesser extent in the pilot plant, so there were not as many bacteria that could perform denitrification there compared to the EDN zone.

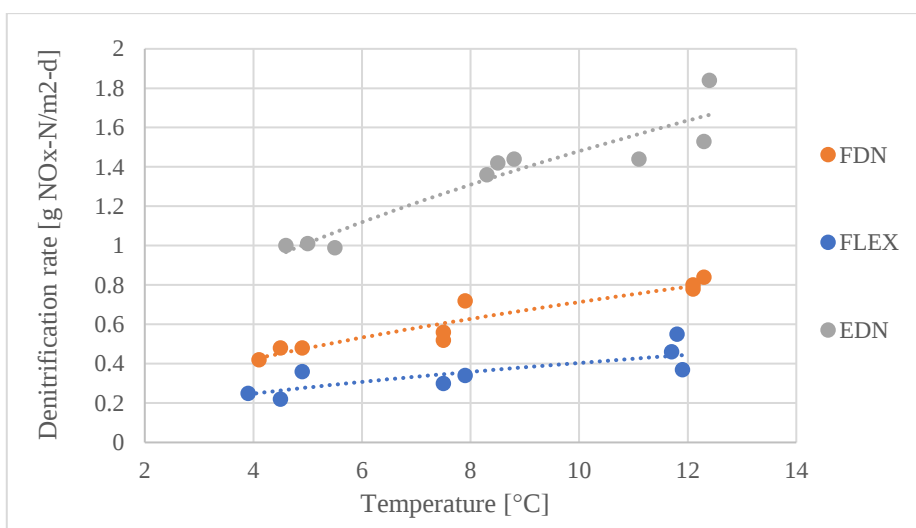


Figure 25 Denitrification rates during the 3 sets of batch activity tests in line 2

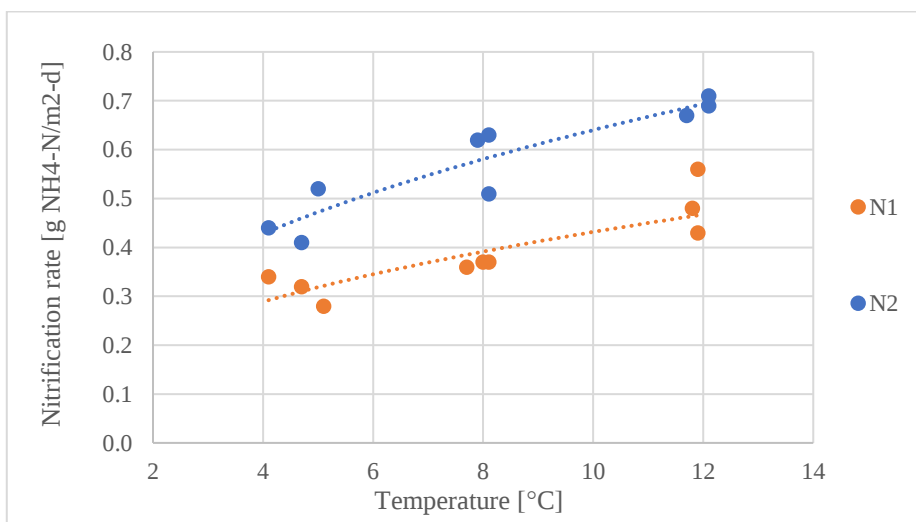


Figure 26 Nitrification rates during the 3 sets of batch activity tests in line 2

4.3.1.5 Temperature dependence coefficient from Arrhenius equation

According to the biomass activity rate calculated during the 3 sets of batch activity tests in line 2, a θ value of 1.086, 1.128, and 1.083 was determined in the FDN, FLEX, and EDN zones. In the N1 and N2 zones, the θ value was 1.110 and 1.063, respectively.

To obtain the values of θ per zone, the predicted activity rate (k_{T_1}) of each of the tests was calculated, applying equation 5. For this, in each zone an arbitrary value of θ was chosen and the maximum measured activity rate was used as a reference (T_{ref} , $k_{T_{ref}}$). Then, the value of θ was iterated until the minimum value of the percentage difference between the measured and predicted activity rates was reached. In Figure 27 and Figure 28, the similarity between the measured and predicted activity rates can be compared for the 3 sets of batch activity tests.

The lowest θ value was found in the N2 zone, suggesting a lower dependence on temperature, which could lead to the conclusion that both nitrifying and denitrifying bacteria show a similar dependence on temperature, which contradicts the literature, as the nitrification process is considered the most temperature-sensitive (Hwang & Oleszkiewicz, 2007). However, as previously mentioned, these trials were carried out under different operational parameters, and, in addition, only temperatures near 4, 8, and 12 °C were analyzed.

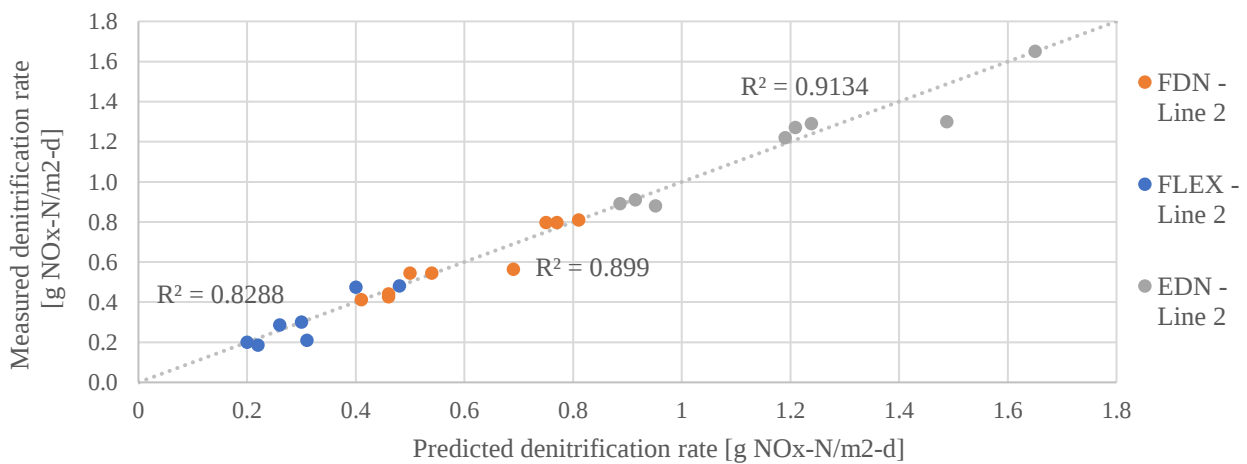


Figure 27 Correlation between measured and predicted values of the denitrification rates in line 2

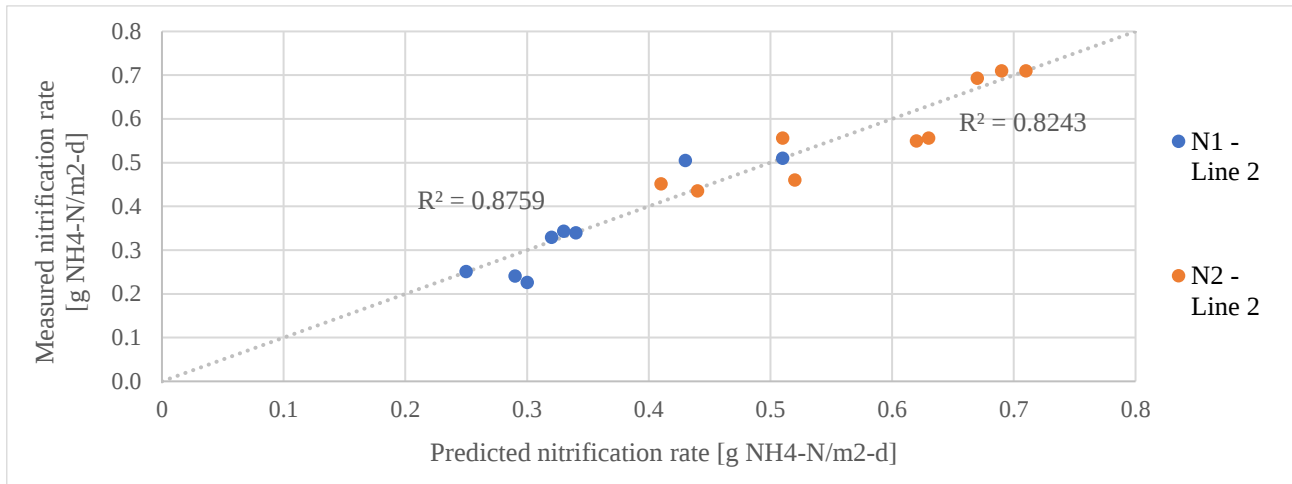


Figure 28 Correlation between measured and predicted values of the nitrification rates in line 2

Due to the disparity between the results obtained during the overall analysis of the 3 sets of batch activity tests, the same method was used to calculate the θ values separately. Each of the 3 sets carried out was evaluated individually in order to minimize the uncertainty generated by the time gap between the sets. The results are shown in Table 8 and show a high variation in the calculation of the Arrhenius temperature dependence coefficient, with values between 1.037 and 1.094 for the denitrification process and 1.040 and 1.083 for the nitrification process. The average values per set were always lower than the θ values calculated previously for the total 9 batch tests.

Table 8 θ values for each set of the batch activity tests and in general for the total 9 tests in line 2

Process	Zone	θ value				
		1st set	2nd set	3rd set	Average per set	Total batch tests
Denitrification	FDN	1.079	1.082	1.066	1.076	1.086
	FLEX	1.037	-	1.073	1.055	1.128
	EDN	1.094	1.057	1.060	1.070	1.083
Nitrification	N1	1.083	1.066	1.042	1.064	1.110
	N2	1.040	1.061	1.073	1.058	1.063

According to the literature and for the nitrification process, Hem et al. (1994) reported a value of θ equal to 1.044 in a temperature range between 8 and 18 °C, while Ahmed et al. 2019 showed a value of 1.049 in a range of 4 – 10 °C. Ødegaard et al. (2023) reported that, in 2019, a θ value of 1.10 was observed at the Nordre Follo WWTP with a temperature range of 7 – 11.5 °C and the Gardemoen WWTP determined a $\theta = 1.015$ with wastewater temperature within 6 and 16 °C. Whereas, the Nedre Romerike WWTP registered a θ value for denitrification and nitrification between 1.07 and 1.11.

4.3.2 Laboratory-scale activity tests

The laboratory-scale test results carried out on line 2, are summarized in Table 9 and Figure 29. It can be concluded that high DO concentrations negatively affected the efficiency of the denitrification process. In addition, it was evident that high DO concentration also favored NO₂-N production in the tests where an external carbon source was used.

Table 9 Results from the laboratory-scale activity tests in line 2

Set	Temperature	DO concentration	Denitrification rate	NO ₂ -N production
	°C	mg/l	g NO _x -N/m ² -d	(After 55 min) mg/l
FDN –	8.2	1.03	0.46	3.5
External	8.3	1.30	0.47	3.4
carbon	8.1	0.08	0.65	1.4
source	9.1	0.04	0.58	1.0
FDN – No	7.9	1.05	0.04	0.0
external	8.7	1.00	0.05	0.0
carbon	8.3	0.25	0.19	0.0
source	9.4	0.11	0.19	0.0
EDN –	8.2	0.98	1.53	3.8
External	9.2	1.00	1.31	4.1
carbon	8.6	0.05	1.62	2.2
source	9.0	0.00	1.76	2.5

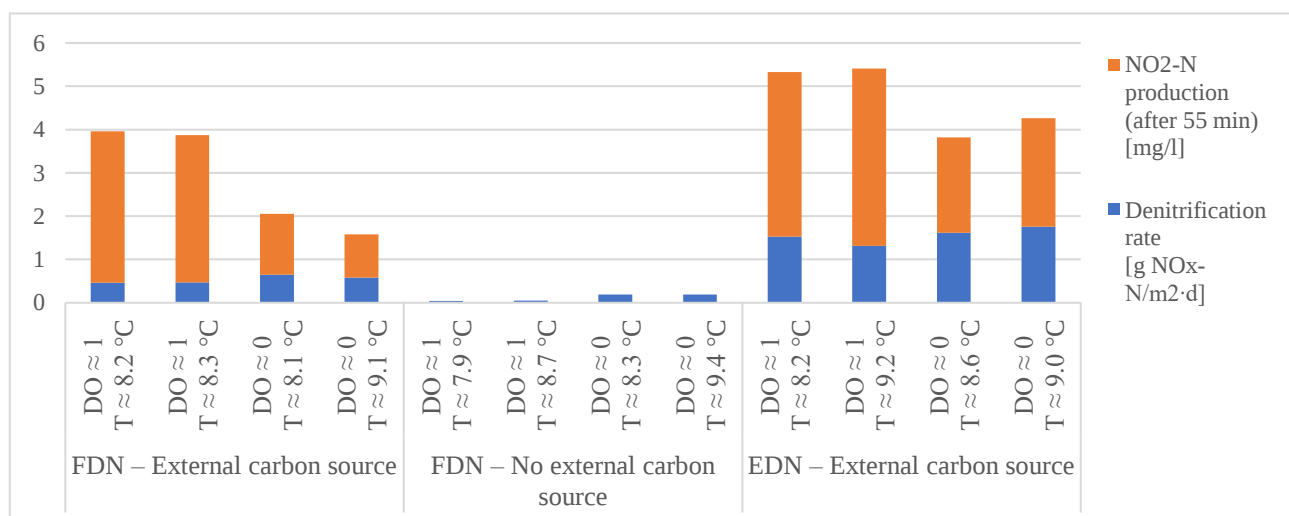


Figure 29 NO₂-N production and denitrification rates from the laboratory-scale activity tests

4.4 Biofilm analysis and additional tests

The biofilm TS were measured in each zone of both lines on two occasions and it is shown in Table 10. In both lines, carriers from the N1 and N2 zones had the highest amount of biofilm TS, while carriers in the FLEX and DE-OX zones showed the lowest values, with a difference of up to 68% in comparison to the biofilm TS in the N2 zone. Additionally, the biofilm TS of line 1 were lower compared to line 2, with a maximum difference of 27% for the N2 zone in the 25/04/23 analysis and except for the FDN zone on the same date. An increase over time in biofilm TS was observed in most zones of both lines when comparing both analyses. FDN and EDN zones had approximately the same TS content in the biofilm, but the biomass in EDN was much more active, showing that biofilm thickness is not an important factor in estimating activity rates.

Table 10 *Biofilm TS in both lines on 14/03/23 and 25/04/23*

Sample	Biofilm total solids [g/m ²]			
	14/03/23		25/04/23	
	Line 1	Line 2	Line 1	Line 2
FDN	8.71	9.70	11.82	9.37
FLEX	7.15	7.35	8.50	8.61
N1	15.15	15.39	14.62	17.67
N2	14.95	16.52	17.53	23.94
DE-OX	6.30	6.89	6.55	7.66
EDN	7.74	9.84	10.02	10.19

The alkalinity of the wastewater in both lines in the different zones was measured on three occasions and the results are shown in Table 11. A reduction in alkalinity is observed which in most cases is sufficient to carry out the nitrification process, however, the measurement on 21/02/23 in the N2 zone of line 1, was below the recommended value of 75 mg/l CaCO₃ to avoid alkalinity limitation (ATV - Handbuch, 1997).

Furthermore, the concentration of PO₄-P was measured on 31/01/23 in the effluent of each line. With a value of 1 mg/l in both cases (see Appendix G), the results suggest that the wastewater in the pilot plant had the necessary concentrations to accomplish the nitrogen removal processes, based on the Nordo Follo WWTP experiment, which recommends maintaining a residual PO₄-P concentration of 0.3 mg/l (Ødegaard et al., 2023).

Table 11 Alkalinity results in both lines on three different occasions

Sample	Alkalinity [mg/l CaCO ₃]					
	21/02/23		11/04/23		10/05/23	
	Line 1	Line 2	Line 1	Line 2	Line 1	Line 2
Fillan WWTP ¹	173	173	127	127	154	154
Inflow to the pilot	192	192	-	-	-	-
FDN	-	-	112	121	132	127
FLEX	153	149	120	116	129	120
N1	-	-	97	117	120	122
N2	73	79	83	101	89	103
EDN	119	104	99	112	109	106

¹ After pre-sedimentation.

4.5 Limitations and uncertainties

Among the limitations of this master's thesis is the lack of consistency in the information collected by the online meters, which was evidenced by continuously comparing the results of the chemical analysis of the wastewater samples and the readings of the online meters in both lines. As a result, the pilot plant performance was influenced by limiting the DO supply in the aerated zones (see Table 3), and consequently, the nitrogen removal. Nonetheless, in order to increase the reliability of the information, the online meters were constantly cleaned and calibrated, and one of them was replaced.

Several technical problems occurred in the pilot plant during the study period, which stopped the heating and cooling systems, the agitators, the inflow to the pilot plant, and the recirculation flow, among others. Therefore, the biological processes along each line were altered and should be considered analytical errors and a source of uncertainty in the results presented in this analysis. Additionally, it was complex to keep a constant DO concentration of 5 mg/l during the nitrification batch activity tests.

To calculate the loss of surface area due to the broken carriers, the level of the carriers within each of the dewatered zones was measured. Although many carriers were broken, others simply changed their shape reducing the volume they occupied, so it was difficult to estimate their influence on the calculation of activity rates.

4.6 Recommendations

In terms of pilot scale, further studies are recommended in the following topics:

- Optimal external carbon source dose for denitrification, not only for economic aspects but also to reduce NO₂-N production.
- Optimal DO supply in the aerated zones, not only for economic aspects but also to take advantage of the deoxygenation zone (DE-OX) and to reduce the DO concentrations sent to the zones where denitrification occurs.
- Nitrification and denitrification batch activity tests to calculate values of the θ with tightly controlled operational parameters, with a smaller magnitude of the temperature shift (from 4 °C to 5 °C, to 6 °C, etc.) and also during cold shock, to better understand the adaption behaviors of bacteria.

In terms of the operation and construction of full-scale WWTPs with low-temperature conditions it is recommended:

- Continuous cleaning and calibration of the online meters.
- Combination of pre- and post-denitrification treatment processes with the possibility of adapting the operation of the tanks (aerated or non-aerated), according to the performance of the process and the temperature variations throughout the year.
- Intermittent addition of an external carbon source in the pre-denitrification zone during the snow melting period, when the concentrations of biodegradable organic matter in the influent are very low.
- The inclusion of an aerated tank at the end of the treatment line to remove the residual COD concentration and to prevent the discharge to receiving water bodies.

5 Conclusions

It is possible to achieve high nitrogen removal levels using the MBBR treatment process during the winter season in Sundsvall, however, during the snow-melting period, the efficiency of the treatment is limited by the denitrification process. During the snow melting period, the N-tot removal efficiency decreased below 50%, however, the concentration in the effluent was in most cases lower than 10 mg/l. Removal efficiency in the nitrification process of more than 80% even during snow-melting and it can be controlled by the oxygen supply.

Reliable online meters and automation systems are crucial aspects during the implementation of MBBR in WWTPs to optimize oxygen supply, nitrate recirculation, and the external carbon source dose. As well as the implementation of flexible reactors that include an aeration system and mechanical mixing at the same time so that they can be adjusted according to the process performance.

The variability in Arrhenius temperature dependence coefficients is expected, as they rely on various factors including the temperature range examined, the magnitude of temperature shifts, the rate at which temperature changes occur, the precision of temperature and DO supply control, and the operational parameters, among others. Nonetheless, the Arrhenius temperature dependence coefficients in this study were generally in the range of what could be expected from the literature, but there was no clear difference between the temperature dependence of nitrification and denitrification processes.

Although line 1 performed 4 °C warmer and with a 35% higher load than line 2, the results of the continuous operation of the pilot plant indicate that both lines had similar nitrogen removal efficiencies. Finally, except for the direct effect of temperature, the major influence on process performance is due to the DO concentration in the denitrification zones, which severely influenced the pre-denitrification process.

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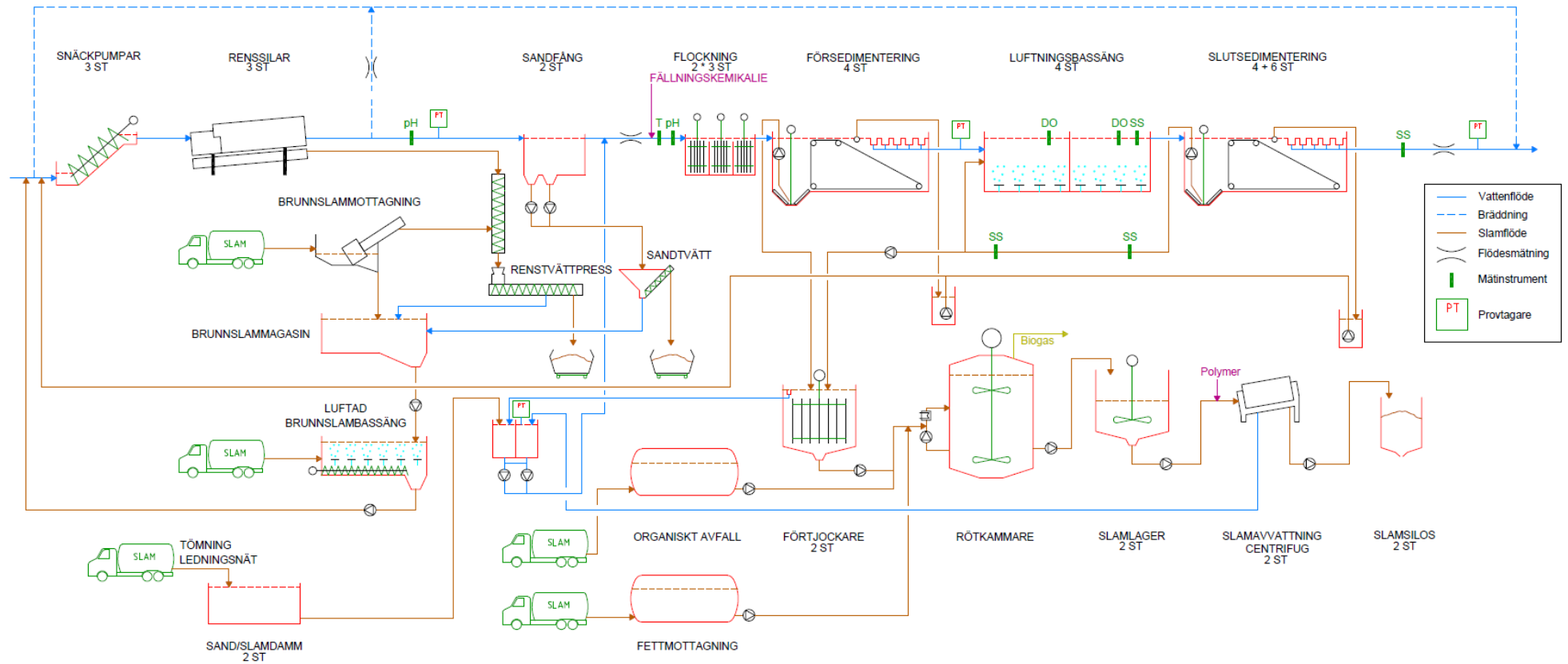
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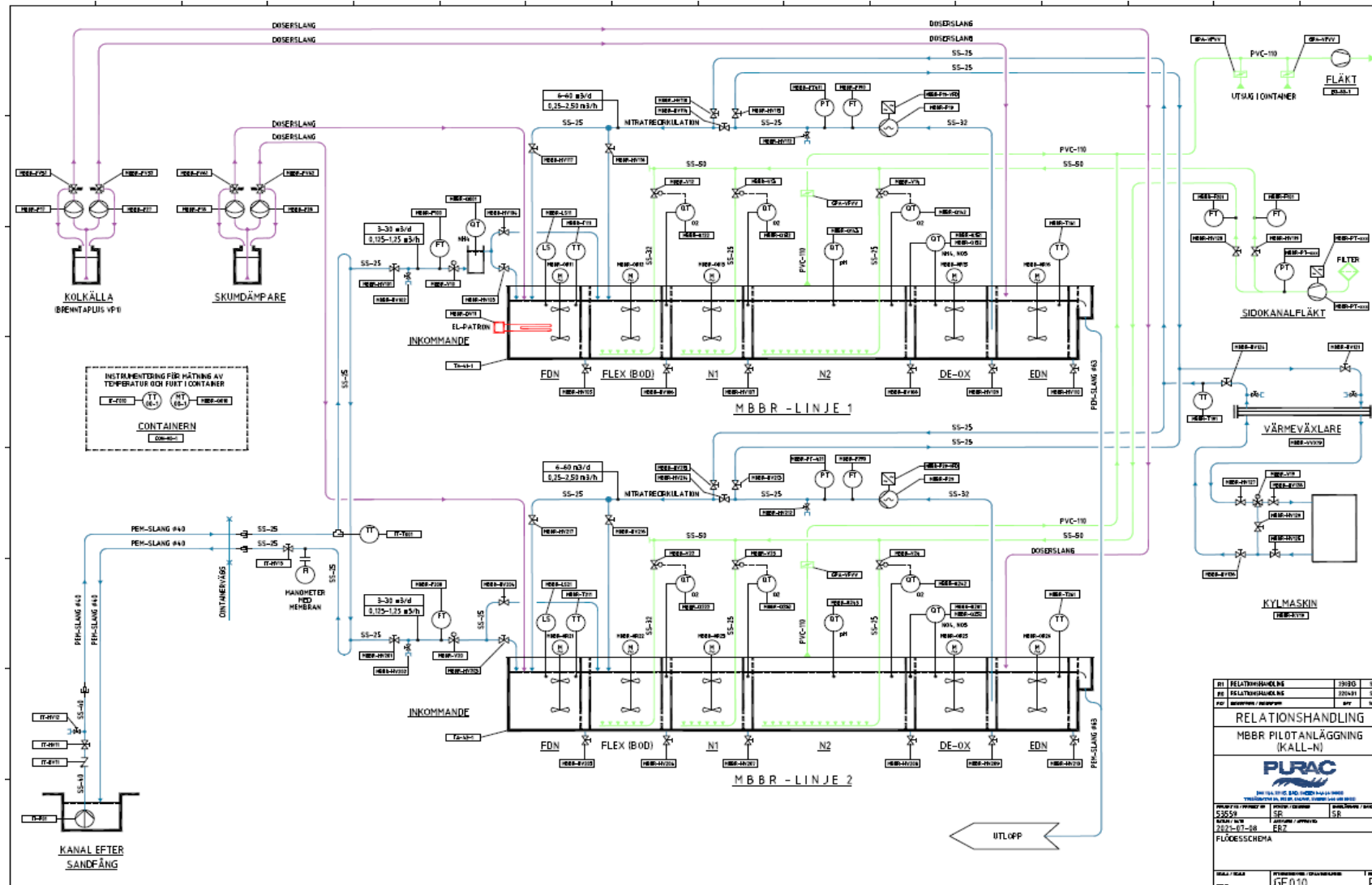
Appendix A

Process scheme in Fillan WWTP from MittSverige Vatten och Avfall files.



Appendix B

Pilot plant flow scheme from PURAC, (2021a).



Appendix C

Documentation of the decisions made regarding the performance of the pilot plant during the study period.

Date	Inflow from Fillan WWTP	Flow and load	Recirculation flow	Temperature	DO per zone				Carbon dose in EDN zone	Agitators frequency
Starting conditions 16/01/23	After pre-sedimentation without chemical precipitation	L1: 0.135% of the incoming to Fillan WWTP. L1: 0.5 to 1.0 m ³ /h. L2: 0.100% of the incoming to Fillan WWTP. L2: 0.4 to 0.7 m ³ /h.	100% of the inflow to the pilot.	L1: 4 °C more than the incoming to the pilot. L2: same temperature as the incoming to pilot.	NH ₄ -N after N2 [mg/l]	FLEX	N1	N2	9 g COD / g NO ₃ -N	20 hz
					< 1.0	-	2.0	4.0		
					1.0 - 2.0	-	3.0	5.0		
					2.0 - 4.0	-	5.0	5.0		
					> 4.0	2.0	5.0	5.0		
19/01/23										30 hz
01/02/23					NH ₄ -N after N2 [mg/l]	FLEX	N1	N2		
					< 2.0	-	2.0	4.0		
					2.0 - 3.0	-	3.0	5.0		
					3.0 - 5.0	-	5.0	5.0		
					> 5.0	2.0	5.0	5.0		
17/03/23				L1: 3 °C more than the incoming to the pilot.						

				L2: 1 °C less than the incoming to the pilot.						
29/03/23				L1: set at the constant 9 °C. L2: set at the constant 5 °C.						
12/04/23		L1: 0.155% of the incoming to Fillan WWTP. L2: 0.120% of the incoming to Fillan WWTP.								
Ending conditions 03/05/23	After pre-sedimentation without chemical precipitation	L1: 0.155% of the incoming to Fillan WWTP. L2: 0.120% of the incoming to Fillan WWTP.	100% of the inflow to the pilot.	L1: set at the constant 9 °C. L2: set at the constant 5 °C	NH ₄ -N after N2 [mg/l]	FLEX	N1	N2	9 g COD / g NO3-N	30 hz
					< 2.0	-	2.0	4.0		
					2.0 - 3.0	-	3.0	5.0		
					3.0 - 5.0	-	5.0	5.0		
					> 5.0	2.0	5.0	5.0		

Appendix D

The main equations used during the evaluation of the continuous operation of the pilot plant are listed below.

The nitrogen removal efficiency including all the zones:

$$N_{rem,eff} [\%] = \frac{Tot - N_{inflow WWTP} - Tot - N_{effluent}}{Tot - N_{inflow WWTP}} * 100 \quad (8)$$

The nitrogen removal efficiency excluding post-denitrification:

$$N_{rem,eff excl EDN} [\%] = \frac{Tot - N_{inflow WWTP} - (NH_4 - N_{after N2} + NO_2 - N_{after N2} + NO_3 - N_{after N2})}{Tot - N_{inflow WWTP}} * 100 \quad (9)$$

The NO₂-N concentration was measured after the N₂ zone on several occasions and the results were always equal to or less than 0.160 mg/l, then it was considered to be neglected.

The nitrification efficiency including all the zones:

$$Nitrification_{eff} [\%] = \frac{Tot - N_{inflow WWTP} - NH_4 - N_{effluent}}{Tot - N_{inflow WWTP}} * 100 \quad (10)$$

The denitrification efficiency including all the zones:

$$Denitrification_{eff} [\%] = \frac{Tot - N_{inflow WWTP} - NO_3 - N_{effluent}}{Tot - N_{inflow WWTP}} * 100 \quad (11)$$

The incoming NH₄-N diluted with recirculation flow:

$$NH_4 - N_{inflow diluted with recirc} \left[\frac{mg}{l} \right] = \frac{Tot - N_{inflow WWTP} * Q_{inflow WWTP} + (NH_4 - N_{after N2} - 1) * Q_{recirculation}}{Q_{inflow WWTP} + Q_{recirculation}} \quad (12)$$

In the previous equation, 1 mg/l of NH₄-N is subtracted from the recirculation flow as a result of the nitrification process that occurs in the DE-OX zone. Since there are no online meters in the DE-OX zone and the specific values are unknown, it is estimated based on the difference in NH₄-N concentration after the N₂ zone and the effluent.

The incoming NO₃-N diluted with recirculation flow:

$$NO_3 - N_{inflow \text{ diluted with recirc}} \left[\frac{mg}{l} \right] = \frac{NO_3 - N_{after N2} * Q_{recirculation}}{Q_{inflow WWTP} + Q_{recirculation}} \quad (13)$$

In the previous equation, it was assumed a 0 concentration of nitrate in the influent.

The carrying area of the carriers:

Carrying area [m²] =

$$Wet \ volume \ of \ reactor \ [m^3] * Surface \ area \ \left[\frac{m^2}{m^3} \right] * Proportion \ of \ carriers \ [%] \quad (14)$$

NH₄-N removal rate after the FLEX zone per day:

$$NH_4 - N_{rem,rate \ after \ FLEX} \left[\frac{gN}{m^2 * d} \right] = \frac{(NH_4 - N_{inflow \text{ diluted with recirc}} - NH_4 - N_{after \ FLEX}) * (Q_{inflow WWTP} + Q_{recirculation}) * 24}{Carrying \ area_{FLEX}} \quad (15)$$

NH₄-N removal rate after the N1 zone per day:

$$NH_4 - N_{rem,rate \ after \ N1} \left[\frac{gN}{m^2 * d} \right] = \frac{(NH_4 - N_{after \ FLEX} - NH_4 - N_{after \ N1}) * (Q_{inflow WWTP} + Q_{recirculation}) * 24}{Carrying \ area_{N1}} \quad (16)$$

NH₄-N removal rate after the N2 zone per day:

$$NH_4 - N_{rem,rate \ after \ N2} \left[\frac{gN}{m^2 * d} \right] = \frac{(NH_4 - N_{after \ N1} - NH_4 - N_{after \ N2}) * (Q_{inflow WWTP} + Q_{recirculation}) * 24}{Carrying \ area_{N2}} \quad (17)$$

NO₃-N removal rate after the FDN zone per day:

$$NO_3 - N_{rem,rate \ after \ FDN} \left[\frac{gN}{m^2 * d} \right] = \frac{(NO_3 - N_{inflow \text{ diluted with recirc}} - NO_3 - N_{after \ FDN}) * (Q_{inflow WWTP} + Q_{recirculation}) * 24}{Carrying \ area_{FDN}} \quad (18)$$

NO₃-N removal rate after the EDN zone per day:

$$NO_3 - N_{rem,rate \ after \ EDN} \left[\frac{gN}{m^2 * d} \right] =$$

$$\frac{(\text{NO}_3 - \text{N}_{after \text{ N}_2} - \text{NO}_3 - \text{N}_{after \text{ EDN}}) * Q_{inflow \text{ WWTP}} * 24}{\text{Carrying area}_{\text{EDN}}} \quad (19)$$

COD dose at the EDN zone:

$$\text{COD dose}_{at \text{ EDN}} \left[\frac{mg}{l} \right] = \frac{\text{Carbon source supply}}{Q_{inflow \text{ WWTP}}} * \text{COD content of Brenntapulus VP1} \quad (20)$$

COD consumption at the EDN zone

$$\text{COD removal}_{at \text{ EDN}} \left[\frac{mg}{l} \right] = \text{COD dose}_{at \text{ EDN}} - (\text{COD}_{after \text{ EDN}} - \text{COD}_{after \text{ N}_2}) \quad (21)$$

Appendix E

The following tables summarize the conditions established for each activity test (including batch and laboratory-scale tests) performed.

Table E1 *Batch tests performed for the denitrification zones during the study period*

Batch test – Date	Zone – Line	Average temperature	Average DO concentration	NaNO ₃ dose	Brenntapplus VP1 dose
		[°C]	[mg/l]	[g]	[ml]
1 09/02/23	FDN – L1	11.6	0.14	56	75
	FLEX – L1	11.5	0.15	62	75
	EFD – L1	12.1	0.12	81	75
2 09/02/23	FDN – L2	7.5	0.15	66	75
	FLEX – L2	7.5	0.11	69	75
	EFD – L2	8.5	0.15	81	75
3 22/02/23	FDN – L2	4.9	0.17	66	75
	FLEX – L2	4.9	0.38	69	75
	EFD – L2	5.5	0.19	85	75
4 24/02/23	FDN – L2	12.3	0.07	69	75
	FLEX – L2	11.7	0.06	72	75
	EFD – L2	12.4	0.09	85	75
5 17/03/23	FDN – L1	10.2	0.15	71	75
	FLEX – L1	10.8	0.34	75	75
	EFD – L1	11.3	0.13	85	75
6 22/03/23	FDN – L2	7.5	0.10	62	75
	FLEX – L2	7.7	0.04	66	75
	EFD – L2	8.8	0.08	85	75
7 24/03/23	FDN – L2	12.1	0.07	71	75
	FLEX – L2	11.8	0.06	75	75
	EFD – L2	12.3	0.10	85	75
8 29/03/23	FDN – L2	4.1	0.15	65	75
	FLEX – L2	3.9	0.11	69	75
	EFD – L2	4.6	0.08	85	75
9	FDN – L1	9.1	0.12	73	75

21/04/23	FLEX – L1	9.1	0.36	77	75
	EFD – L1	9.4	0.05	85	75
10 26/04/23	FDN – L2	7.9	0.15	76	75
	FLEX – L2	7.9	0.13	80	75
	EFD – L2	8.3	0.09	85	75
11 28/04/23	FDN – L2	4.5	0.11	73	75
	FLEX – L2	4.5	0.09	77	75
	EFD – L2	5.0	0.09	85	75
12 03/05/23	FDN – L2	12.1	0.16	71	75
	FLEX – L2	11.9	0.06	75	75
	EFD – L2	11.1	0.07	85	75

Table E2 Batch tests performed for the nitrification zones during the study period

Batch test - Date	Zone - Line	Average temperature	Average DO concentration	NH ₄ HCO ₃ dose	NaHCO ₃ dose
		[°C]	[mg/l]	[g]	[g]
1 09/02/23	N1 – L1	11.7	5.85	23	12
	N2 – L1	11.7	5.32	151	80
2 09/02/23	N1 – L2	7.7	4.18	23	12
	N2 – L2	7.9	4.78	142	76
3 22/02/23	N1 – L2	5.1	5.43	23	12
	N2 – L2	5.0	5.64	149	79
4 24/02/23	N1 – L2	11.8	6.06	23	12
	N2 – L2	12.1	4.51	150	80
5 17/03/23	N1 – L1	11.4	5.75	15	8
	N2 – L1	11.1	6.03	154	82
6 22/03/23	N1 – L2	8.0	5.63	15	8
	N2 – L2	8.1	4.45	130	69
7 24/03/23	N1 – L2	11.9	6.56	59	31
	N2 – L2	12.1	4.19	150	80
8 29/03/23	N1 – L2	4.1	5.47	68	36
	N2 – L2	4.1	5.20	151	80

9	N1 – L1	9.2	7.40	60	32
21/04/23	N2 – L1	9.2	5.46	154	82
10	N1 – L2	8.1	5.14	58	31
26/04/23	N2 – L2	8.1	5.18	144	76
11	N1 – L2	4.7	4.90	55	29
28/04/23	N2 – L2	4.7	5.13	150	80
12	N1 – L2	11.9	5.35	53	28
03/05/23	N2 – L2	11.7	5.00	149	79

Table E3 *Laboratory-scale tests performed during the study period*

Date	Wastewater and carriers from the zone	Dose [ml]		Average DO concentration [mg/l]		Average temperature [°C]	
		NaNO ₃	COD	Beaker 1	Beaker 2	Beaker 1	Beaker 2
01/03/23	FDN – Line 2	3.2	0.42	1.2	0.0	8.3	9.1
02/03/23	FDN – Line 2	3.0	0	1.0	0.1	8.8	9.4
02/03/23	EDN – Line 2	3.8	0.42	1.0	0.0	9.2	9.0
06/04/23	FDN – Line 2	3.8	0.42	1.03	0.08	8.2	8.1
06/04/23	FDN – Line 2	3.8	0	1.05	0.25	7.9	8.3
05/05/23	EDN – Line 2	3.0	0.42	0.98	0.05	8.2	8.6

The following equations were used to determine the rate and capacity of the nitrification and denitrification processes during the activity tests (batch and laboratory-scale tests). The term "Free water volume" refers to the actual volume of water inside the reactor after removing the volume occupied by the carriers.

The nitrification rate is determined as:

$$Nitrification_{rate} \left[\frac{gN}{m^2 * d} \right] = \frac{\left(\frac{\Delta NH_4 - N}{\Delta Time} \right) * Free\ water\ volume}{Carrying\ area} * 24 \quad (22)$$

The nitrification capacity is determined as:

$$Nitrification_{capacity} \left[\frac{gN}{d} \right] = Nitrification_{rate} * Carrying\ area \quad (23)$$

The denitrification rate is determined as:

$$Denitrification_{rate} \left[\frac{gN}{m^2 * d} \right] = \frac{\left(\frac{\Delta NO_3 - N}{\Delta Time} \right) * Free\ water\ volume}{Carrying\ area} * 24 \quad (24)$$

The denitrification equivalent rate is determined as:

$$Denitrification_{equivalent\ rate} \left[\frac{gN}{m^2 * d} \right] = \frac{\left(\frac{\Delta NO_3 - N - 0.6 * (\Delta NO_2 - N)}{\Delta Time} \right) * Free\ water\ volume}{Carrying\ area} * 24 \quad (25)$$

The equivalent denitrification rate was calculated when high NO₂-N production was observed during the activity tests, specifically in the EDN zones. For this, 60% of the NO₂-N production was subtracted from the NO₃-N reduction during the denitrification process, as recommended by van Loosdrecht et al. (2016).

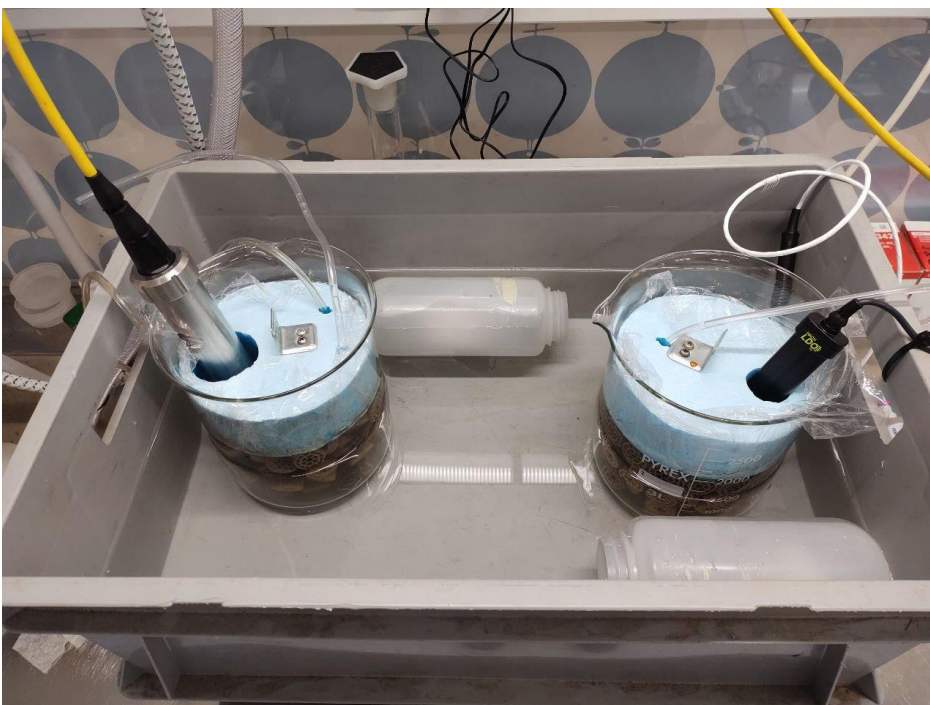
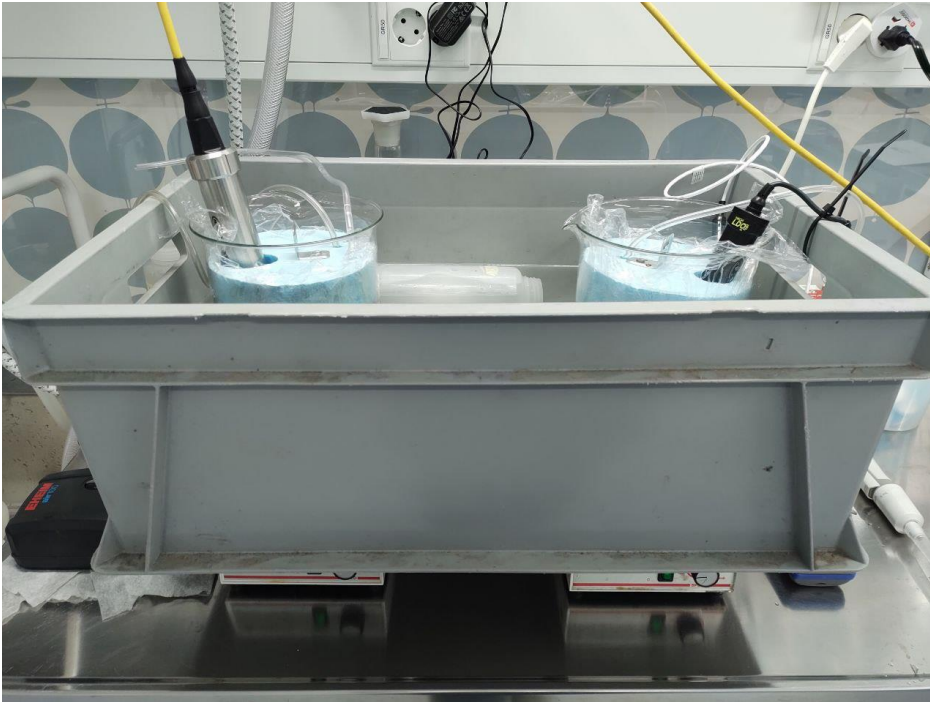
The denitrification capacity is determined as:

$$Denitrification_{capacity} \left[\frac{gN}{d} \right] = Denitrification_{rate} * Carrying\ area \quad (26)$$

Appendix F

Laboratory-scale tests setup

This setup was repeated in each of the laboratory-scale tests.



Appendix G

Phosphate tests

Date: 31/01/23

Sample	PO ₄ -P [mg/l]
Fillan WWTP ¹	1,2
Line 1 effluent - filtrated	1,0
Line 2 effluent - filtrated	1,0

¹After pre-sedimentation.



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