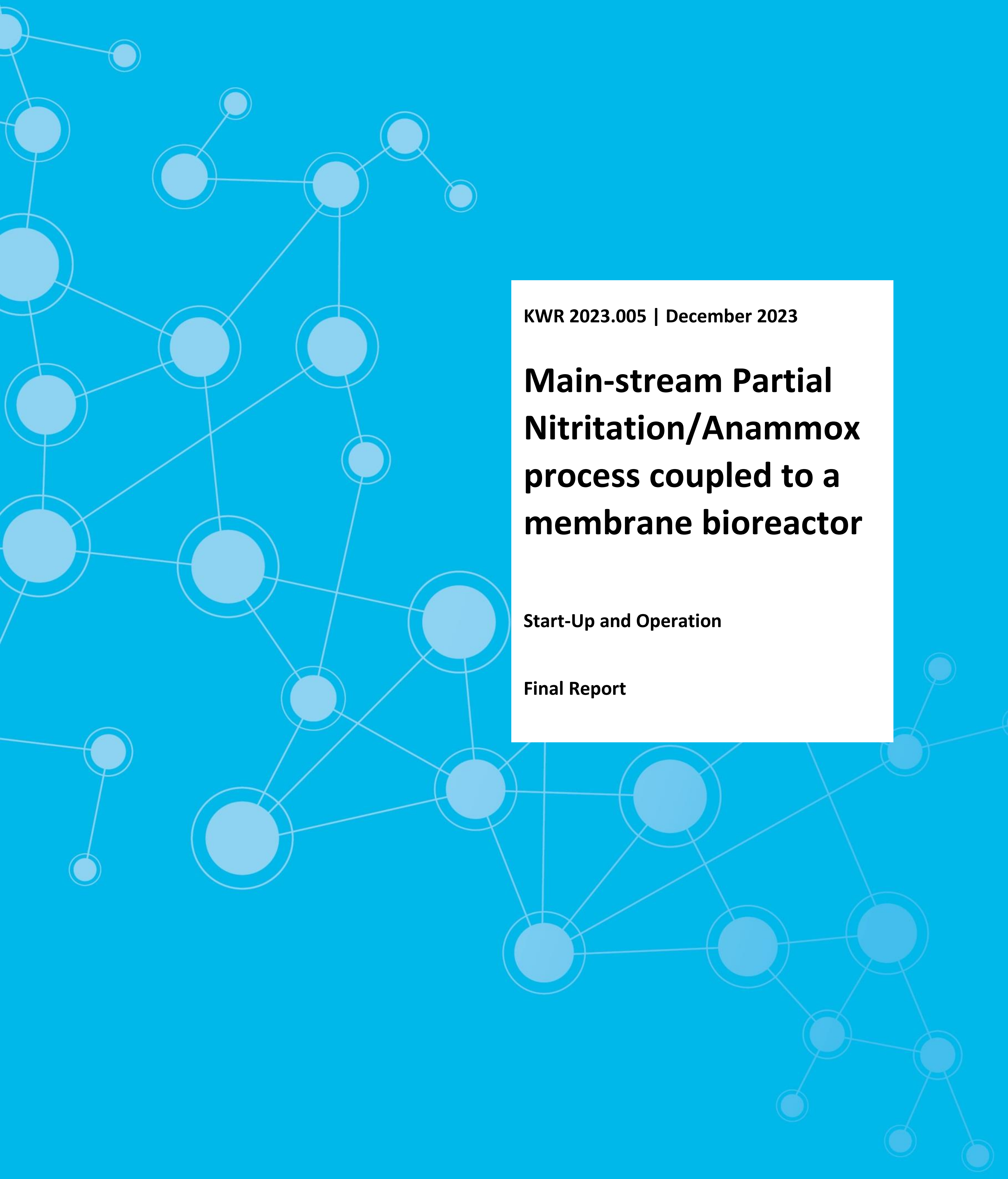

KWR 2023.005 | December 2023

Main-stream Partial Nitritation/Anammox process coupled to a membrane bioreactor

Start-Up and Operation

Final Report

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Report

Main-stream Partial Nitritation/Anammox process coupled to a membrane bioreactor
Start-Up and Operation

KWR 2023.005 | December 2023

Project number
402147

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Client
TKI Water Technology

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Keywords

Start-up, partial nitritation, anammox, pilot plant, low temperature

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Summary

In this project, a two-stage (Partial Nitritation-Anammox; PN/A) deammonification pilot plant was operated with municipal wastewater to investigate the long-term stability of the PN/A process at mainstream conditions and the impact of low temperature on the anammox process. The pilot plant with suspended biomass was operated at the WWTP in Simmern, Germany, for 214 days with the start-up in winter, and 80 days with a start-up in autumn. The piilot plant layout consisted of an influent finescreen, followed by a PN reactor, Anammox reactor, membrane bioreactor (MBR) (with ultrafiltration (UF) membranes) and a sieve for the waste sludge to retrieve anammox bacteria and return it to the system. The dissolved oxygen (DO) concentration was used to control the aeration in PN reactor.

During the experimental period, the pilot plant showed a stable COD removal efficiency between 90 and 95%. The nitrogen removal of the pilot plant was unstable due to issues with influent wastewater and in air supply caused by fine sieve, influent pump and blower failures.

In phase 1 (0-97 days), the abnormal performance of the original blower resulted in a low DO concentration of 0.1 mg/L in the PN reactor. The sludge recirculation was adjusted several times to increase the DO concentration in the PN reactor to generate nitrite, which was unsuccessful. The nitrogen conversion changed with the adopted adjustments, leading to varying nitrogen removal. Denitrification was identified in the anammox reactor in this phase.

In phase 2 (98-155 days), a blower with fixed capacity supplied excess air to the PN reactor, which led to a high DO concentration of over 7 mg/L. The influent load was increased to reduce the DO concentration in the PN reactor to produce nitrite, which failed due to rain weather flow, which caused strong dilution of the influent concentrations. It was found that the sludge recirculation from the MBR and the effluent from the PN reactor, both containing dissolved oxygen, had a combined impact on the DO concentration in the anammox reactor. While the PN reactor was supplied with excess airflow, the DO concentration in the anammox reactor increased substantially, resulting in the loss of an anoxic environment in the anammox reactor. The overall nitrogen removal could not be determined due to the absent TN measurement. During phases 1 and 2, the sludge recirculation and the feed had to be adjusted accordingly to adapt to the unfavourable oxygen supply. Based on the adjustments and associated DO variations, it became evident that the DO concentration in the reactors was influenced by multiple factors: e.g. airflow rate, the sludge concentration in the reactor, oxygen diffusion via the sludge recirculation, varying wastewater temperature, and organic load in the feed (mostly influenced the PN reactor). Despite the multiple influencing factors of the DO concentration, failed attempts to produce nitrite demonstrated that suitable airflow coupled with another control strategy was a prerequisite for establishing a stable partial nitritation.

In phase 3 (156-214), the air supply was automatically controlled by the detected DO concentration in the PN reactor. Although no nitrite production was detected in the PN reactor and anammox reactor, simultaneous denitrification and deammonification in the anammox reactor were determined by the mass balance calculation. The low NH_4-N removal rate in the anammox reactor indicated a low Anammox activity, which was attributed to the limited nitrite production, low wastewater temperature (10,72±1,11 °C) and a small fraction of anammox bacteria in the system (granule concentration: sludge concentration in the MBR=0.024 g MLSS/L: 11.20 g MLSS/L). The absent nitrite production in the PN reactor could be attributed to the combined effect of high C/N-ratio (between 5-30 gCODtotal/gN) and NOB overgrowth in the system. Therefore, nitrite production was considered the limiting step in this two-stage deammonification process. To further improve the overall performance of the system, sludge was extracted frequently to wash out the NOB of the system and reduce the SRT.

In phase 4 (0-80 days), after reinoculation, reduction of SRT, and DO intermittent aeration implementation in the PN reactor, still nitrite build-up was not observed, nor was evidence of significant anammox activity via NO_2 with complete deammonification/denitrification. Anammox activity might have followed another hypothesized pathway via NO instead of NO_2, as reported by Fritsch 2022.

During the whole experimental period, the pilot plant experienced several operational issues, including blower failures, the clogging of the fine sieve, pump failures and setup issues. Due to these incidents, the plant operation was frequently interrupted and severely limited by its effects. Therefore, the pilot plant did not reach a relatively stable long-term operation. The operation of this plant proved that not only aeration control is enough to achieve a deammonification process. The intercepted anammox granules demonstrated and proved the feasibility of the fine sieve to selectively retain the granular biomass. The application of the MBR ensured that the effluent had a stable COD concentration and suspended solids free effluent. An attempt to investigate the impact of low temperature on the Anammox mainstream process was challenging due to diverse troubleshooting during the start-up and operation. The stability of the pilot plant was very limited due to operational restrictions and challenges.
A COD pre-treatment might still be necessary to reduce the influent COD and the COD:N ratio. The main challenge of the pilot plant remains the establishment of nitritation under mainstream conditions. The experienced troubleshooting and adopted measures were documented. Optimization possibilities were stated from the perspectives of operation, performance and evaluation. Optimization suggestions, especially on control strategies, could be used to improve the operation and the performance of the process and pilot plant in the future.

Content

1 Introduction

Nitrogen in municipal wastewater, which mainly originates from urine, is currently removed by biological process (Hoekstra 2017). Nitrification and denitrification (N/DN) as the traditional route to remove nitrogen have been applied as core stages in biological treatment trains in the past decades (Lackner et al. 2014). However, the oxidation of ammonium to nitrate (autotrophic nitrification), which requires aeration and the subsequent reduction of nitrate to nitrogen gas (heterotrophic denitrification), which requires additional carbon source, are associated with high costs (Lackner et al. 2014). The anaerobic ammonium oxidation (anammox) discovered by (Mulder et al. 1995) is seen as a novel nitrogen removal approach. Compared to the traditional N/DN process the partial nitrification and anammox (PN/A) (also termed deammonification) have three advantages: strong reduction of the oxygen demand, no additional carbon demand and significant reduction of excess sludge (Cao et al. 2017). Due to these unique advantages, the deammonification process is used to treat high-strength ammonium wastewater with low C/N ratios at elevated temperatures (sidestream treatment of sludge digestion centrate), and its application on large scales has been widely reported (Lackner et al. 2014). Since the early 2010s, deammonification has been intensively investigated for its application in mainstream conditions because of its energetic and associated cost advantages (Cao et al. 2017). The application of deammonification process in a mainstream is, however, still challenging for the following reasons: high influent C/N ratio, low wastewater temperature with seasonal variation and associated imbalance of the key bacteria groups due to slow growth of Anammox (Cao et al. 2017).

The aim of this project is to investigate a two-stage deammonification process (PN/Anammox) in the mainstream in combination with a membrane bioreactor (MBR) and a screen-assisted recirculation of the anammox biomass of a municipal wastewater treatment plant (WWTP). Main attention is given to anammox biomass retention in the system, temperature effect on anammox bacteria and long-term stability of the deammonification process. The research questions are stated as follows:

1. What is the achievable nitrogen conversion at low temperatures by a PN/A-MBR pilot plant?
2. What is the effectiveness of separating the anammox biomass from the waste sludge to keep it in the process?
3. What are the stability and challenges expected in the operation of the PN/A-MBR pilot plant with anammox granules sieving from surplus sludge?

1.1 Theoretical background

1.1.1 Deammonification (Partial nitrification/Anammox Process)

Since the 1990s, the anaerobic ammonium oxidation process (anammox) has been considered a novel way to remove nitrogen from wastewater (Mulder et al. 1995, Strous et al. 1997). Its performance is comparable with the conventional nitrification and denitrification system in terms of nitrogen removal rate, the nitrogen volumetric loading rate and energy requirement (Graaf et al. 1995, Mulder et al. 1995, Strous et al. 1997). In the anammox process, ammonium can be converted to nitrogen gas under anoxic conditions in the presence of nitrite, which further serves as an electron acceptor (Strous et al. 1998, van de Graaf et al. 1996). Along with the biomass formation from carbon dioxide, small amounts of nitrate are formed during the reaction (Third et al. 2001).

$$1\,NH_4{}^+ + 1.3\,NO_2{}^- \rightarrow 1.02N_2 + 0.26\,NO_3{}^- + 2H_2O$$

Since the majority of nitrogen in the wastewater exists in the form of ammonium, the anammox process is usually combined with preceding partial nitrification.

$$1\ NH_4{}^{+} + 1.5\ O_2 \rightarrow NO_2{}^{-} + 2H^{+} + H_2O$$

In the partial nitrification-anammox (PN/A) process, a portion of the ammonium is first oxidized to nitrite, which further reacts with the remaining ammonium to form dinitrogen gas (Sliekers et al. 2002, Strous et al. 1997, van de Graaf et al. 1996)

$$1\ NH_3 + 0.85\ O_2 \rightarrow 0.11\ NO_3{}^{-} + 0.44N_2 + 0.14H^{+} + 1.43H_2O$$

The anammox stoichiometry was re-iterated by Lotti et al. (2014a) as:

$$1.00NH4^{+} + 1.146NO_2{}^{-} + 0.071HCO_3{}^{-} + 0.057H^{+} \rightarrow 0.986N_2 + 0.161NO_3{}^{-} + 0.071\mathrm{CH_{1.74}O_{0.31}N_{0.20}} + 2.002\mathrm{H_2O}$$

1.1.2 Advantages of the PN/A process

Compared with conventional combined nitrification and denitrification reactions, the novel PN/A process brings its individual cost advantages. Firstly, oxygen is no longer required for the anammox reaction (Graaf et al. 1995). As only part of the ammonium needs to be oxidized to nitrite instead of nitrate in the nitrification, the PN/A process reduces around 50% to 63% of the oxygen demand, resulting in a significant reduction in aeration costs (Strous et al., 1997; Third et al., 2001; Cao et al., 2017). Secondly, anammox biomass is an autotrophic population which takes carbonate as the carbon source (van de Graaf et al., 1996). Thus, an additional chemical dosing to provide COD, which is sometimes required in conventional wastewater treatment, including Denitrification, is unnecessary for the PN/A process. An alkalinity addition is required as well in the treatment of ammonium-rich wastewater e.g. sludge digestate by nitrification (Fux and Siegrist 2004). Therefore, the cost of the chemical addition and the cost for the handling of the increased sludge amount caused by the chemical dosing do not occur in a PN/A process. As a result of the low growth rate of anammox populations, the sludge production of the anammox process is relatively low, which further reduces the operational costs (Strous et al., 1997). The combined advantages could lead to a total cost reduction of up to 40% (Fux & Siegrist, 2004).

In addition, anaerobic ammonium oxidation has advantages from an ecological point of view. No undesirable gaseous intermediates that promote ozone depletion are released during anaerobic ammonium oxidation, whereas heterotrophs produce carbon dioxide (CO_2) from organic carbon and significant nitrous oxide (N_2O) at elevated nitrite concentration during denitrification (Fux & Siegrist, 2004). Anammox bacteria are not expected to emit N_2O since they do not reduce nitrate through denitrification over N_2O (Kampschreur et al. 2009, Kartal et al. 2010). However, N_2O can still be measured during PN/A process, which is attributed to the presence of nitrifying and denitrifying bacteria in the system (Lackner and Welker 2019). Compared to traditional nitrogen removal, PN/A process might produce less N_2O (Kuypers et al. 2018) with proper a proper control strategy, but this is still under discussion.

1.1.3 Balance of key microorganisms

The partial nitrification and anammox process relies on the balance between three bacteria groups: aerobic ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anaerobic ammonia-oxidizing bacteria (AnAOB). Part of the influent ammonium is oxidized to nitrite under the function of AOB, and the produced nitrite is further provided with AnAOB. A stable nitrite production by AOB organisms is valuable to achieving a successful PN/A process. Therefore, inhibition of the AOB should be prevented in the process. In the meanwhile, NOB activity must be suppressed to avoid further oxidation of nitrite to nitrate.

Inhibition of AOB

Joss et al. (2011) proved the reduced oxygen consumption caused by the inhibition of AOB, which may favour the

NOB growth with the surplus oxygen under stable air supply. In addition, the organic matter in the wastewater plays an important role for the AOB groups. In the system where aerobic heterotrophs and autotrophic nitrifying bacteria coexist, they compete with each other for oxygen. In the presence of a considerable amount of organic compounds, nitrifying bacteria are unable to compete with aerobic heterotrophs, which will consequently be abundant in the system (Mulder et al., 1995). It was proven that an influent C/N ratio of a nitrification reactor between 1 and 2 could lead to a decline in AOB activity of approximately 70% (Zhu and Chen 2001).

Suppression of NOB

In the PN/A process, nitratation is always closely coupled with nitritation, as both nitrite and oxygen are present. The responsible NOB compete not only with AOB for oxygen but also with AnAOB for nitrite, which affects both nitritation and anammox processes. Moreover, the produced nitrate cannot be removed during the PN/A process. Thus, the suppression of NOB populations is imperative.

A restricted DO is often used to suppress the growth of NOB microorganisms. It is generally considered that AOB has a lower oxygen affinity constant $K_{DO,}$ which represents a higher growth rate of AOB at low DO concentrations (Arnaldos et al. 2015, Rittmann and McCarty 2007). This means the AOB group grows faster than the NOB group under oxygen-limiting conditions. The NOB organisms can be washed out of the system with the additional extraction of the activated sludge. Thus, DO limitation has been widely used as a powerful strategy to outcompete the NOB from the system.

In nature, however, diverse NOB genera prefer different DO levels. *Nitrobacter*-like NOB (r-strategists) and *Nitrospira*-like NOB (K-strategists) were found to coexist and are considered the key nitrite-oxidizers in nitrifying wastewater treatment plants (Kim and Kim 2006, Siripong and Rittmann 2007). *Nitrospira* species, as the predominant NOB genus, have high substrate affinity and prefer low nitrite and oxygen concentrations (Liang et al. 2015). Therefore, Nitrospira-like NOB will theoretically remain in the system under DO limitations, while *Nitrobacter*-like NOB could be outcompeted by AOB. This was proved by the presence of small amounts of *Nitrospira* species and the absence of *Nitrobacter* species in autotrophic nitrogen removal systems under oxygen limitation (Lv et al., 2010; Jeanningros et al., 2010). A complete washout of NOB will be difficult. The control of the aerobic sludge retention time (SRT) is used to wash out NOB groups either by controlling hydraulic retention time (HRT) in continuous operation or by excess sludge removal in an SBR reactor (Fux and Siegrist, 2004). Due to the low decay rate of the NOB organisms, it would be difficult to reduce the abundance of NOB without extraction of activated sludge from the system (Joss et al. 2011) In addition, Joss et al. (2011) suggested that an NH_3-N concentration of up to 10 mg/L is not sufficient to suppress the growth rate of NOB and ensure a successful PN/A process (Joss et al. 2011). In the completely autotrophic nitrogen removal over nitrite (CANON) process, it was proved that the bioactivity of NOB could be effectively inhibited by a high free ammonia (FA) concentration (>11.8 mg/L) and the adoption of organic materials into the system (Liang et al. 2015)

1.1.4 Characteristics of anammox bacteria

Van de Graaf et al. (1995) demonstrated that the anammox reaction is a microbiological process which requires specific microorganisms. This specific microorganism is termed anaerobic ammonium-oxidizing bacteria (anammox bacteria (AnAOB)). As isolation of its pure culture is yet impossible, processes based on the anammox reaction are currently conducted by microbial communities that include various microbial populations (Kallistova et al. 2016). To achieve a successful PN/A process, it is essential to identify the properties of the anammox groups at first and then create suitable conditions for them to achieve a prolonged stable operation. The most notable property of the anammox population is the extremely low growth rate varying from 0.02 d^{-1} to 0.09 d^{-1} (van de Graaf et al., 1996; Strous et al., 1998; Cao et al., 2017). For this reason, the enrichment of these specific microorganisms during the process, on the one hand, and their retention in the system, on the other hand, are of great value. Meanwhile, the

inhibition of the anammox activity by various substances or environmental changes that may occur in the wastewater must be prevented during operation.
The change in environmental factors such as pH and temperature could influence the activity of AnAOB. They have their growth optimum when the temperature is between 20°C to 40°C, and the pH is around 8 (Kallistova et al., 2016). However, the range that allows their growth for both factors is wider: temperature from 15°C to 45°C, pH in the range of 6.5 and 8.8 (Kallistova et al., 2016). Recent studies have shown a stable anammox activity even at temperatures of 10°C or lower (Hoekstra et al. 2018, Isaka et al. 2008a, Lotti et al. 2014b).

Direct inhibition
To ensure the growth of the anammox biomass, ammonium and nitrite must be present in the system as necessary substrates (van de Graaf et al., 1996). The ammonium to nitrite NH4+:NO2- ratio ranges between 1.3 and 1.32 (Strous 2000). However, a high nitrite concentration has been proven to inhibit the anammox activity (van de Graaf et al., 1996). The reported nitrite concentration that could cause an inhibitory effect on the reaction varies over a wide range from 10 mg N/L to 350 mg N/L, resulting in inhibition of different degrees(Lackner et al., 2014). The loss of the anammox activity increased with nitrite concentrations and the exposure time, with the exposure time having a greater influence (Lotti et al. 2012). The inhibitory effect caused by high nitrite concentrations has been proven reversible after the nitrite removal (Lotti et al., 2012). Compared to that, ammonium of 55 mM and nitrate of 45 mM were found to inhibit 50% of anammox activity, indicating that inhibition of AnAOB activity by ammonium and nitrate occurs at very high concentrations (Dapena-Mora et al. 2007).

Dissolved oxygen (DO) has been shown to inhibit the activity of AnAOB (Sliekers, 2002; Strous et al., 1997; Third et al., 2001). A DO concentration of 0.2 mg O_2/L could already result in a complete inhibition of the anammox activity. The inhibition by high DO concentrations is proved reversible once the oxygen is depleted (Joss et al. 2011).
In addition, sulfide and phosphate were proven to inhibit the anammox activity at high concentrations (Dapena-Mora et al., 2007).

Indirect inhibition
The presence of organics in the wastewater is considered to have a negative effect on the anammox process for the following reasons. Firstly, organic matter serves as the substrate of the heterotrophs, which compete with AOB for oxygen and AnAOB for nitrite, decreasing populations and activities of AOB and AnAOB (Jenni et al. 2014). Secondly, organics such as methanol can be toxic for AOB and AnAOB (Isaka et al. 2008b). Thirdly, some anammox species could be induced to switch from an autotrophic metabolism to a heterotrophic metabolism in the presence of organic acid, which leads to a decline in the deammonification rate (Hausherr et al. 2021, Kartal et al. 2007).

1.1.5 Enrichment of anammox biomass

As the growth rate of the Anammox biomass is very slow, the technique for its enrichment has to be chosen properly to achieve a successful Anammox process. In this case, efficient biomass retention is regarded as the prerequisite for the enrichment of slowly growing microorganisms. Strous et al. (1998) suggested that SBR with around 90% biomass retention is a practical technique that enables a high enrichment of Anammox biomass under stable conditions to make a reliable long-term operation possible. By 2014, 50% of the full-scale installations applying the PN/A concept were SBR (Lackner et al., 2014).
To enable a quick startup of the anammox process, AnAOB biomass is usually used for the inoculation (Sliekers et al. 2002, Wett 2007). However, AnAOB biomass inoculation is not indispensable (Jeanningros et al. 2010). Nitrifying biomass under restricted aeration operations (DO in the range of 0.3 to 0.8 mg/L) could also develop AnAOB to initiate an autotrophic nitrogen removal process successfully, even within several months (Jeanningros et al. 2010, Lv et al. 2010).

1.2 Mainstream anammox application

1.2.1 Main challenges

The anaerobic ammonium oxidation is applied more often to treat ammonium-rich wastewater with low COD concentrations, such as the side stream of WWTP at elevated temperatures (above 30°C). Given its major cost and environmental benefits, deammonification has been intensively investigated in recent years for its application under mainstream conditions (Li et al. 2018b). Until 2017 mainstream PN/A has been proven feasible; however, with a low nitrogen loading rate (NLR) and relatively high nitrogen concentration in the effluent at low T (15-10°C) (Cao et al., 2017). The mainstream PN/A process in municipal wastewater treatment is very different from the sidestream PN/A process because municipal wastewater tends to have rather low ammonium concentrations (20-60 mg/L) and low temperatures (10-20°C) that vary greatly from season to season (Gilbert et al. 2015). This poses several difficulties for the application under mainstream conditions.

Firstly, municipal wastewater contains a high COD concentration of 250-800 mg/L and a low nitrogen concentration of 20-70 mg/L, leading to a high C/N ratio in the influent (Metcalf and Eddy, 2014). A high C/N ratio could lead to the out-competition of AnAOB by heterotrophs (Jenni et al., 2014). Therefore, the C/N ratio should be kept as low as possible in the PN/A process (Xu et al. 2015). A C/N ratio was suggested to be 0.5 (Daigger 2014). In addition, NOB suppression is more difficult in mainstream municipal wastewater treatment. NOB groups are inhibited by free ammonia (FA) and free nitrous acid (FNA), which normally result from high ammonium concentrations between 500 and 1500 mg NH_4-N/L in sidestreams. However, it cannot be generated in untreated municipal wastewater with low ammonium concentrations (12 and 45 mg NH_4-N/L) (Metcalf and Eddy, 2014; Lackner et al., 2014;Cao et al., 2017).

Secondly, the low temperature of municipal wastewater with seasonal fluctuations between 10-15°C, compared to the high sidestream temperature (~30 °C), is a major challenge for the application of the PN/A process in the mainstream. The AnAOB activity strongly depends on the temperature and declines sharply below 15°C (Figure 1) (Dosta et al. 2008, Sobotka et al. 2021). Considering the low growth rate of AnAOB, the retention of AnAOB within the system becomes exceedingly critical at low temperatures (Cao et al., 2017). The temperature decrease altered the predominant bacteria in the microbial populations and resulted in a decline in both anammox activity and nitrogen removal rate/efficiency (Isanta et al. 2015, Le et al. 2022, Li et al. 2018a, Wang et al. 2018). A recent study proved that the activity of hydrazine dehydrogenase (HDH), a key enzyme of the anammox reaction, was affected by temperature and directly impacted anammox activity and nitrogen removal (Le et al., 2022). From 10°C to 15°C, the NOB activity is greater than the AOB activity, which makes partial nitrification more difficult to achieve (Yang et al. 2007). In addition, the growth rate of AnAOB, AOB and NOB is dependent on the temperature to varying degrees, resulting in an imbalance between the main groups as temperature changes (Cao et al., 2017).

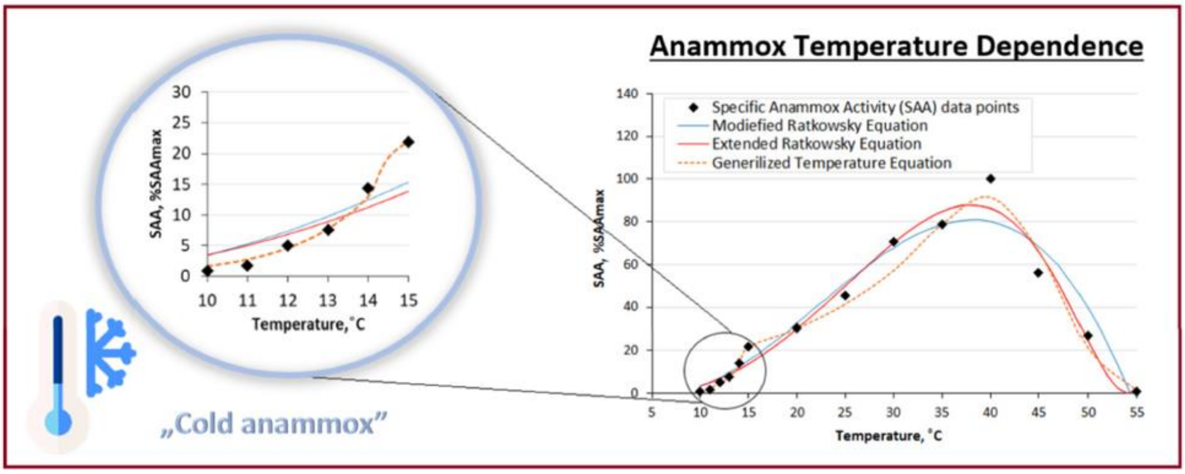


Figure 1 Anammox temperature dependence model (Sobotka et al., 2021)

1.2.2 Technological solutions to the challenges

Due to the undesirable growth of heterotrophs caused by the presence of soluble COD in municipal wastewater, the COD/N ratio must be reduced to achieve the deammonification process. Therefore, a process configuration consisting of two stages (A-B stage) is often used to implement the deammonification process under mainstream conditions (Xu et al. 2015). At stage A, organic matter is captured through different mechanisms so that the effluent of stage A with a lower COD/N ratio could be fed to stage B, where a mainstream deammonification is implemented (Xu et al. 2015). The COD removal efficiency of stage A significantly influences the performance of the subsequent deammonification (Hoekstra 2017). de Graaff et al. (2016) suggested an optimal SRT of 0.3 days for maximal sludge production and a short contact time (minimum: 15 min) combined with sufficient aeration to remove soluble COD. The obtained sludge could be used to generate biogas for energy production, which makes the mainstream PN/A process a step forward in achieving energy neutrality in WWTP (Hoekstra, 2017).

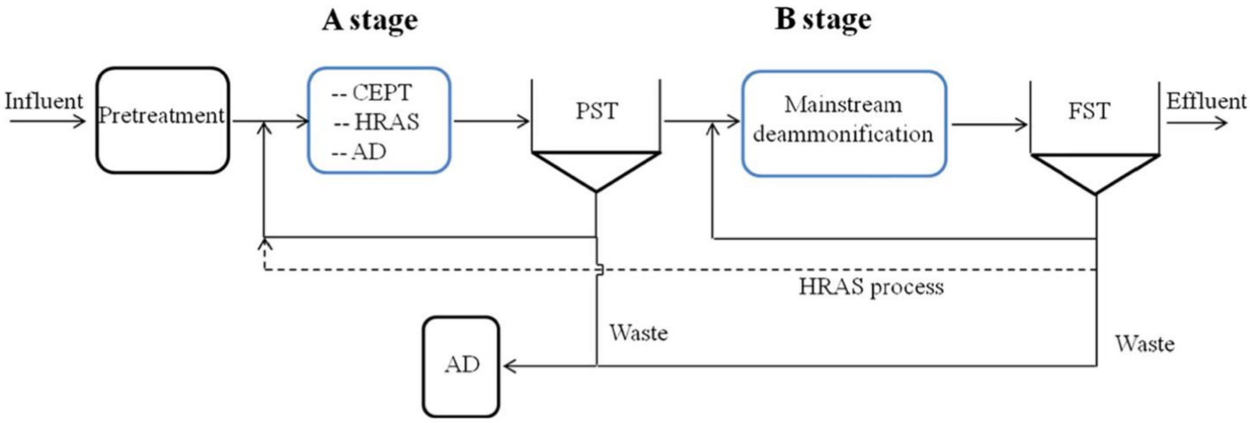


Figure 2 A-B stage-based deammonification process (CEPT: chemically enhanced primary treatment; HRAS: high rate activated sludge; AD: anaerobic digester; PST: primary sedimentation tank; FST: final sedimentation tank) (Xu et al. 2015)

Due to the extremely low growth rate of AnAOB, the retention of AnAOB is of great value in mainstream applications. Anammox bacteria could be retained within the system either by selective AnAOB enrichment in the aggregated biomass or by selective recycling of the anammox biomass using cyclones or screens (Wett 2007, Xu et al. 2015).

1.2.3 Strategies to achieve PN/A

There are several strategies to oxidize part of the ammonium to nitrite required by the anammox biomass and suppress NOB at the same time.

DO is often used as a control parameter to suppress NOB. Since NOB has a lower affinity for oxygen compared to AOB, a low DO concentration of 0.5 mg/L could strongly inhibit nitrite oxidation, while ammonium oxidation was not influenced (Hanaki et al. 1990). A continuous-flow reactor without sludge retention was proven to enrich AOB and wash out NOB (*Nitrobacter* sp.) at a low DO concentration of 0.4 mg/L, while only 70% of the ammonium was oxidized to nitrite, and the extent of nitritation was unstable and highly sensitive to process disruptions (Blackburne et al. 2008). Coupling the DO control with another selection factor was suggested to perform the nitritation (Blackburne et al. 2008). In some studies, a high DO concentration (≥1.5 mg/L) was applied to suppress NOB (especially *Nitrospira* sp.) (Ge et al. 2014, Regmi et al. 2014).

In practice, intermittent aeration has been found to be effective for NOB suppression (Xu et al. 2015). The principle is that NOB requires a longer lag phase to start their metabolism after transitioning from anoxic to aerobic conditions, which enables NOB suppression by frequent alternation between anoxic and aerobic conditions (Jardin and Hennerkes 2012, Katsogiannis et al. 2003). The anoxic time should be able to induce a lag phase, and the aeration time should not exceed the lag time so that NOB can be sufficiently suppressed (Gilbert et al. 2014).The intermittent aeration could be coupled with DO control (Ma et al. 2015, Wett et al. 2013, Xu et al. 2020), with step-feed strategy (Chen et al. 2020, Ge et al. 2014), or with SRT (Regmi et al. 2014).

pH could also be used as a control parameter for nitrogen removal in the deammonification process. The pH value declines during nitritation due to the production of protons and increases under anoxic conditions due to the reduction of protons by the denitritation process (Claros et al. 2012). The intermittent aeration was ruled by the online pH signal combined with the DO/ORP (redox potential) signal to achieve transient anoxic conditions (Claros et al. 2012, Jiang et al. 2018). The pH control is often applied in a real-time control strategy.

Free ammonia (FA) and free nitrous acid (FNA) were proven to selectively inhibit nitrite oxidation, so they have been frequently applied to control the nitritation in sidestream deammonification with high ammonium concentrations (Park and Bae 2009, Xu et al. 2015). Compared with the sidestream, the lower ammonium and nitrite concentrations in municipal wastewater make the inhibition of the NOB population by FA and FNA impossible (Ma et al. 2015). However, a recent study has shown that NOB suppression in the mainstream deammonification could be achieved by adding high FA and controlling low DO concentrations, where the ratio of DO to total ammonia nitrogen (DO/TAN=DO/(NH_4-N+NH_3-N)) was used as a control parameter. A complete NOB suppression occurred at a DO/TAN ratio of 0.003 (Le et al. 2020).

1.2.4 Long-term stability

The production of nitrite for the anammox biomass is considered a crucial step in the PN/A process, as the availability of nitrite tends to limit the anammox activity (Joss et al., 2011). Despite its inhibition by a high nitrite concentration, the anammox activity can be restored by reducing the nitrite concentration (van de Graaf et al., 1996). This allows the system to recover from nitrite accumulation which may occur in the prolonged operation of the PN/A process. Furthermore, the successful start-up of an Anammox process to treat wastewater with high nitrite concentrations of 100 to 250 mg NO_2-N/L showed that nitrite toxicity is not pivotal for its long-term operation (Lv et al., 2010; Joss et al., 2011).

For the PN/A application in municipal wastewater treatment, variation of influent concentrations is a big challenge. The aeration rate must be adapted to the incoming ammonium concentrations. Otherwise, either an excessive or an insufficient air supply could lead to undesirable consequences. Third et al. (2001) reported that the continued ammonium limitation leads to an increase in dissolved oxygen, thereafter the development of NOB groups and eventually a decrease in the total nitrogen removal rate (NRR) of the CANON system. This is similar to the seasonal variation in municipal wastewater influent: ammonium concentrations remain at low levels during the rainy season. Therefore, aeration control is of great importance.

As mentioned above, the DO limitation has been used as the essential strategy to suppress NOB growth. However, the aeration control based on the DO concentration cannot ensure a successful NOB suppression since the NOB organism can also consume the dissolved oxygen when nitrite is present (Joss et al., 2011). Therefore, the control of the volumetric air flow rate is seen as a more reliable control strategy for long-term NOB suppression (Joss et al., 2011).

1.2.5 Process configurations

To remove nitrogen from the wastewater via the PN/A process, it is essential to create an oxygen gradient, either spatially or temporally, which provides a proper anoxic microniche for the AnAOB (Vlaeminck et al. 2012). Thus, depending on the process configuration, the partial nitrification and anammox can be arranged either in one reactor with temporal oxygen gradient, termed single-stage PN/A process, or separately in two reactors with individual DO levels, termed two-stage PN/A process.

The single-stage PN/A is marked by its low space requirement and associated low investment costs, relatively straightforward process control, and lower risk of inhibitory effect on the AnAOB activity caused by nitrite accumulation (Fux and Siegrist, 2004; Vlaeminck et al., 2012). As a result, most of the existing full-scale PN/A plants are single-stage processes (Vlaeminck et al., 2012). The common single-stage process includes completely autotrophic nitrogen removal over nitrite (CANON) (Sliekers et al. 2002) oxygen-limited autotrophic nitrification/denitrification (OLAND) (Vlaeminck et al., 2012), DEMON (Wett, 2007).

In comparison with the single-stage configuration, the two-stage configuration is more favourable to achieving a successful PN/A process under mainstream conditions (Chen et al., 2020). In the two-stage process, individual optimization of the partial nitritation and the anammox step is feasible, whereby the operational conditions are more suitable for the growth of AOB and AnAOB, respectively (Cao et al., 2017; Vlaeminck et al., 2012).

2 Pilot Plant operation and monitoring

2.1 Description of the pilot plant

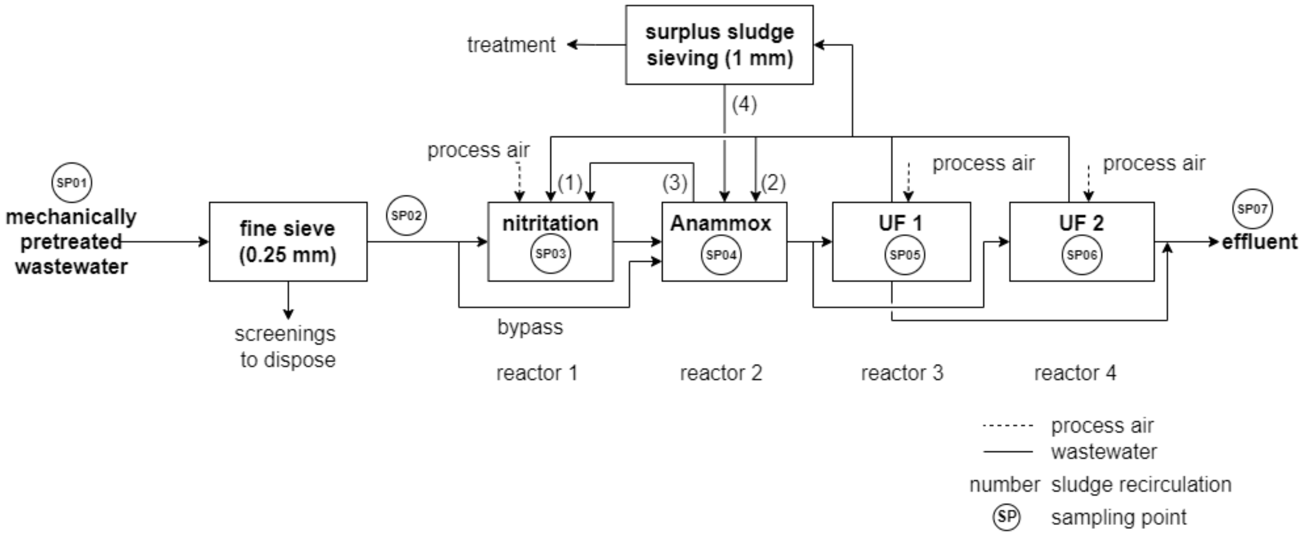


Figure 3. Process flow diagram of the pilot plant with an indication of sampling points. UF: submerged Ultrafiltration membranes modules as part of the membrane bioreactor (MBR) system.

Figure 3 shows the configuration of the pilot plant in the form of a two-stage PN/A process. The influent of the pilot plant is taken from the grit chambers and pumped into an intermediate bulk container (IBC) by two wastewater pumps (450/12 IX-S, TIP). The IBC could be used for flocculation of particulate COD, but this has not been applied during the pilot plant operation. The influent wastewater passes through a pretreatment stage by using a rotary drum with a mesh size of 0.25 mm. This screening stage aimed to intercept coarse solids larger than 0.25 mm, mainly to protect the ultrafiltration membranes. Due to the 24 h-rotation of the drum, the solids captured on the screen surface automatically fell down through a plastic hose and were collected in a container. The screenings were regularly disposed of at the landfill together with the screenings from the wastewater treatment plant. Since the flocculation stage is not used, the fine sieve serves as the first treatment stage of the entire pilot plant.

The biological stages of the pilot plant consist of four reactors: an aerated reactor (nitritation-/PN-stage), a completely stirred reactor (Anammox stage), and two activated sludge reactors, each of them with submerged ultrafiltration hollow-fiber (HF) membrane cassette installed (Supratec) working in parallel as membrane bioreactors (MBRs). The partial nitritation (PN) reactor and anammox reactor are each 9 m^3, while two MBRs each are 7 m^3. The effluent from the fine sieve is fed purely hydraulically by gravity into the partial nitritation/PN- reactor (reactor 1), which is continuously (or intermittently in the last phase) aerated with the process air occurring in the form of fine bubbles. The originally installed air blower had a capacity of 228 m^3/h and was operated to maintain a DO concentration between 0.3 mg/L and 0.8 mg/L in the PN reactor. After passing through the partial nitritation/PN-reactor, the wastewater is fed to the anammox stage (reactor 2), which is continuously stirred by an agitator. Depending on the wastewater's quality (COD, N-NH4), the fine sieve's effluent can alternatively be fed to the anammox reactor. The distribution of the feed to nitritation/PN-reactor and the anammox-reactor can be adjusted manually with a valve. After passing through the anammox stage, biomass is fully retained by the HF ultrafiltration membranes (pore size: 0.03 µm, membrane area: 160 m^2/cassette, size: 2025 mm*790 mm*1010 mm/cassette) in the MBRs (reactor 3 and 4). To keep membranes without fouling issues, the process air is diffused into coarse bubbles to maintain a continuous tangential flow across the membrane. Meanwhile, the mixed liquor of the MBR is pumped back to the preceding nitritation (recirculation 1) or anammox stages (recirculation 2) to avoid high mixed liquor suspended solids (MLSS) concentrations in the MBR which result in complete ammonium and COD conversion. Therefore, the MLSS concentrations in the other reactors should be controlled as well. This requires regular sludge extraction from the pilot plant. Similarly, the mixed liquor of the anammox stage can be returned to the nitritation/PN- stage for the required sludge recirculation when the ammonium conversion in the PN reactor is not sufficient.

The treated wastewater is pumped to a permeate tank by an eccentric screw pump for intermediate storage and then flows under gravity into a pump sump, from where it is further pumped back to the grit chamber of the WWTP by a wastewater pump (450/12 IX-S, TIP). The permeate stored in the tank is used for operational procedures such as the backflush of the fine sieve and membranes. The surplus sludge out of the biological treatment system is passed through a fine sieve (1mm) to retain the slow-growing anammox granules, which are further returned into the anammox reactor.

2.2 The pilot plant installed in Simmern, Germany

The pilot plant was designed and constructed in Shanghai, China, Supratec. After the pre-commissioning with tap water in China, it was dismantled and then shipped to Simmern, Germany. The pilot plant was transported in three containers: one contained four water tanks that further operated as above-described reactors (reactor 1 to 4) (see Figure 3), one contained permeate tank, barrels storing chemicals for the membrane back flush procedures and other equipment including the control cabinet and high-pressure pump for the automatic cleaning of the fine sieve, one contained fine sieve and the flocculation IBC as well as small parts for the installation. Three containers arrived at the end of September 2021 and were then installed on the grounds next to the grit chamber of the municipal wastewater treatment plant of Simmern. The pilot plant during the experimental period operated in the form of two containers, one fed with municipal wastewater taken from the WWTP and the other as an equipment container accessible to operators (see Figure 4). The fine sieve and an IBC intended for flocculation were placed on the top (see Figure 5).

Figure 4 Front view (left) and side view of the pilot plant (right) of the pilot plant

Figure 5 Side view of the pilot plant

2.3 Characteristics of the WWTP influent

The two-stage PN/A MBR pilot plant was installed at the wastewater treatment plant (WWTP) in Simmern, Germany. The WWTP has a capacity of 42,000 p.e. (size class: 4 based on AbwV). The municipal wastewater is first treated mechanically via the screens (opening size: 6 mm), grit chambers and primary sedimentation tanks, then is treated biologically by a conventional activated sludge system. In addition, phosphate is removed by adding ferric chloride through precipitation. The mechanically pretreated municipal wastewater was taken as the feed of the pilot plant, so two feed pumps were placed underwater near the outlet of the rectangular grit chambers.

The influent of the WWTP at Simmern was characterized between October 2021 and March 2022, with the main parameters summarized in Table 1. For this characterization, 24-hour volumetric composite samples were also collected from the grit chamber.

Table 1 Influent data of the WWTP at Simmern in the period October 2021 - March 2022 (data from WWTP Simmern)

	T_{inflow} [°C]	PH_{inflow} [-]	TS_{inflow} [ml/L]	$BOD_{5,inflow}$ [mg/L]	COD_{inflow} [mg/L]	$NH4\text{-}N_{inflow}$ [mg/L]	TN_{inflow} [mg/L]	P_{inflow} [mg/L]
data amount	182	143	127	27	27	27	26	27
average	10.79±2.14	7.11±0.37	6.15±4.39	177.44±84.84	400.85±254.06	18.62±7.57	33.8±10.78	5.01±2.29
min.	7.0	6.0	1.0	20.0	69.0	4.1	14.6	1.1
max.	15.9	7.6	26	330	1258	33.8	50.2	10.9

As shown in Figure 6, the wastewater temperature gradually decreased from 16°C to 9°C and increased again from day 165 (around mid-March). The experimental period of the pilot plant allowed the study of the challenges of having low temperatures for the anammox process. In addition, the influent COD/N-ratio calculated based on the influent data is also shown in Figure 6. The COD/TN ratio of the influent varied between 5 and 15. The average values of calculated different C/N ratios were COD/TN 11.8±7.5, COD/NH_4-N 24.1±19.4, and BOD_5/NH_4-N 10.7±8.9.

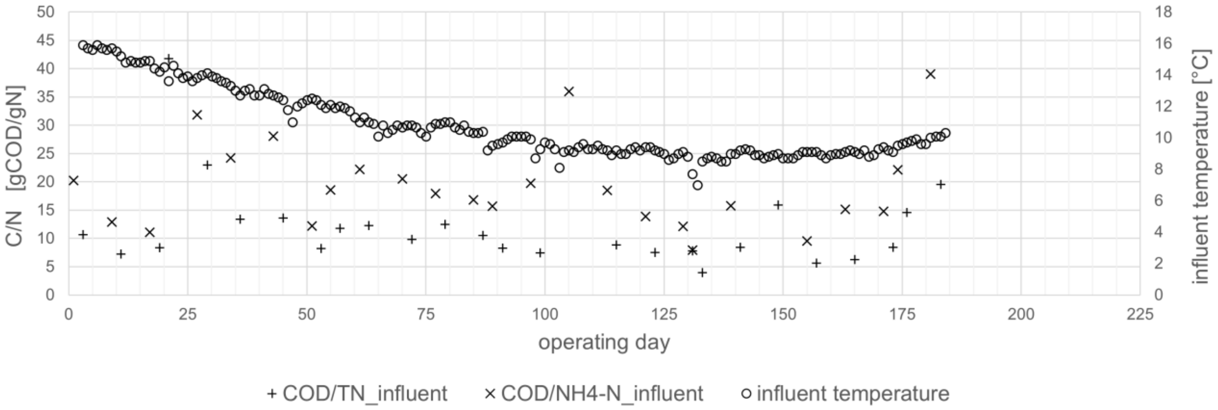


Figure 6 Temperature and COD/N-ratio of the influent wastewater between October 2021 and March 2022

Fluctuations over time in COD and TN concentrations in the influent were observed (Figure 7). Both influent concentrations were found to vary greatly over time, which was comprehensive given the diurnal fluctuations in municipal wastewater. From day 85 to 175, both COD and TN concentrations were significantly lower than those at the beginning of the operation. In this period, dry weather days of the individual month also decreased substantially. Therefore, it is presumed that the municipal wastewater was strongly diluted by rain and snow water between day 85 and day 175.

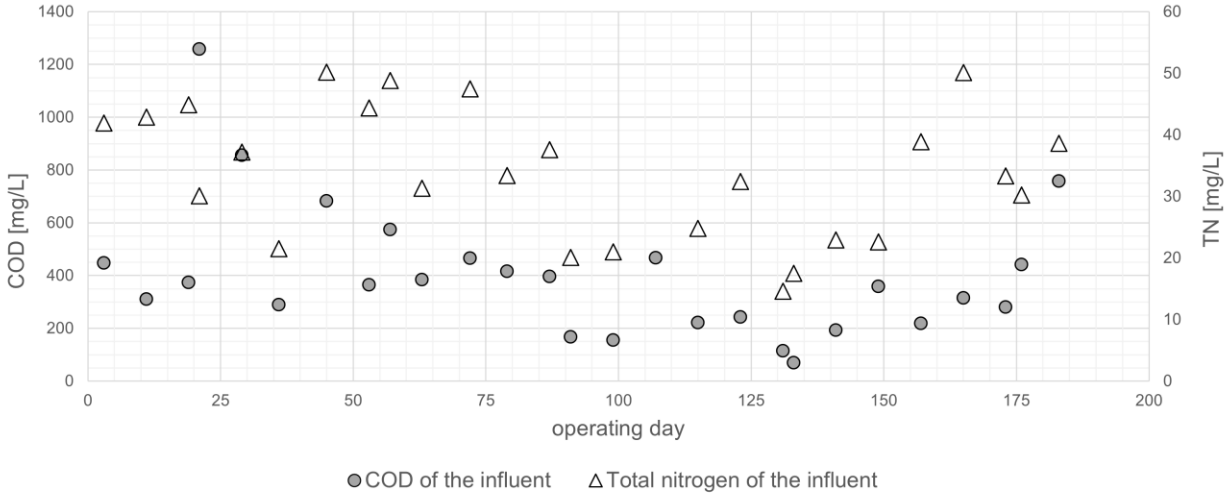


Figure 7 Fluctuation of COD and TN influent concentrations during the experimental period (data from WWTP Simmern)

2.4 Start-up of the pilot plant

After around two weeks of installation in Germany, the commissioning, including a hydraulic test of the tanks and pipelines, single function tests and complex function tests, was performed from 29th to 30th Sep. 2021. After this, the pilot plant was inoculated with 1 m^3 Anammox seeding sludge on 30th Sep. and then with 1 m^3 activated sludge in two days (Figure 8). The seeding sludge characteristics are summarized in Table 2.

Table 2 Key parameters of seeding sludges

	Anammox sludge	Activated sludge
Source	Industrial plant treating food processing wastewater	WWTP at Simmern
TSS [g/L]	58	-

VSS [g/L]	54	-
morphology	granules with a diameter of around 2 mm	flocs
Inoculation time after being taken [days]	2-3	0.5

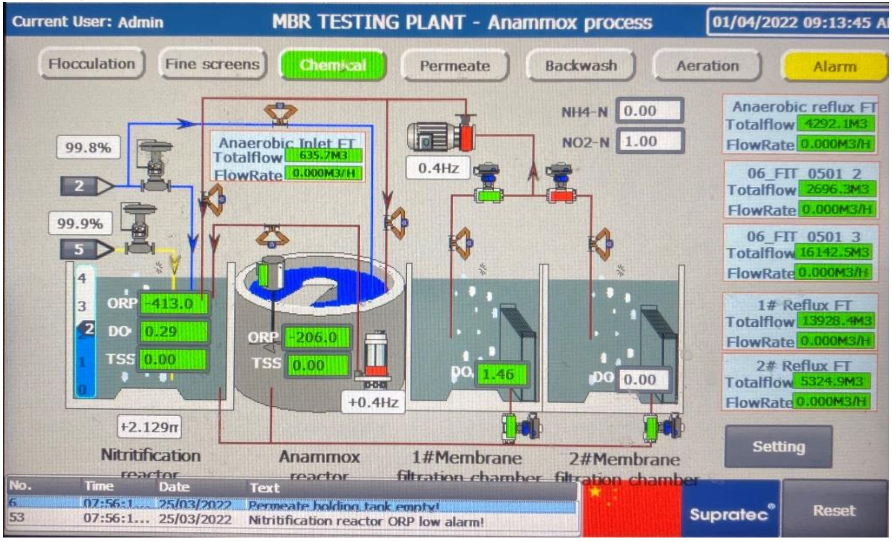
Due to the incompatible technical equipment and required adaptations to the software, the commissioning of the pilot plant was not completed until several weeks after the sludge inoculation and therefore took a total of about 5 weeks (from 13th September to 22nd October). After commissioning, the pilot plant could be operated partially in automatic mode.

Figure 8 Granules in the inoculum of the pilot plant (granules > 1 mm)

In phase 4, a similar anammox sludge was used for reinoculation. However, it was reported that the structure of the anammox received appeared to be, this time, less granular and more flocculant.

Control of the pilot plant

The programmable Logic Controller (PLC) display controls the pilot plant. The changes required during the commissioning and operation of the pilot plant were made by Chinese automation engineers and incorporated into the program via remote access.



NH4-N 0.00
NO2-N 1.00

Anaerobic Inlet FT
Totalflow 635.7M3
FlowRate 0.000M3/H

Nitrification reactor: ORP -413.0, DO 0.29, TSS 0.00
Anammox reactor: ORP -206.0, TSS 0.00
1#Membrane filtration chamber: DO 1.46
2#Membrane filtration chamber: DO 0.00

Anaerobic reflux FT
Totalflow 4292.1M3
FlowRate 0.000M3/H

06_FIT_0501_2
Totalflow 2696.3M3
FlowRate 0.000M3/H

06_FIT_0501_3
Totalflow 16142.5M3
FlowRate 0.000M3/H

1# Reflux FT
Totalflow 13928.4M3
FlowRate 0.000M3/H

2# Reflux FT
Totalflow 5324.9M3
FlowRate 0.000M3/H

```
No.   Time         Date         Text
6     07:56:1...   25/03/2022   Permeate holding tank empty!
53    07:56:1...   25/03/2022   Nitritification reactor ORP low alarm!
```

Figure 9 Process control via PLC-display: control of biological stages as an example

2.5 Sampling and Analysis

There was no operational online monitoring of the pilot plant. The operation's monitoring, evaluation and optimization are mainly based on parameters determined by laboratory analyses.

2.5.1 Sampling

All sampling points are indicated in Figure 3. Due to the lack of a sampling point in the fine sieve outlet (SP02) at the beginning of the operation, water samples taken at SP01 were used as influent samples until day 83, when SP02 was installed. Since then, water samples taken from SP02 have been used for monitoring.

Liquid samples are taken at respective sampling points daily to determine the key parameters in Table 3. While the water samples can be taken at SP02 by opening a sampling valve and at SP07 directly from the permeate tank, water samples at SP01 and the mixed liquor sludge samples at SP03-06 must be taken by hand using a plastic dipper. It should be noted that all samples are random samples and are collected in bottles for further analysis. Despite that, most samples were collected between 8:40 and 9:30 am to ensure certain consistency of samples, with few exceptions.

2.5.2 Analytical procedures for liquid samples

Several important parameters are routinely determined in the laboratory to monitor biological processes that occur in the pilot plant and further improve its operation (Table 3). During the operation, total Chemical Oxygen Demand (COD_{total}) and nitrogen compounds concentration (NH_4-N, NO_3-N, NO_2-N) are measured three times per week (Monday, Wednesday, Friday) while sludge concentrations (MLSS) of individual reactors are determined twice per week (Tuesday and Thursday). The detailed measurement procedures are described in the following text.

- For the determination of COD_{total}, samples were taken from bottles immediately after the bottles had been shaken vigorously. COD_{total} is measured colourimetrically with Aqualabo test tubes (Detection range: 50-1500 mg/L for influent, 5-150 mg/L for effluent).
- To measure the nitrogen compounds, the fluid samples are first filtered with a folded paper filter (MN 615, Macherey-Nagel, Germany). The filtered samples are determined for nitrate, nitrite and ammonium colourimetrically using Test Kits from Aqualabo (NH4-N: 0.02-5 mg/L, 0.5-50 mg/L; NO_2-N: 0.01-1 mg/L, 0.1-5 mg/L; NO_3-N: 0.1-20 mg/L). The measured COD_{total} and nitrogen concentrations are acquired on a portable field photometer consisting of ODEON (Aqualabo, France) field equipment and an accessory with a silicon photodiode (PHOTOPOD, Aqualabo).
- The pH value is measured for the influent and effluent three times a week using a combined pH electrode (Ag/AgCl, Aqualabo), and turbidity is measured for the effluent three times a week using an NTU numerical sensor (measure range: 0-4000 NTU, Aqualabo). The measured pH values and turbidity are read on the ODEON unit after the sensor has been connected to it, respectively.
- The MLSS concentration is determined using the standard method according to APHA (2017). The ceramic crucibles and the filter papers (MN 615, Macherey-Nagel) were dried in a drying cabinet for 1 h and then cooled down to room temperature in the desiccator. The weight of the crucibles and the filter papers were determined. Liquid samples were taken from individual reactors and filtered through filter paper. Biomass dry weight, including the weight of the crucibles and the filter papers, was determined by drying the filtered sample at 105°C for at least 12 h. The total dry weight minus the weight of the crucibles and the filter papers is hereafter termed mixed liquor suspended solids (MLSS). The sludge measurement was conducted since 08th Dec. 2021(from day 71).
- In addition, temperature and dissolved oxygen (DO) are measured using a combined sensor (FDO®925, WTW, Germany) (DO range: 0-20 mg/L, T range: 0-50°C). The measured values are indicated on a portable oxygen meter (Oxi 3310, WTW). The measurement was carried out every day at the same time, between 12:30 to 13:00.
- From day 178, the DO of the nitritation reactor was measured using an optical DO sensor (optod, Aqualabo), which was cleaned every day and calibrated every week. The online monitoring of the DO level was carried

out via the PLC and served for automatic air blower control. The measurement was performed in intervals of 30 s.

Table 3 Routine measurement of key parameters related to sampling points

	Location	Indication for	Type	Sampling frequency	purpose (Determination of)
SP01	grit chamber (WWTP)	influent	wastewater	3/week	COD_{total}, N compounds (NH_4-N, NO_3-N, NO_2-N), PH, TS
SP02	outlet of the fine sieve	influent	wastewater	3/week	COD, N compounds (NH_4-N, NO_3-N, NO_2-N),
SP03	nitritation reactor	nitritation/PN	mixed liquor	3+2/week	N compounds (NH_4-N, NO_3-N, NO_2-N), MLSS, T, DO
SP04	anammox reactor	anammox	mixed liquor	3+2/week	N compounds (NH_4-N, NO_3-N, NO_2-N), MLSS, T, DO
SP05	UF 1	MBR 1	mixed liquor	2/week	MLSS, T, DO
SP06	UF 2	MBR 2	mixed liquor	2/week	MLSS, T, DO
SP07	permeate tank	effluent	wastewater	3/week	COD_{total}, N compounds (NH_4-N, NO_3-N, NO_2-N), pH, turbidity

2.6 Operational conditions of the pilot plant

The pilot plant operation started on 29th September 2021. The pilot operation between 29th September 2021 and 30th April 2022 are indicated as days 1 to 214, respectively. As low temperatures occurred during the experimental period, attention was paid to the fine sieve located in the pilot plant's top open space. To prevent it from freezing, a tent was erected on day 83 to insulate the entire pilot plant and has maintained a temperature above 2°C with an additional heating system using fuel oil during the winter months. The tent was dismantled on day 211.
After, the plant was out of normal operation with minimum load due to operational and logistic issues. A reconstruction of the fine sieve into a 1 mm mesh (a fine sieve with 0.25 mm was used in phases 1-3) size was carried out, and inlet pump failures were solved. About 83 m^3 of waste sludge was taken out during 12 consecutive weeks to decrease the SRT of the plant aiming for NOB washout. After 1 m^3 anammox seeding sludge was added to the wastewater plant on 16-9-2022, this day was considered as day 0 for the final operational phase 4.

2.6.1 Operation of the fine sieve

The fine sieve with a mesh size of 0.25 mm is designed to protect the subsequent membrane from coarse particles. To prevent clogging of the fine screening stage (mesh size of 0.25 mm), the treated wastewater stored in the permeate tank was used for its backwashing. During the experimental period, the fine sieve was clogged several times, so the feed had to be reduced to avoid the overflow of wastewater from the fine screening stage. The operation of the pilot plant was, therefore, significantly affected. To maintain the performance of the fine sieve, the cleaning strategy had to be adjusted accordingly. Based on the cleaning strategy, the operation of the fine sieve is divided into the following operating days (see Table 4).

Table 4 Cleaning strategy of the fine sieve

Operating phase	Operating day	Cleaning strategy of the fine sieve
1&2	0-124	automatic backwashing with permeate water at a set interval
2&3	125- 184	• automatic backwashing with permeate water • manual backwashing with KOH-solution once a week
3	185- 214	• automatic backwashing with permeate water • manual backwashing with KOH-solution regularly • manual backwashing with HCl-solution regularly
4	0-80	• automatic backwashing with permeate water • manual backwashing with KOH-solution regularly • manual backwashing with HCl-solution regularly

From day 0 to day 118, backwashing was conducted every five hours for 2 minutes at a flow rate of 40 L/min. On day 119, the fine sieve was clogged by wastewater contaminants that were presumed to be oil and grease. On day 125, 50% potassium hydroxide (KOH) solution was dosed with permeate during normal backwashing and diluted to 1%-KOH to remove the clogging. After that, the fine sieve's performance was restored, proving the presumption. From day 125, the normal backwashing frequency was increased to every 2 hours and a 1-minute backwashing with KOH-solution was performed every week to maintain the performance of the fine sieve.

During operating days 174 to 176, influent wastewater contained much higher solid due to the cleaning procedures of the rain back hold basins in the WWTP. It led to an overload of the fine sieve. The chemical backwashing with KOH-solution was conducted more frequently to restore its performance. However, the cleaning effect was very limited. On day 178, the fine sieve was clogged again, and the feed of the pilot plant had to be reduced from 3 m^3/h to 0.5 m^3/h. Despite several attempts to recover its performance (including cleaning the fine sieve surface with high-pressure-water, the interior of the fine sieve and the downstream pipe), it was presumed that the fine sieve was clogged by inorganic deposits that should be removed with hydrochloric acid (HCl)-solution. After backwashing with 0.5% HCl-solution, which was diluted with permeate from 25%-HCl solution during a normal backwashing procedure, the performance of the fine sieve was recovered on day 185. Since then, it was decided to carry out backwashing procedures with chemicals regularly, first with KOH-solution for 3 minutes and then with HCl-solution for 3 minutes, to restore the performance. The dosing system (dosing pump and connected tubing) was sufficiently cleaned with water before and after dosing one chemical.

2.6.2 Operation of PN- anammox reactors

The main task of the nitritation reactor is to achieve stable partial nitrification (oxidation of ammonium mainly to nitrite) and supply the subsequent anammox reactor with sufficient nitrite and ammonium. The nitritation reactor was continuously aerated. The DO concentration should be maintained at a low level in the nitritation reactor to suppress the NOB activity by adjusting the air flow rate. However, the operation of the PN reactor was severely restricted during the entire operating period due to failures of the air blower. To compensate for the deficit caused by the blower, the operating strategy had to be adjusted, mainly by changing the sludge recirculation. As a result, the operation of the anammox reactor was greatly influenced by the operation of the nitritation reactor. Therefore, the operation of these two reactors is described together. Based on the challenges presented with the air blower, the operation was divided into three phases: Phase 1 from day 0 to 97 (operation with the original air blower supplying air to PN reactor and MBR), Phase 2 from day 98 to 155 (operation with two blowers with fixed capacity, each supplying air to the PN reactor and MBR) and Phase 3 from day 156 to 214 (operation with new blower supplying air to the PN reactor, two blowers with fixed capacity to each MBR). An overview of the DO concentration during the total operation is depicted in Figure 10.

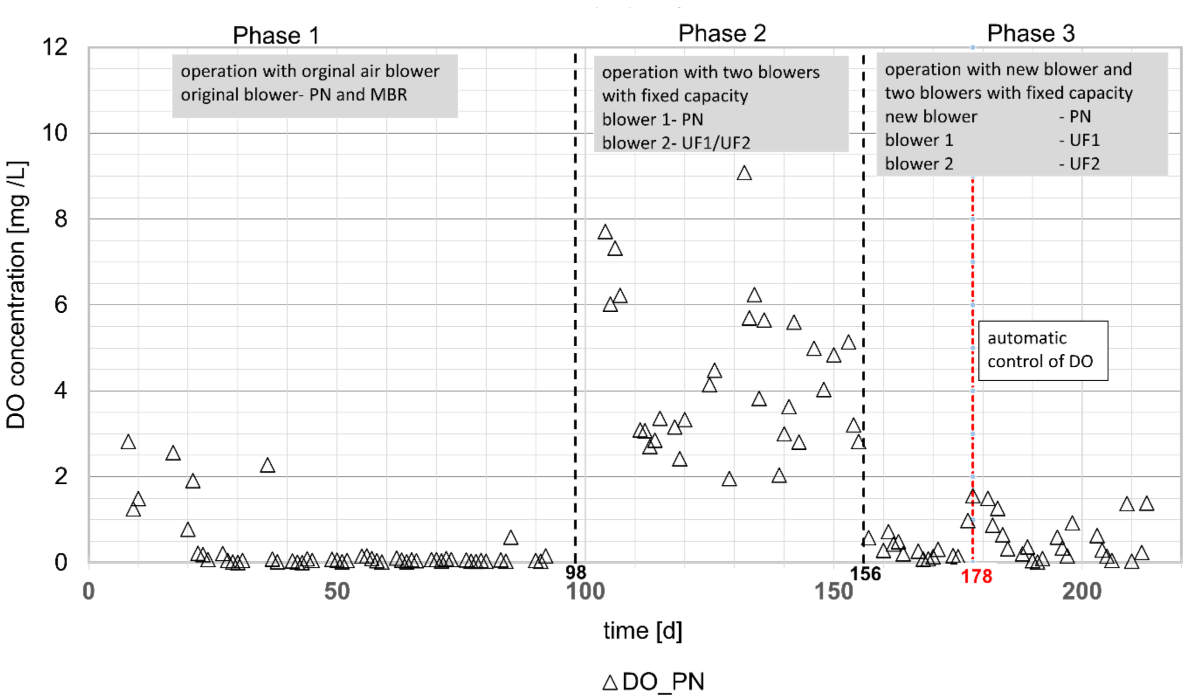


Figure 10 DO concentration in the PN reactor over the total operating period. From day 178 automatic control of DO occurred.

Operation phase 1 from day 0 -day 97

At the beginning of the operation, the air blower showed an atypical performance. From day 14, the air blower was operated at 100% performance (50 Hz) due to the failure of the frequency converter. The air blower's maximum airflow rate of about 40 m^3/h generated was far below the specified capacity (228 m^3/h). The limited air was supplied not only for the nitritation stage but also for the air scouring system in the MBR at the same time. As the membrane operation required a fixed airflow rate of 32 Nm^3/h ($Q_{airscouring}=160\ m^2 \times 0.2\,\frac{Nm^3}{(m^2 \cdot h)} = 32\ \frac{Nm^3}{h}$) to diminish fouling, it was not possible to increase the airflow rate to the nitritation reactor during operating phase 1. Despite several adjustments to the operating strategy, the DO concentration during this phase was approximately 0.1 mg/L (see Figure 10). The operating strategies are summarized in Table 5 and described in more detail as follows:

- At the beginning of the operation, the effluent of the fine sieve was fed to the PN reactor (Q_{feed}=0.5 m^3/h), and the sludge was recirculated from the MBR to the PN reactor (sludge recirculation: 1; Q_1=3.5m^3/h) and to the anammox reactor (sludge recirculation: 2; Q_2=3.5m^3/h). On day 9, a submerged pump was installed as an agitator in the nitritation reactor to avoid the existing settling of the sludge.
- On day 24, the effluent of the fine sieve was fed 50% into the PN reactor and 50% to the anammox reactor (bypass). In the meantime, the sludge was returned from MBR to the anammox reactor (Q_2=3*Q_{feed}) and from the anammox reactor to the PN reactor (sludge recirculation: 3; Q_3=Q_{feed}). During this phase, the feed flow rate was increased stepwise from 0.5 to 1 m^3/h.
- On day 45, the sludge was fully recirculated from the MBR to the nitritation/PN reactor (Q1=3*Qfeed) to provide more oxygen for nitrogen conversion via oxygen diffusion. In the meantime, the bypass was switched off due to insufficient nitrogen conversion. Thereafter, the detected DO concentration remained extremely low (about 0 mg/L). Since additional air supply was not possible at this point, reducing the sludge concentration in the nitritation/PN reactor was considered to produce more nitrite. As a high MLSS concentration is one of the main advantages of the MBR technology, it is unfavourable to extract the sludge directly from the pilot plant to reduce the sludge concentration. Instead, the sludge concentration in the PN reactor was reduced by a two-step sludge recirculation.
- From day 71, the sludge was returned from MBR to the anammox reactor (Q_2=3*Q_{feed}) and from the anammox reactor to the PN reactor (Q_3=0.5*Q_{feed}), respectively. During this phase, the feed flow rate varied from 0.3 to 0.6 m^3/h depending on the operational adjustment.

Table 5 Development of the operating strategy of reactors during phase 1

Days	Bypass	Sludge recirculation			Operating strategy
		1	2	3	
0-23		x	x		increase of the feed
24-44	x		x	x	increase of the nitrogen conversion via bypass
45-70		x			supply of the PN reactor with more oxygen from MBR via the sludge recirculation (1)
71-98			x	x	increase of the DO by reducing $MLSS_{PN}$

Operation phase 2 from day 98 -day 155

On day 98, the air blower failed overnight. Therefore, the pilot plant was shut down for three days. From day 101, two air blowers were installed as temporary substitutes to continue the pilot plant operation. Both blowers have a capacity of 60 Nm^3/h airflow at 400 mbar. One air blower supplied air for the nitritation reactor, and one for MBR. After installing two air blowers, the high DO concentration (above 7 mg/L) detected in the PN reactor indicated an excessive air supply. Since the air supply of these two blowers was not adjustable as required for proper control of the DO, the feed of the pilot plant was increased stepwise to adapt the fixed air supply. Although the increase of the feed was interrupted twice (once due to the failure of the feed pump and once due to the clogging of the fine sieve), the feed was eventually increased from 0.5 m^3/h to 3 m^3/h on day 133. Due to the strong dilution of the wastewater by rains during this period, the adaption strategy did not succeed in achieving a suitable DO level to generate nitrite. A further increase of the feed was no longer possible after reaching 3 m^3/h since one filtration lane was not in order due to the failure of the permeate pump. The DO concentration was initially greatly reduced by the increase of the feed and then fluctuated widely between 2-6 mg/L during this phase (see Figure 10).

Operation phase 3 from day 156 -day 214

From day 156, a new air blower supplied the air exclusively for the PN reactor, and two existing air blowers with fixed air supply for the respective filtration lanes of the MBR (UF1, UF2) were installed. On day 157, an optical DO sensor was also installed and used to monitor the DO concentration in the PN reactor online. Since the optical DO sensor monitoring was frequently interrupted by the accumulation of suspended solids on the sensor surface, a mechanical wiper system (hydroclean_P, Aqualabo) was mounted on the sensor and automatically cleaned the sensor surface from day 171. In addition, the DO sensor was cleaned with tap water every day and calibrated every week. From day 178, the frequency of the blower was regulated by the detected DO concentration via the PLC to adapt the air supply to the fluctuations of the influent fractions. In order to present a general development of the DO concentrations in the PN reactor and to visualize the differences between the three operating phases, the DO concentration was measured manually as usual in addition to the online monitoring. During this phase, the DO concentration remained at about 0.5±0.1 mg/L (see Figure 10).

Operation phase 4 (0 – 80 days)

After the restart of the plant, from day 0 to 10 in phase 4, it was operated at an average flow rate of 1.4 m^3/h, then increased to about 3 m^3/h until day 24 On day 24 the inflow rate was increased to 3.5 m^3/h. However, operational issues arose at this inflow rate with the ultrafiltration units (only one filtration/MBR lane in operation) and with the check valves of the inflow pump, and therefore, the flow was decreased/varied between 1.5 – 3.4 until day 39. From day 40 to 59, the flow rate was kept at 4 m^3/h. From day 60 – 80, the inflow rate varied between 3 and 3.8 m^3/h.

A new DO sensor was installed and automated in the partial nitritation reactor on day 25 and was initially set up to 2.5 mgO_2/L and reduced to 0.8 mg/L once the controller was fine-tuned. The controller was set up to be ON/OFF, providing aeration for 10 min and no aeration for 20 min.

Extraction of the surplus sludge

During the operation, the activated sludge should be regularly removed from the filtration reactor to control the SRT. A fine sieve was used to retain the anammox granules from the surplus sludge, and the granules were further recycled into the anammox reactor. Due to the difficulties with the mesh size of the original fine sieve (0.25 mm), it had to be replaced by one with a mesh size of 1 mm. The extraction of the sludge was performed from day 168. After that, the sludge extraction was performed when the measured sludge concentration in the MBR was greater than 10 g/L or actions to diminish the SRT of the whole plant were taken.

Before the starting day of phase 4, the waste sludge was always sieved; however, retention of anammox granules was not observed, concluding that because of the low load and intermittently operational conditions, the anammox was lost, and new seeding sludge was necessary. Nevertheless, after the reinoculation in phase 4, anammox granules retained in the sieve were not representative or comparable to what was observed during the first 211 days (phases 1-3).

2.6.3 Operation of membrane ultrafiltration units

The filtration cycle of the membrane units was set to 8-minute filtration and 1-minute relaxation. This means that the treated wastewater was sucked by the permeate pump via the hollow fiber membrane for 8 minutes with 1 minute pause. On day 102, the operation of the pilot plant was interrupted due to the failure of the permeate pump in MBR 2, so only MBR 1 was in operation from day 102.

To minimize the membranes' fouling issues, the ultrafiltration process contained continuous air scouring and operational backflush procedures with chemicals. The required air was calculated to be 32 Nm^3/h and was provided from day 0 to day 98 by the air blower that also served for the PN-stage and from day 98 to day 214 by an independent air blower with fixed performance. To ensure a stable operation of membranes, the enhanced backflush with chemicals was conducted manually at regular time intervals or additionally when the transmembrane pressure (TMP) was higher than 100 mbar (see Table 6). The operational back flush with chemicals is illustrated in Figure 40 (Appendix I). In the procedure, the cleaning solutions (NaClO or citric acid) were diluted with permeate by chemical injection and simultaneous backwashing with permeate. The dosing amount of individual chemicals is given in Appendix I.II.

Table 6 Chemical enhanced backflush frequency of UF-membranes

Backflush with chemicals	Frequency
sodium hypochlorite (NaClO)	1 /week or at high TMP
citric acid	½ weeks or at high TMP

2.7 Mass balance and removal calculations

The calculation of the COD removal efficiency was divided into 2 phases. For phase 1, it was calculated as the ratio of the difference between the influent and effluent concentrations to the influent concentrations. For phase 2, the COD concentrations in the outlet of the fine sieve were taken for the calculation instead of influent concentrations.

$$\text{COD removal efficiency phase1 [\%]} = \frac{(COD_{total,in} - COD_{total,ef})}{COD_{total,in}}$$

$$\text{COD removal efficiency phase2 [\%]} = \frac{(COD_{total,ef,fs} - COD_{total,ef})}{COD_{total,ef,fs}}$$

To compare the COD removal performance with other plants, the volumetric loading rate and the sludge loading rate were calculated as follows:

$$\text{Volumetric loading rate COD [kg COD total/m3/d]} = \frac{\frac{COD_{total,in}}{1000} * Q_{in} * 24}{V}$$

$$\text{sludge loading rate COD [kg COD total/kg MLSS/d]} = \frac{\frac{COD_{total,in}}{1000} * Q_{in} * 24}{m_{s,total}}$$

To evaluate the influence of the oxygen diffusion on the anammox reactor, the oxygen mass was calculated as follows:

$$\text{Oxygen mass [kg O2 /d]} = \frac{\frac{DO_k}{1000} * Q_i * 24}{V_{amx}}$$

To determine the nitrogen conversion, the ammonium consumption (removal) was calculated as follows:

$$\text{Ammonium consumption [\%]} = \frac{c_{NH4-N,in} - c_{NH4-N,ef}}{c_{NH4-N,in}}$$

The nitrite consumption and the nitrate consumption were calculated as the concentration difference between influent and effluent divided by the consumed ammonium:

$$\text{Nitrate/nitrite consumption [\%]} = \frac{c_{i,in} - c_{i,ef}}{c_{NH4-N,in} - c_{NH4-N,ef}}$$

Since the pilot plant was operated with a sludge recirculation in two steps from day 71, the recirculated sludge flow was normally set to three to four times of the inflow rate. Thus, the nitrogen concentrations in the reactors were strongly influenced by the return flow from the MBR. The internal recirculation from the anammox reactor to the nitritation-PN reactor also had a similar impact, although it had a minor flow rate. Given this dilution effect, the nitrogen concentrations could not be directly compared between the individual reactors. Instead, a mass balance was calculated based on a simplified process diagram (Figure 11). The mass of specified nitrogen fractions was calculated as shown below. Due to the continuous measurement error of the total nitrogen (TN) concentration in the wastewater, the TN concentration could not be measured directly. Therefore, TN was calculated as the sum of NH_4-N, NO_3-N, and NO_2-N.

$$m_i \text{ [kg N/d]} = \frac{c_i}{1000} * Q_i * 24$$

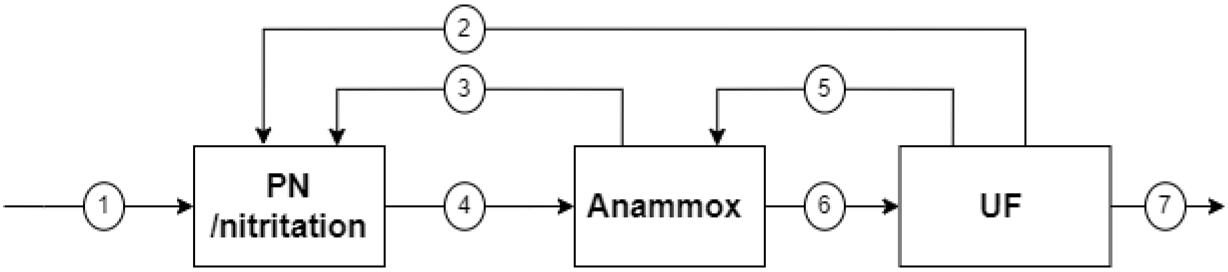


Figure 11 Simplified process diagram for the calculation

Due to the strong dilution effect of fraction concentrations by the recirculated flow, the input mass and output mass were used to calculate the ammonium consumption in a single reactor, for example, in the PN:

$$\text{Ammonium consumption PN [\%]} = \frac{m_{NH4-N,1}+m_{NH4-N,2}+m_{NH4-N,3}-m_{NH4-N,4}}{m_{NH4-N,1}+m_{NH4-N,2}+m_{NH4-N,3}}$$

$$\text{Nitrate/nitrite production PN [\%]} = \frac{m_{i,1}+m_{i,2}+m_{i,3}-m_{i,4}}{m_{NH4-N,1}+m_{NH4-N,2}+m_{NH4-N,3}}$$

To compare the nitrogen removal performance with other plants, the volumetric loading rate, NH4-N removal rate and TN removal rate were calculated as follows:

$$\text{Volumetric loading rate NH4-N [kg NH4-N/m3/d]} = \frac{m_{NH4-N,1}}{V_k}$$

$$\text{NH4-N removal rate [kg NH4-N/kg MLSS/d]} = \frac{\Delta m_{NH4-N,k}}{m_{s,k}}$$

$$\text{TN removal rate [kg N/kg MLSS/d]} = \frac{\Delta m_{TN,k}}{m_{s,k}}$$

Where:

$COD_{total,in}$ = COD concentration in the pilot plant influent [mg/L]
$COD_{total,ef}$ = COD concentration in the pilot plant effluent [mg/L]
$COD_{total,ef,fs}$ = COD concentration in the fine sieve effluent [mg/L]
Q_{in} = inflow rate [m3/h]
V = volume of the pilot plant [m3]
$m_{s,total}$ = total sludge mass in the pilot plant [kg MLSS]
DO_k = dissolved oxygen concentration in the reactor [mg/L]
Q_i = flowrate to the anammox reactor [m3/h]
$c_{i,in}$ = concentration in the pilot plant influent [mg/L]
$c_{i,ef}$ = concentration in the pilot plant effluent [mg/L]
c_i = concentration in the flow (i:1-7) [mg/L]
$Q_{i,j}$ = flow rate related to flow number j, fraction i [m3/h]
$m_{i,j}$ = mass of specified nitrogen fractions related to flow number j, fraction i [kg N/d]
V_k = volume of the reactor k (PN or Anammox reactor) [m3]
$m_{s,k}$ = sludge mass in the reactor k [kg MLSS]
$\Delta m_{i,k}$ = difference of the input mass and output mass related to fraction i [kg/d]

For phase 4, an additional calculation was introduced since TN and TKN were measured analytically. Therefore, an organic fraction of the TN was determined by subtracting NH_4-N, NO_2-N, and NO_3-N.

3 Results

3.1 Performance of the fine sieve

The fine sieve is the first treatment stage of the pilot plant. Coarse particles were intercepted by this screening stage which, on the one hand, protects membranes from fouling and disruption and, on the other hand, reduces the particulate organic material in the wastewater. Since the sampling point in the fine sieve outlet was not available at the beginning of the operation, and the influent data was provided by the WWTP until March 2022, the performance of the fine sieve was evaluated based on data between days 85 and 185. The performance of the fine sieve was evaluated based on the influent COD concentrations measured by WWTP and the weekly average of effluent COD concentrations presented in Figure 12. Due to the different sampling times and data resolution, a direct comparison of concentrations in the fine sieve inlet and outlet was not possible. However, it can be seen from Figure 12 that the majority of influent concentrations were higher than effluent concentrations, indicating proper COD removal by the fine screening stage. It can be quantified by a simultaneous sampling of the influent and effluent, which was performed only occasionally during the entire operation. The existing measurement results show that the fine sieve has a total COD removal efficiency between 30% and 45%.

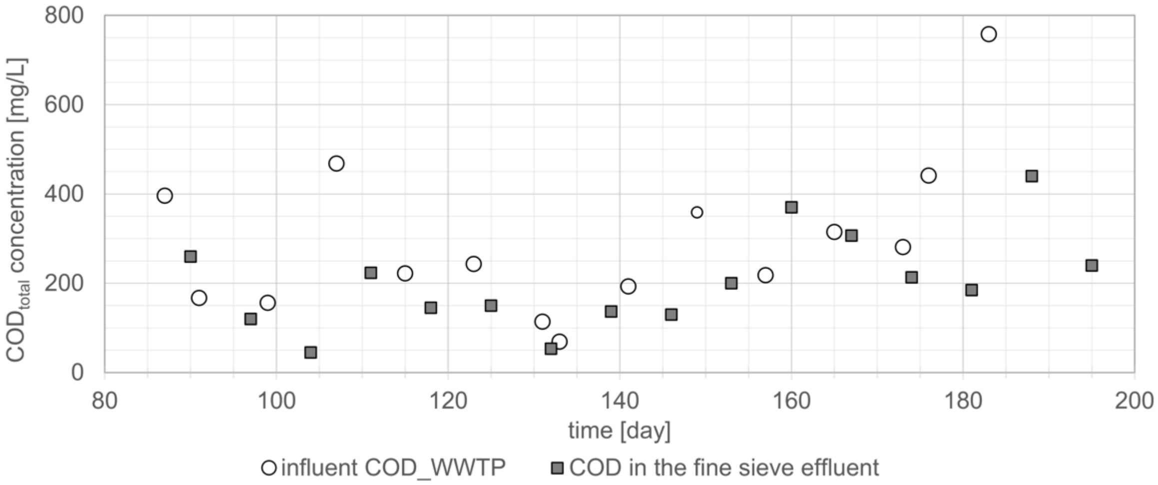


Figure 12 COD_{total} concentrations in the influent and effluent of the fine sieve over time (influent concentrations provided by WWTP and calculated weekly average values as effluent concentrations)

Both COD and ammonium concentrations in the effluent of the fine sieve were subject to strong fluctuations. The C/N ratio is considered one of the most important parameters for the operation of the anammox process; the calculated C/N-ratios ($gCOD_{total}/gN$) in the influent and effluent of the fine sieve are summarized in Figure 13. The C/N ratio in the influent was between 10 and 30, and in the effluent varied from 5 to 30. The mean value of C/N ratios in the fine sieve effluent was 16.92±10.16 $gCOD_{total}/gN$, which was lower than that in the influent with 22.99±19.84 $gCOD_{total}/gN$, suggesting that the particulate COD removal achieved decreased the C/N ratio about 26%.

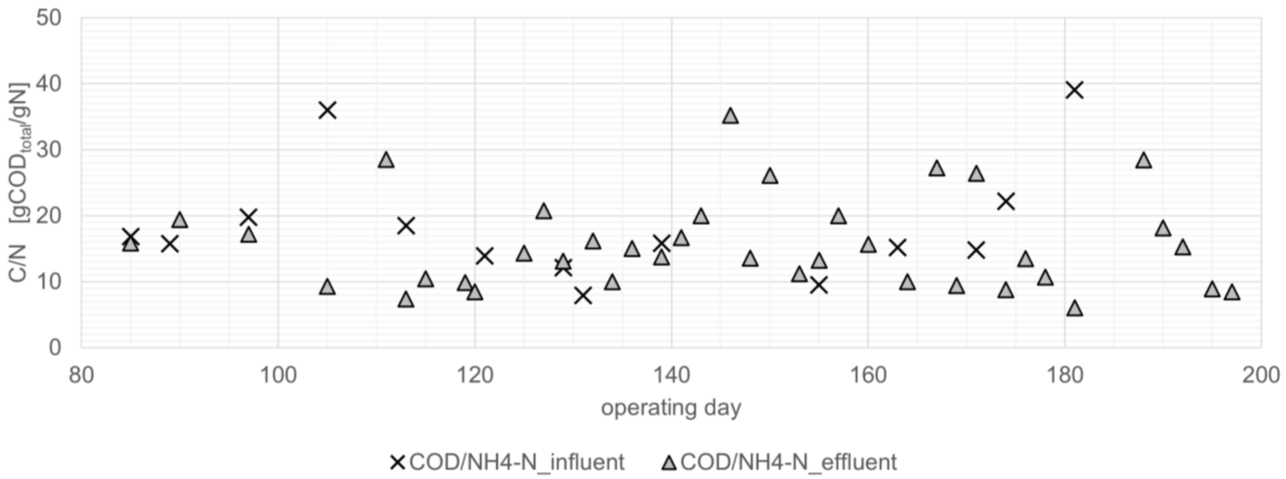


Figure 13 C/N-ratio in the WWTP and effluent of the fine sieve

During phase 4, the COD removal by the fine sieve was about 34±18%, with a maximum of 67%. The mean value of C/N-ratio in the fine sieve effluent was 5.28±2.43 $gCOD_{total}/gN$, about three times lower than observed in phases 1 - 3; and which, in principle, it is more favourable for anammox mainstream application.

3.2 Pilot plant overall COD removal

Due to the later installed sampling point in the fine sieve outlet, the overall pilot plant COD removal efficiency is divided into two phases: phase 1 from day 0 to 85 and phase 2-3 from 85 to 214. The measured COD concentrations and the calculated COD removal efficiency are summarized in Figure 14. From day 21, the COD concentration in the effluent of the pilot plant remained below 30 mg/L and was stable during the operation. The influent concentrations were characterized by significant fluctuations and relatively low values between day 90 and day 155. As the effluent concentrations were maintained at a low level by the membrane filtration, the COD removal efficiency was mainly influenced by the fluctuations of the influent concentrations. On average, the COD removal efficiency was 93±5% in phase 1, and 90±8% in phases 2-3.

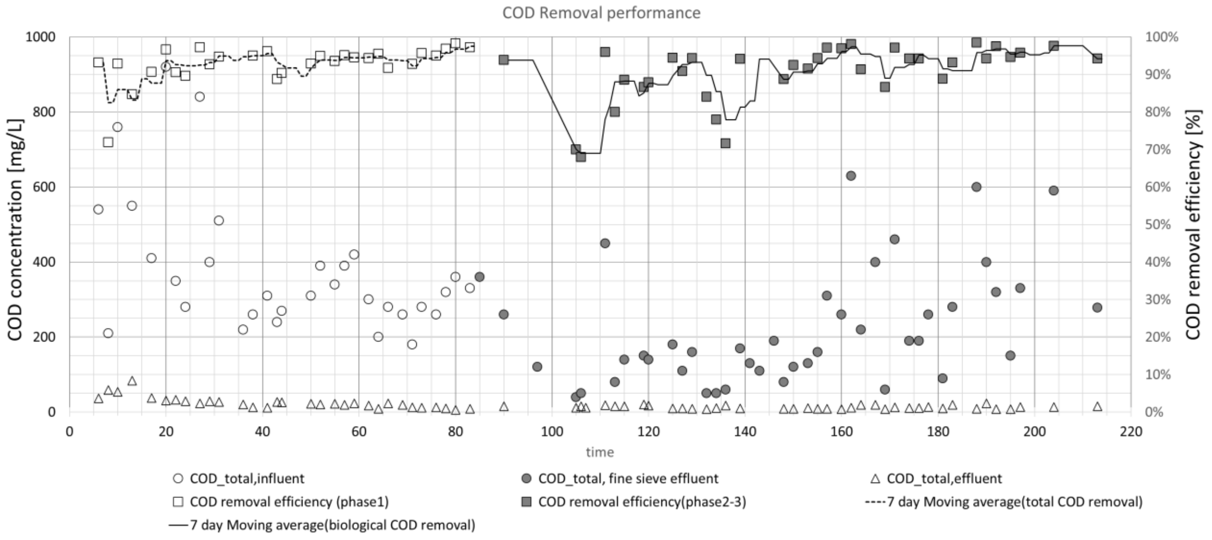


Figure 14 Overall COD removal efficiency of the pilot plant over the experimental period

In phase 4 (0-80 days), after restarting the plant, the COD removal efficiency was about 86.3% (Figure 15).

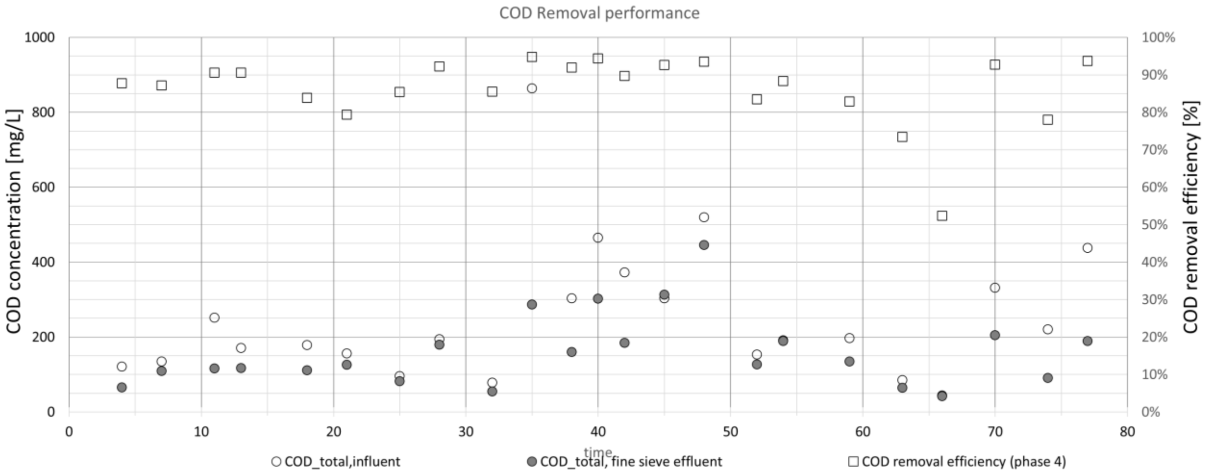


Figure 15 Overall COD removal efficiency of the pilot plant over the experimental period of phase 4.

Although the pilot plant had a high COD removal efficiency of about 90%, the volumetric loading rate was considered to be low, with an average of approximately 0.4 kg $COD_{total}/(m^3*d)$ during phases 2-3 and 0.5 kg $COD_{total}/(m^3*d)$ in phase 4. In Figure 16 and Figure 17, the organic volumetric and sludge loading rates were plotted with the inflow rate. During the period from day 120 to 160, the inflow rate was increased stepwise to adapt to the excess air supply. Despite the increase in the feed, the volumetric loading rate did not increase accordingly. The reason for this was the strong dilution of the influent by rain and snow water. The average sludge loading rate of 0.07±0.05 kg $COD_{total}/(kg$ MLSS*d) during phases 1-3 was in the low range compared to the load of the WWTP (average value during the experimental period: 0.41±0.11 kg $COD_{total}/(kg$ MLSS*d)). In phase 4, the sludge loading rate increased to an average of 0.12±0.08 kg $COD_{total}/(kg$ MLSS*d) mainly because SRT was reduced and, therefore, the sludge concentrations in the reactors.

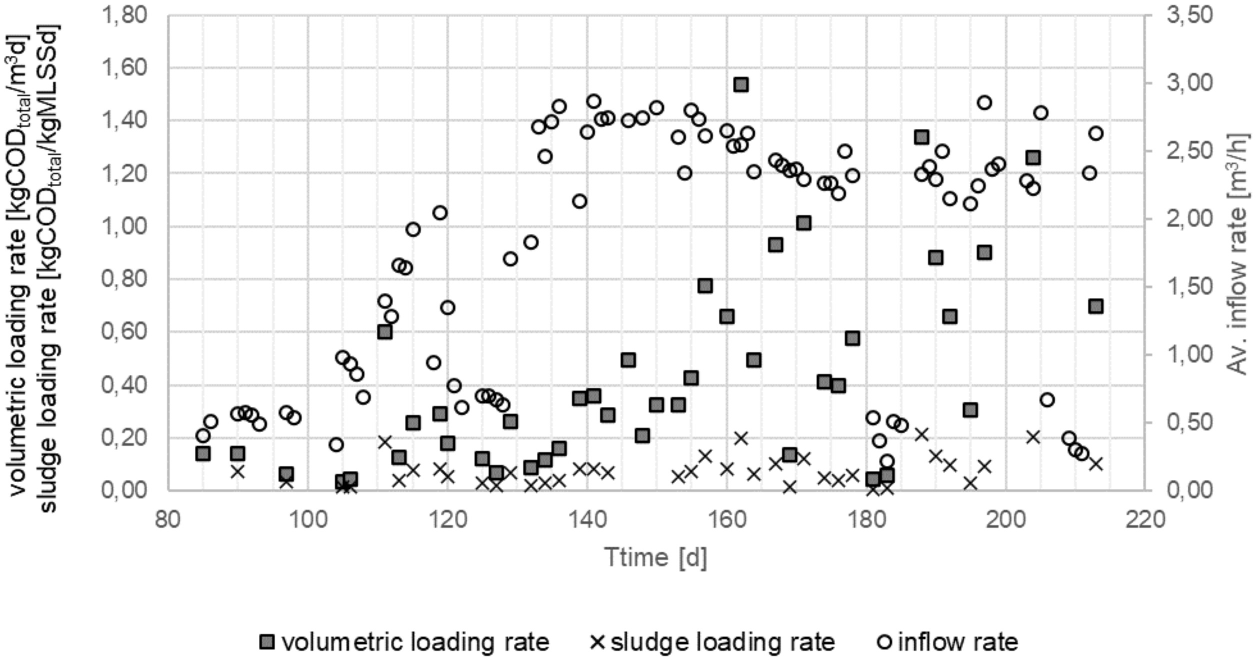


Figure 16 COD volumetric loading rate [kgCOD/m^{3}d], sludge loading rate [kgCOD/kg MLSS d], and the inflow rate of the pilot plant over time (phases 2-3)

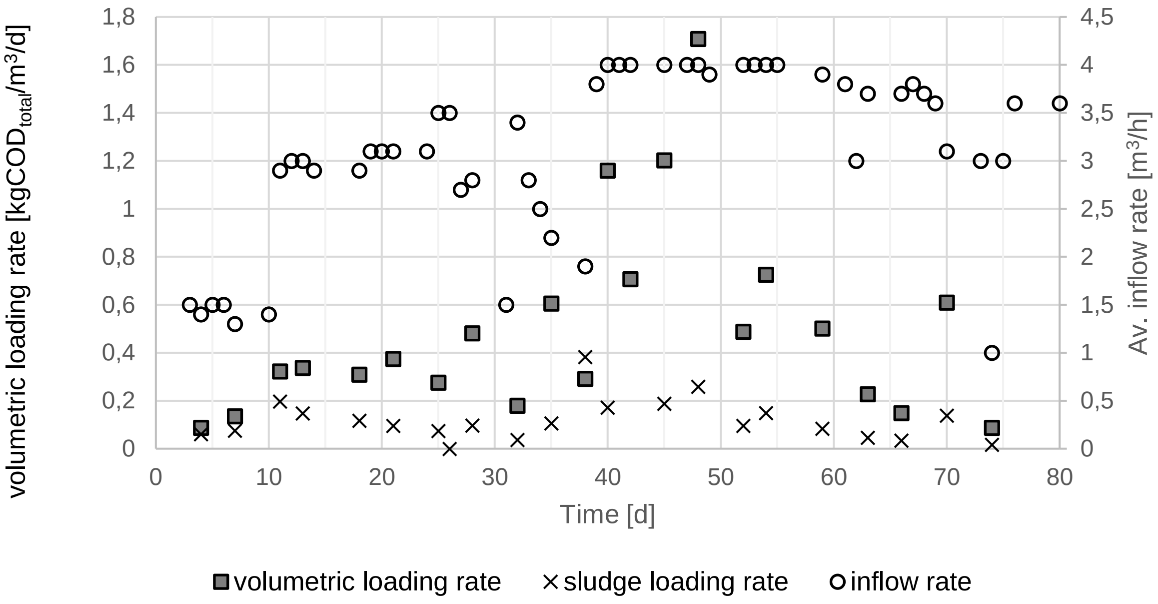


Figure 17 COD volumetric loading rate [kgCOD/m³d], sludge loading rate [kgCOD/kg MLSS d], and the inflow rate of the pilot plant over time (phase 4)

3.3 DO variations in individual reactors

In the PN/A process, the nitrogen removal was strongly related to the DO concentrations in the reactor. Thus, the development of DO concentration in each reactor will be discussed at fist to support the following nitrogen removal evaluation.

Although the DO concentration in the PN reactor was used as a critical control parameter for the operation of this pilot plant, its adjustment was severely limited during most of the experimental period due to the malfunction of the air blower (phase 1 and phase 2) and the inevitable manual setting of the new air blower (at the beginning of phase 3). The air blower failures affected DO concentrations in the anammox reactor and MBR to a different extent. Therefore, the DO variation in each reactor is presented according to the operating phases of the PN reactor combined with the adopted sludge recirculation. The experimental period was divided into phases: phase 1-1 (from day 0 to 44), phase 1-2 (from day 45 to 97), phase 2 (from day 98 to 155) and phase 3 (from day 156 to 214). DO concentrations in PN-, anammox-reactor and MBR as well as the wastewater temperature are shown in Figure 18.

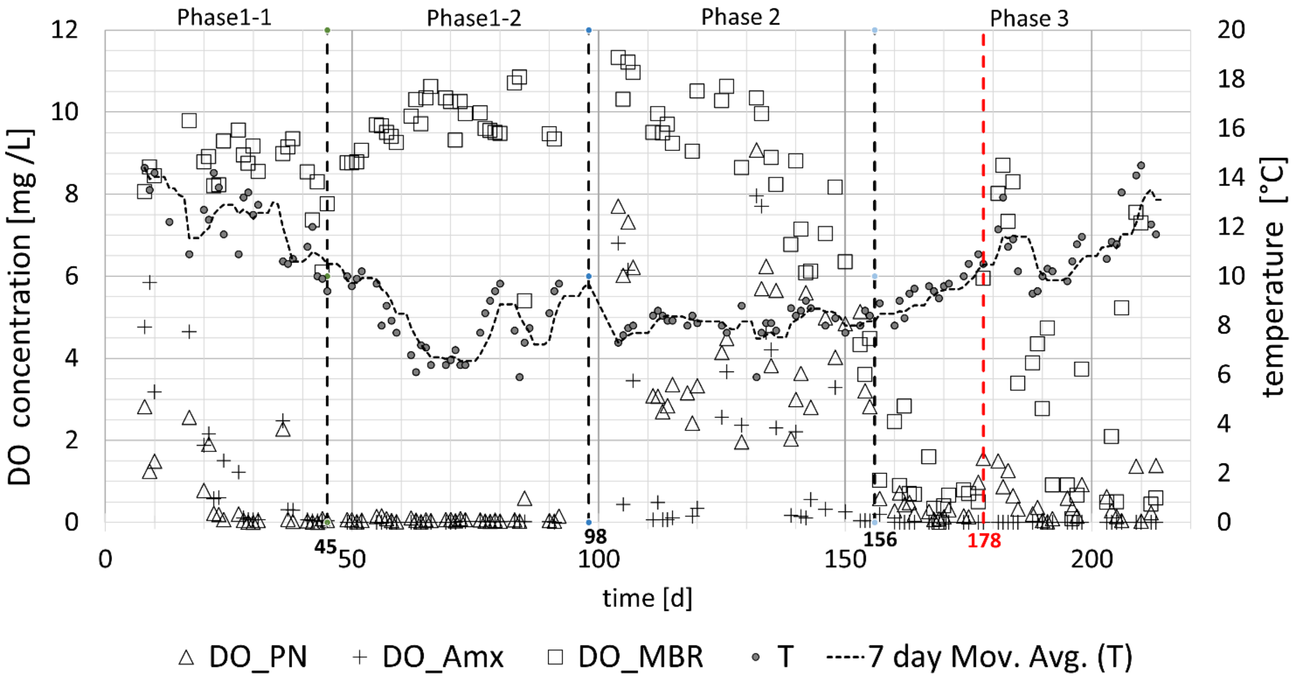


Figure 18 DO concentration in individual reactors and wastewater temperature over the experimental period (phases 1 to 3)

3.3.1 Phase 1-1 and Phase 1-2

During phase 1-1, the DO concentrations in the PN- and anammox reactor decreased as the DO concentration in the MBR remained over 8 mg/L. The gradual decrease of the DO implied biomass growth at the beginning of the operation.

In phase 1-2, the abnormal performance of the air blower resulted in an insufficient air supply to the PN stage, while a fixed airflow had to be supplied to the MBR air scouring system, leading to an average DO concentration in the PN reactor of 0.1 mg/L. In addition, the DO concentration in the anammox reactor was maintained at approximately 0 mg/L.

During phase 1-2, the DO concentration in the MBR first rose and then dropped despite a constant air supply to the MBR. A decreasing trend was observed in temperature at the same time (see Figure 18). The DO concentration in the MBR and the calculated volumetric loading rate of organic matter are shown in Figure 19. Meanwhile, the nitrogen volumetric loading during phase 1-2 proved to be rather stable. However, the impact of sludge recirculation on the DO concentration cannot be excluded. The sludge recirculation was adjusted to supply more oxygen for the PN reactor on day 71, which corresponded to a decrease of the DO concentration in the MBR (Figure 20).

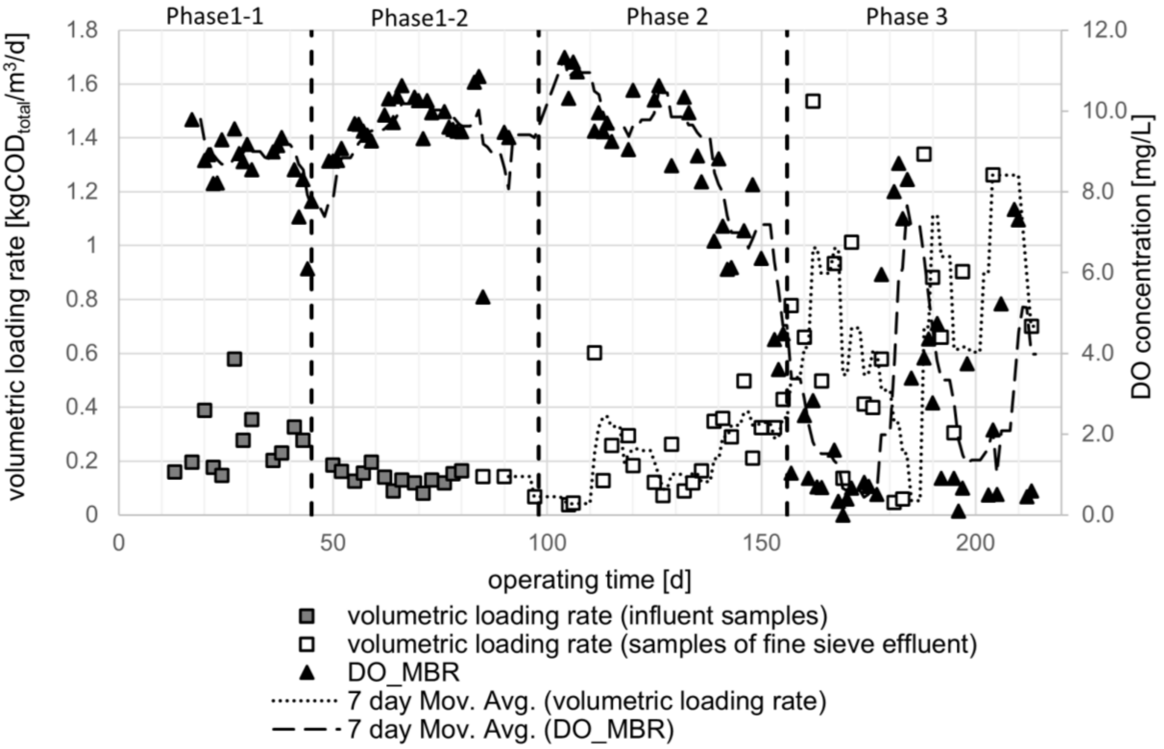


Figure 19 Volumetric loading rate and DO concentrations in the MBR over time

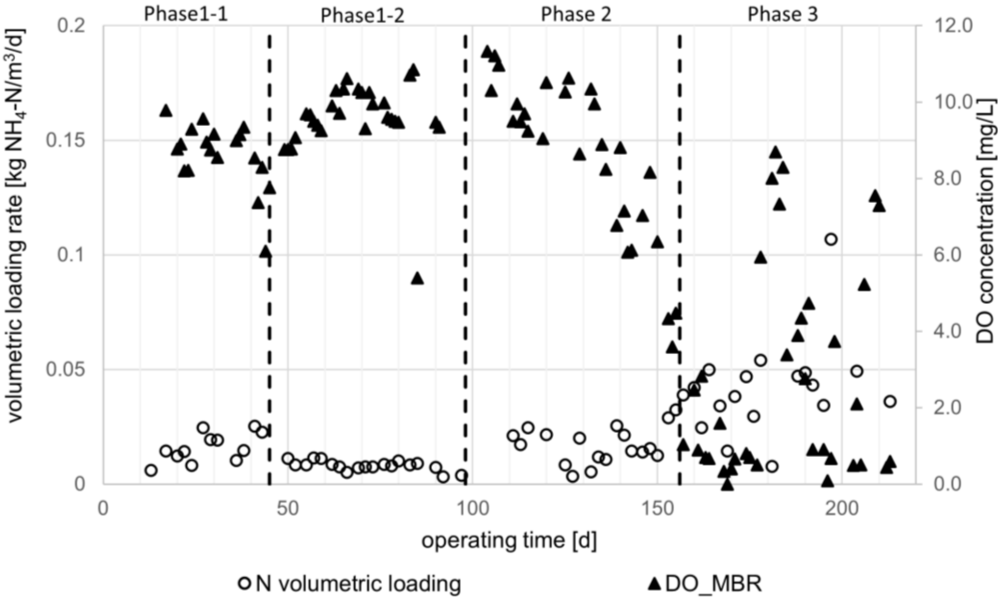


Figure 20 Nitrogen volumetric loading of the pilot plant and DO concentration over time

3.3.2 Phase 2

From day 101, two air blowers with a fixed capacity as temporary substitutes provided the PN reactor and the MBR with an airflow of 60 Nm^3 per hour, respectively, which was higher than the previous air flow rate to the PN reactor (about 16 Nm^3/h) and to the MBR (about 30 Nm^3/h). In order to adapt to the excessive air in the PN reactor, the feed of the pilot plant was stepwise increased to decrease the DO concentration in the PN reactor. However, it decreased only at the beginning of phase 2 and then fluctuated widely between 2 and 6 mg/L (Figure 18). This was attributed to the strong dilution effect by rain and snow water on the one hand, and to the fluctuation of influent concentrations on the other hand, as the sludge concentration in the PN reactor varied between 1.5 and 3.2 g MLSS/L (see Figure 21).

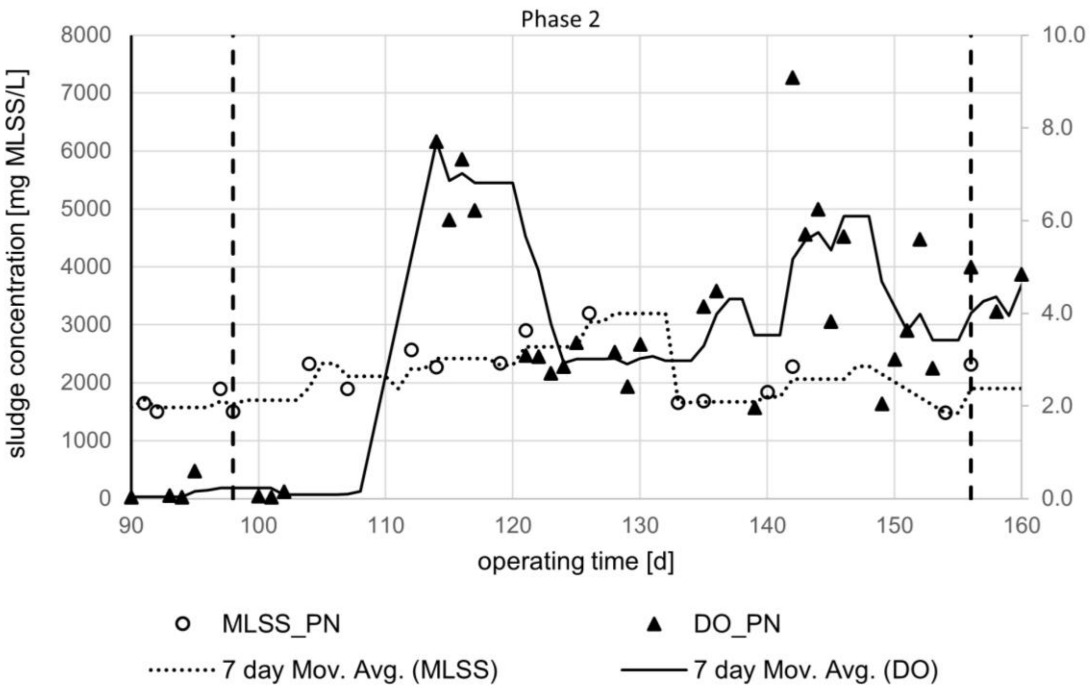


Figure 21 Sludge concentration and DO in the PN reactor during phase 2 over time

During phase 2 a considerable decline of DO concentration in the MBR compared to its relatively stable state in the previous phases was observed. Despite the airflow twice as much as in phase 1-1 and 1-2, the DO concentration in the MBR decreased sharply in phase 2 and, by the end, was below 4 mg/L. As the sludge concentration in the MBR increased substantially, the DO concentration dropped correspondingly (see Figure 22)

A remarkable point is the high DO concentration in the Anammox reactor. Since the anammox reactor was not aerated, the dissolved oxygen originated from the PN or MBR. The high DO concentration in the Anammox reactor was attributed to a combined consequence of the excessive air supply to the PN reactor and the high return flow from the MBR, which was increased accordingly with the feed.

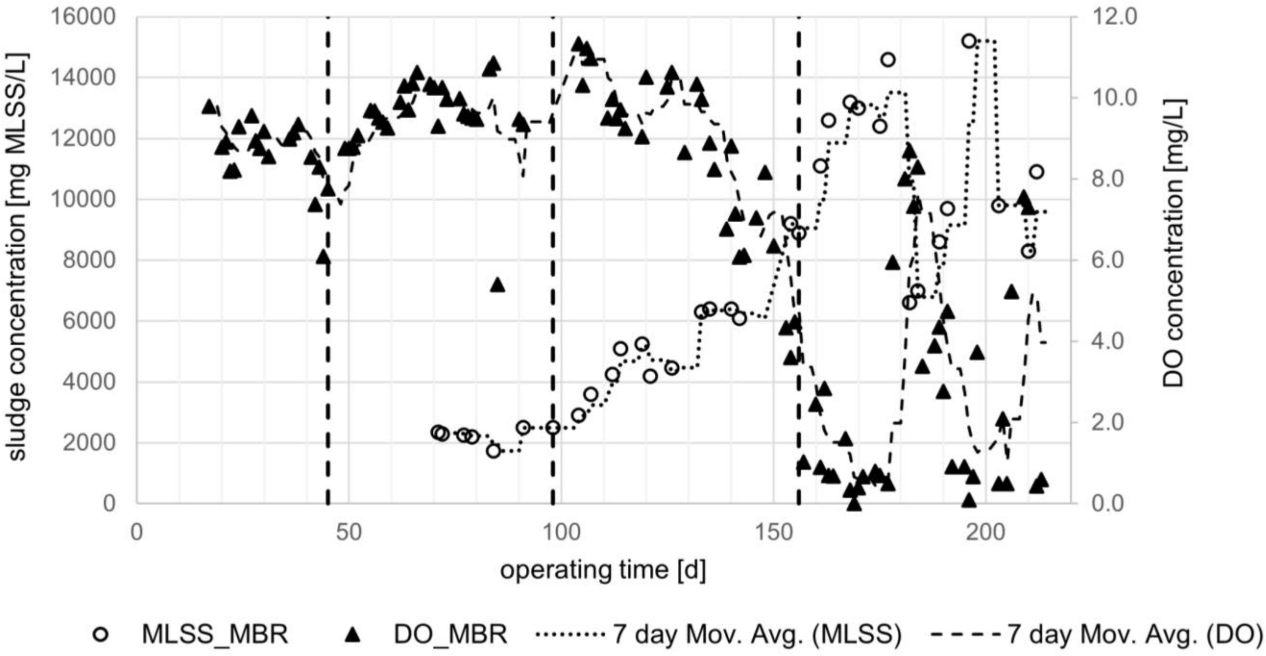


Figure 22 DO and sludge concentration in the MBR during phases 1-3.

3.3.3 Phase 3

On day 156, the new air blower was installed to supply air for the PN reactor, and two air blowers with fixed capacity continued to supply air to MBRs. Between days 156 and 177, the air blower was manually adjusted, leading to diurnal fluctuations below 1 mgO_2/L. From day 178, the air blower was automatically controlled to maintain a set DO concentration. Without an excess air supply to the PN reactor, the DO concentration in the anammox reactor was back to 0 mg/L. In addition, the DO concentration in the MBR was below 3 mg/L until sludge began to be extracted from the pilot plant. As the DO concentration increased when the sludge concentration in the MBR decreased and decreased when it increased, this indicated that the DO variation was dependent on the sludge concentration since biomass consumes oxygen (see Figure 22).

3.3.4 Phase 4

In phase 4, a new DO sensor was installed, and the airflow into the PN reactor was automated based on time in an ON (10 min) / OFF (20 min) mode (see Figure 23).

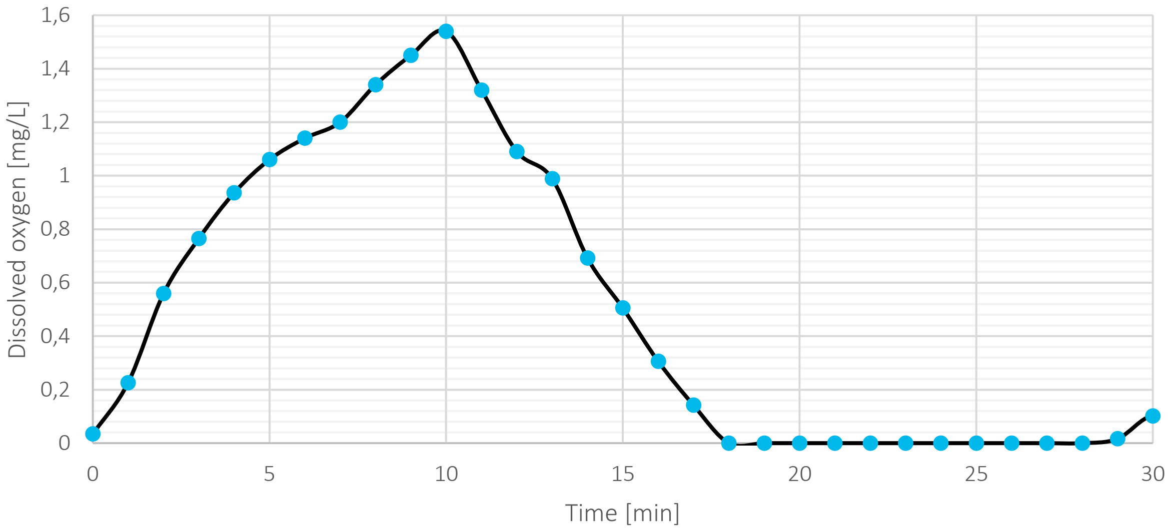


Figure 23 DO On/Off mode in the PN reactor. DO set point was decreased to 0.8 at the end of the operation.

The DO concentration and average temperature in all reactors during phase 4 are presented in Figure 24. The average dissolved oxygen concentration in the partial nitritation reactor was 0.7±1.1 mgO_2/L, 0.25±0.6 mgO_2/L in the anammox and 6.8±2.0 mgO_2/L in the MBR. An average temperature of 13.6±1.6 °C was determined during this phase of operation.

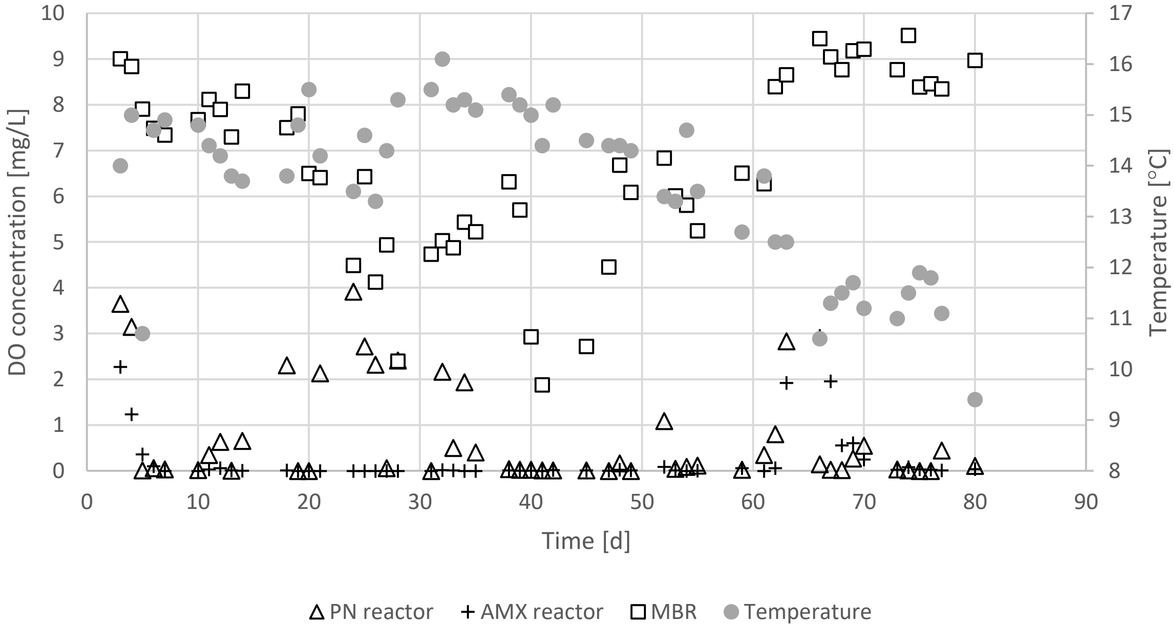


Figure 24 DO concentration in individual reactors during phase 4.

3.4 Nitrogen removal

The weekly average values of measured nitrogen concentrations in the influent and effluent of the pilot plant are presented in Figure 25. It was found that the effluent concentrations were occasionally higher than the influent concentrations. The hydraulic retention time (HRT) of the wastewater in single reactors varied greatly (from 2.3 to 30 hours) in the experimental period due to frequent changes in the feed.

It was found that the nitrite and ammonium concentrations remained at around 0 mg N/L stably during the experimental period except for the period from day 0 to 45 (Figure 26). The low concentrations were attributed to the complete oxidation in the PN reactor or MBR depending on the air supply in different operating phases. Combined with the development of the observed nitrogen species in the effluent, the nitrogen conversion was divided into four phases: phase 1-1 (from day 0 to 44), phase 1-2 (from day 45 to 97), phase 2 (from day 98 to 155) and phase 3 (from day 156 to 214) (see Figure 26). It should be stated that the large data gap between days 139 and 187 was caused by inadequate test kits for nitrate and nitrite measurements.

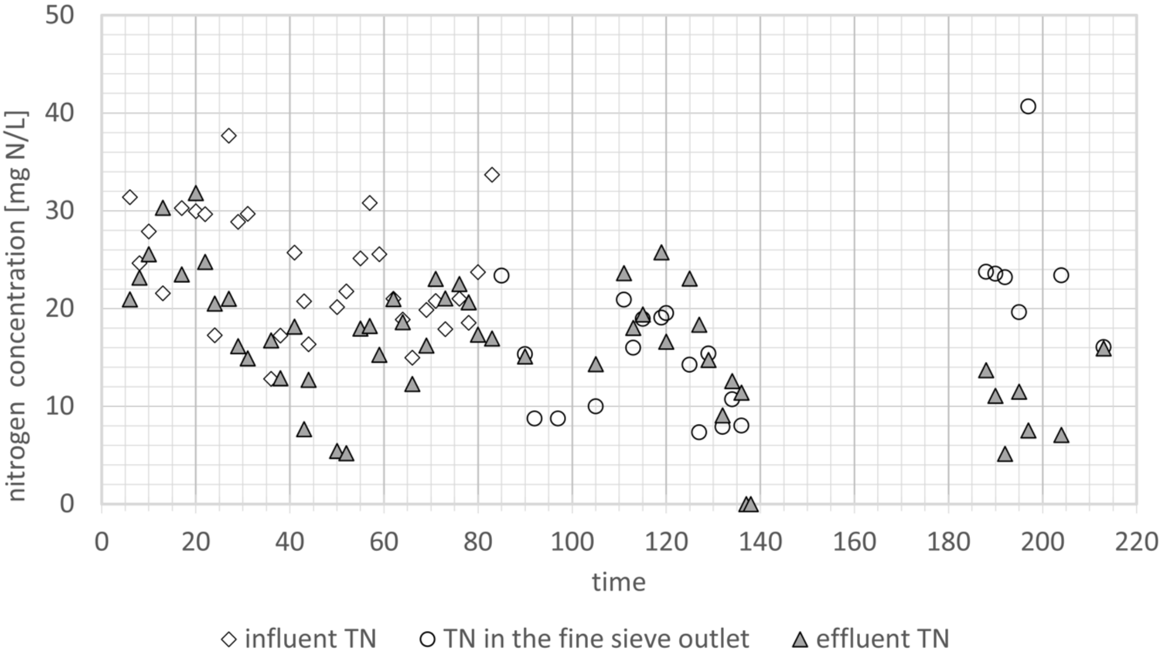


Figure 25 Total nitrogen (TN) concentrations in the influent and effluent (TN as the sum of NH4-N, NO3-N, NO2-N) over time (phases 1-3)

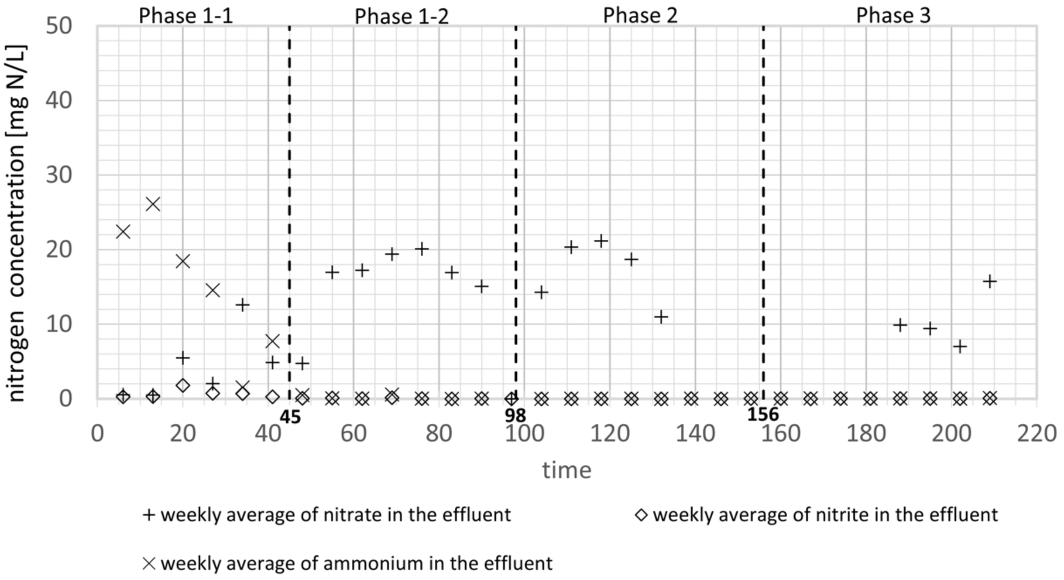


Figure 26 Weekly average of nitrogen concentrations in the effluent over time (phases 1-3)

TN concentrations in the effluent were, on average, 16.4±5.5 mg N/L, which was relatively high compared to the average influent concentrations of 20.78±6.8 mg N/L, suggesting poor nitrogen removal in the pilot plant. Figure 27 shows the weekly average of TN concentrations in the influent and effluent. It was found that the effluent TN concentration decreased gradually during phase 1-1, while the influent concentration fluctuated between 15 and 35 mg/L. In phase 1-2, TN concentrations in the effluent were almost equal to the concentrations in the influent. In phase 2, it was observed that TN concentrations in the effluent were slightly higher than in the influent. In phase 3, there was a significant difference between influent and effluent TN concentrations, indicating nitrogen removal.

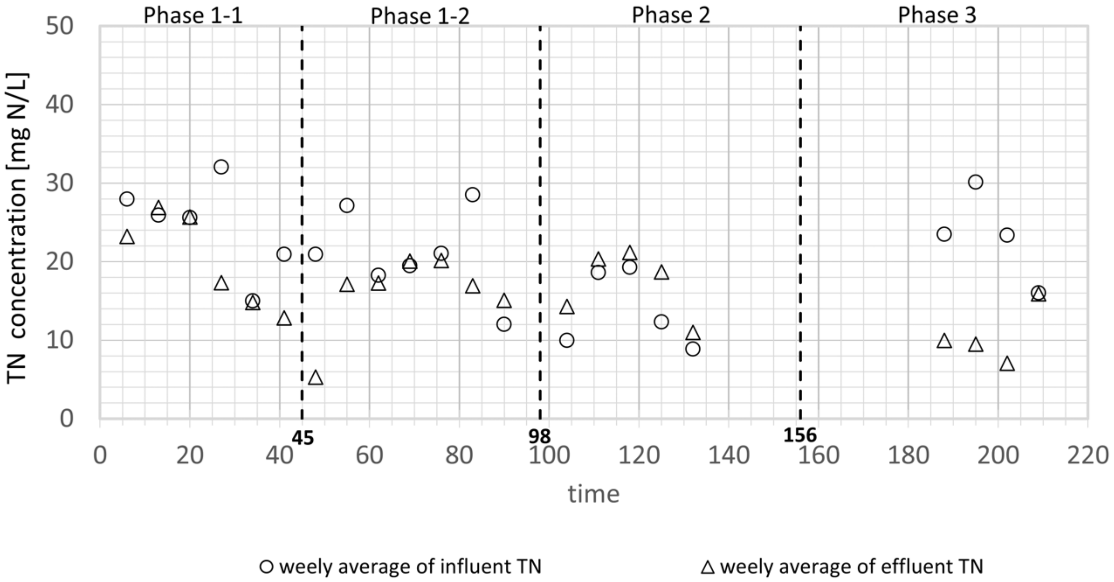


Figure 27 Weekly average of TN concentrations in the influent and effluent (TN as a sum of NH4-N, NO3-N and NO2-N) (phase 1-3)

The NH_4-N volumetric loading rate and NH_4-N specific removal rate of the PN reactor are shown in Figure 28. It was observed that the volumetric loading rate increased over time even after the increase of the feed was stopped on day 132. The volumetric loading rates in phase 3 were substantially higher than in other phases (0.11±0.06 kg NH_4-N/m^3/d). In comparison, the overall average loading rate was lower (0.05±0.05 kg NH_4-N/m^3/d). Similarly to that, NH_4-N removal rates of the PN reactor were also found to be higher in phase 3. However, the overall development of the NH_4-N specific removal rate could not be identified due to the limited data amount. This was because the sludge measurement was only possible until day 71 due to lab limitations, and the removal rate calculation was based on the weekly average. The overall specific NH_4-N removal rate averaged at 0.02±0.01 kg NH_4-N/kg MLSS/d.

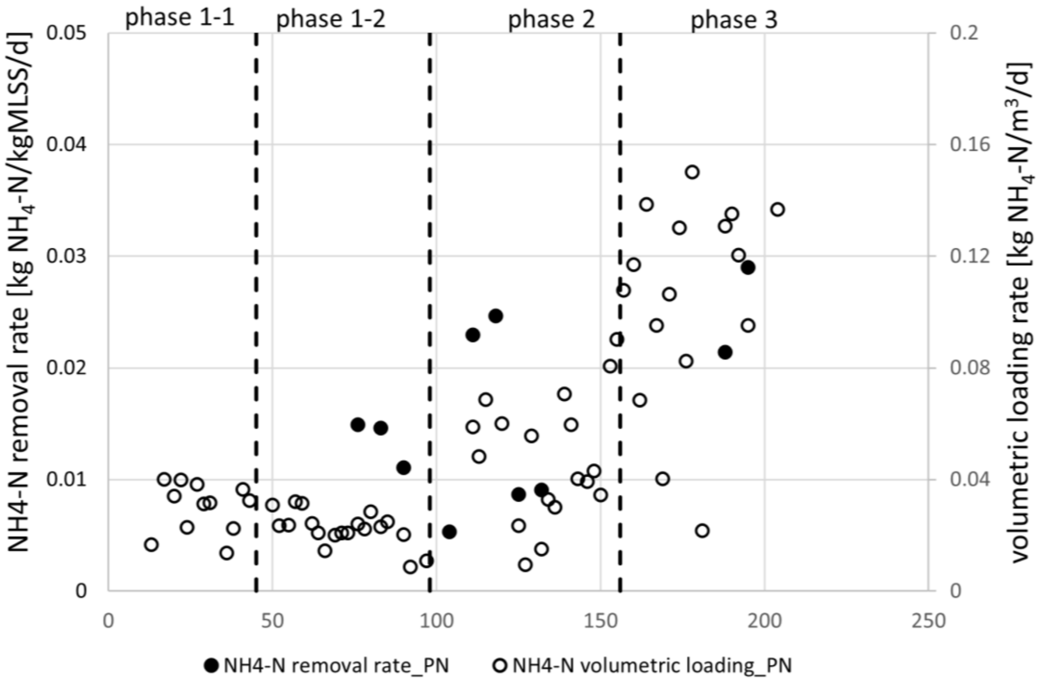


Figure 28 NH_4-N volumetric loading rate and NH_4-N removal rate of the PN reactor (phases 1-3)

3.4.1 Phase 1-1 from day 0 to 44

Figure 29 shows the development of effluent nitrogen concentrations, including NH_4-N, NO_3-N and NO_2-N, from day 0 to 44. Ammonium was observed to be the major part of the effluent nitrogen concentrations. The average ammonium concentration of 15.13±9.22 mg N/L in the effluent was slightly lower than that in the influent of 23.64±5.87 mg N/L. During this phase, the ammonium concentration in the effluent decreased gradually over time while the nitrate concentration increased. During phase 1-1, the TN removal efficiency of the pilot plant varied from 1% to 46%.

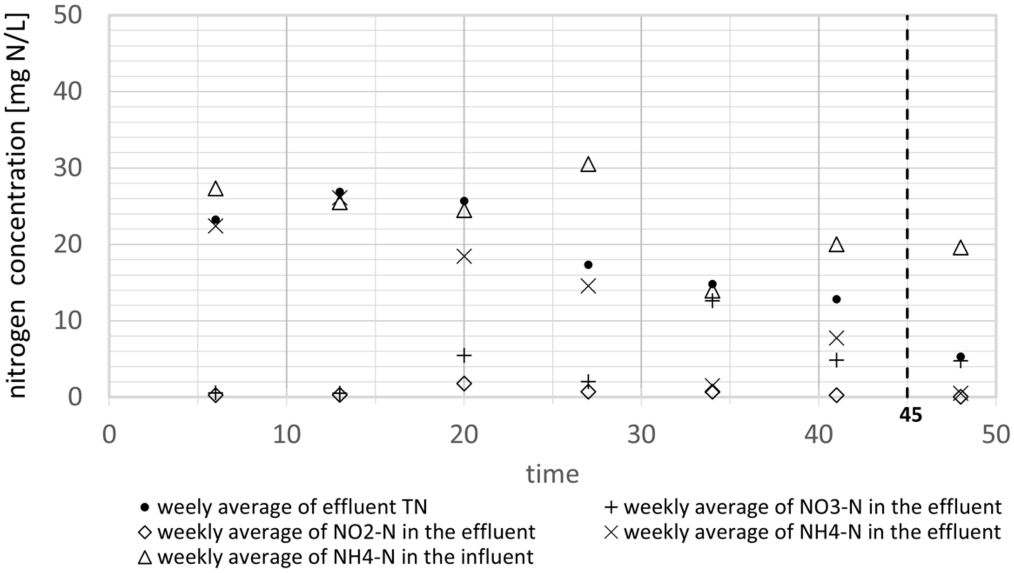


Figure 29 Effluent Nitrogen concentrations during Phase 1-1 (TN as a sum of NH4-N, NO3-N, NO2-N)

The average value of ammonium consumption in phase 1-1 was 41±33%.

3.4.2 Phase 1-2 from day 45 to 97

During phase 1-2, nitrate concentrations in the effluent were relatively high, with an average value of 16.41±5.29 mg/L, while the nitrite and ammonium concentrations were close to 0 mg/L. The effluent's high nitrate concentration was comparable with the TN concentration in the influent (average: 21.9± 4.91 mg/L), indicating that little denitrification occurred.

While the ammonium consumption remained around 100%, the nitrate production increased at first from 20% to 100% and then dropped to 60%. The decrease in nitrate production was concomitant with the adjustment of sludge recirculation on day 71.

Figure 30 shows the mass flow of the pilot plant based on the data obtained in phase 1-2. It was found that the majority of influent ammonium (74%) was consumed in the PN reactor, and 88% of it was oxidized to nitrate despite the low DO concentration of 0.1 mg/L. No nitrite was generated in the PN reactor. A large amount of nitrate entered the anammox reactor via the sludge recirculation from MBR to anammox. In the anammox reactor, a nitrate loss of 0.11 kg NO_3-N/d was calculated (see Table 7), inferring that denitrification took place. Some ammonium was still present in the effluent of the anammox reactor. The remaining ammonium of 0.07kg NH_4-N/d was further oxidized to nitrate in the MBR. Only nitrate and COD were still present in the pilot plant's effluent. The mass balance showed that the pilot plant had an overall COD removal of 3.19 kg COD/d and nitrogen removal of almost zero. The latter is indicated when comparing the ammonium nitrogen in the influent and the nitrate nitrogen in the effluent.

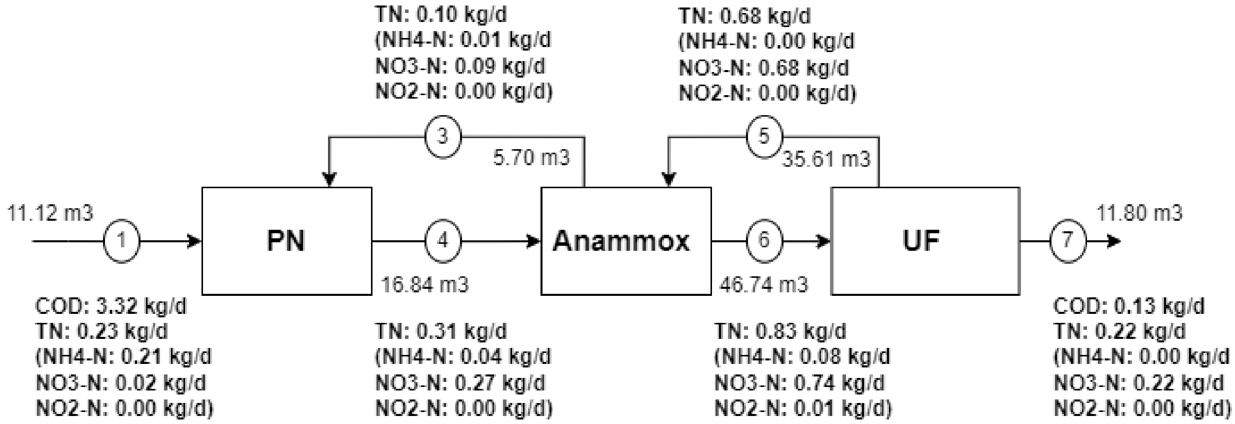


Figure 30. Daily average mass flow of the pilot plant in phase 1-2 (based on the average value from days 71-98) (UF (ultrafiltration) refers to the membrane bioreactor (MBR))

Table 7 Mass balance of the pilot plant in phase 1-2 (based on the average value from day 71-98)

Phase1-2	NH_4-N	NO_3-N	NO_2-N	TN
	[kg/d]	[kg/d]	[kg/d]	[kg/d]
PN	-0.17	0.15	0.00	-0.02
AMX	0.04	-0.11	0.00	-0.06
MBR	-0.07	0.16	0.00	-0.51
overall	-0.2	0.2	0.00	0.00

3.4.3 Phase 2 from day 98 to 155

Combined with the calculated results (Table 8, Figure 31), it was found that most ammonium (92%) was consumed in the PN reactor in this phase. There was still limited nitrite detected in the PN reactor. The other part of ammonium was consumed in the anammox reactor. Since the DO concentration in the anammox during this phase was, on average, 2.03±2.51mg/L, it was uncertain whether the anammox reaction occurred. Combined with the nitrate production at the same time, it is expected that the left ammonium was further oxidized to nitrate in the anammox reactor. A part of nitrate was consumed in the MBR, inferring there was denitrification. Furthermore, the total nitrogen of nitrate in the effluent was found to be higher than in the influent. This was consistent with the observation that the nitrate concentration in the effluent (average value: 17.22±5.14 mg N/L) was higher than the TN concentration in the influent (average value: 9.19±4.49 mg N/L).

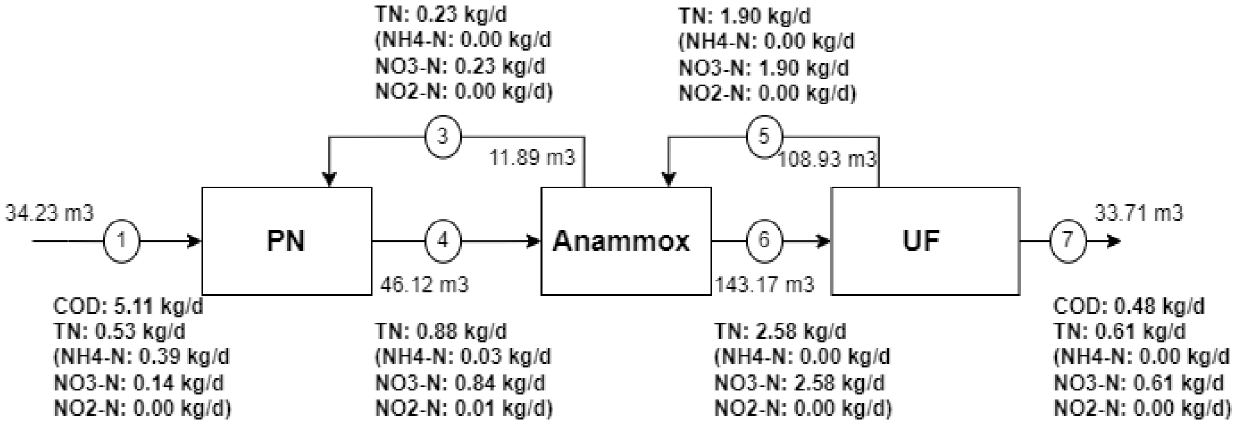


Figure 31 Daily average mass flow of the pilot plant in phase 2 (based on the average value). (UF (ultrafiltration) refers to the membrane bioreactor (MBR))

Table 8 Mass balance of the pilot plant in phase 2 (based on the average value)

Phase1-2	NH_4-N	NO_3-N	NO_2-N	TN
	[kg/d]	[kg/d]	[kg/d]	[kg/d]
PN	-0.36	0.48	0.00	0.12
AMX	-0.03	0.06	0.00	0.03
MBR	0.00	-0.07	0.00	-0.07
overall	-0.38	0.47	0.00	0.08

During Phase 2, it was observed that the sludge concentrations in all reactors increased considerably over time (Figure 32). Surplus sludge was extracted from the pilot plant only from day 168.

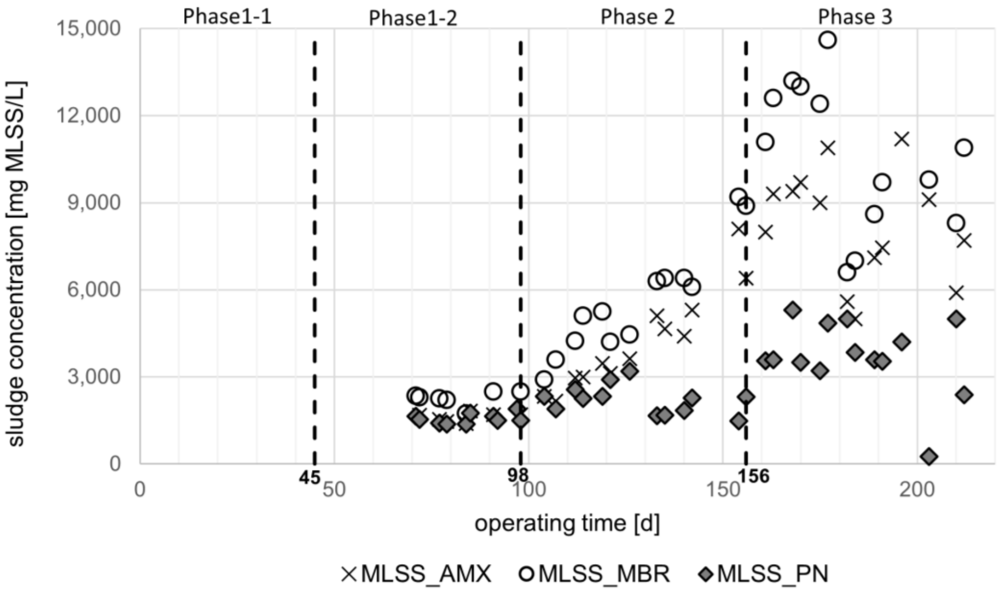


Figure 32 Development of the sludge concentration in the reactors with time

3.4.4 Phase 3 from day 156 to 214

The pilot plant was operated relatively stable from day 188 for three weeks, which allowed three sets of data for the evaluation. Figure 33 shows the mass flow of the pilot plant based on the data set between days 195 and 201. The results are listed in Table 9. It was found that the ammonium nitrogen decreased step by step as the wastewater flowed through the pilot plant. It was found that 62% of influent ammonium was consumed in the PN reactor, and 42% of consumed ammonium was oxidized to nitrate. No significant nitrite generation was identified in the PN reactor in terms of the low nitrite mass in the effluent of the PN reactor. 38% of the influent ammonium remained in the effluent of the PN reactor and entered the anammox reactor. A high amount of nitrate entered the anammox reactor via the recirculation from the MBR. In the anammox reactor, a nitrogen loss of 1.15 kg N/d and an ammonium loss of 0.22 kg N/d were determined. The effluent of the anammox reactor still contained 0.46 kg N/d of ammonium, 0.02 kg N/d of nitrite and 1.98 kg N/d of nitrate. The ammonium and nitrite were oxidized to nitrate in the MBR. The mass balance based on the data from 195 to 201 showed a COD decrease of 14.45 kg COD/d (96% COD removal) and a nitrogen decrease of 1.34 kg N/d (71% nitrogen removal) in the pilot plant.

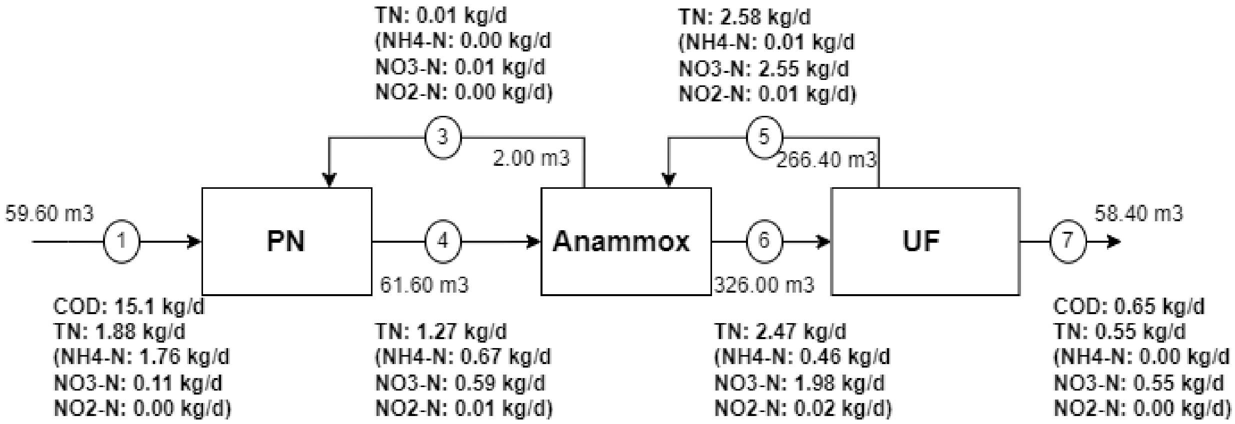


Figure 33 Daily average mass flow of the pilot plant in phase 3 (based on the data from day 195 to 201). (UF (ultrafiltration) refers to the membrane bioreactor (MBR))

Table 9 Mass balance of the pilot plant in phase 3 (based on the data from day 195 to 201)

Phase 3	NH_4-N	NO_3-N	NO_2-N	TN
	[kg/d]	[kg/d]	[kg/d]	[kg/d]
PN	-1.10	0.47	0.01	-0.62
AMX	-0.22	-1.15	0.00	-1.37
MBR	-0.45	1.12	-0.01	0.66
overall	-1.76	0.43	0.00	-1.32

The results of the mass balance for the PN reactor and anammox reactor were summarized in Table 10. In the PN reactor, ammonium consumption varied between 59% and 75%, while nitrate production was around 40%. The nitrite production was 1%, indicating no significant nitritation occurred in the PN reactor. Approximately 40% of input ammonium (mass flow 4) and 30% of input nitrate were consumed in the anammox reactor, indicating simultaneous denitrification and anammox, or simulataneous nitrification/denitrification.

Table 10 Mass balance for the PN-reactor in phase 3 (based on the data from day 188 to 208)

Nitritation/ PN reactor							
Day	NH_4-N	NO_3-N	NO_2-N	TN	ammonium consumption*	nitrate production*	nitrite production*
	[kg/d]	[kg/d]	[kg/d]	[kg/d]	[%]	[%]	[%]
188-194	-0.69	0.29	0.01	-0.39	59	42	1
195-201	-1.10	0.47	0.01	-0.62	62	42	1
202-208	-0.93	0.41	0.01	-0.51	75	44	1
Anammox reactor							
Day	NH_4-N	NO_3-N	NO_2-N	TN	ammonium consumption*	nitrate consumption*	nitrite production*
	[kg/d]	[kg/d]	[kg/d]	[kg/d]	[%]	[%]	[%]
188-194	-0.24	-0.73	0.00	-0.98	49	26	0
195-201	-0.22	-1.15	0.00	-1.37	32	37	0
202-208	-0.11	-0.74	0.01	-0.84	37	33	10

* *Calculation based on the mass fraction*

The nitrogen loading rate and removal rate were calculated and presented in Table 11. The specific NH_4-N removal rate in the PN reactor was, on average, 0.025 kgNH_4-N/kg MLSS/d, while it was 0.0035 kgNH_4-N/kg MLSS/d in the anammox reactor, which is in accordance with the low loading rate in the anammox (average: 0.006 kgNH_4-N/m^3/d). In comparison, the TN removal rate in the anammox reactor was significantly higher than the NH_4-N removal rate, indicating denitrification.

Table 11 Nitrogen loading rate and removal rate of the PN/Anammox reactor in Phase 3

PN reactor				
day	NH_4-N volumetric loading rate	NH_4-N volumetric removal rate	NH_4-N specific removal rate	TN removal rate
	[kgNH_4-N/m^3/d]	[kgNH_4-N/m^3/d]	[kgNH_4-N/kg MLSS/d]	[kg N/kg MLSS/d]
188-194	0.130	0.077	0.021	0.012
195-201	0.196	0.122	0.029	0.016
202-208	0.140	0.103	-*	-*
**Operational problems led to extremely low MLSS in the PN reactor*				
Anammox reactor				
day	NH_4-N volumetric loading rate	NH_4-N volumetric removal rate	NH_4-N specific removal rate	TN removal rate
	[kgNH_4-N/m^3/d]	[kgNH_4-N/m^3/d]	[kgNH_4-N/kg MLSS/d]	[kg N/kg MLSS/d]
188-194	0.055	0.027	0.004	0.015
195-201	0.076	0.024	0.002	0.014

202-208	0.035	0.013	0.001	0.010

3.4.5 Phase 4

Because mechanical and loading issues, the pilot plant was operated still without reaching the desired stability with an average higher volumetric load. In some days the load was very diluted, and therefore the organic/ammonium load was not that high.
The daily average mass flow is shown in Figure 34. From the average overall balance, it can be inferred that COD, TKN, NH4-N, NO3-N and NO2-N were removed by about 91%, 98%, 98%, -462% and 90%, correspondingly. The latter means that denitrification/anammox were poorly achieved in the pilot plant since a significantly higher amount of nitrate was in the effluent.
The mass balance separately on the partial nitritation and anammox reactors, which are the core of the process, is shown in Figure 35 to be observed more clearly which processes were likely to occur in each.

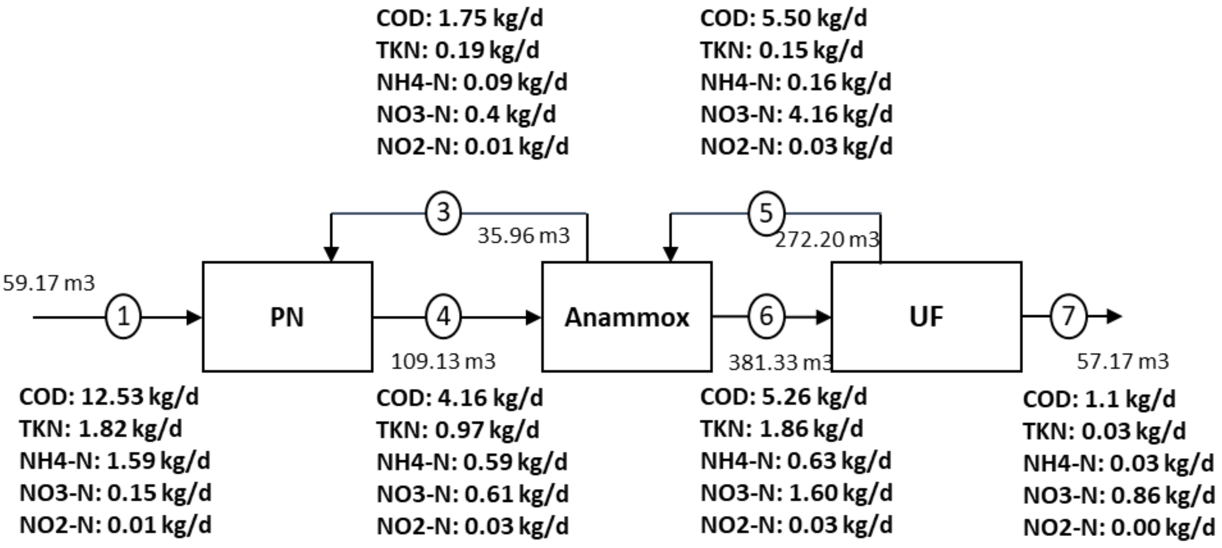
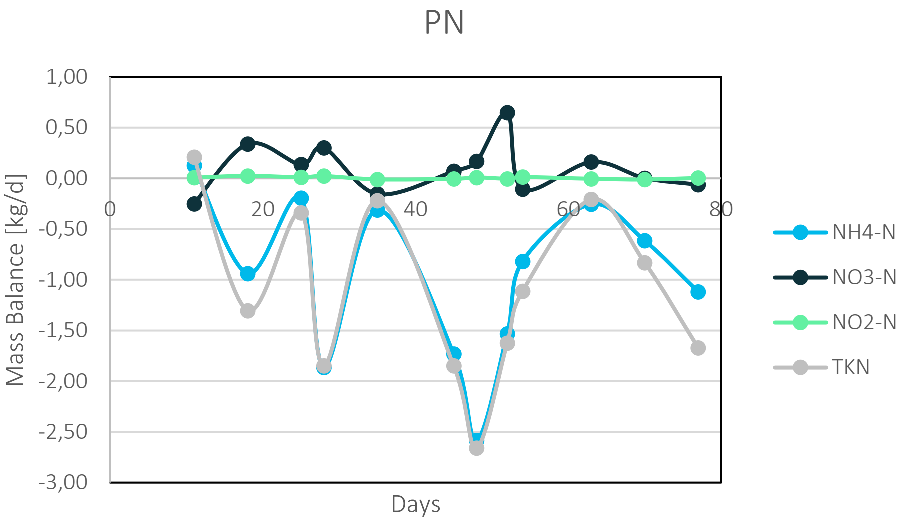


Figure 34 Daily average mass flow of the pilot plant in phase 4. (In the figure UF (ultrafiltration) refers to the membrane bioreactor (MBR))

According to the results in the PN reactor (Figure 35 A), in most of the dates, NH4-N was consumed (21-97%) with a daily average mass balance of 0.99 kg/d, and nitrate was produced (even though in 4 days consumption was observed, indicating denitrification). A similar trend to NH4-N was observed for TKN. However, NO2-N was not determined to be higher than 0.0025 kg/d. The latter indicated that NH4-N was converted to NO3-N and N2 via nitrification/denitrification in the PN reactor, and significant production of NO2-N was never achieved with the pilot conditions, suggesting that nitrite oxidizing bacteria (NOB) were highly active and dominant and not suppressed despite the reduction of SRT and DO. The prevalence of AOB would have resulted in a positive mass flow of NO2-N in the PN reactor. The On-Off DO control in the PN was not enough to promote the NO2-N build-up and prevent denitrification due to the high COD available.

A.



B.

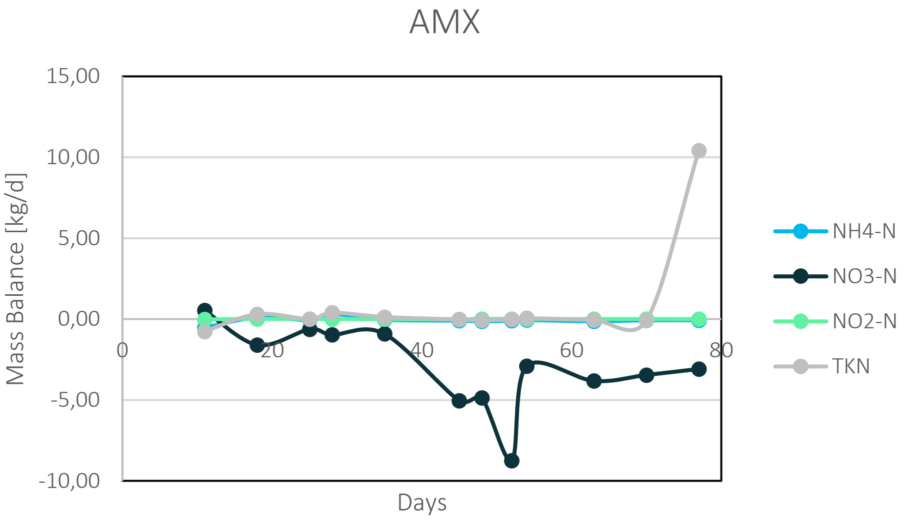


Figure 35 Partial NItritation and Anammox reactor mass balances. Negative values indicate consumption (removal), and positive values indicate production/increase of each of the N-compounds within the different days.

In the case of the anammox reactor, the very low consumption of NH4-N (0.08 – 0.053 kg/d) and NO2-N (0.00-0.02 kg/d) suggested that anammox activity was not present. Nitrate was consumed, inferring denitrification was taking place in this stage of the process. At the end of the operation, a large amount of TKN was determined, even though NH4-N did not show the same trend, inferring a high concentration of organic nitrogen entered the plant.

An overall balance of the pilot plant of specific days is presented in
Table 12.

Table 12 Mass balance for the PN, AMX, UF(MBR) reactors and overall pilot plant in phase 4 (specific days)

Day	Phase 4	NH_4-N	NO_3-N	NO_2-N	TKN	NH_4-N consumption*	NO_3-N production/consumption *	NO_2-N production/consumption
		[kg/d]	[kg/d]	[kg/d]	[kg/d]	[%]	[%]	[%]
18	PN	-0,94	0,34	0,03	-1,30	71%	36%	3%
	AMX	0,10	-1,60	0,01	0,30	22%	29%	17%
	MBR	-0,28	2,13	-0,05	-0,48	69%	73%	95%
	overall	-1,12	0,87	-0,02	-1,48	97%	-248%	96%
28	PN	-1,86	0,30	0,02	-1,85	88%	16%	1%
	AMX	0,31	-0,97	0,00	0,39	84%	47%	0%
	MBR	-0,14	1,13	-0,04	-0,26	46%	107%	72%
	overall	-1,69	0,46	-0,01	-1,72	98%	-387%	77%
45	PN	-1,73	0,07	-0,01	-1,85	47%	4%	0%
	AMX	-0,10	-5,05	-0,02	-0,02	4%	63%	44%
	MBR	-1,63	6,36	0,02	-1,98	79%	265%	78%
	overall	-3,46	1,38	-0,01	-3,85	98%	-773%	55%
52	PN	-1,53	0,65	0,00	-1,62	58%	42%	0%
	AMX	-0,09	-8,74	0,01	-0,02	7%	67%	28%
	MBR	-0,95	10,01	-0,02	-1,25	82%	275%	84%
	overall	-2,58	1,92	-0,02	-2,90	99%	-1062%	96%
63	PN	-0,25	0,16	0,00	-0,21	87%	63%	2%
	AMX	-0,13	-3,83	0,00	-0,06	63%	67%	2%
	MBR	0,13	4,14	0,00	-0,13	203%	274%	53%
	overall	-0,25	0,48	-0,01	-0,40	91%	-181%	94%
77	PN	-1,12	-0,06	0,00	-1,67	62%	6%	0%
	AMX	-0,07	-3,09	0,00	10,41	9%	65%	6%
	MBR	-0,55	4,11	-0,01	-10,92	77%	306%	61%
	overall	-1,73	0,96	-0,01	-2,18	98%	-1776%	95%

3.5 Extraction of surplus sludge

During the extraction of surplus sludge, it was observed that a large number of granules were intercepted by the 1mm fine sieve (see Figure 36 A). The majority of granules were brownish and small fractions were black. The intercepted granules proved that the fine sieve selectively retained bigger biomass, potentially anammox bacteria, in the system. The brownish colour could indicate anammox as well.

On day 196, the granules of 5 L surplus sludge were retained using 1 mm fine sieve and used to determine the concentration of the potentially anammox biomass (Figure 36 B). The measured value was 0.024 g MLSS /L. It was found some of the retained granules were black and therefore did not belong to anammox bacteria. The anammox granule concentration in the surplus sludge is at least lower than 0.024 g MLSS /L.

Figure 36 A. Retention of the anammox granules by the fine sieve 1mm. B. Determination of anammox granule concentration on day 196

In phase 4, the sieved surplus activated sludge did not contain many granules, which was not understood by the operators. The granules from the separated WAS (waste activated sludge) were defined as less than 0.1% by volume. Granules size of less than 1 mm could have been the reason, but different than expected, the sieving of surplus sludge was not effective in retaining the granules during this phase.

4 Discussion

4.1 Performance of the pilot plant

Due to various incidents, the operation of the pilot plant was interrupted multiple times, so the performance of the pilot plant was impacted substantially.

4.1.1 COD removal performance

The pilot plant showed a stable COD removal efficiency of over 90% during the experimental period. The average value of COD concentration in the effluent was 14.75±5.82 mg/L, which was far below the required COD value of 90 mg/L in the effluent of the WWTP (AbwV, 2004). The good effluent quality was attributed to the application of the ultrafiltration hollow fiber (HF) membranes. However, the COD sludge loading rate of 0.07±0.05 kg COD_{total}/ (kg MLSS*d) was relatively low in comparison to that of the WWTP (0.41±0.11 kg COD_{total}/ (kg MLSS*d)). This is due to the strong dilution of the influent by rains, and the increase of the inflow rate during the experimental period was strictly limited: first due to the clogging of the fine sieve (from day 119 to 125), then due to the aggregated consequence of the fine sieve clogging, the limited membrane surface area (one permeate pump was broken) and abnormal feed pump performance (from 178 to 214). The original target inflow rate was 4.2 m^3/h and then had to be reduced to 3 m^3/h due to the limitations described. On the other hand, the surplus sludge was extracted from the pilot plant until day 168 of phase 3, leading to high sludge concentrations in reactors. As the influent concentrations cannot be influenced, the COD sludge loading can be improved in the future by controlling the sludge retention time with the extraction of surplus sludge. However, the increase of the volumetric loading can only be achieved by increasing the feed to the pilot plant.

4.1.2 Nitrogen removal performance

Due to the various operation problems, the aeration of the pilot plant was under automatic control only from day 178. During the experimental period, no significant nitrite generation in PN was observed, questioning whether there is anammox activity in the anammox reactor. The nitrogen removal of the pilot plant is discussed by phase as follows.

Phase 1-1

In phase 1-1, the nitrogen conversion was incomplete, as indicated by the presence of ammonium in the plant effluent, despite the high DO concentration of more than 8 mg/L in the MBR. Considering the constant air supply and high DO concentration, the incomplete nitrogen conversion was attributed to the low sludge seed amount in the pilot plant at the start-up. During this phase, nitrate production rose with ammonium consumption, indicating an increasing nitrogen conversion over time. This was consistent with biomass growth over time. During this phase, the nitrogen removal efficiency varied from 1% to 46%.

Phase 1-2

In phase1-2, the pilot plant had high nitrate concentrations and a rather low nitrite and ammonium concentrations in the effluent. The ammonium consumption of the pilot plant was increased from 41±33% (phase 1-1) to 99±1% (phase 1-2), indicating a complete ammonium conversion in phase 1-2. Despite the stable ammonium consumption during 1-2, nitrate production first increased (days 45-70) and then decreased (days 70-98). The change in nitrate production could be caused by the adjustment of the sludge recirculation. From day 45 to 70, sludge was recirculated from the MBR to the PN reactor to provide more oxygen to the PN reactor due to the low DO concentration of around 0.1 mg O_2/L. After the adjustment on day 45, it was observed that nitrate production increased substantially. Between day 45 and day 70, the nitrogen removal efficiency varied between 1% and 73%.

On day 71, sludge was returned from the MBR to the anammox reactor and from the anammox reactor to the PN reactor, respectively, to increase DO concentration in the PN reactor under air supply limitation. After that, it was found that the nitrate production rate decreased again. However, an increase in the DO concentration was not observed in the PN reactor. The mass balance showed that 74% of influent ammonium was consumed in the PN reactor, and 88% of it was oxidized to nitrate. Considering the low concentration of 0.1 mg NO_2/L and the nitrate production of 88%, it was unlikely that anammox activity had already occurred in the PN reactor. Furthermore, a nitrate loss of 0.11 kg NO_3-N/d was identified in the anammox reactor. Since no ammonium loss occurred at the same time, the nitrate loss was attributed to denitrification. Considering the effluent's remaining nitrate, the denitrification process did not completely consume the available nitrate. This could be attributed to an insufficient supply of organic matter. In addition, the mass balance showed that no total nitrogen was removed (considered as the addition of NH_4-N, NO_2-N, NO_3-N), which is not consistent with the identified denitrification. This was probably because organic nitrogen was present in the influent in the form of proteins that were ammonified or the influence of leachate discharge in the plant, and this nitrogen is not considered in the calculations. The presence of organic nitrogen in the influent could lead to a higher TN concentration in the effluent (mostly nitrate) than in the influent. The actual nitrogen removal could not be determined without measuring TN concentration in the influent.

Phase 2

Similar to phase 1-2, it was found that effluent nitrate concentrations were higher than the influent TN concentrations. The mass balance showed that more ammonium (92%) was consumed in the PN reactor in this phase than in Phase 1-2 (74%). The remaining ammonium was oxidized in the anammox reactor due to the high DO concentration of 2.03±2.51mg/L, which was even higher than the DO concentration in the PN reactor during phase 1-1 and 1-2. In addition, the excess air supply to the PN reactor led to a strong growth of microorganisms, indicated by the rising sludge concentrations in all reactors from day 98. While the sludge concentration in the MBR increased significantly, the DO concentration decreased accordingly. As the DO concentration in the reactor was over 2 mg/L, it was expected that the NOBs would be the dominant microorganism group. Moreover, the mass balance indicated a nitrate loss of 0.07 kg N/d in the MBR. Although the oxygen in the MBR was severely depleted over time, the DO concentration dropped to 4 mg/L by the end of phase 2. It was unlikely that substantial denitrification or anammox reaction, both of which require anoxic conditions, occurred in the MBR.

Phase 3

From day 178, the air supply to the PN reactor was regulated by the online detected DO concentration. Due to the absent test kits for nitrite and nitrate measurement, the evaluation of the pilot plant was not possible until day 188. Based on the available data, the pilot plant showed an overall TN removal efficiency of 58% to 70%.

The mass balance showed that in the PN reactor, about 65% of influent ammonium was consumed in the PN reactor. 40% of consumed NH_4-N was oxidized to NO_3-N, only 1% to NO_2-N. It was found that most ammonium was converted to nitrate instead of nitrite. Based on the measurement results, there are three possible reasons for the absent nitrite production. First of all, the set DO concentration of 0.8±0.1 mg/L might already favour the growth of NOB. To improve the nitrite production, the set DO concentration was adjusted to 0.6±0.1 and 0.4±0.1 mg/L, respectively, for several days after the new air blower was automatically regulated. However, no significant nitrite production was detected in the PN reactor. Secondly, there probably has been a long period of overgrowth of NOB in the pilot plant, given the aeration with excess air for 2 months. The excess air supply could result in rapid growth of NOB. The sludge removal was manually conducted based on the MBR's sludge concentration from day 168. It was interrupted several times for multiple reasons, leading to a varying average SRT from 25 to 175 days. Therefore, a regular sludge extraction to wash out NOB could improve nitrite production. As a fine sieve was used in the pilot plant to retain the anammox granules, NOB might be washed out of the system without the loss of anammox bacteria. The measured C/N- ratio of 5-30 $gCOD_{total}$/gN in the effluent of the fine sieve is exceedingly high compared with the recommended C/N-ratio for the anammox-based process with suspended sludge (BCOD/N ratio≤ 2-3) (Cao et al., 2017). The high C/N- ratio could lead to a substantial presence of heterotrophs in the activated sludge (Cao et al., 2017).

In the anammox reactor, it was found that both nitrate and ammonium were consumed. The nitrate consumption was attributed to denitrification. As only 30%-40% of nitrate was consumed in the anammox reactor, and the average effluent nitrate concentration was 10.17±3.84 mg/L, it is assumed that there was not sufficient organic matter as the substrate to further support the denitrification. The incomplete denitrification was also observed in another anammox process treating wastewater with an elevated influent COD/N ratio of 1.4 gCOD/gN and was attributed to COD degradation and insufficient COD addition (Jenni et al., 2014). The observed ammonium loss in the anammox reactor might be attributed to deammonification. It was hypothesized that nitrite was formed during the denitrification and continued to react with the existing ammonium. In addition, the average wastewater temperature during this period was 10,7±1,1 °C, which also limited the anammox activity in the reactor. On day 196, anammox granule concentration was determined to be less than 0.024 g MLSS/L in the surplus sludge, which is extremely low compared to the sludge concentration in the MBR, being 11.20 g MLSS/L. This indicated a very small fraction of the anammox bacteria in the activated sludge in the system. It was hypothesized that the excess air supply for almost 2 months led to the overgrowth of aerobic bacteria in the pilot plant, and the growth of anammox bacteria was probably limited by the undesirable high DO concentration in the anammox and the low temperature at the same time.

Phase 4

In phase 4, no very different results were observed compared with what was experienced in phase 3. Most of the NH4-N was converted to NO3-N in the PN reactor, and both ammonium and nitrate were consumed in the anammox reactor mainly via nitrification/denitrification. However, full denitrification did not take place even though, on average, there was enough COD available, and a significant amount of nitrate in the effluent was always present.

The nitrite production could be improved by reducing the COD concentration in a pre-treatment stage, for example, by using a flocculation/coagulation unit. Since no significant nitrite production was detected in the reactor, the nitrite could have been immediately consumed or further oxidized to nitrate.

The average NH_4-N removal rate in the PN reactor was 0.025 kgNH_4-N/kg MLSS/d, and in the anammox reactor 0.0035 kgNH_4-N/kg MLSS/d. The NH_4-N removal rate of 0.0035 kgNH_4-N/kg MLSS/d in the anammox reactor is in the low range compared to the removal rate in different studies on deammonification at mainstream conditions (Table 13).

Table 13 Evaluation of ammonium removal rate in main stream configurations.

Reactor	Wastewater	NH_4-N removal rate	
Granular sludge fluidized bed (1.8 L)	Municipal wastewater	50±7 mg N/g VSS.d (10°C)	Lotti et al. (2014b)
Gas-lift reactor (4.2 L)	Synthetic (80-100mg COD/L; 40-80 NH_4-N/L)	30-44 mg N/g VS.d (10°C)	Hendrickx et al. (2014)
Two-stage PN/A ($1m^3$+$1m^3$)	Municipal wastewater	4-20 mg N/g TS.d (in-situ,7-25°C) 35-60 mg N/g TS.d (ex-situ, 7-25°C))	Lackner and Welker (2019)
Two-stage PN/A pilot plant (9 m^3+ 9 m^3)	Municipal wastewater	3.5 mg N/g MLSS.d	This study

Based on the results, it was hypothesized that another pathway different than via NO_2-N could have taken place if anammox activity was present (Not Determined). Nitrifiers can also produce NO, which is usually minimal and sometimes may be higher (about 10 % of ammonium converted), and it cannot be excluded that under some conditions, there is even more produced. It is known that anammox can grow on NO; it has been described as an organotrophic anammox (Fritsch 2022). It is assumed that via this pathway, Anammox bacteria have the capacity to oxidize volatile fatty acids with nitrate as an electron acceptor (and likely NO or NO_2-N) while forming ammonium. The latter would make a COD sink. In general, a low COD:N ratio is key for enrichment (Yin et al. 2019).

4.2 Control strategies for getting a stable process operation

Nitrite production/build-up was not really achieved; sometimes, a slight increase in nitrite-concentration was seen, but never a structural increase. In theory, several control strategies (or combinations of control strategies) are available to introduce and maintain nitrification/nitritation in a biological wastewater treatment plant. A summary of possible control strategies is presented in the table below.

Control strategies	Maintaining low DO concentration?	Maximizing ammonium removal?	Preventing nitrate build-up?	Compatible with fluctuation?	Other limitations
SRT	No	No	Mostly yes	No	Suitable for the two-stage anammox process and biofilm PN-A process, maintaining low DO concentration is required.
Fixed-DO setpoints	Yes	No	No	No	Need to be combined with other strategies.
Nitrogen-based aeration control	Yes	Yes	Yes	No	Ammonium sensors are not as reliable as pH sensors, and their accuracy is affected by the presence of potassium; pH sensors are also needed to monitor the alkalinity dynamics.
Intermittent aeration	No	No	May not	No	Promote N_2O production; it need to be combined with other methods to ensure long-term NOB suppression.
pH-nitrogen-aeration control	Yes	Yes	Yes	Yes	Extensive online instruments are needed.
DO-nitrogen-aeration control	Yes	Yes	Yes	No	Extensive online instruments are needed; the alkalinity dynamics is unknown.

The plant has been operating with a very high COD/N ratio between 5 and 15, significantly higher than what is recommended for the mainstream. In the pilot plant, a fine sieve (1 mm) with flocculation was foreseen in order to minimize the COD-load to the biological treatment. The fine sieve was implemented (but not always running), and the flocculation has not been in operation. It is recommended to decrease the COD-load to the mainstream Anammox as much as possible on one hand in order to reduce the sludge-production and hence make it easier to maintain low SRT and, on the other hand, to prevent denitrification of the produced nitrite.

4.2.1 Remarks on applied pilot plant control philosophy

The actual control philosophy to maintain the N-NO_2 / N-NH_4 ratio for the anammox reaction was not based on a DO control/air-flow depending on the ammonium influent concentration, as was initially planned.

The control strategy was initially based on a flow by-pass between the nitritation and the anammox reactor. Therefore the fine-tuning of the set-point of the DO will be very critical to be able to control the desired ratio of N-NO_2/N-NH_4= 1.3. The stochiometric of anammox reaction requires a molar ratio N-NH_4/N-NO_2= 1 / 1.32.
If the N-NO_2/N-NH_4 (C07/C06) ratio is higher than 1.3, then the by-pass valve will be closed with 5%. If the ratio is lower than 1.3, the bypass valve will be opened with 5%. This bypass valve allows the wastewater to flow directly to the anammox reactor.

The air throughput into the nitritation reactor was not based on the N-NH_4 influent concentration or N-NO_2/N-NH_4 ratio. Adjusting flow with the bypass valve was the actual control action to maintain a ratio of 1.3 (N-NO_2/N-NH_4) in the effluent of the partial nitritation reactor.

In the blower control, it is stated that in order to ensure the proper residual dissolved oxygen concentration values in the nitritation reactor, a dissolved oxygen probe (04-AIT-0601-0) is installed. The measured dissolved oxygen concentration value (M_{01}) shall be compared with its set-point values ($M_{01\text{-min}}$ and $M_{01\text{-max}}$, adjustable on the PLC):

- if $M_{01} < M_{01\text{-min}}$, the blower's (08-B-0601-0) frequency (f_{05}) shall be increased by a set value ($f_{05\text{-delta}}$, adjustable on the PLC);
- if $M_{01} < M_{01\text{-max}}$, the blower's (08-B-0601-0) frequency (f_{05}) shall be decreased by a set value ($f_{05\text{-delta}}$, adjustable on the PLC).

The blower frequency also changes the airflow and the DO concentration in the MBR reactor for nitrification, which probably is not needed.

The current function of valve (04-FV-0605-0, according to design speficifications) is expected to vary the airflow into the nitritation tank, based either on the DO set-point but also possibly linked to the N-NO_2/N-NH_4 ratio or even the (N-NO_2+N-$NO_{3)}$ / N-NH_4 ratio, especially when inducing transient anoxic conditions.

In phase 4 of the operation, an intermittent ON-OFF aeration DO control was setup (10 min aeration, 20 min without). Intermittent aeration is indeed considered as a better way to control AOB/NOB balance but does not guarantee long-term NOB suppression. Besides, in current side-stream anammox it is claimed that intermittent aeration could promote N_2O production, so the fine-tuning of this control strategy to prevent that can be even more important in mainstream anammox applications.

4.2.2 Control strategy options

Based on the state of the art for side-stream anammox, stable operation is key, independently of activated sludge configuration (MBR, AGS, CAS, MBBR). A stable operation is defined as a period without any disruptions. Disruptions include equipment failure and reactor operation that lead to an imbalance of the deammonification biological reactions. (Ochs et al. 2020) determined root causes for all individual imbalances following the schematic in Figure 37. In summary, imbalances were related to the following:
– Partial nitritation, the nitrate produced to ammonia removed ratio, was used to evaluate the partial nitritation. Based on deammonification stoichiometry, the ideal ratio is 0.08.
– Accumulation of nitrite, analysing the residual nitrite to ammonia ratio. Based on deammonification stoichiometry, the ideal nitrite-to-ammonia ratio is 0.53.

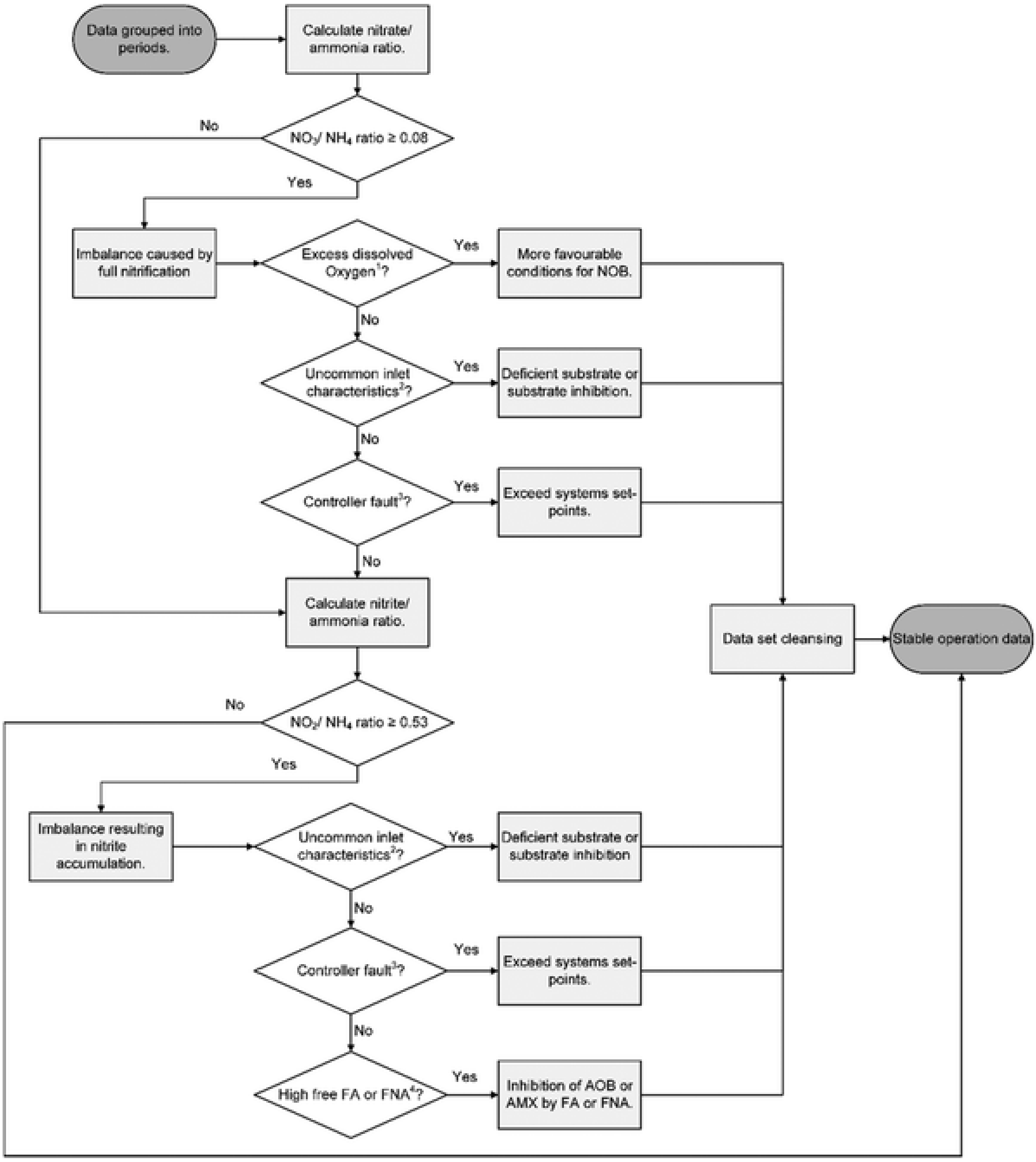


Figure 37. Flowchart for evaluation of process imbalances (Ochs et al. 2020). Excess dissolved oxygen was when oxygen concentration exceeded reactor setpoints. Uncommon inlet characteristics were defined as the either high or low influent concentrations of soluble COD, ammonia, pH or alkalinity. Controller fault was when control setpoints were exceeded, resulting in ammonia (>200 mgN L^{-1}), nitrite (>50 mgN L^{-1}) accumulation or high pH (>7.2). High free ammonia (FA) or free nitrous acid (FNA) was defined as inhibition by AOB or AMX. Free ammonia inhibition ranges were 8–120 mgN L^{-1} and 20–50 mgN L^{-1} for AOB and AMX, respectively. Free nitrous acid inhibition ranges were 0.2–2.8 mgN L^{-1} and 0.01–0.2 mgN L^{-1} for AOB and AMX, respectively.

4.2.3 Control strategies applied in full-scale anammox plants

One of the most promising control strategies for mainstream Anammox is intermittent aeration due to the lag-phase of the NOB after restarting the aeration. By consequently starting and stopping the aeration, the growth of

the NOB is inhibited compared to the growth of the AOB. The length of the aeration's on- and off times can be fixed but can also be controlled by the ratio of NH_4/NO_x in the aeration tank. The so-called AVN-control (AVN = Ammonium versus NO_x) (Regmi et al. 2015). This ratio then controls the on-time within a pre-set cycle length. In the case of an increasing ratio, the on-time is increased, and in the case of decreasing ratio, the on-time is decreased. During on-time, the aeration is controlled by a DO-set-point of approximately 1,5 mg/l.
The disadvantage of this control strategy is the need for on-line measurement of NH_4 and NO_x.
During the design of the pilot plant, it was initially the plan to install this control strategy, but it was eventually skipped due to the need for the online-measurements. Moreover, during the first phases of the pilot plant investigation, it was not possible to apply intermittent aeration.

Another very promising control strategy is strict SRT-control. NOB need, especially at higher temperatures, a higher SRT to “survive” than the AOB. By controlling the SRT very close to the minimum SRT needed for AOB to “survive”, a second (next to the intermittent aeration) mechanism is introduced to inhibit the NOB growth, resulting in the production of nitrite instead of nitrate.

The intermittent aeration was gradually applied during phase 4. The SRT-control has been attempted to apply during all phases but was very hard due to the variation of the influent.

4.3 Limitations of the evaluation

The evaluation of the pilot plant performance has been limited for several operational reasons.
One limiting factor was the absence of TN measurement. Since only NH_4-N, NO_3-N and NO_2-N concentrations were measured to monitor the pilot plant, the TN concentration was calculated as the sum of NH_4-N, NO_3-N and NO_2-N during three phases. However, the presence of organic nitrogen in the influent often resulted in a higher TN concentration in the effluent than in the influent, so a proper overview of the total nitrogen removal efficiency was not completely clear. This also influenced the mass balance based on the influent and effluent concentrations. In phase 4, this was changed, and more insights were gained on the N-balance.
Another limiting factor was the absence of an airflow meter for the PN reactor aeration. Since the original airflow meter was identified as the cause of the abnormal blower performance, it was removed on day 176. However, the airflow rate was normally considered a critical parameter to control the aeration for the anammox process. Without the measurement of the airflow, it is difficult to know and adjust the air flowrate. Hence, an airflow meter should be available on-site, not only to support the evaluation of results but also to improve the performance of the pilot plant.
There were very limited periods without any disruptions, and that made the whole evaluation of the system challenging to stir the process properly.

4.4 Lessons learned from pilot plant troubleshooting

During the experimental period of 214 days, a total of 44 operational difficulties, adjustments or problems occurred in the pilot plant, making the operation challenging.
Table 14 gives an overview of the main incidents encountered during the operation and the corresponding countermeasures.

Table 14 Operational problems during the operation of the pilot plant

Incident type	Number of occurrences	Duration	Measures
Blower failure	4	up to 147 days	• Shutdown of the pilot plant

			• Replacement of the blower • Remove the flow meter that caused the blower blockage
Fine sieve clogging	5	7-77 days	• Reduction of the feed flow rate • More frequent automatic backwashing of the fine sieve with permeate • Enhanced backwashing with chemicals • Additional cleaning with water under high-pressure (A lifting platform must be available)
Pump failure	7	1-22 days	• Shutdown of the pilot plant • Replacement of the pump • Pump venting
Pilot setup issues	28	1-170 days	• Programming adjustment • Reparation of the defect parts • Setup adjustment, e.g. installation of the sampling point • Installation of a submerged pump as a mixer in the PN reactor (in case of a mixing problem) • Replacement of the DO sensors and daily manual DO measurement (in case of failure of the DO signal) • Adjustment of the water level switch

4.4.1 Blower failure

The blower failure was reported four times throughout the entire operation. However, it had the most significant influence on the pilot operation in terms of duration. The operational difficulties caused by the blower failure revealed aspects to take into account in future operation. Firstly, a second air blower as a spare part is of great importance. A replacement of the blower for the pilot plant required a long period of time (147 days in this case) due to the long delivery time from the supplier. This limited the aeration of the pilot plant to the blowers with excessive airflow for up to 57 days. An appropriate air supply to the PN reactor was first performed after installing the new blower on day 156. An abnormal performance of the blower was indicated by its low airflow rate since the start of the pilot operation. After checking the connected pipes and the blower itself, the cause at that time was considered to be the malfunction of the old blower, and therefore it was decided to replace the blower. After the new air blower was installed, it was found that the new blower also supplied a lower airflow than the specified value. Then it was determined that the air flowmeter for the PN reactor (DN 40) was not compatible with the connected pipes (DN 50), resulting in the blockage of the air blower. Furthermore, the air supply for the PN reactor and for the membrane air scouring systems should be performed independently. In operating phase 1 of the PN reactor, a fixed air flowrate was supplied to the MBR to ensure its stable operation. Due to the limited total flow by the blower, the airflow to the PN reactor could not be increased to achieve a proper DO concentration, severely limiting the PN reactor's operation.

4.4.2 Fine sieve clogging

The clogging of the fine sieve that occurred also had a great influence on the pilot operation. A clear indication of the clogging was that wastewater flowed over the fine sieve though the blue plastic hose into the container. When the fine sieve was clogged, the feed of the pilot plant had to be reduced until there was no overflow to 0.4 m^3/h. The loading of the pilot plant was reduced accordingly. This strongly affected the reactors' biological conversion since the air supply adjustment was limited during most of the experimental period.

In addition to the consequences caused by the clogging, the fine sieve required high maintenance. Despite the automatic backwashing with permeate, the fine sieve had to be cleaned with chemicals manually to prevent clogging. Since the enhanced backwashing with chemicals was not included in the original plan, it had to be conducted by the personnel every week. In the case of severe clogging, the fine sieve had to be cleaned additionally using a high-pressure cleaner, for which a lifting platform must be available. Since the organization of the lifting platform normally required 2-3 days, the fine sieve performance could not be restored immediately after the clogging was identified. Based on the severe consequences of the fine sieve clogging and associated operational difficulties, there are two possibilities to improve the operation of the fine sieve. The first possibility is to add a treatment prior to the fine sieve to remove solids from the wastewater so that the fine sieve does not clog so easily. For this, the IBC intended for flocculation could be used directly without additional construction changes to remove the colloidal/suspended solids. The second possibility involves the position of the fine sieve. The fine sieve should be arranged on the ground next to the pilot plant so that it is more easily accessible to personnel and the cleaning can be performed without a lifting platform. The same should apply to the flocculation tank as well.

4.4.3 Pump failures

The pump failures involved the feed pump, discharge pump, and permeate pump of MBRs. Depending on the function of the pump, its malfunction affected the pilot operation to varying extents.

As the permeate pump was broken, the filtration cycle did not stop but continued under hydraulic pressure due to the siphon effect. This led to a significantly lower outflow rate than the inflow rate, and the feed pump was frequently switched off by the high water level indication in the MBR. After the siphon was identified in the pilot plant, a valve was used to allow air to penetrate, and filtration would stop automatically when the permeate pump stopped. The failure of the permeate pump was reported once and did not cause a long interruption since two MBRs were available.

In comparison, the failures occurred more frequently in the feed and discharge pumps. In case of a failure of the discharge pump that pumped treated wastewater from the sump back to the WWTP, filtration stopped automatically, followed by the turn-off of the feed pump, resulting in a shutdown of the pilot plant. Although the discharge pump could be replaced within a few hours, the pilot operation was interrupted due to the lack of inflow and outflow. When the failure occurred on weekends, the interruption lasted longer, significantly impacting the biological processes. The failure of the feed pump also had the same consequences. However, in contrast to the stable performance of the discharge pump, the performance of the feed pump decreased distinctly with time. When the inflow rate decreased, and the outflow rate did not change with time, the filtration stopped automatically due to the low water level in the MBR. As inflow continued to drop to 0 m^3/h, the pilot plant was considered as shutdown even though the feed pump was still on. The reason for this was the clogging of the feed pump by solids, as the wastewater from the grit chamber still contained a large number of solids. Furthermore, the clogging problem was combined with the unfavourable pump position. Two feed pumps were placed directly at the bottom of the grit chamber near the outlet for safety reasons, as the water level in the grit chamber fluctuated greatly. The solid concentration at the bottom was much higher than that near the water surface due to the sedimentation of solids. Therefore, changing the pump position could be a good solution to maintain the performance of the feed pump, or implement mixing in the grid chamber. The outlet of the primary sedimentation tank, for example, would be an alternative since the total suspended solids (TSS) can be removed via the primary sedimentation tanks (up to 55% within one hour retention time (Metcalf and Eddy et al., 2014). The effluent of the primary sedimentation tanks with lower TSS could protect the feed pump from the solids and reduce the solid load on the fine sieve. Another possibility to improve the pilot operation in terms of the pump failure was to change the pump. Both the discharge pump and the feed pump were the same wastewater pump (450/12 IX-S, TIP) purchased from the “Globus” construction market in Simmern. Considering the frequency of discharge pump failures (three times within three months) and the limited feed pump performance by the solids, this pump may not be suitable for the pilot operation in the long term. Thus, this pump should be replaced with one that is more resistant to solids intrusion.

4.4.4 Pilot setup issues

The pilot issues included various operational problems. Some were caused by insufficient information about the plant installation and setup, and others by the deficiency of the pilot plant itself. The first one related strongly to the construction of the pilot plant in China and operation in Germany. Although the pilot plant was originally designed by Supratec Germany, a large number of details were changed during the construction by Supratec China. Not all changes made were documented, and some existing instructions still needed to be translated at the time, which severely hampered the operation at the beginning of the experimental period and extended the time to achieve a stable operation. Therefore, changes made during the construction should have been documented to support future installation and operation.

Although the majority of the problems were solved by adjusting the programming or modifying the plant construction, several problems persisted during the operation. The most remarkable issue was the mixing in the PN reactor. This was first identified by low sludge volume (SV) values in all the reactors on day 7. Then a submerged pump was installed in the PN reactor as a mixer to create more turbulence, which directly improved the sedimentation of the activated sludge. However, the observed DO gradients inside the PN reactor implied that the mixing problem persisted. In addition, the mixing problem was aggravated after the automatic control of the air blower began. Since the failure of the submerged pump occurred on day 46, mixing the PN reactor depended solely on aeration. While the sludge concentration in the PN reactor was decreased to generate more nitrite, the airflow to the PN reactor decreased accordingly, allowing the activated sludge to settle more easily. The sedimentation of the activated sludge resulted in further decreased airflow, which deteriorated the mixing in the PN reactor. An extremely low MLSS concentration of 256 mg/L was measured in the PN reactor on day 203. This indicated that mixing solely depending on aeration was insufficient. To improve the operation and eliminate the influence of the mixing problem on the performance, the mixing and aeration of the PN reactor should be decoupled, which requires an additional agitator in the PN reactor. Considering the great effort to incorporate an agitator into the pilot plant, another submerged pump could be installed as a substitute to improve the mixing.

Another issue with lasting effects was the failure of the oxygen sensor. This resulted in a manual DO measurement that caused a time delay in adjusting the air supply to the influent concentrations. Maintaining a proper DO concentration in the PN reactor without the oxygen sensor was difficult. Therefore, at least four oxygen sensors should be available at the site to prevent the lasting effects: three are for the DO monitoring (PN reactor, anammox reactor and the MBR), and one is stored as a spare part for replacement.

5 Insight into industrial Hybrid anammox system

5.1 Industrial Full-scale Hybrid anammox system

5.1.1 Overall system description

The results of this research are compared to a full-scale aerobic system, treating the anaerobic effluent of slaughterhouse wastewater. The wastewater originating from a slaughterhouse is collected in a buffering tank after being sieved. From the buffering tank, the wastewater is transported to a Dissolved Air Flotation unit (DAF). The effluent of the DAF unit is transported to a anaerobic UASB (Upflow Anaerobic Sludge Blanket) reactor. In this reactor, the anaerobic bacteria convert organic waste into biogas. The effluent of the anaerobic reactor is treated in the aerobic system.

The aerobic system is a hybrid anammox system consisting of three separate zones (Figure 38):

The first zone, or the PN (partial nitritation), is aerated with a fine bubble aerating system. AOB activity converts NH_4^+ partially into NO_2. The second zone (anammox and conventional denitrification) is anoxic and not aerated. A mixer is installed to maintain the sludge in suspension. Here, anammox bacteria convert the formed NO_2 from the first zone and NH4 into nitrogen gas (N_2). Besides, conventional denitrification takes place in the same zone.

The third zone is an aerated zone and is operated as an MBR. The required air is partially injected through a fine bubble aerating system. The remaining part is injected via the headers on which the MBR membrane modules are installed. The remaining nitrogen is converted into NO_3 by AOB and NOB. A recirculation occurs between the third MBR zone and the second anoxic zone.

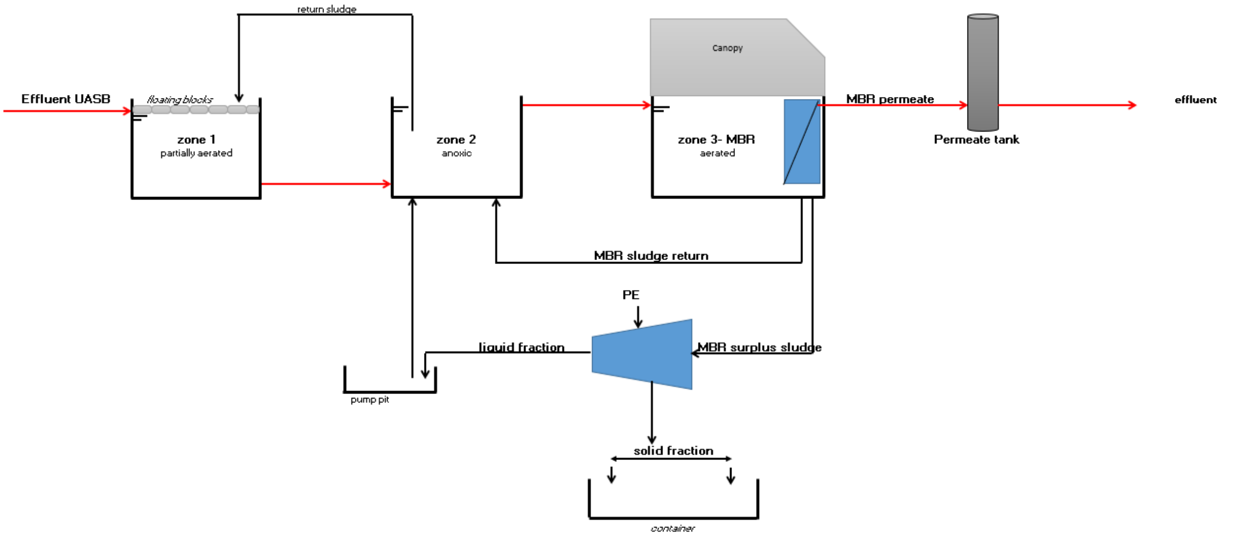


Figure 38 PFD of the full-scale mainstream Anammox

5.1.2 Design Basis

Zone 1: PN

The first zone receives the effluent from the UASB system. Design flow is a maximum of 125 m³/hour. The effluent from the PN is transported to the second zone (Anammox) by gravitational flow. There is a recycle from the second zone to the PN to ensure an appropriate suspended solids concentration. Also, the biological biogas treatment is

discharged to the PN with a design flow of 0.1 m^3/h (in batches). Suspended solids concentration is controlled by daily analyses. The PN is aerated with a blower and a fine bubble aeration system.
PN has an effective volume of 2,200 m^3 and 7,5 meter of water column (mwc). The average HRT is 16 hours. The design temperature is 30°C. With a design sludge concentration of 1,4 kg MLSS/m^3, the design sludge loading rate equals 0.80 kg COD.kg MLSS.day^{-1}. The design nitrogen volumetric loading rate is 0,36 kg NH_4-N/m^3.day^{-1}. The design assumed a nitrogen conversion rate of 0.17 kg NH4-N/kg MLSS.day-1. According to the design, the nominal aeration requirement equals an airflow of 24 Nm^3/min.

Zone 2: Anammox + DN
The effluent of the PN flows under gravitational forces towards the second zone, and an internal circulation flow from the third zone (MBR). Also, the centrate flow from the surplus sludge dewatering decanter enters the second zone. The second zone is mixed with one submerged banana-leaf mixer. The second zone has an effective volume of 2,000 m^3 and 7.5 mwc. The average HRT is 4.1 hours. The design sludge concentration equals 9.7 kg MLSS/m^3.

Zone 3: MBR
The third zone is divided into 2 equal compartments receiving the effluent from the second zone gravitationally. There is a recycle from the MBR to the second zone for additional denitrification. Membrane units are installed, which separate the effluent (permeate) from the sludge.
The aeration of the third aerobic zone is provided by MBR aeration and fine bubble aeration. The MBR-aeration has somewhat more coarse bubbles and is less efficient for mass transfer.
Each compartment has a volume of 644 m3 and 6.5 mwc. The HRT is designed to be 2.5 hours.
The surplus sludge is transported to the decanter centrifuge from the MBR compartments. The sludge concentration, therefore, is slightly higher in the third zone than in the second zone, namely 12.5 kg MLSS/m3.

The average SRT of the aerobic system equals 23.4 days, based on average data over 2022.

The average pH, temperature and suspended solids concentrations of the different zones in practice are depicted in Table 15:

Table 15 Parameters of the full-scale hybrid system (based on average data over 2022)

	pH	T	MLSS
	[-]	[°C]	[g/l]
Zone 1 (PN)	7,11	37,8	3,44
Zone 2 (Anammox + conventional DN)	7,71	35,0	10,8
Zone 3 (MBR)	7,41	35,2	14,4

5.1.3 Mass balances and effluent quality achieved

Based on the average analysis results of the quick tests (Macherey + Nagel) in 2022 in practice, the mass balance over the full-scale hybrid system is shown in Table 16:

Table 16 Mass balance for the full-scale hybrid system (based on average data over 2022)

Influent (anaerobic effluent)					
COD	TN	NH4-N	TP		
[kg/d]	[kg/d]	[kg/d]	[kg/d]		
1.310	626	592	82,3		
Effluent (MBR permeate)					
COD	TN	NH_4-N	NO_3-N	NO_2-N	TP
[kg/d]	[kg/d]	[kg/d]	[kg/d]	[kg/d]	[kg/d]
187	64,4	27,9	25,0	6,18	71,0

Based on the mass balance, the overall average removal efficiency of the system is about 96% for NH4-N, and 90% for total nitrogen, indicating a highly efficient mainstream process. The overall efficiencies of the aerobic treatment system determined in practice are presented in Table 17.

Table 17 Removal efficiency of the full-scale hybrid system (based on average data over 2022)

COD	TN	NH_4-N	TP
[%]	[%]	[%]	[%]
85,7	89,7	95,6	13,7

A similar mass balance to the Pilot Plant in Simmern for the industrial full scale plant is presented in Figure 39.

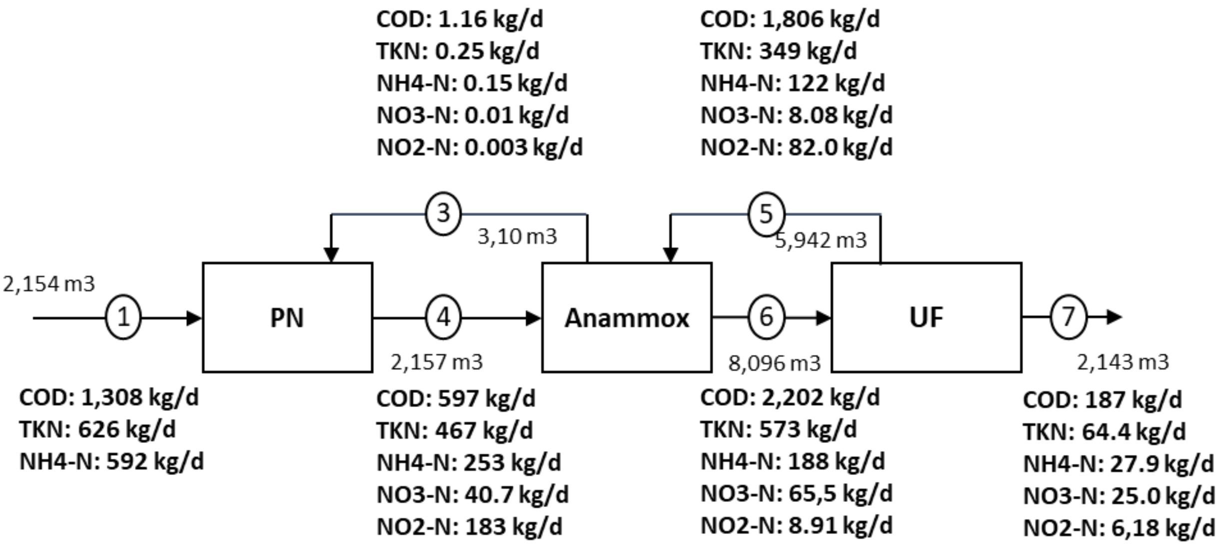


Figure 39 Mass balance at full scale PN/A system based on the averages of 2022. UF: ultrafiltration refers to membrane bioreactor (MBR)

During the partial nitritation of the full scale system already 339 kg/d of the NH4-N is converted, resulting in 183 kg/d of NO2-N and 40.7 kg/d NO3-N. With this average balance, a ratio of NH4-N/NO2-N of 1.38 entering the anammox reactor is achieved, which is very close to the stoichiometric ratio recommended. After the anammox (+ DN) zone, 95% of the nitrite build-up in the PN zone is converted.

The ammonium removal rate achieved within the full-scale hybrid PN/Anammox and conventional nitrification/denitrification has been calculated as 12,5 mg N/g MLSS.d.

Table 18 Ammonium removal rate in full-scale water treatment system

Reactor	Wastewater	NH_4-N removal rate
Full-scale hybrid PN/A & conventional N/DN	Slaughterhouse waste water (anaerobically treated)	12,5 mg N/g MLSS d

On average, the TP uptake in the surplus sludge of the aerobic system corresponds to 5,52 mg TP/kg $MLSS.day^{-1}$. The remaining TP is removed in a polishing step of the MBR permeate. The effluent quality achieved after this step, which is discharged to the public sewer system, is shown in Table 19.

Table 19 Average discharge quality (based on average data over 2022)

Effluent (discharge to sewer)					
COD	TN	NH_4-N	NO_3-N	NO_2-N	TP
[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]
62,8	32,9	16,8	11,3	2,72	6,07

5.1.4 Applied control strategy

Zone 1: PN
The PN is equipped with a DO-sensor in order to regulate the dissolved oxygen content. Next to this, the NH_4, NO_2 and NO_3 concentrations are monitored online to be able to have closer control of the nitrogen conversion process and ratios. When nitrite or nitrate formation is too high, the oxygen setpoint is decreased automatically. When nitrite concentration is low, the oxygen setpoint is increased. Based on the measured DO concentration, the frequency of the blower is adjusted in a variable step with a PID regulation, depending on the actual difference between the actual DO and the setpoint DO.

Zone 3: MBR.
The DO of the third zone is measured in each compartment, and the aeration is controlled in each compartment separately with a separate blower. Each blower provides 50% required oxygen. The frequency of the blowers, which provide the air for the fine bubble aeration system, is adjusted based on the measured DO with a PID regulation.

6 Conclusions

This project aimed to investigate a two-stage deammonification process (PN/Anammox) in the mainstream in combination with a membrane bioreactor (MBR) and a screen-assisted recirculation of the anammox biomass of a municipal wastewater treatment pilot plant (WWTP) build up and installed in Simmern, Germany. The following concluding remarks from the results were formulated:

- A pilot plant was designed, built up and commissioned, which consisted mainly of four reactors: an aerated reactor (nitritation-/PN-stage), a completely stirred reactor (Anammox stage), and two activated sludge reactors, each of them with submerged ultrafiltration hollow-fiber (HF) membrane cassettes installed (Supratec) working in parallel as membrane bioreactors (MBRs). Moreover, the influent wastewater passed through a pretreatment stage by using a rotary drum with a mesh size of 0.25 mm to intercept coarse solids larger than 0.25 mm. The surplus sludge out of the biological treatment system was passed through a fine sieve (1mm) to retain the slow-growing anammox granules, which were returned to the anammox reactor.

- The existing pretreatment with the fine sieve resulted in a total COD removal efficiency between 30% and 45%. The mean value of C/N-ratio in the fine sieve effluent was 16.92±10.16 gCODtotal/gN during phases 1-3 and reduced to about 5.28±2.43 $gCOD_{total}$/gN in the last phase to favor anammox mainstream application.

- The COD removal efficiency of the pilot plant was mainly influenced by the fluctuations of the influent concentrations achieving an average value in the range of 90±8% to 93±5%, with a volumetric loading rate between 0.4 – 0.5 kg CODtotal/(m^3*d) and a sludge loading rate in average between 0.07±0.05 kg CODtotal/(kg MLSS*d) and 0.12±0.08 kg CODtotal/(kg MLSS*d).

- Multiple DO variations in the PN reactor during the operation did not allow the partial nitritation process to occur as desired. Also, some unwanted DO concentration in the Anammox reactor were attributed to a combined consequence of the excessive air supply to the PN reactor and the high return flow from the MBR, which was increased accordingly with the feed.

- It was found that the majority of influent ammonium was consumed in the PN reactor, and the majority of it was oxidized to nitrate despite some low DO concentrations. In general, during the whole operation of the plant, no nitrite was built up in the PN reactor, which might have limited the anammox activity. The On-Off DO control in the PN was not enough to promote the NO_2-N build-up and prevent denitrification due to the high COD available, suggesting that nitrite-oxidizing bacteria (NOB) were highly active and dominant and not suppressed despite the reduction of SRT and DO. The most likely dominant process taking place was nitrification-denitrification in the anammox reactor, even though some of the mass balances inferred simultaneous denitrification and anammox, with a nitrogen removal efficiency of about 70% at some time of operation. However, despite the high removal of ammonium achieved of about 98%, the plant did not achieve complete denitrification, resulting in high concentrations of nitrate in the effluent.

- The average NH_4-N removal rate in the PN reactor was 0.025 kgNH_4-N/kg MLSS/d, and in the anammox reactor 0.0035 kgNH_4-N/kg MLSS/d. The NH_4-N removal rate of 0.0035 kgNH_4-N/kg MLSS/d in the anammox reactor is in the very low range compared to the removal rate in different studies on the deammonification at mainstream conditions.

- The waste sludge was sieved, and it was initially observed that a large number of granules were intercepted by the 1mm fine sieve. The intercepted granules proved that the fine sieve selectively retained bigger bacteria, presumably anammox bacteria, in the system. However, the measured value of anammox from the retained sludge was about 0.024 g MLSS /L. Furthermore, later the retention of anammox granules was not significantly observed, concluding that because of the low load and intermittently operational conditions, the anammox was lost, and seeding sludge was necessary. Nevertheless, after reinoculation in phase 4, anammox granules retained in the sieve were not representative.

- Since there is no control of the NH_4-N/NO_2-N ratio as initially planned, nitrate production and COD oxidation are mainly occurring in the PN reactor. Since only DO control might not be the best way of controlling nitritation, there are other control strategies to trigger nitrite production or inhibit NOB, such as the implementation of intermittent aeration and SRT reduction. The latest was applied gradually during phase 4, but without success.

- When compared with a full-scale industrial anammox mainstream system, all NH_4, NO_2 and NO_3 concentrations are monitored online in that wastewater treatment plant to have closer control of the nitrogen conversion and ratios. Based on the concentrations, the oxygen setpoint is automatically decreased or increased using a PID control, which allows for a stable partial nitritation.

References

Arnaldos, M., Amerlinck, Y., Rehman, U., Maere, T., Van Hoey, S., Naessens, W. and Nopens, I. (2015) From the affinity constant to the half-saturation index: Understanding conventional modeling concepts in novel wastewater treatment processes. Water Research 70, 458-470. https://doi.org/10.1016/j.watres.2014.11.046

Blackburne, R., Yuan, Z. and Keller, J. (2008) Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 19(2), 303-312. 10.1007/s10532-007-9136-4

Cao, Y., van Loosdrecht, M.C.M. and Daigger, G.T. (2017) Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies. Applied Microbiology and Biotechnology 101(4), 1365-1383. 10.1007/s00253-016-8058-7

Chen, Y., Zhao, Z., Liu, H., Ma, Y., An, F., Huang, J. and Shao, Z. (2020) Achieving stable two-stage mainstream partial-nitrification/anammox (PN/A) operation via intermittent aeration. Chemosphere 245, 125650. https://doi.org/10.1016/j.chemosphere.2019.125650

Claros, J., Serralta, J., Seco, A., Ferrer, J. and Aguado, D. (2012) Real-time control strategy for nitrogen removal via nitrite in a SHARON reactor using pH and ORP sensors. Process Biochemistry 47(10), 1510-1515. https://doi.org/10.1016/j.procbio.2012.05.020

Daigger, G.T. (2014) Oxygen and Carbon Requirements for Biological Nitrogen Removal Processes Accomplishing Nitrification, Nitritation, and Anammox. Water Environment Research 86(3), 204-209. https://doi.org/10.2175/106143013X13807328849459

Dapena-Mora, A., Fernández, I., Campos, J.L., Mosquera-Corral, A., Méndez, R. and Jetten, M.S.M. (2007) Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. Enzyme and Microbial Technology 40(4), 859-865. https://doi.org/10.1016/j.enzmictec.2006.06.018

de Graaff, M., van den Brand, T., K;, R., M;, Z., Duin O and Van Loosdrecht, M.C.M. (2016) Full-Scale Highly-Loaded Wastewater Treatment Processes (A-Stage) to Increase Energy Production from Wastewater: Performance and Design Guidelines. Environmental Engineering Science 33(8), 571-577. 10.1089/ees.2016.0022

Dosta, J., Fernández, I., Vázquez-Padín, J.R., Mosquera-Corral, A., Campos, J.L., Mata-Álvarez, J. and Méndez, R. (2008) Short- and long-term effects of temperature on the Anammox process. Journal of Hazardous Materials 154(1), 688-693. https://doi.org/10.1016/j.jhazmat.2007.10.082

Fux, C. and Siegrist, H. (2004) Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economical considerations. Water Science and Technology 50(10), 19-26. 10.2166/wst.2004.0599

Fritsch S. Deammonification in the main stream under extreme conditions. KA Korrespondenz Abwasser, Abfall, 2022 (69), No. 9. www.dwa.de/KA

Ge, S., Peng, Y., Qiu, S., Zhu, A. and Ren, N. (2014) Complete nitrogen removal from municipal wastewater via partial nitrification by appropriately alternating anoxic/aerobic conditions in a continuous plug-flow step feed process. Water Research 55, 95-105. https://doi.org/10.1016/j.watres.2014.01.058

Gilbert, E.M., Agrawal, S., Brunner, F., Schwartz, T., Horn, H. and Lackner, S. (2014) Response of Different Nitrospira Species To Anoxic Periods Depends on Operational DO. Environmental Science & Technology 48(5), 2934-2941. 10.1021/es404992g

Gilbert, E.M., Agrawal, S., Schwartz, T., Horn, H. and Lackner, S. (2015) Comparing different reactor configurations for Partial Nitritation/Anammox at low temperatures. Water Research 81, 92-100. https://doi.org/10.1016/j.watres.2015.05.022

Graaf, A.A.v.d., Mulder, A., Bruijn, P.d., Jetten, M.S., Robertson, L.A. and Kuenen, J.G. (1995) Anaerobic oxidation of ammonium is a biologically mediated process. Applied and Environmental Microbiology 61(4), 1246-1251. doi:10.1128/aem.61.4.1246-1251.1995

Hanaki, K., Wantawin, C. and Ohgaki, S. (1990) Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. Water Research 24(3), 297-302. https://doi.org/10.1016/0043-1354(90)90004-P

Hausherr, D., Niederdorfer, R., Morgenroth, E. and Joss, A. (2021) Robustness of mainstream anammox activity at bench and pilot scale. Science of The Total Environment 796, 148920. https://doi.org/10.1016/j.scitotenv.2021.148920

Hoekstra, M. (2017) Mainstream anammox, potential and feasibility of autotrophic nitrogen removal, Delft University of Technology. https://doi.org/10.4233/UUID:94B5831B-D22D-4FB2-8122-4D4300AE4526

Hoekstra, M., de Weerd, F.A., Kleerebezem, R. and van Loosdrecht, M.C.M. (2018) Deterioration of the anammox process at decreasing temperatures and long SRTs. Environmental Technology 39(5), 658-668. 10.1080/09593330.2017.1309078

Isaka, K., Date, Y., Kimura, Y., Sumino, T. and Tsuneda, S. (2008a) Nitrogen removal performance using anaerobic ammonium oxidation at low temperatures. FEMS Microbiology Letters 282(1), 32-38. 10.1111/j.1574-6968.2008.01095.x

Isaka, K., Suwa, Y., Kimura, Y., Yamagishi, T., Sumino, T. and Tsuneda, S. (2008b) Anaerobic ammonium oxidation (anammox) irreversibly inhibited by methanol. Applied Microbiology and Biotechnology 81(2), 379-385. 10.1007/s00253-008-1739-0

Isanta, E., Bezerra, T., Fernández, I., Suárez-Ojeda, M.E., Pérez, J. and Carrera, J. (2015) Microbial community shifts on an anammox reactor after a temperature shock using 454-pyrosequencing analysis. Bioresource Technology 181, 207-213. https://doi.org/10.1016/j.biortech.2015.01.064

Jardin, N. and Hennerkes, J. (2012) Full-scale experience with the deammonification process to treat high strength sludge water – a case study. Water Science and Technology 65(3), 447-455. 10.2166/wst.2012.867

Jeanningros, Y., Vlaeminck, S.E., Kaldate, A., Verstraete, W. and Graveleau, L. (2010) Fast start-up of a pilot-scale deammonification sequencing batch reactor from an activated sludge inoculum. Water Science and Technology 61(6), 1393-1400. 10.2166/wst.2010.019

Jenni, S., Vlaeminck, S.E., Morgenroth, E. and Udert, K.M. (2014) Successful application of nitritation/anammox to wastewater with elevated organic carbon to ammonia ratios. Water Research 49, 316-326. https://doi.org/10.1016/j.watres.2013.10.073

Jiang, H., Liu, G.-h., Ma, Y., Xu, X., Chen, J., Yang, Y., Liu, X. and Wang, H. (2018) A pilot-scale study on start-up and stable operation of mainstream partial nitrification-anammox biofilter process based on online pH-DO linkage control. Chemical Engineering Journal 350, 1035-1042. https://doi.org/10.1016/j.cej.2018.06.007

Joss, A., Derlon, N., Cyprien, C., Burger, S., Szivak, I., Traber, J., Siegrist, H. and Morgenroth, E. (2011) Combined Nitritation–Anammox: Advances in Understanding Process Stability. Environmental Science & Technology 45(22), 9735-9742. 10.1021/es202181v

Kallistova, A.Y., Dorofeev, A.G., Nikolaev, Y.A., Kozlov, M.N., Kevbrina, M.V. and Pimenov, N.V. (2016) Role of anammox bacteria in removal of nitrogen compounds from wastewater. Microbiology 85(2), 140-156. 10.1134/S0026261716020089

Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M. and van Loosdrecht, M.C.M. (2009) Nitrous oxide emission during wastewater treatment. Water Research 43(17), 4093-4103. https://doi.org/10.1016/j.watres.2009.03.001

Kartal, B., Kuenen, J.G. and van Loosdrecht, M.C.M. (2010) Sewage Treatment with Anammox. Science 328(5979), 702. 10.1126/science.1185941

Kartal, B., Kuypers, M.M.M., Lavik, G., Schalk, J., Op den Camp, H.J.M., Jetten, M.S.M. and Strous, M. (2007) Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. Environmental Microbiology 9(3), 635-642. https://doi.org/10.1111/j.1462-2920.2006.01183.x

Katsogiannis, A.N., Kornaros, M. and Lyberatos, G. (2003) Enhanced nitrogen removal in SBRs bypassing nitrate generation accomplished by multiple aerobic/anoxic phase pairs. Water Science and Technology 47(11), 53-59. 10.2166/wst.2003.0586

Kim, D.-J. and Kim, S.-H. (2006) Effect of nitrite concentration on the distribution and competition of nitrite-oxidizing bacteria in nitratation reactor systems and their kinetic characteristics. Water Research 40(5), 887-894. https://doi.org/10.1016/j.watres.2005.12.023

Kuypers, M.M.M., Marchant, H.K. and Kartal, B. (2018) The microbial nitrogen-cycling network. Nature Reviews Microbiology 16(5), 263-276. 10.1038/nrmicro.2018.9

Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H. and van Loosdrecht, M.C.M. (2014) Full-scale partial nitritation/anammox experiences – An application survey. Water Research 55, 292-303. https://doi.org/10.1016/j.watres.2014.02.032

Lackner, S. and Welker, S. (2019) Betrieb der Pilotanlage. In Entwicklung eines Verfahrens zum Einsatz der Deammonifikation im Haupt-strom kommunaler Kläranlagen zur energie-effizienten Stickstoffelimination.

Le, L.-T., Jeon, J., Dang, B.-T., Bui, X.-T. and Jahng, D. (2022) Influence of temperature on anammox reaction and microbial diversity in a bio-carriers reactor under mainstream conditions. Environmental Technology & Innovation 25, 102178. https://doi.org/10.1016/j.eti.2021.102178

Le, L.-T., Lee, S., Bui, X.-T. and Jahng, D. (2020) Suppression of nitrite-oxidizing bacteria under the combined conditions of high free ammonia and low dissolved oxygen concentrations for mainstream partial nitritation. Environmental Technology & Innovation 20, 101135. https://doi.org/10.1016/j.eti.2020.101135

Li, Q., Wang, S., Zhang, P., Yu, J., Qiu, C. and Zheng, J. (2018a) Influence of temperature on an Anammox sequencing batch reactor (SBR) system under lower nitrogen load. Bioresource Technology 269, 50-56. https://doi.org/10.1016/j.biortech.2018.08.057

Li, X., Klaus, S., Bott, C. and He, Z. (2018b) Status, Challenges, and Perspectives of Mainstream Nitritation–Anammox for Wastewater Treatment. Water Environment Research 90(7), 634-649. 10.2175/106143017x15131012153112

Liang, Y., Li, D., Zhang, X., Zeng, H., Yang, Z., Cui, S. and Zhang, J. (2015) Stability and nitrite-oxidizing bacteria community structure in different high-rate CANON reactors. Bioresource Technology 175, 189-194. https://doi.org/10.1016/j.biortech.2014.10.080

Lotti, T., Kleerebezem, R., Lubello, C. and van Loosdrecht, M.C.M. (2014a) Physiological and kinetic characterization of a suspended cell anammox culture. Water Research 60, 1-14. https://doi.org/10.1016/j.watres.2014.04.017

Lotti, T., Kleerebezem, R., van Erp Taalman Kip, C., Hendrickx, T.L.G., Kruit, J., Hoekstra, M. and van Loosdrecht, M.C.M. (2014b) Anammox Growth on Pretreated Municipal Wastewater. Environmental Science & Technology 48(14), 7874-7880. 10.1021/es500632k

Lotti, T., van der Star, W.R.L., Kleerebezem, R., Lubello, C. and van Loosdrecht, M.C.M. (2012) The effect of nitrite inhibition on the anammox process. Water Research 46(8), 2559-2569. https://doi.org/10.1016/j.watres.2012.02.011

Lv, Y., Wang, L., Sun, T., Wang, X., Yang, Y. and Wang, Z. (2010) Autotrophic nitrogen removal discovered in suspended nitritation system. Chemosphere 79(2), 180-185. https://doi.org/10.1016/j.chemosphere.2010.02.007

Ma, B., Bao, P., Wei, Y., Zhu, G., Yuan, Z. and Peng, Y. (2015) Suppressing Nitrite-oxidizing Bacteria Growth to Achieve Nitrogen Removal from Domestic Wastewater via Anammox Using Intermittent Aeration with Low Dissolved Oxygen. Scientific Reports 5(1), 13048. 10.1038/srep13048

Mulder, A., van de Graaf, A.A., Robertson, L.A. and Kuenen, J.G. (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiology Ecology 16(3), 177-183. 10.1111/j.1574-6941.1995.tb00281.x

Ochs, P., Martin, B.D., Germain, E., Stephenson, T., van Loosdrecht, M.C.M. and Soares, A. (2020) Ammonia removal from mixed dewatering liquors by three different deammonification technologies. Environmental Science: Water Research & Technology 6(12), 3440-3450. 10.1039/D0EW00875C

Park, S. and Bae, W. (2009) Modeling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid. Process Biochemistry 44(6), 631-640. https://doi.org/10.1016/j.procbio.2009.02.002

Regmi, P., Holgate, B., Fredericks, D., Miller, M., Wett, B., Murthy, S. and Bott, C. (2015) Optimization of a mainstream nitritation-denitritation process and anammox polishing Pusker Regmi, Becky Holgate, Dana Fredericks, Mark W. Miller. Water science and technology : a journal of the International Association on Water Pollution Research 72, 632-642. 10.2166/wst.2015.261

Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S. and Bott, C.B. (2014) Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation. Water Research 57, 162-171. https://doi.org/10.1016/j.watres.2014.03.035

Rittmann, B.E. and McCarty, P.L. (2007) Environmental biotechnology: Principles and applications (Internat. ed., [Nachdr.]). McGraw-Hill.

Siripong, S. and Rittmann, B.E. (2007) Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. Water Research 41(5), 1110-1120. https://doi.org/10.1016/j.watres.2006.11.050

Sliekers, A.O., Derwort, N., Gomez, J.L.C., Strous, M., Kuenen, J.G. and Jetten, M.S.M. (2002) Completely autotrophic nitrogen removal over nitrite in one single reactor. Water Research 36(10), 2475-2482. https://doi.org/10.1016/S0043-1354(01)00476-6

Sobotka, D., Zhai, J. and Makinia, J. (2021) Generalized temperature dependence model for anammox process kinetics. Science of The Total Environment 775, 145760. https://doi.org/10.1016/j.scitotenv.2021.145760

Strous, M. (2000) Microbiology of anaerobic ammonium oxidation, Delft University of Technology

Strous, M., Heijnen, J.J., Kuenen, J.G. and Jetten, M.S.M. (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Applied Microbiology and Biotechnology 50(5), 589-596. 10.1007/s002530051340

Strous, M., Van Gerven, E., Zheng, P., Kuenen, J.G. and Jetten, M.S.M. (1997) Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. Water Research 31(8), 1955-1962. https://doi.org/10.1016/S0043-1354(97)00055-9

Third, K.A., Sliekers, A.O., Kuenen, J.G. and Jetten, M.S.M. (2001) The CANON System (Completely Autotrophic Nitrogen-removal Over Nitrite) under Ammonium Limitation: Interaction and Competition between Three Groups of Bacteria. Systematic and Applied Microbiology 24(4), 588-596. https://doi.org/10.1078/0723-2020-00077

van de Graaf, A.A., de Bruijn, P., Robertson, L.A., Jetten, M.S.M. and Kuenen, J.G. (1996) Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142(8), 2187-2196. https://doi.org/10.1099/13500872-142-8-2187

Vlaeminck, S.E., De Clippeleir, H. and Verstraete, W. (2012) Microbial resource management of one-stage partial nitritation/anammox. Microbial Biotechnology 5(3), 433-448. https://doi.org/10.1111/j.1751-7915.2012.00341.x

Wang, W., Yan, Y., Song, C., Pan, M. and Wang, Y. (2018) The microbial community structure change of an anaerobic ammonia oxidation reactor in response to decreasing temperatures. Environmental Science and Pollution Research 25(35), 35330-35341. 10.1007/s11356-018-3449-1

Wett, B. (2007) Development and implementation of a robust deammonification process. Water Science and Technology 56(7), 81-88. 10.2166/wst.2007.611

Wett, B., Omari, A., Podmirseg, S.M., Han, M., Akintayo, O., Gómez Brandón, M., Murthy, S., Bott, C., Hell, M., Takács, I., Nyhuis, G. and O'Shaughnessy, M. (2013) Going for mainstream deammonification from bench to full scale for maximized resource efficiency. Water Science and Technology 68(2), 283-289. 10.2166/wst.2013.150

Xu, G., Zhou, Y., Yang, Q., Lee, Z.M.-P., Gu, J., Lay, W., Cao, Y. and Liu, Y. (2015) The challenges of mainstream deammonification process for municipal used water treatment. Applied Microbiology and Biotechnology 99(6), 2485-2490. 10.1007/s00253-015-6423-6

Xu, Z., Zhang, L., Gao, X. and Peng, Y. (2020) Optimization of the intermittent aeration to improve the stability and flexibility of a mainstream hybrid partial nitrification-anammox system. Chemosphere 261, 127670. https://doi.org/10.1016/j.chemosphere.2020.127670

Yang, Q., Peng, Y., Liu, X., Zeng, W., Mino, T. and Satoh, H. (2007) Nitrogen Removal via Nitrite from Municipal Wastewater at Low Temperatures using Real-Time Control to Optimize Nitrifying Communities. Environmental Science & Technology 41(23), 8159-8164. 10.1021/es070850f

Yin, X., Zhai, J., Hu, W., Li, Y., Rahaman, M.H. and Mąkinia, J. (2019) A fast start-up of the organotrophic anammox process inoculated with constructed wetland sediment. Ecological Engineering 138, 454-460. https://doi.org/10.1016/j.ecoleng.2019.07.039

Zhu, S. and Chen, S. (2001) Effects of organic carbon on nitrification rate in fixed film biofilters. Aquacultural Engineering 25(1), 1-11. https://doi.org/10.1016/S0144-8609(01)00071-1

I Appendix

I.I Operational backflush procedure of membranes

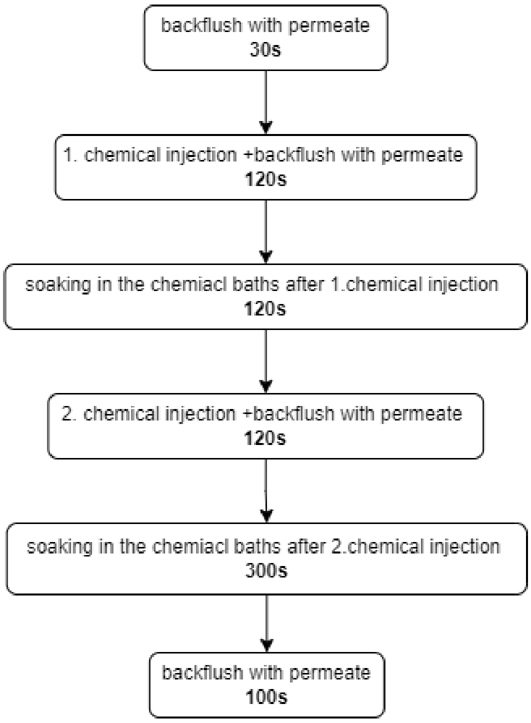


Figure 40. Membrane backflush procedure with chemicals

I.II Chemical-dosing for the membrane backflush

Dosing of NaClO bulk solution

bulk solution (NaClO):	120 g/L
backwash flux:	20 L/m²/h (10-40LMH provided by the supplier)
backwash flow rate:	$Q_{\text{backwash}} = 160\ m^2 \times 0.02\frac{m^3}{(m^2 \cdot h)} = 3.2\ \frac{m^3}{h}$

desired chemical concentration in the backwash water:

$c_{\text{NaClO}} = 200$ mg/L

dosing capacity of the NaClO dosing pump:

$$Q_{\text{NaClO, dosing}} = \frac{3.2\frac{m^3}{h} * 200\frac{mg}{L}}{120\frac{g}{L}} = 5.3\frac{L}{h}$$

Dosing of citric acid

bulk solution (citric acid):	500 g/L
backwash flux:	20 L/m²/h (10-40LMH provided by the supplier)
backwash flow rate:	$Q_{\text{backwash}} = 160\ m^2 \times 0.02\frac{m^3}{(m^2 \cdot h)} = 3.2\ \frac{m^3}{h}$

desired chemical concentration in the backwash water:

$c_{\text{NaClO}} = 1$ g/L

dosing capacity of the citric acid dosing pump:

$$Q_{\text{NaClO, dosing}} = \frac{3.2\,\frac{m^3}{h} * 1\,\frac{g}{L}}{500\,\frac{g}{L}} = 6.4\,\frac{L}{h}$$