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STUDY ON COLD PARTIAL NITRITATION INOCULATED BY NITRIFYING MUNICIPAL SLUDGE

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ABSTRACT

This thesis develops an alternative process to nitrification/denitrification (N/DN) for nitrogen removal from municipal sewage at temperatures between 13-26ºC, due to drawbacks of N/DN systems: high consumption of energy and organic carbon, and production of a great amount of sludge. The partial nitritation (PN) start-up from fully nitrifying municipal sludge was studied through a pilot-scale sequencing batch reactor (SBR) fed with contaminated tap water by ammonium sulfate ($(NH_4)_2SO_4$), which simulated real sewage from anaerobic digestion. The analysis of the samples by using several tests (nitrogen test, kinetic test and volatile suspended solids test) and the control of certain parameters (pH, dissolved oxygen (DO), ammonium concentration in the influent (TAN), etc.) were carried out to keep the process under stable conditions and to know the system behaviour in the nitrogen removal at low temperatures. The final challenge was to generate a suitable effluent for the subsequent anammox step with an ammonium conversion between 50-57% and a high suppression of the activity of nitrite oxidizing bacteria (NOB) by the growth of ammonium oxidizing bacteria (AOB), the limitation on the duration of the aerobic SBR phase and the control of the solid retention time (SRT).

After an exhaustive analysis of the results, the success of the research was confirmed since the effluent from the pilot plant showed an acceptable NOB inhibition with an average nitrite accumulation (NAR) of 89% and a partial conversion of ammonium to nitrite through a final ammonium nitrogen conversion between 41-62% and a $N\text{-}NO_2^-/N\text{-}NH_4^+$ ratio of 1.20±0.30; in spite of a low NOB activity in the reactor and the loss of biomass due to excess sludge washout in the effluent. To overcome these small limitations in the future, several improvements have been suggested. On the basis of the above, this study provides a good background for the next research on the anammox reaction at low temperatures, which is going to be carried out by the Department of Water Technology and Environmental Engineering of University of Chemistry and Technology (UCT) from Prague (Czech Republic).

Keywords: partial nitritation; wastewater treatment; municipal sludge; nitrogen removal; low temperatures.

LIST OF ABBREVIATIONS

ANAMMOX:	Anaerobic Ammonium Oxidation
AOB:	Ammonia Oxidizing Bacteria
AMX	Anammox bacteria
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FA	Free Ammonia
FNA	Free Nitrous Acid
HB	Heterotrophic Bacteria
NOB	Nitrite Oxidizing Bacteria
PN/A	Partial Nitritation-Anammox
N/DN	Nitrification-Denitrification
SRT	Solid Retention Time
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant
MBBR	Moving bed biofilm reactor
SBR	Sequencing batch reactor
NLR	Nitrogen loading rate

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1. MOTIVATION OF THE RESEARCH

Currently, wastewater treatment has an important role due to the worry and interest of the population for the environment, which needs useful and innovative technologies that guarantee the protection of rivers and seas, as well as the ecosystem which encloses them, with a high efficiency and low energy demand.

During the last years one of the most important techniques for the treatment of water is the anaerobic digestion as it offers fundamental benefits, such as:

- The volume of excess sludge produced in anaerobic treatment generally is significantly lower as compared to aerobic systems.
- It can be employed at very low costs, because technically plain and relatively inexpensive reactors are used, and anaerobic treatment systems generally can be operated with little if any consumptive use of high grade energy.
- It can be applied at practically any place and at any scale.
- Anaerobic organisms can be preserved unfed for long periods of time (exceeding one year) without any serious deterioration of their activity, while also other important characteristics of anaerobic sludge generally remain almost unaffected.

The drawback of this process is the need of combining it with post-treatment methods to remove nutrients (nitrogen and phosphorus) from the effluent since the anaerobic digestion cannot carry it out [1]. The solution of this issue is the partial nitritation and subsequent anammox reaction, as an alternative to nitrification and denitrification, and it will be the focus of this thesis in which this first part of the process will be developed to contribute to the future researches about anammox.

Moreover, this work let me increase my knowledge and continue to learn about wastewater treatment, field of chemical engineering in which I focused my degree and my final bachelor project with the title “Analysis of hydraulic performance and proposals for improvement for the San Rafael irrigation network (sector 4 canal Jucar-Turia)”.

2. INTRODUCTION

2.1. NUTRIENTS FROM WASTEWATER

Wastewater contains suspended solids, biodegradable organics, pathogens, nutrients, heavy metals and dissolved inorganic. One of the most important constituents are the nutrients which organic carbon, nitrogen and phosphorus can be highlighted [2]:

- Organic carbon: the main component of the organic substances found in sewage. COD or BOD_5 are measurement of this element.

- Nitrogen: it is present in organically bonded form (organic N) and as ammonium nitrogen ($N\text{-}NH_4^+$). They can be known with total ammonium nitrogen (TAN).

- Phosphorus: in sewage, there are orthophosphate ($PO_4\text{-}P$), polyphosphate and organic phosphorus compounds. They can be known with total phosphorus (P_{tot}) parameter.

The origin of nutrients in sewage may be due to anthropogenic causes of domestic and industrial uses, atmospheric dissolution or geological deposits rich in these materials, surface and subterranean runoff, use of fertilisers mainly in agriculture, fixation of prokaryotic organisms and decomposition of organic material, and lastly, atmospheric deposition due to air pollution.

For the correct start-up of the anaerobic method [3], C:N:P ratio should be around 400:5:1-300:5:1, while 100:10:1-100:5:1 appears suitable for activated sludge process [4]. Thus, the nutrient removal is necessary through their simultaneous or subsequent treatment in the wastewater treatment plants as the high concentrations of nitrogen and phosphorus-based nutrients in water lead to eutrophication, reduction in sunlight, lower dissolved oxygen levels, changing rates of plant growth, reproduction patterns, and overall deterioration of water quality. Furthermore, nitrate is a risk to human health, especially as a possible cause of infant methaemoglobinemia [5], [6].

Due to all of the above, the next pages are focused on the nitrogen removal in sewage, so it can be incorporated to the nitrogen cycle again.

2.2. NITROGEN CYCLE

Nitrogen is the most abundant element in the Earth´s atmosphere and it constitute 78% of the troposphere. However, this element cannot be absorbed by animals and plants thus it is necessary to convert nitrogen into compounds they can use. This process is called nitrogen cycle and it has four stages [7]:

1)Nitrogen Fixation: The nitrogen gas (N2) is transformed to ammonia (NH3) by special bacteria. Plants are able to use this ammonia.

2)Nitrification: Process in which ammonia is converted into nitrite ions which the plants can take in as nutrients.

3)Ammonification: Decomposer bacteria convert the nitrogen-rich waste compounds (after all of the living organisms have used it) into simpler ones.

4)Denitrification: This is the last step in the nitrogen cycle where the reduction of soil nitrate to nitrogen-containing gases, such as nitric oxide, nitrous oxide, and nitrogen gas, that return to the atmosphere to start the cycle again, takes place.

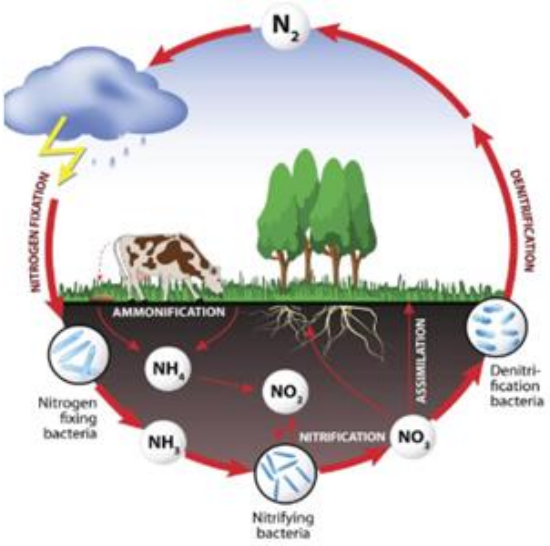


Figure 1. Stages of the nitrogen cycle: Nitrogen fixation, nitrification, ammonification and denitrification. Source [7].

The wastewater treatment consists of several steps that reduce nitrogen concentration until reaching to permitted limits. This process uses the natural nitrogen cycle and implement it by using bacteria [8].

2.3. NITRIFICATION AND DENITRIFICATION (N/DN)

Currently, biological nitrification-denitrification is the main method to remove nitrogen from wastewaters, though its use is going to decrease in the future due to the development of new methods more effective. This treatment presented several improvements in the past such as simultaneous nitrification-denitrification (SND), where nitrification and denitrification happen at the same time within the same reactor. Some advantages of this process against normal nitrification-denitrification (N/DN) are [9], [10]:

- Achieves removal of 80-96% total nitrogen.
- Eliminates the need to build separate tanks.
- Produce less nitrous oxide emissions than conventional nitrogen removal processes.
- Simpler process design with a smaller total tank size.
- Maintains a relatively neutral pH in the bioreactor without the addition of an external acid or base because alkalinity consumed by nitrification is partially recovered by alkalinity production in denitrification.

Nevertheless, the most common configuration is still nitrification-denitrification in two different stages at the wastewater treatment plant (WWTP):

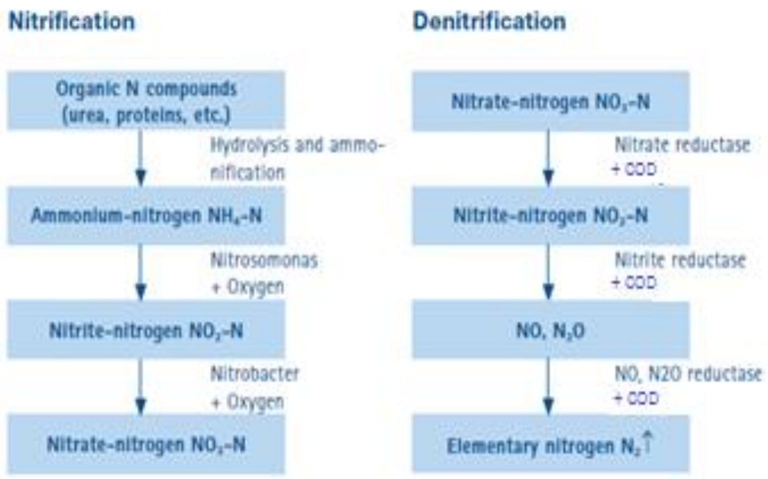


Figure 2. All stages of the nitrification and denitrification process for the nitrogen removal in sewage. Source: [2].

Nitrification is the first step and it consists of an aerobic oxidation called nitritation of ammonium (NH_4^+) to nitrite (NO_2^-) thanks to ammonium oxidizing bacteria (AOB) like *Nitrosomonas* bacteria, which use ammonium as energy source and oxygen as electron acceptor, and then to nitrate (NO_3^-) with nitrite-oxidizing bacteria (NOB) like *Nitrobacter* bacteria that compete with AOB for oxygen in nitratation. These microorganisms get their energy from the oxidation of these nitrogen compounds and they are autotrophic hence they derive their carbon source from inorganic carbon (for example: bicarbonate and carbon dioxide).

Temperature, solids retention time, nitrogen loading rate, inhibitors, alkalinity, pH, organic loading, dissolved oxygen concentration… affect the nitrification of the activated sludge plants.

$$NH_4{}^+ + 1.5O_2 \rightarrow NO_2{}^- + 2H^+ + H_2O \text{ (a)}$$

$$NO_2{}^- + 0.5O_2 \rightarrow NO_3{}^- \text{ (b)}$$

$$NH_4{}^+ + 2O_2 \rightarrow NO_3{}^- + 2H^+ + H_2O \text{ (c)}$$

Reaction 1. Nitrification: a) ammonium oxidizing bacteria (AOB) transform ammonium to nitrite (Nitritation); b) nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate (Nitratation); c) global nitrification reaction. Source: [11].

The next stage is denitrification, where nitrate formed in the previous step is anoxically reduced to nitrite (NO_2^-), then into nitrous oxide (N_2O), nitric oxide (NO) and lastly into gaseous nitrogen (N_2, non-reactive) because of denitrifying microorganisms (DNF), which are heterotrophic and they require organic matter (electron donor) but not molecular oxygen or dissolved oxygen. They use nitrate or nitrite as the final electron acceptor.

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$$

Reaction 2. Global denitrification: Nitrate is transformed to gaseous nitrogen through denitrifying microorganisms (DNF). Source:[11].

The main drawbacks of the nitrification-denitrification process are:

- High operating cost because it is related with the electric consumption by aeration.
- Production of a great amount of sludge which has to be managed.
- Sensitive process to the presence of toxins and inhibitors.

These disadvantages have caused the development of other methods in order to optimize biological nitrogen removal, like the partial nitritation-anammox process, which has been recognized as a promising nitrogen removal technology for wastewater treatment [12], [13].

2.4. PARTIAL NITRITATION – ANAMMOX (PN/A)

Anaerobic ammonium oxidation (anammox) is a completely autotrophic oxidation of ammonium with nitrite as an electron acceptor and without organic electron donors. This process is combined with partial nitritation therefore in this first stage approximately 50-57% of the influent ammonium is oxidized to nitrite by ammonium oxidizing bacteria (AOB; like the previous step in nitrification/denitrification). Then, the remaining ammonium reacts with the produced nitrite in the anammox process and nitrogen gas (N_2) is obtained thanks to anammox bacteria that utilize nitrite as electron acceptor and ammonium as electron donor. These microorganisms belong to the *phylum Planctomycetes* and up to now five genera have been described: *Candidatus* Brodacia, *Ca.* Kuenenia, *Ca.* Anammoxoglobus, *Ca.* Jettenia and *Ca.* Scalindua [14], [15]. Moreover, they can be inhibited by high nitrite concentration (despite the fact that it is the substrate of the reaction) or even low concentration of dissolved oxygen (DO) (though this is required in partial nitritation) [16].

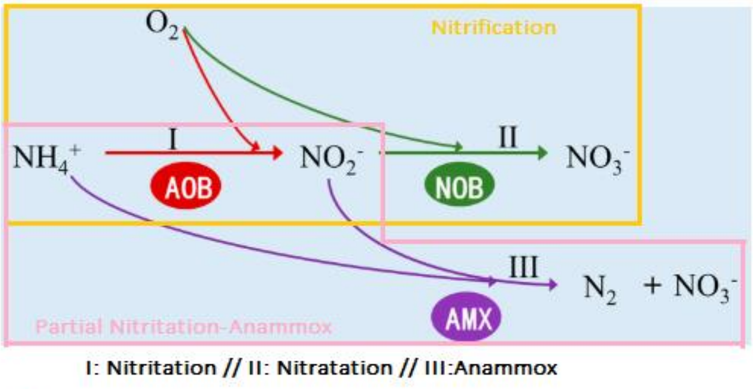


Figure 3. Diagram about the technique of nitrogen removal in wastewater treatment partial nitritation-anammox (PN/A), with all the species which take part in each reaction. Source: [17].

PN/A can occur through two different configurations (Tab. 1): The first type involves a two-steps process, i.e., nitritation reactor and anammox reactor are combined in series, one example of this is SHARON (single reactor system for high activity ammonium removal over nitrite) + ANAMMOX. The second type is a one-step process, i.e., nitritation and anammox are mixed in one reactor, like CANON (completely autotrophic nitrogen-removal over nitrite). The reactions of both methods are shown in the next figure [18]:

$$NH_4^+ + 1.5\, O_2 \rightarrow NO_2^- + H_2O + 2\, H^+ \quad \text{(a)}$$

$$NH_4^+ + 1{,}32\, NO_2^- + 0{,}066\, HCO_3^- + 0{,}13\, H^+ \rightarrow 1{,}02\, N_2 + 0{,}26\, NO_3^- + 0{,}066\, CH_2O_{0,5}N_{0,15} + 2{,}03\, H_2O \quad \text{(b)}$$

Reaction 3. Partial nitritation-anammox: a) ammonium oxidizing bacteria (AOB) transform ammonium to nitrite up to 50-57% (partial-nitritation, see I in figure 3); b) the remaining ammonium reacts with nitrite in order to produce nitrogen gas (anammox, see III in figure 3), by using anammox bacteria (AMX). Source: [19].

In this process, nitrate is not a desirable product of reaction though it is produced in low amounts in partial nitritation-anammox reactions. Therefore, the success of PN/A depends on the inhibition of the nitratation reaction (React. 1.b) that can occur after nitritation, as this reaction would produce nitrate and consume nitrite so finally anammox reaction could not be carried out (it competes with NOB for nitrite) [20]. For this reason, there are methods which allow to inhibit NOB and to guarantee simultaneously optimal conditions for the ammonium oxidising bacteria (AOB) so as to favour partial nitritation-anammox (PN/A), for instance:

- Inhibitors: inhibition depends on the kind of microbial communities as inhibition constants (KI) are different among species. The smaller the K_I, the greater the binding affinity and the smaller amount of inhibitor is needed in order to decrease the activity of bacteria. Currently, one of the more promising appears to be sulphide due to the fact that researches show that NOB are more vulnerable to sulphide inhibition than AOB as they have a lower K_I value (KI_{AOB} = 150 mg-S/L, KI_{NOB} = 10 mg-S/L [21]).

  In some cases, it is not necessary to add sulphide so that inhibition happens because the amount of hydrogen sulphide come from sulfate content in wastewater, whose reaction is favoured by several reason, such as anaerobic conditions in long separate sewer system or anaerobic reactor. Sulfate is variable and it depends on factors such as industrial wastewater discharges, drinking water source water, etc. In regions near the coast, sulfate concentrations increase in wastewater due to seawater infiltration into both groundwater reservoirs used as drinking water sources and sewage collection systems, a phenomenon that will be magnified by global climate change, sea level rise, and projected population growth along coasts. These increases all lead to increased sulphide loads to wastewater treatment plants [22].

- Temperature and SRT: The focus of this inhibition method is that AOB are favoured at temperatures above 25ºC since though at 30-35ºC NOB is not inhibited, the specific growth rate of AOB is slightly higher that the growth rate of NOB at 30ºC. However, an increase in temperature is not effective enough for the completed inhibition, it is necessary to join this temperature rise with a decrease in solid retention time (SRT) because *Nitrosomonas* spp. (typical AOB) have a minimum doubling time of 7–8 h, while the value for *Nitrobacter* spp. is 10–13 h. Thereby, the SRT should be set lower than the duplication time of NOB, thus ensuring the washout of these bacteria and keeping a large amount of AOB in the reactor [23] [24].

- Dissolved oxygen: In this method, is necessary to create an oxygen limiting environment since at low dissolved oxygen (DO) concentrations, AOB are more dominant than NOB. This can be seen when the value of DO is lower than 0.5mg/L in a suspended growth reactor [25].

- Free compounds-pH: High free ammonia (FA) and/or free nitrous acid (FNA) concentration may induces to the inhibition of AOB and NOB [26]. FA completely inhibits AOB and NOB at a concentration of 78 mg/L, and at FA concentrations between 14 and 17 mg/L only nitrite oxidation is selectively inhibited, whereas ammonium is oxidised to nitrite. In the case of FNA, a concentration of 0.22–2.8 mg/L inhibits the nitrification process and 0.02–0.04 mg/L inhibits only NOB.

  Secondly, the pH is an alternative parameter for controlling nitrification for ammonium rich wastewater as it has a strong influence on FA and FNA concentration. Thus, it is necessary to arrange pH to inhibit NOB: free nitrous acid (HNO_2) formation is associated with a decrease in pH thus equilibrium (React. 4b) shifts towards it at low pH (from 7.5 to 6), while free ammonia (NH_3) is proportional to the pH increase as equilibrium (React. 4a) shifts towards free ammonia at high pH (from 7.5 up to 8.5) [27].

Equilibrium reaction of NH_4^+/NH_3 and NO_2^-/HNO_2 acid are shown in the next reactions [28]:

$$\mathrm{NH_4}^+ + \mathrm{OH}^- \leftrightarrow \mathrm{NH_3} + \mathrm{H_2O} \quad \text{(a)}$$

$$H^+ + NO_2{}^- \leftrightarrow HNO_2 \quad \text{(b)}$$

Reaction 4. Equilibrium reactions according to pH in sewage: a) equilibrium ammonium/ammonia reaction; b) equilibrium nitrogen dioxide/nitrous acid reaction. Source: [28].

In addition to the bacterial activity, the temperature also controls the amount of FA and FNA, as it can be seen in the next equations [29]:

$$\mathrm{FA}\left(\mathrm{mg\,L^{-1}}\right) = \frac{17}{14} \times \frac{\rho\,\mathrm{NH_4-N} \times 10^{\mathrm{pH}}}{\exp\left[6334/(273+T)\right] + 10^{\mathrm{pH}}} \quad \text{(eq.1)}$$

$$\mathrm{FNA}\left(\mathrm{mg\,L^{-1}}\right) = \frac{46}{14} \times \frac{\rho\,\mathrm{NO_2^{-}-N}}{\exp\left[-2300/(273+T)\right] \times 10^{\mathrm{pH}}} \quad \text{(eq.2)}$$

Where:

T: wastewater temperature (ºC).

ρNH_4^+-N: ammonium nitrogen concentration (mg/L).

ρNO_2^--N: nitrite nitrogen concentration (mg/L).

Finally, the most important advantages of partial nitration-anammox technology are [30]:

- Energy consumption for aeration is nearly 59% lower than in nitrification-denitrification process.
- Completely autotrophic process, so up to 80% less excess sludge.
- Nearly 100% elimination of carbon demand for denitrification.

Nevertheless, depending on the kind of PN/A (Fig. 4a/b) the advantages or disadvantages can vary, as Tab. 1 shows:

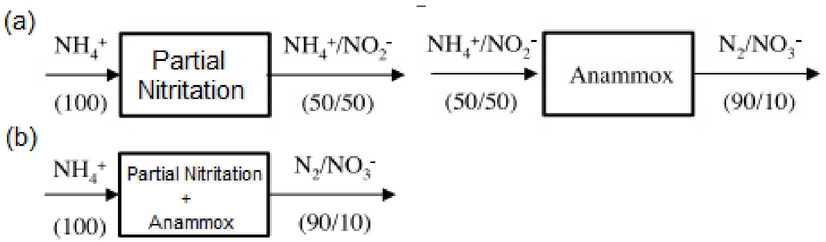


Figure 4. Flux diagrams of wastewater treatment in basis of partial nitritation-anammox process: a) two steps (PN-A), b) one-step (PNA). Source: [31].

Table 1. Advantages and disadvantages of the different combinations of PN/A processes for the nitrogen removal in wastewater. Source: [18].

	ADVANTAGES	DISADVANTAGES
TWO STEPS	AOB and AMX are cultured separately, each maximizing their role in microbial nitrogen transformation	To control pH fluctuation in each reaction, a large amount of HCO_3^- needs to be added.
		The nitritation reactor often loses stability due to NOB growth.
		The need to constantly adjust partial nitritation to meet the requirements of subsequent anammox reactions complicates the process.
		The reactor requires a large area
ONE STEP	The base produced in anammox can buffer the acid produced in the partial nitritation process	The conflict in DO needs between the nitritation process and anammox cannot be resolved.
	The NO_2^- produced in nitritation can be immediately consumed by AMX, inhibiting the need of NOB for substrate	It is difficult to achieve stable control of a hypoxic environment via limiting oxygen.
	The reactor requires a small area	

3. BACKGROUND

Since the discovery of partial nitritation/anammox (PN/A) in 1990s as new biological wastewater treatment, many innovative technologies have been developed and studied in Europe and North America for their applicability to the PN/A concept, and several have made it into full-scale with one or two stages (Tab. 1). According to different studies more than 50% of all PN/A installations are sequencing batch reactors (SBR; specially DEMON configuration [32], [33]) followed by granular systems [34] and MBBR [35], though granular systems treat by far the most nitrogen; 88% of all plants being operated as single-stage systems, and lastly 75% for side-stream treatment of municipal wastewater (Fig. 5) [13].

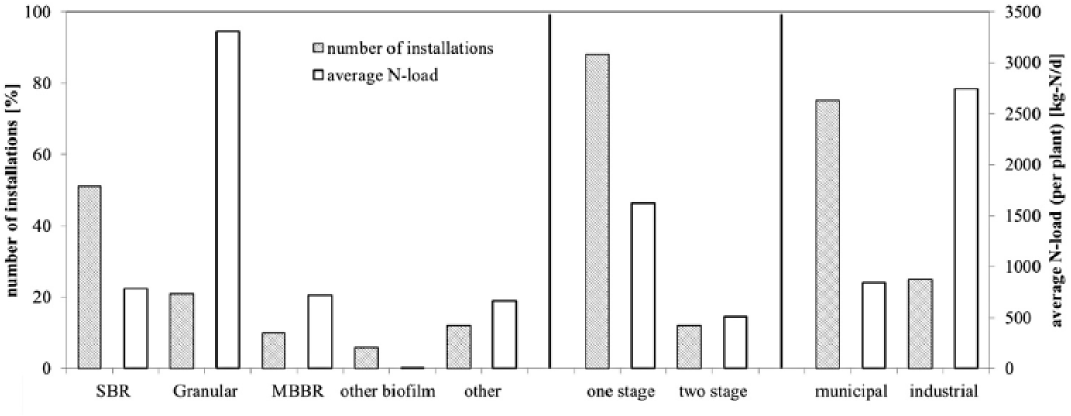


Figure 5. Distribution of PN/A applications, with the number of installations and information on the total nitrogen load in each category. Source:[13].

In the past, the study and application of the partial nitritation-anammox process has been usually focused on the treatment of wastewater with temperatures around 30ºC in order to operate under optimum conditions since the growth rates of ammonium oxidizing bacteria are usually higher than those of nitrite oxidizing bacteria at elevated temperatures (>30Cº), hence the nitratation inhibition is favoured [36]; besides, the maximum activity of anammox bacteria (AMX) and ammonium oxidizing bacteria (AOB) is found at 35-40ºC [37]. Nonetheless, several researches showed that nitrogen removal at a lower temperature by an anammox process can work [38]–[40] despite the fact that the activities of anammox bacteria (AMX) and ammonium oxidizing bacteria (AOB) decreased from 20 to 15°C [37], [38] and that partial nitrification is difficult to accomplish in winter due to the varying temperature of municipal wastewater [41].

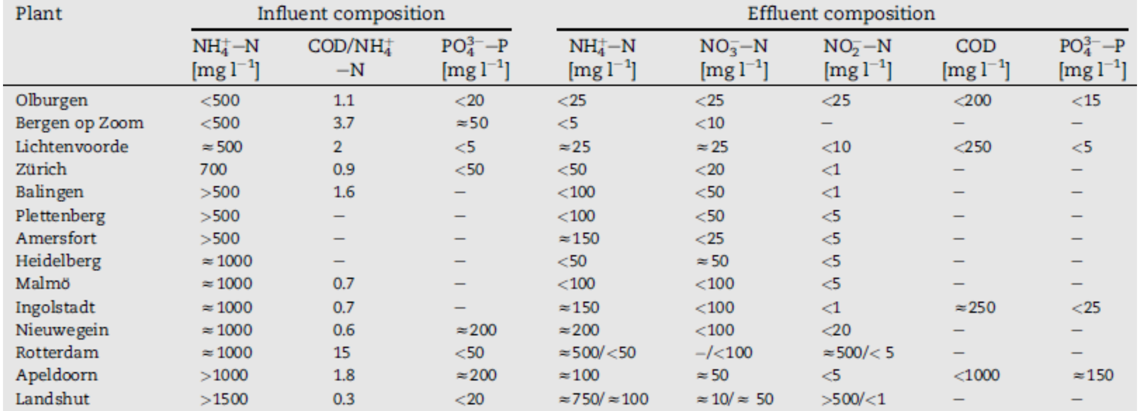
3.1. HIGH TEMPERATURES (30-40ºC)

Different authors have studied the feasibility of the partial nitritation-anammox (PN/A) process at high temperatures for nitrogen removal from wastewater. The most relevant researches are:

Christian Fux et al. [42] in 2002, studied this method at 30ºC and a pH around 7.5 to treat digester effluents from two different wastewater treatment plants (Werdhoelzli WWTP in Zurich and the Au WWTP in St.Gallen) in two stages. Partial nitritation was carried out in a tank reactor without sludge retention and the subsequent anammox reaction in a sequencing batch reactor (SBR) with suspended biomass inoculated by nitrifying sludge from Koelliken WWTP. The results of this research indicated the success of the system since 58% of the ammonium was converted to nitrite during PN with a N-NO_2^-/N-NH_4^+ratio of 1.3 and over 90% of this inlet nitrogen load to the anammox reactor was removed. Besides, the produced nitrate was low due to denitrification by denitrifying microorganisms (DNF) and the sludge production was negligible.

In 2014, Susanne Lackner et al. [13] carried out an exhaustive comparison among 14 full-scale PN/A facilities (SBR, MBBR, granular and 2-stage systems) at 30ºC with a pH and dissolved oxygen (DO) concentration between 6.7-8 and 0.05-1.5 mg/L, respectively; and nitrogen loading rates (NLR) between 1-7 kg-N/m^3*d for biofilm systems (1- and 2-stage) and 0.04-0.65 kg-N/m^3*d for SBR systems. Its results (Tab. 2) showed a nitrogen removal efficiency over 90% in all the questioned plants, though 50% of them reported accumulation of nitrite oxidizing bacteria (NOB) and therefore nitrate (NO_3^-), despite the actions that was performed to counteract it, such as: reduction of the air flow rate, the DO set-point, blower frequency or runtime (increased anoxic phases) and intermittent aeration (changes in on/off times).

Table 2. Influent and effluent comparison of all surveyed plants to remove nitrogen from wastewater through the partial nitritation-anammox system. Source [13].

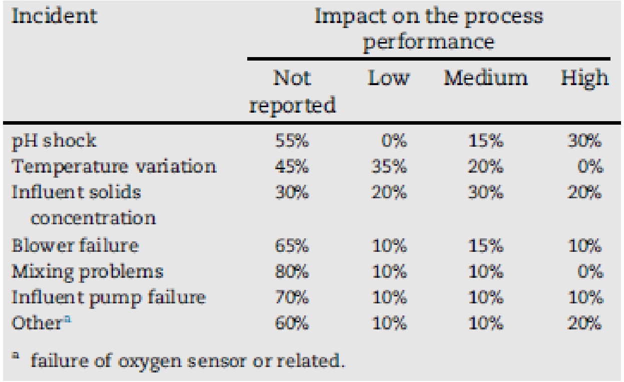


Plant	Influent composition			Effluent composition				
	NH_4^+–N [mg l^{-1}]	COD/NH_4^+ –N	PO_4^{3-}–P [mg l^{-1}]	NH_4^+–N [mg l^{-1}]	NO_3^-–N [mg l^{-1}]	NO_2^-–N [mg l^{-1}]	COD [mg l^{-1}]	PO_4^{3-}–P [mg l^{-1}]
Olburgen	<500	1.1	<20	<25	<25	<25	<200	<15
Bergen op Zoom	<500	3.7	≈50	<5	<10	–	–	–
Lichtenvoorde	≈500	2	<5	≈25	≈25	<10	<250	<5
Zürich	700	0.9	<50	<50	<20	<1	–	–
Balingen	>500	1.6	–	<100	<50	<1	–	–
Plettenberg	>500	–	–	<100	<50	<5	–	–
Amersfort	>500	–	–	≈150	<25	<5	–	–
Heidelberg	≈1000	–	–	<50	≈50	<5	–	–
Malmö	≈1000	0.7	–	<100	<100	<5	–	–
Ingolstadt	≈1000	0.7	–	≈150	<100	<1	≈250	<25
Nieuwegein	≈1000	0.6	≈200	≈200	<100	<20	–	–
Rotterdam	≈1000	15	<50	≈500/<50	–/<100	≈500/< 5	–	–
Apeldoorn	>1000	1.8	≈200	≈100	≈50	<5	<1000	≈150
Landshut	>1500	0.3	<20	≈750/≈100	≈10/≈ 50	>500/<1	–	–

After analysing different parameters, Susanne Lackner et al. [13] concluded that biofilm/granular based systems present a behavior more robust than sequencing batch reactors at temperatures around 30ºC, but the energy demand is significantly lower in SBRs, since these facilities worked within a range of 0.8-2 kWh/kg-N whereas for the other systems the energy consumption was 1.05-4.17 kWh/kg-N, as other authors showed as well [43]. Nevertheless, compared to the nitrification-denitrification (N/DN) process which reaches 11.7 kWh/kg-N during nitrogen removal [44], there is a clear savings in energy consumption of at least 50% with PN/A systems.

In addition, this study collected for the first time information on the difficulties/problems that commonly happed in PN/A and the impact of them on the process yield (Tab. 3), in order to know weaknesses of this technology and consider them in future facilities:

Table 3. Number of plants (in percentage of total number, n=14) having experienced these factors with a rating of the impact on process performance. Source: [13]



Incident	Impact on the process performance			
	Not reported	Low	Medium	High
pH shock	55%	0%	15%	30%
Temperature variation	45%	35%	20%	0%
Influent solids concentration	30%	20%	30%	20%
Blower failure	65%	10%	15%	10%
Mixing problems	80%	10%	10%	0%
Influent pump failure	70%	10%	10%	10%
Other[a]	60%	10%	10%	20%

[a] failure of oxygen sensor or related.

Another important research on the partial nitritation-anammox process at 30ºC was performed in 2019 when Songkai Qiu et al. [45] studied this wastewater treatment by using a new technique, based on intermittently aerated sequencing batch reactor (IASBR), i.e. 6 successive 15 min non-aeration-30 min aeration intervals, a nitrogen loading rate of 0.105 kg-N/m^3*d, HRT of 2.9 days, and a DO concentration and pH between 0-0.05 mg/L and 7-8, respectively. The success of this system was achieved since partial nitritation was stable with a nitrite accumulation ratio (NAR) of about 80% and a total nitrogen removal of up to 81.5%. Besides, NOB was effectively restrained (abundance <1.6%) by the optimization of ammonium oxidizing bacteria (AOB) conditions.

Finally, these researches show a complete control of the PN/A process because they reached good values of nitrite accumulation ratio (>82%), nitrogen loading rate (0.1-7 kg-N/m^3*d), nitrogen removal efficiency (>90%), etc. in the effluent, together with a great NOB inhibition in the reactor under different conditions (pH, HRT, DO, intermittently aeration etc.) and configurations (SBR, MBBR, granular and 2-stage systems). The success of all of this studies demonstrates the viability of partial nitritation-anammox for the nitrogen removal at high temperature (>30ºC).

3.2. LOW TEMPERATURES (10-20ºC)

Over recent years, researches on partial nitritation-anammox (PN/A) were focused at low temperatures (<20ºC) after the good results that were obtained at high temperatures (>30ºC, see point 3.1) and the start-up of several full scale PN/A facilities. The first studies on PN/A at low temperatures showed a contradictory behaviour of the system at temperatures <18ºC, as it can be observed in the next researches:

In 2007, Qing Yang et al. [41] worked with a PN/A system between 11.9 to 26.5°C in a pilot-scale SBR under normal dissolved oxygen condition (≥ 2.5 mg/L) and a pH variation between 7-8.5. The average nitrogen loading rate was 0.2 kg-N/m^3*d and the effective SRT was about 13 days. The results were suitable and they showed for a long period (180 days) an average nitrite accumulation rate (NAR) above 95%, a nitrogen removal efficiency constantly higher than 90%, and an important NOB inhibition (these bacteria did not detect with the FISH analysis) through real-time control that effectively prevented excessive aeration.

Nonetheless in 2008, J. Dosta et al. [37] reported in their study that the system lost totally its efficiency when the temperature started to decrease from 18ºC to 15ºC because the nitrite accumulation caused the inhibition of anammox process and the decreased of the capacity of the system. The decrease in nitrogen loading rate from 0.3 to 0.05 kg-N/m^3*d was necessary to recover the correct operation of the SBR.

In both researches, NOB inhibition was accomplished so the difference in the PN/A operation at temperatures <18ºC happened because bacteria activity decrease at low temperatures, so in the case of J. Dosta et al. [37] the activity of anammox bacteria (AMX) decreased more than the activity of ammonium oxidizing bacteria (AOB) whereas the amount of nitrite started to increase. The mixed effect of the low temperature and the nitrite accumulation provoked the complete inhibition of AMX. Nevertheless, Qing Yang et al. [41] avoided this situation and worked with a stable PN/A system through the long-term application of real-time control, which changes the process according to specific parameters, such as: pH, nitrite accumulation, etc. to ensure the correct operation of PN/A under any circumstances [46].

On the basis of these studies, the research of partial nitritation-anammox at low temperatures was continued by other authors at the same time that its operation at high temperatures was optimized. Tim L.G. Hendrickx et al. [39] and Ramon Vazquez-Padin et al. [36] (2011) reported on the correct operation of two different PN/A facilities at <20ºC and an average NLR of 0.09 kg-N/m^3*d and 0.0009 kg-N/m^3*d, respectively.

In 2015, Susanne Lackner et al. [47] carried out an important contribution through the comparison different reactor configurations (SBR with suspended and granulated biomass and MBBR with two different carrier geometries) for PN/A at low temperatures (10-20ºC) and with DO concentrations ≤ 0.3 mg/L. Susanne Lackner et al concluded that MBBR with the 10 mm-carriers is the best configuration to performed PN/A between 10-

20ºC since the ammonium conversion was higher than in other cases at low temperature and the production of nitrite and nitrate was lower, hence the nitrite accumulation was also low.

Although this study on different reactor configurations at low temperatures shows that high nitrite concentrations in SBR compromises the effluent quality [41], it would be very important to continue with an intensive research on partial nitritation in sequencing batch reactor (SBR) to know its operation in detail and collect more information on this method. For this reason, the present thesis will continue the study on the partial nitritation process with SBR, by using all this background and the different articles on this topic, in order to provide relevant information on partial nitritation at low temperatures (10-20ºC).

4. OBJECTIVES

As the introduction indicates, liquid effluents from anaerobic digester supernatants, landfill leachates, and some industrial waste streams are rich in nitrogen (N), mostly as ammonium (NH_4^+), and contain low amount of readily biodegradable organic carbon (OC). The conventional bioprocess that is used for removing nitrogen from sewage is nitrification-denitrification (N/DN), but in many cases it is inefficient in such scenario owing to the high energy demand for aeration, the requirement of an external biodegradable OC source for completing denitrification and high excess sludge. This situation has caused the development of a new alternative called partial nitritation-anammox (PN/A), which solves the problems of nitrification-denitrification and removes distinct species of nitrogen in the effluent from anaerobic reactors treating municipal sewage under different conditions of temperature, nitrogen concentration, pH, etc. As it can be observed, for example, in previous researches for diluted and low-temperature streams and in this project which studied PN at temperatures such as 10-15°C in the pilot plant from the University of Chemistry and Technology (UCT) in Prague.

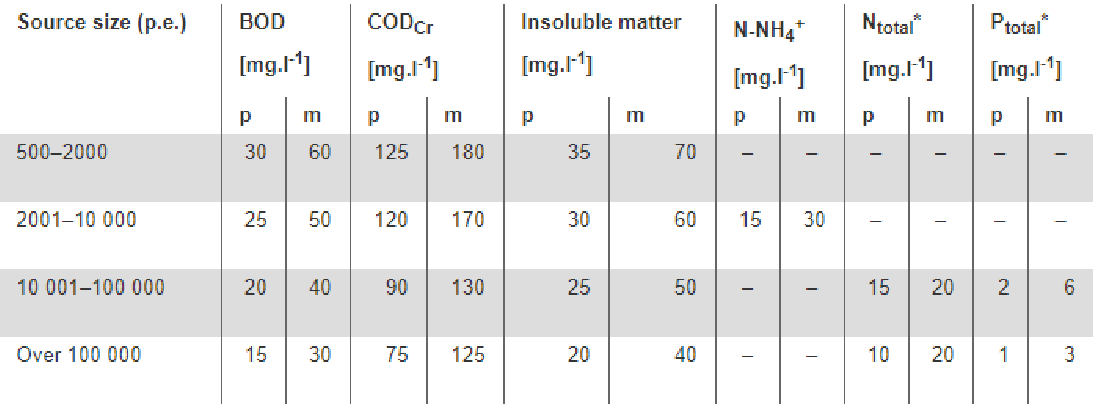
The main challenge and objective of this project is to optimize through the best work condition for ammonium oxidizing bacteria (AOB) and the measurement of different nitrogen species which exist in municipal wastewater at temperatures close to 10-15ºC, the partial nitritation start-up for the nitrogen removal after the anaerobic digestion in wastewater treatment plants (WWTP). Moreover, complementary tests were performed like suspended solids together with the analysis of other parameters e.g.: pH, FA/FNA, DO (dissolved oxygen) … in order to control and know the state of system in every moment, and produce a suitable effluent for the subsequent anammox process (treatment in two steps). At present, the results of this research are an important contribution for the next study on anammox at this same university, but also for other similar projects on a larger scale.

Finally, the academic aim of this thesis is to reach a high knowledge about the new wastewater treatments, the fundament that constitutes these processes and the conditions that affect them. Moreover, it allowed to carry out the application of the knowledge acquired during the degree and master in chemical engineering performed at the Polytechnic University of Valencia (UPV), improving the capacity for self-learning and decision-making.

5. NORMATIVE

The Member States of the European Union are bound by the Council Directive 91/271/EEC of 21 May 1991 on treatment of urban waste water (Directive 91/271/EEC). In the Czech Republic regulations on water management have been established through the consideration of EU directives and regulations by means of the Act No. 254/2001 Code, on water and amending certain acts (hereinafter as the Water Act), which became effective on 1 January 2002 and has been changed several times since then. Pursuant to Section 38, paragraph 5 of the Water Act the Government of the Czech Republic indicates acceptable values of pollution for the waste water discharge into watercourses and the Government shows them in the Order of Government No. 61 on indicators and values of acceptable pollution of surface water and waste water, according to the permit for the waste water discharge into surface water and into sewerage systems, and on sensitive areas, which became valid on 1 March 2003.

Table 4. Emission standards of indicators of acceptable wastewater pollution pursuant to the Order of the Government of the Czech Republic No. 61/2003 code. Source: [48]



Source size (p.e.)	BOD [mg.l^{-1}]		COD$_{Cr}$ [mg.l^{-1}]		Insoluble matter [mg.l^{-1}]		N-NH$_4^+$ [mg.l^{-1}]		N$_{total}$* [mg.l^{-1}]		P$_{total}$* [mg.l^{-1}]	
	p	m	p	m	p	m	p	m	p	m	p	m
500–2000	30	60	125	180	35	70	–	–	–	–	–	–
2001–10 000	25	50	120	170	30	60	15	30	–	–	–	–
10 001–100 000	20	40	90	130	25	50	–	–	15	20	2	6
Over 100 000	15	30	75	125	20	40	–	–	10	20	1	3

Where:

-p: acceptable concentrations. It may be exceeded within a tolerable extent, which is established in the Annex No. 5 to the OG No. 61; approx. in 10% of all determinations (mg/L).

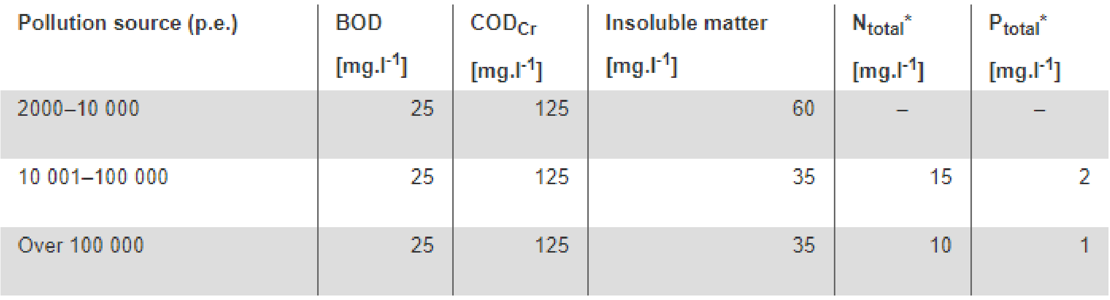
-p*: values for N_{total}* and P_{total}* are yearly averages (mg/L)

-m: they are maximum concentrations, which may not be exceeded (mg/L).

Note: p.e.: unit that means the population equivalent load of one inhabitant.

Comparing Tab. 4 with next table (Tab. 5) about the EU Directive, this last one is less strict than those imposed in the Czech Republic. The OG No. 61 carried out this action to lead to retrofits and improvements of waste water treatment plants with capacity over 10,000 p.e., which would otherwise meet the requirements of the Council Directive 91/271/EEC (except for sensitive areas) [48].

Table 5. The council Directive 91/271/EEC on the limits of nutrient concentrations in water from wastewater treatment plant. Source: [48]



Pollution source (p.e.)	BOD [mg.l^{-1}]	COD_{Cr} [mg.l^{-1}]	Insoluble matter [mg.l^{-1}]	N_{total}* [mg.l^{-1}]	P_{total}* [mg.l^{-1}]
2000–10 000	25	125	60	–	–
10 001–100 000	25	125	35	15	2
Over 100 000	25	125	35	10	1

Note: The tolerable extent is the same as that in the OG No. 61.

6. EXPERIMENTAL PART

6.1. PILOT PLANT

To carry out the research on partial nitritation process, the Department of Water Technology and Environmental Engineering from the University of Chemistry and Technology (UCT) from Prague moved to the university the pilot plant that they had built in the wastewater treatment plant (WWTP) from Pilsen, through the management of the assoc. prof. Jan Bartáček and the work of the PhD. student Vojtěch Kouba. This facility simulates part of a real WWTP based on an anaerobic digestion through a system with one tap water inlet (Tab. A.1) that flows into the accumulation tank for later it is led to SBR where nitrifying activated sludge from Prague's central WWTP was inoculated, and ammonium is added to start with partial nitritation process (PN) in every cycle; and one outlet which is joined to the drainage system.

On the other hand, this works at low temperatures (though they are higher than 10-15ºC because the temperature is not controlled, it is mainly given by the ambient temperature of Prague in May and June) by cycles which try to create the best conditions for ammonium oxidizing bacteria (AOB) in the reactor, so that their growth and partial nitritation reaction are favoured against nitratation (see point 2.4) e.g.: controlling the duration of aerobic phase in sequencing batch reactors (SBR) and solids retention time (SRT), adding inorganic matter, with the temperature etc. These are the steps of each cycle:

1. Pump in: tap water from accumulation tank flows into reactor (40-120 L, 1-2 min).

2. Wait: the system is stabilized for subsequent stage where nitritation reaction is carried out (0-5 h).

3. Nitritation: this is the state more important of the cycle. Partial nitritation reaction is produced and part of the ammonium that has been added is converted to nitrite. It is the aerobic phase with manually adjusted duration (aerobic duration control) in order to achieve desired ammonium conversion (0.3-20.5 h).

4. Sludge waste: period when excess sludge is removed, so that the solids retention time (SRT) is kept consistent in the system (0-30 L, 10 min).

5. Sedimentation: sludge from the reactor by using gravity in a physical water treatment process that uses gravity so that sludge stays on the bottom of the reactor (1 h).

6. Pump out: part of the supernatant that is above the sedimentation of the reactor is pumped to the effluent. This would be the stream that should keep to the normative on nutrients in sewage, so it has to be specially controlled process (1 h).

7. Transition: time between the end of one cycle and the start-up of the next one (1-2 min).

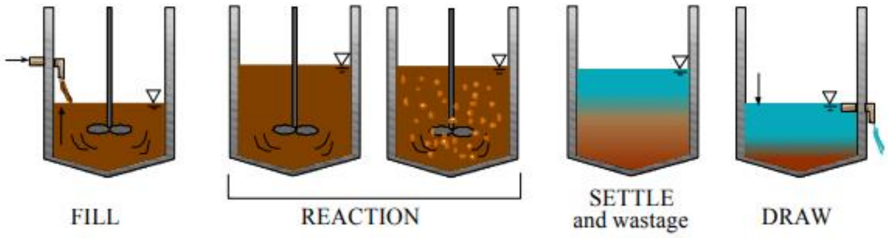


Figure 6. Sequence of phases in an SBR operation. Source: [49].

All the components of this pilot plant can be seen in Fig. 7, where each element is indicated by using one number in red colour and explained in the next page:

Figure 7. Pilot plant located in University of Chemistry and Technology, Prague (UCT) and used in the research of partial nitritation reaction which takes part in PN/A process for nitrogen removal in sewage: a) front part, b) back part, c) inside of front part, d) inside of back part. Source: author.

1. Inlet pipe: through this pipe the tap water from a laboratory flows into pilot plant until accumulation tank.

2. Outlet pipe: this pipe contains partially treated water of the effluent from the reactor, but it could flow water from accumulation in the case that its level is high. It is used to pour water to drainage system near pilot plant.

3. Toolbox: it keeps all the necessary tools for the maintenance of the pilot plant.

4. Computer and electronic system: the probes, pumps, aerators and stirrer were connected to control module Compact RIO run by LabVIEW software (National Instruments, US). it is employed to analyse, control and modify some parameters of the system. Furthermore, it allows to carry out changes in its stages (times, order of the steps, etc.) and monitor the cycle in real time.

5. Reactor: in this 200 L tank with normally a volume of 130 L (effective volume 70-180) happens the partial nitritation reaction (React. 3a). It is the most important part of the pilot plant as it is the place where ammonium oxidizing bacteria (AOB) grows and ammonium is transformed to nitrite. The reactor works as sequencing batch reactor (SBR).

6. Reactor cover: in this part, there are one blade stirrer, aeration element connected to aerator (AirMac DBMX80) and probes for continuous measurement of dissolved oxygen (Hamilton VisiFerm DO Arc 120), ammonium concentration, pH and temperature. Also, the addition of sodium bicarbonate ($NaHCO_3$) and ammonium sulfate ($(NH_4)_2SO_4$)) is carried out.

7. Reactor outlet pipe: for this pipe, partially treated water (effluent from reactor) and excess sludge are withdrawn using pump Verder V-MD20.

8. Effluent: Store of partially treated water by partial nitritation (PN). This simulates the inlet stream for the anammox process at a wastewater treatment plant based on PN/A.

9. Accumulation-Reactor pipe: during “pump in” state, tap water from accumulation tank flows into reactor to start with the process.

10. Accumulation: 200 L tank where tap water (Tab. A.1.) from laboratory is stored in order to supply later this water to the reactor by using one sludge pump (AL-KO DRAIN 12000).

11. Accumulation outlet pipe: this pipe would remove water from this tank if level of water was too high. In this way, overflow may be avoided.

12. Pumps: the pumps that are located on accumulation tank are used to lead ammonium sulphate and sodium bicarbonate to the reactor, so as to ammonium oxidizing bacteria

(AOB) work in the ideal conditions and the yield of the partial nitritation reaction can be as high as possible (around half ammonium conversion).

13. Drums of HCO_3^- and NH_4^+: to supply to the reactor sodium bicarbonate ($NaHCO_3$) which adds the enough amount of inorganic carbon to favour the growth of AOB (as it is autotrophic) and increase the alkalinity at the reactor; and ammonium sulfate ($(NH_4)_2SO_4$), for contaminating tap water and simulating wastewater from the anaerobic digestion in a WWTP. The concentrations of HCO_3^- and NH_4^+ in this drums are 80g/L and 75g/L respectively.

Finally, based on the results that will be achieved with this facility the University of Chemistry and Technology Prague (UCT), it will be able to study anammox reaction and lastly develop all the completed system of partial nitritation-anammox (PN/A) on larger scale for treating huge amount of wastewater from anaerobic digestion at low temperatures.

6.2. ANALYSIS

The samples from the accumulation, reactor and effluent of the pilot plant are analysed through several tests which allow to know the concentration of the different nitrogen species (ammonium, nitrite and nitrate) that exist in each one of the stages of the partial nitritation system, the volatile suspended solids concentration (VSS), pH, etc. Moreover, it lets perform a continue control of the process and analyse the effect of parameters like dissolved oxygen (DO), ammonium concentration, etc., on the performance of the facility.

Secondly, it is important to emphasise that the laboratory coat, gloves and the safety glasses (in the case of using the solution of amidosulphuric acid) are necessary to keep the security and decrease the possible negative effects chemicals and instruments from the laboratory might have on humans.

This research has been focused on three tests whose aims, experimental procedures, equipment/instruments and chemicals, and lastly their periodicity are shown in the next points.

6.2.1. NITROGEN TEST

This test shows how to vary the concentration of ammonium, nitrite and nitrate nitrogen from the accumulation, reactor and effluent over time. The expected results for this test are:

-Accumulation: this tank stores tap water so the concentration of ammonium, nitrite and nitrate nitrogen should be similar to the concentrations of Tab. A.1.

-Reactor: depending on the time when the sample is taken and the phase of the cycle, the results of the test could change.

-Effluent: The outlet stream of the pilot plant, which is partially treated water, simulates the inlet stream into the anammox process which should ideally have around 44% of ammonium nitrogen and 56% of nitrite nitrogen. However, there is always a small percent of nitrate nitrogen.

The measuring of this different species of nitrogen which are in sewage provides information on the activity of the biomass from the reactor and the pilot plant yield, as it can be seen in the points 7.1 and 7.2.

The analysis is carried out once a day/every day and it is necessary to take three samples to start it, one of each part of the system (1x accumulation, 1x reactor and 1x effluent; Fig. 8). In addition, each method (see point 6.2.1.2) that is employed to determine each one of the different forms of nitrogen in wastewater (ammonium, nitrite and nitrate) is repeated 3 times for the same sample in order to increase the reliability of the results. Thereby, the final result on the concentration of each specie is the average of these three measurements that have been obtained for each sample by using a spectrophotometer (Fig. 10b).

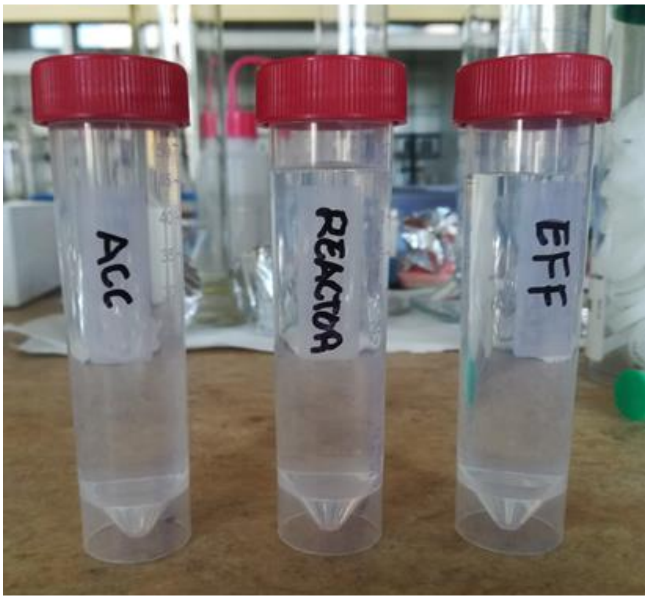


Figure 8. Samples of the accumulation, reactor and effluent from the pilot plant at UCT for the nitrogen test. Source: author.

6.2.1.1. EQUIPMENT/ INTRUMENTS AND CHEMICALS

Then, the reagents that have been used in this test and their CAS number are:

- Solution of amidosulphuric acid (CAS: 5329-14-6)
- DMP (2,6-dimethylphenol) (CAS: 576-26-1)
- Mixture of H_3PO_4 and H_2SO_4 (1:1) (CAS: 7664-38-2 // 7664-93-9)
- Nessler (CAS: 7783-33-7)
- Ethanol (CAS: 64-17-5)
- Destilated water (CAS: 7732-18-5)
- SANED
- Seignet

Figure 9. Reagents which are used to carry out the nitrogen/kinetic test of the collected samples: accumulation, reactor and effluent. Source: author.

Secondly, the next list shows the necessary instruments and equipment which have been employed in the nitrogen test:

- Personal protective equipment (PPE): laboratory coat, safety glasses and gloves.
- Volumetric flasks 25-50 mL
- Manual pipettes of 5000,2000,1000, 500,100 and 50 µL
- Beaker 100-50 mL
- Spectrophotometer
- 30x Test tubes and plastic caps
- 1x Test-tube rack
- 2x Filter pore size 0.45 nm
- 1x Syringe
- 3x Plastic tubes
- 1x Permanent
- 2x Chronometre

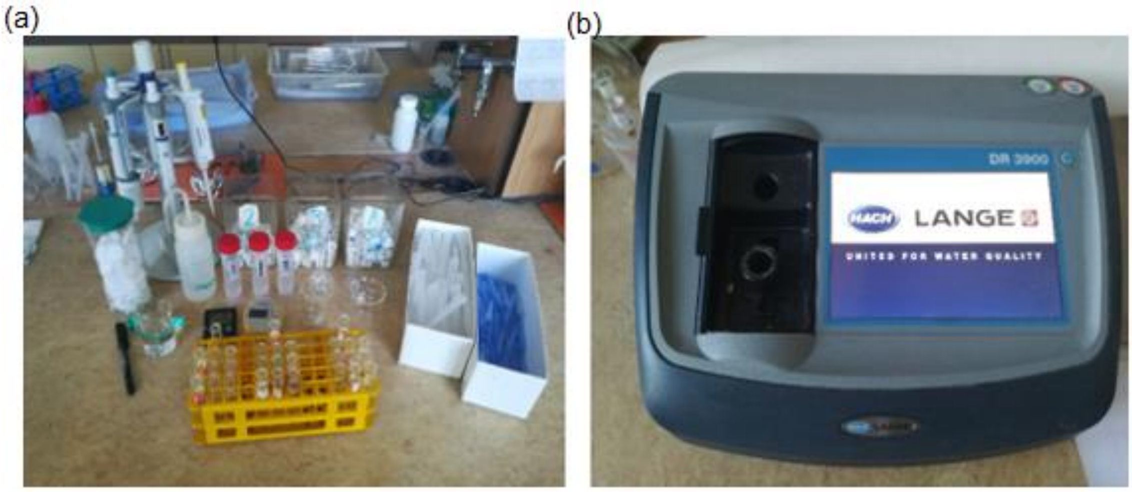


Figure 10. Instruments and devices for the nitrogen test: a) necessary instruments for analysing the three collected samples, b) spectrophotometer used to know the concentration of different nitrogen species in the accumulation, reactor and effluent from the pilot plant. Source: author.

6.2.1.2. EXPERIMENTAL PROCEDURE

To measure nitrogen in each species (nitrite NO_2^-, nitrate NO_3^- and ammonium NH_4^+) a different method should be followed [50]:

- NITRITE NITROGEN (N-NO_2^-)

Spectroscopic determination. Nitrite is determined through formation of a reddish purple azo dye produced at pH 1.9 by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride):

1) Membrane filtration of sample (average filter pore size 0.45 nm).
2) Dilute the sample for target concentration N-NO_2^-: **0 – 0.375 mg L^{-1}**.
3) Put 5 mL of sample into the test tube (ideally a tube labelled “2”).
4) Add 100 µL of SANED.
5) Add 1100 µL of DI water.
6) Close using a cap ideally labelled “2”, mix and measure absorbance after approximately 20 minutes (Fig. 11).

- NITRATE NITROGEN (N-NO_3^-)

Spectroscopic determination. Nitrate is determined using 2,6-dimethylphenol in acidic environment (created by adding the mixture of H_2SO_4 and H_3PO_4), producing 4-nitro,2,6-dimethylphenol that can be assessed at 320 nm:

1) Membrane filtration of sample (average filter pore size 0.45 nm).
2) Dilute the sample for a target concentration N-NO_3^-: **4-12 mg L^{-1}** (range 0-13.5 mg L^{-1}).
3) Put 0.5 mL of the diluted sample into a spectrophotometric tube (ideally a tube labeled “3”).
4) Add 50 µl of amidosulphuric acid (for samples with N-NO2- concentrations of 100 add 2-3x more and do additional blank sample with same conc.).
5) Add 3.5 mL of mixture of acids.
6) Add 0.5 mL of DMP.
7) Close the tube using a cap ideally labelled “3”, then mix.
8) measure absorbance after 10 minutes precisely after the mixing of the tube (error > 30 s is not acceptable) (Fig. 11).

TOTAL AMMONIUM NITROGEN (TAN OR NAMON)

Spectroscopic determination. Ammonia reacts with hydroxides of alkali metals with Nessler reagent (potassium tetraiodomercurate(II)) forming a yellowish precipitate $HgO \cdot Hg(NH_2)I$:

1) Membrane filtration of sample (average filter pore size 0.45 nm).
2) Dilute the sample for target concentration TAN: **1.5-3.0 mg L^{-1}** of TAN (range 0-3.5 mg L^{-1}).
3) Put 5 mL of the diluted sample into the spectrophotometric tube.
4) Add 100 μL of Seignet reagent.
5) Add 100 μL of Nessler reagent and start measuring 10 minutes immediately (use 8 sec intervals between Nessler addition into each of the tubes).
6) Close the spectrophotometric tube, then mix.
7) Measure absorbance precisely after 10 minutes from adding the Nessler (Fig. 11).

Note: These methods are also used to make the blanks of each one of species so they can be employed as zero in spectrophotometer.

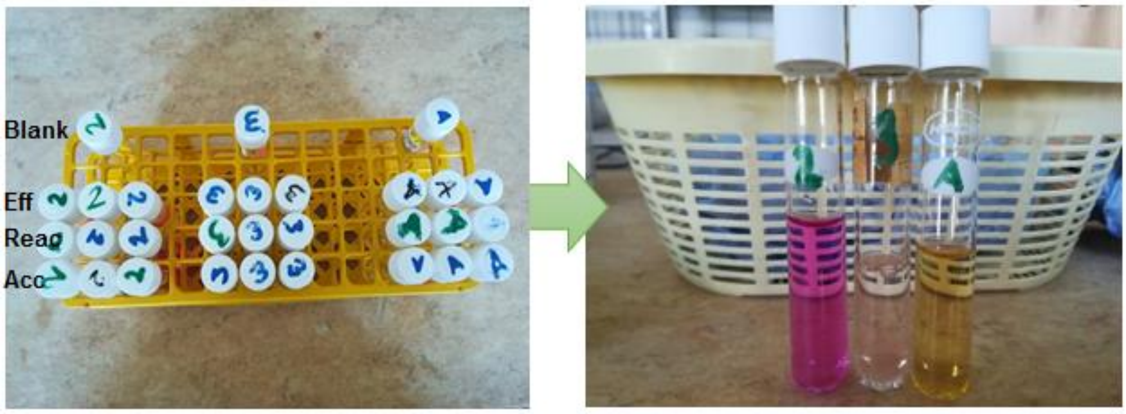


Figure 11. Results of the nitrogen test on the concentration of nitrite nitrogen (N-NO_2^-), nitrate nitrogen (N-NO_3^-) and ammonium nitrogen (N-NH_4^+) in the accumulation, reactor and effluent from the pilot plant. Source: author.

6.2.2. KINETIC TEST

This test presents the same experimental procedure, instruments and chemicals as nitrogen test. There are only two differences:

-The sampling is carried out every hour during all one day. Once a week.

-The measuring is only performed for the reactor (neither accumulation nor effluent) while the nitritation reaction happens.

Throughout this analysis, the detailed study on the partial nitritation reaction in the reactor from the pilot plant can be achieved, hence the changes in the different nitrogen species can be observed every hour. The desired result is that the ammonium nitrogen concentration decreases, the nitrite nitrogen concentration increases (up to reach an ammonium conversion of 50-57%) and the value of nitrate nitrogen concentration is kept constant and low (nitratation should be inhibited) over time.

The data which are collected thanks to the kinetic test are one measure about the performance of the reaction and the pilot plant, but also for the activity of bacteria that take part in the reactions of the reactor (AOB and NOB).

Figure 12. Reactor sample from the pilot plant at UCT for the kinetic test. Source: author.

6.2.2.1. EQUIPMENT/ INTRUMENTS AND CHEMICALS

For this analysis, the same reagents and equipment/instruments are used as for the nitrogen test (see point 6.2.1.1) but with some differences that are show below:

- 12x Test tubes and plastic caps
- 1x Filter pore size 0.45 nm
- 1x Plastic tubes

Note: it is not necessary as many test tubes, filters and plastic tubes as for the nitrogen test.

Figure 13. Necessary instruments for carrying out the kinetic test of the reactor sample from the pilot plant every hour. Source: author.

6.2.2.2. *EXPERIMENTAL PROCEDURE*

The procedure that should be followed is the same as for the point 6.2.1.2 (it has been shown previously).

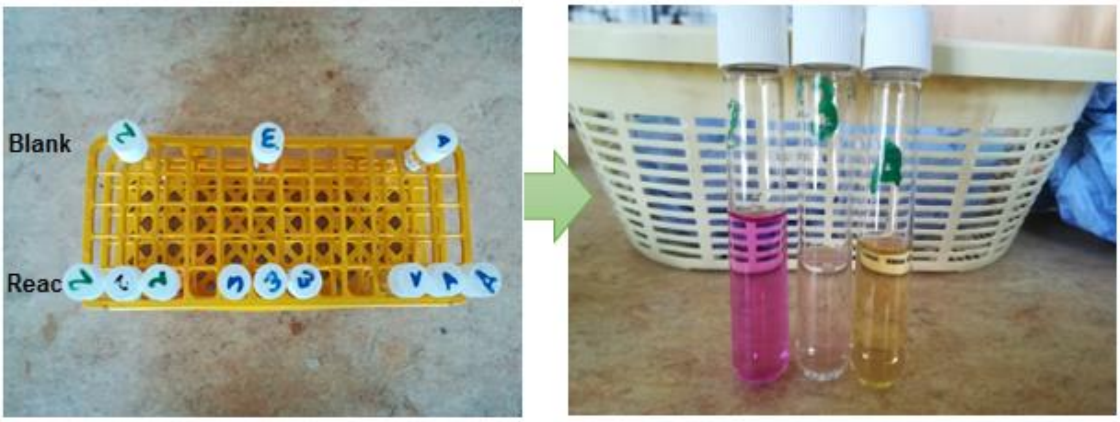


Figure 14. Results of the kinetic test on the concentration of nitrite nitrogen (N-NO$_2^-$), nitrate nitrogen (N-NO$_3^-$) and ammonium nitrogen (N-NH$_4^+$) in the reactor from the pilot plant. Source: author.

6.2.3. VOLATILE SUSPENDED SOLIDS TEST

This analysis lets know the amount of organic matter and biomass from the reactor. Its procedure is different to the two methods that are shown above (see 6.2.3.2) and instruments and equipment that are used as well. Besides, it only is necessary to take one samples from the reactor once a week. The expected result is that the amount of biomass and organic matter remain constant, since the excessive decrease in the concentration of volatile suspended solids (VSS) would mean a decrease in the bacterial population, so the operation of the pilot plant would not be correct; whereas an increase in the concentration of volatile suspended solids (VSS) represents an increase in the bacterial population and the need of removing sludge in the state of the cycle sludge waste [51].

A complementary test would be the fluorescence in situ hybridization (FISH), it is a molecular biology technique that can be used to detect microorganisms known to biodegrade contaminants, like: ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). When combined with traditional measuring of changes in contaminant concentration over time, FISH provides valuable information for site management, including site conceptual model development, remedy selection, and optimization and determination of contaminant attenuation rates [52]. However, this analysis could not be carried out owing to the fact that there was not the required sludge during the last days of operation at the pilot plant (Fig. 26).

6.2.3.1. EQUIPMENT/ INTRUMENTS AND CHEMICALS

The next list shows the necessary instruments and equipment which have been employed in the volatile suspended solid test:

- Personal protective equipment (PPE): laboratory coat and gloves.
- Manual pipettes of 5 mL
- 1x Filter pore size 0.45 nm
- 1x Syringe
- 1x Plastic tubes
- 1x Permanent
- Oven and
- Laboratory tongs
- Dryer
- Laboratory balance
- Desiccator
- Aluminium foil

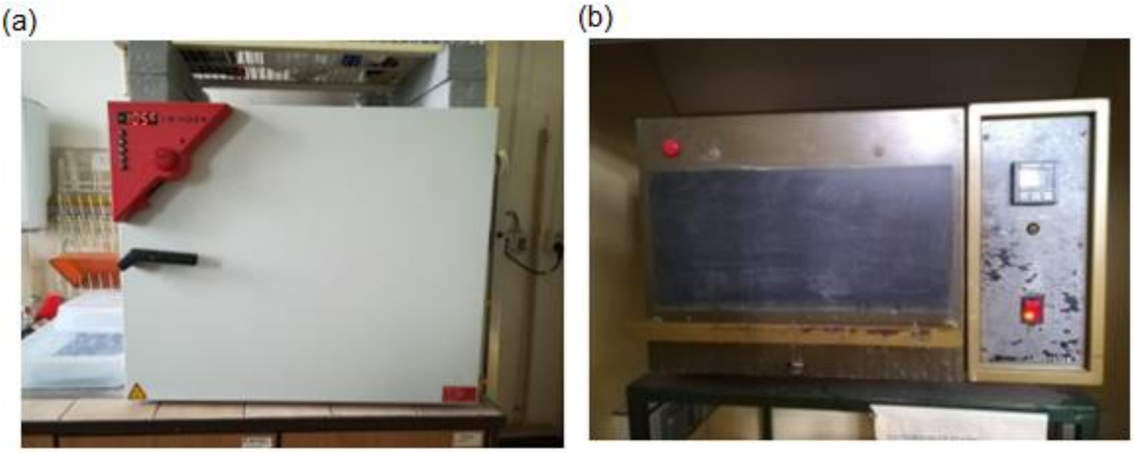


Figure 15. Devices for volatile suspended solids test: a) dryer at 105ºC that is used to evaporate the water from the reactor sample, b) oven at 550ºC that is used to remove the organic fraction from reactor sample. Source: author.

6.2.3.2. *EXPERIMENTAL PROCEDURE*

The procedure for this analysis, that let know the concentration of volatile suspended solids in the reactor, is:

1) The pieces of aluminium foil (1x homogenized sample and 1x filtered sample) are tagged, for example: origin of the sample, name, date and homogenized or filtered (Fig. 17).

2) Weigh the empty pieces of aluminium foil by using a laboratory balance (Fig. 16).

3) Pour the sample of the reactor into the piece of aluminium: Take 10-50 mL of the sample through a syringe depending on suspended solid concentration (if the concentration is high, take 10 mL, however if this is low, take 50 mL). It necessary to use a filter in order to made the filtered sample.

4) Put the samples into a dryer at 105ºC during 2h.

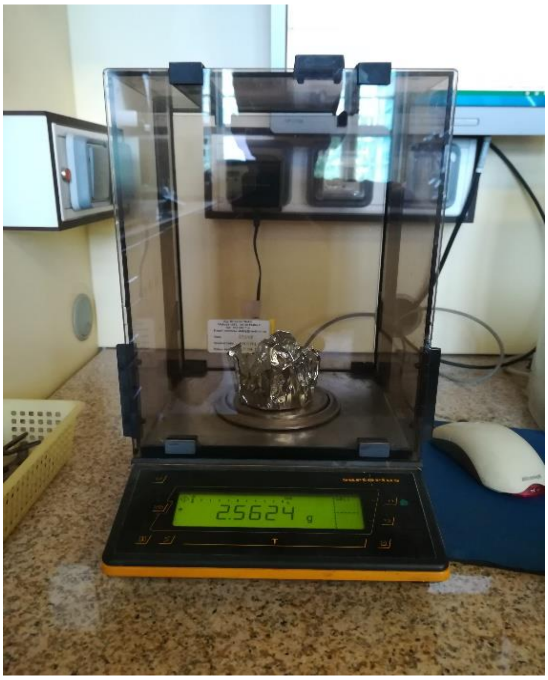


Figure 16. Laboratory balance that allows to know the weights of the homogenized and filtered sample: one measurement after 2h in the dryer and another measurement after 1:30h in the oven. Source: author.

5) Take the samples out and weigh them by using a balance again.

6) Put the samples into an oven at 550ºC, during 1:30h.

7) After this, put the samples in a desiccator, during 5 min.

8) Weigh the samples with a balance.

Figure 17. Piece of aluminium foil referenced where the reactor sample is poured: 1x filtered sample and 1x homogenized sample. Source: author.

With these data, the concentration of volatile suspended solids (VSS) can be calculated as:

$$VSS(mg/L^{-1}) = \frac{W_d - W_c}{V} \quad \text{(eq. 3)}$$

Where:

W_d: mass difference between filtered and homogenized sample after drying at 105ºC without aluminium foil (mg).

W_c: mass difference between filtered and homogenized sample after combustion at 550ºC without aluminium foil (mg).

V: sample volume (L).

7. RESULTS

The analysis that are shown above let obtain information that will be used to study the yield of the pilot plant and accomplish conclusions about the partial nitritation process at low temperatures, that has been researched during one month and presented in this project by the department of water technology and environmental engineering from University of Chemistry and Technology, Prague (UCT). All of this will develop the background which is necessary for the next study on the anammox reaction.

7.1. NITROGEN TEST

The results of the nitrogen test can be seen below, where the variation of nitrogen species, ammonium conversion and the nitrite accumulation ratio (NAR) can be analysed over time through several graphics:

Fig. 18 shows as the concentrations of different nitrogen species change over time during the sampling time. It is important to remove the nitrogen concentration from the accumulation for studying the results as it comes from the tap water, which should be around 18.5 mg/L for nitrate nitrogen, 0.01 mg/L for nitrite nitrogen and 0.02 mg/L for ammonium nitrogen (according to Tab. A.1), and in this research was searched to know how the nitrogen concentration changes by adding ammonium and the partial nitritation reaction:

$$\rho N - NO_{2EFF}^{-}(mgL^{-1}) = \rho N - NO_{2\,eff}^{-} - \rho N - NO_{2\,acc}^{-} \quad \text{(eq. 4)}$$

$$\rho N - NO_{3EFF}^{-}(mgL^{-1}) = \rho N - NO_{3\,eff}^{-} - \rho N - NO_{3\,acc}^{-} \quad \text{(eq. 5)}$$

$$\rho N - NH_{4EFF}^{+}(mgL^{-1}) = \rho N - NH_{4\,eff}^{+} - \rho N - NH_{4\,acc}^{+} \quad \text{(eq. 6)}$$

Where:

$\rho N\text{-}NO_2^-{}_{eff}$: nitrite nitrogen concentration measured in the effluent (mg/L).

$\rho N\text{-}NO_3^-{}_{eff}$: nitrate nitrogen concentration measured in the effluent (mg/L).

$\rho N\text{-}NH_4^+{}_{eff}$: ammonium nitrogen concentration measured in the effluent (mg/L).

$\rho N\text{-}NO_2^-{}_{acc}$: nitrite nitrogen concentration measured in the accumulation (mg/L).

$\rho N\text{-}NO_3^-{}_{acc}$: nitrate nitrogen concentration measured in the accumulation (mg/L).

$\rho N\text{-}NH_4^+{}_{acc}$:ammonium nitrogen concentration measured in the accumulation(mg/L).

$\rho N\text{-}NO_2^-{}_{EFF}$: operational nitrite nitrogen concentration in the effuent (mg/L).

$\rho N\text{-}NO_3^-{}_{EFF}$: operational nitrate nitrogen concentration in the effuent (mg/L).

$\rho N\text{-}NH_4^+{}_{EFF}$: operational ammonium nitrogen concentration in the effuent (mg/L).

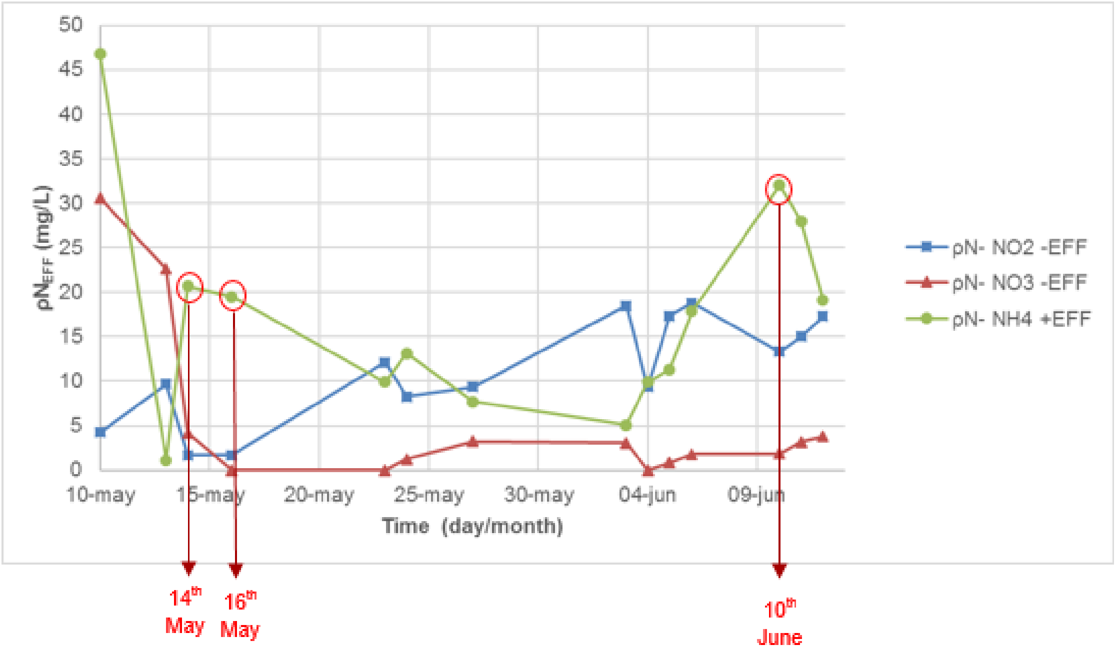


Figure 18. Variation of the nitrogen concentration in different species ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$) over time in the effluent from the pilot plant after partial nitritation. Source: author.

The first days of start-up (until 14th May), when parameters of the pilot plant were not totally adjusted as the cycle was too long (long aerobic phase) and the dose of ammonium sulfate ($(NH_4)_2SO_4$) was excessive, showed that the concentration of ammonium nitrogen and nitrate nitrogen were high and they reached values of 46.73 mg/L and 30.62 mg/L respectively, whereas nitrite nitrogen concentration was lower with a maximum value of 9.67 mg/L. After 14th May, the tendency of nitrogen species changed and the nitratation reaction was limited thanks to the optimization of the ammonium oxidizing bacteria (AOB) conditions: enough oxygen, inorganic carbon, SRT... Thereby, the partial nitritation was the main reaction in the reactor and nitrate nitrogen was kept low, nitrite nitrogen increased and ammonium nitrogen decreased over time. Due to this reaction, the concentrations of $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$ were inverse throughout the research, i.e., when ammonium nitrogen concentration in effluent increased, nitrite nitrogen decreased and vice versa.

Further, in Fig. 18 three points have been highlighted in red because they do not follow the correct tendency. On 14th May ammonium nitrogen concentration increased up to 20.67 mg/L and it was kept until 16th May with 19.47 mg/L. Simultaneously, in these days, the concentrations of nitrite nitrogen (1.72 and 1.71 mg/L, respectively) and nitrate nitrogen (4.20 and 0 mg/L, respectively) were much lower. The reason was that the velocity of the ammonium pump was increased in this period to adjust the system as on 13th May a very small quantity of ammonium nitrogen was achieved in the effluent (1.13 mg/L). On 10th June, owing to the fact that the pump dosed too much ammonium sulfate again, there was loss of biomass and the cycle length was not enough, the ammonium nitrogen concentration had a value of 32 mg/L and the concentrations of nitrate nitrogen and nitrite nitrogen were 1.83 mg/L and 13.31 mg/L respectively. With the objective of adjusting the system, the duration of nitritation phase was increased and the velocity of the ammonium pump was decreased in the next days.

Regarding analysis more exhaustive, from 16th to 23rd May and on 4th June the nitrate nitrogen concentration showed the lowest values, although since 14th May its value was below 3.77 mg/L. Besides, it is important to highlight that there was the same or similar amount of ammonium nitrogen as nitrite nitrogen (~50-50%) in the effluent as it can be seen on 23rd and 27th May, and from 4th to 6th and on 12th June with a $N\text{-}NO_2^-/N\text{-}NH_4^{+-}$ ratio of 1.20±0.30.

The next Fig. 19 shows the ammonium nitrogen conversion over time during this research. The equation that was used is:

$$N - NH_4^+ conversion = \frac{\rho TN_{EFF} - \rho N - NH_{4EFF}^+}{\rho TN_{EFF}} \quad \text{(eq. 7)}$$

$$\rho TN_{EFF}\,(mgL^{-1}) = \rho N - NH_{4EFF}^+ + \rho N - NO_{3EFF}^- + \rho N - NO_{2EFF}^- \quad \text{(eq. 8)}$$

Where:

$N\text{-}NH_4^+{}_{conversion}$: ammonium amount that is converted in the reactor (dimensionless).

ρTN_{EFF}: total operational nitrogen concentration in the effluent (mg/L).

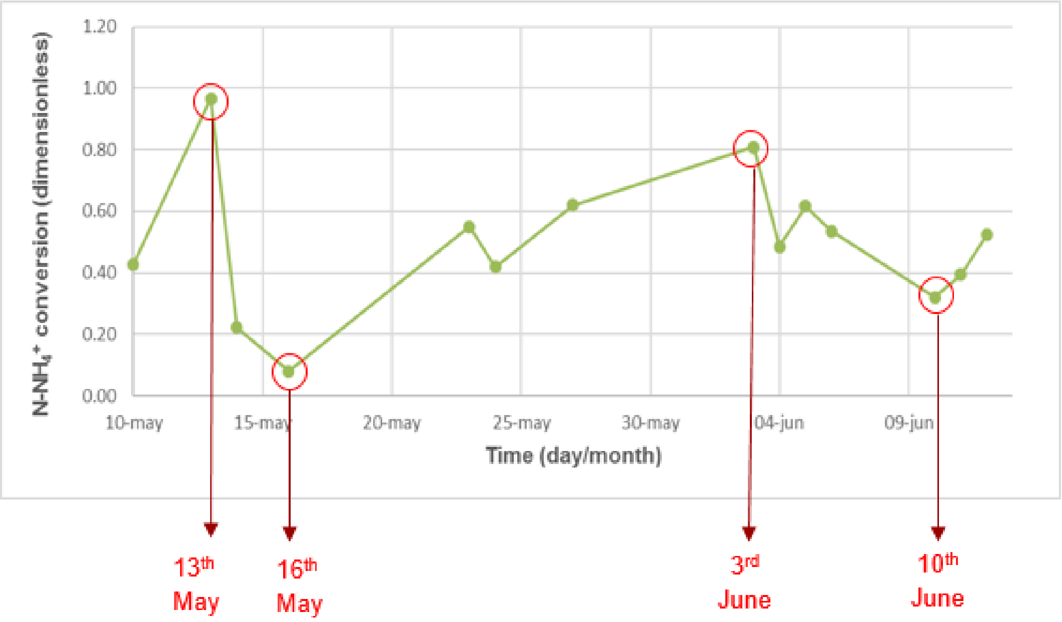


Figure 19. Variation of the amount of ammonium nitrogen which was converted to nitrite nitrogen and nitrate nitrogen over time through the partial nitritation reaction. Source: author.

This graphic is very related with Fig. 18, since it shows the ammonium amount that was transformed to nitrite and nitrate in the reactor from the pilot plant over time through partial nitritation. The first days, the ammonium nitrogen conversion was too high, such as on 13th May with a value of 97% and an ammonium nitrogen concentration in the effluent of 1.13 mg/L (Fig. 18). For this reason, with the aim of increasing the ammonium nitrogen concentration in effluent, the velocity of the ammonium pump was increased and ammonium nitrogen conversion decreased until 16th May with an ammonium nitrogen concentration in the effluent of 19.47 mg/L and an ammonium nitrogen conversion of 8.06%. After this adjustment, the ammonium nitrogen conversion started to stabilize with values between 41-62%, except on 3rd June when ammonium nitrogen conversion was 81% due to the fact that the cycle length was too long and ammonium was transformed to nitrite and then to nitrate by nitrification (5.1 mgN-NH$_4^+$/L, 3.04 mgN-NO$_3^-$/L,18.46 mgN-NO$_2^-$/L).

Secondly, as this has been explained above, the excessive addition of ammonium sulfate, the loss of biomass and the incorrect cycle length caused that the ammonium nitrogen conversion decreased up to 32% on 10th June.

The nitrite accumulation ratio (NAR) is presented in Fig. 20. This parameter shows the fraction of nitrite nitrogen respect nitrate nitrogen in the effluent, thus it can be a measurement of the inhibition of nitrite-oxidizing bacteria (NOB) and the nitratation reaction in the pilot plant:

$$NAR = \frac{\rho N - NO_{2EFF}^{-}}{\sum \rho N - NO_X} = \frac{\rho N - NO_{2EFF}^{-}}{\rho N - NO_{2EFF}^{-} + \rho N - NO_{3EFF}^{-}} \quad \text{(eq. 9)}$$

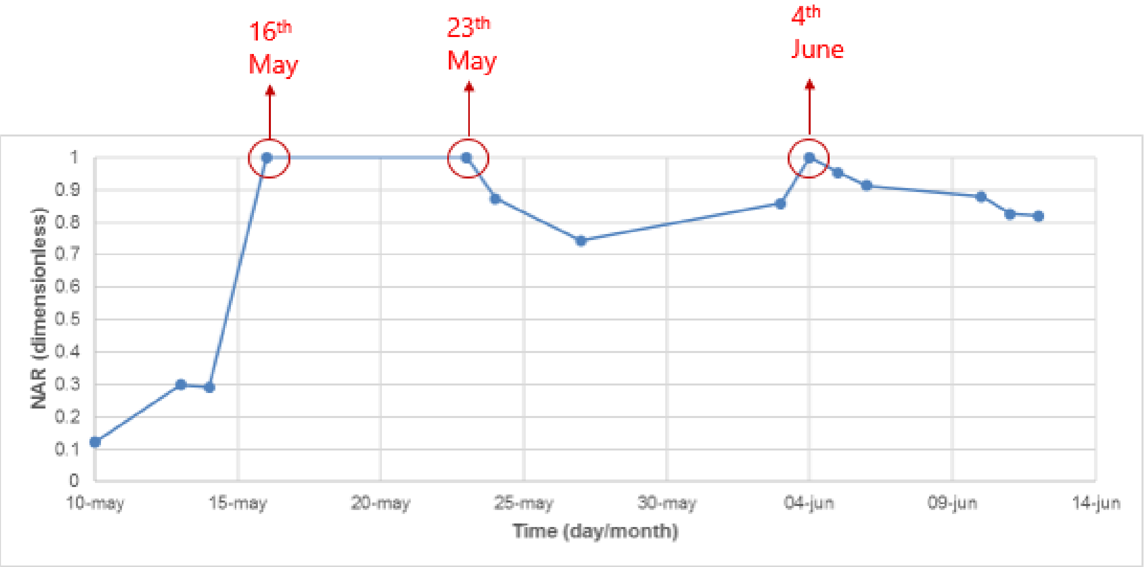


Figure 20. Evolution of nitrite accumulation ratio (NAR) over time in the effluent from the pilot plant after partial nitritation. Source: author.

The nitrite nitrogen concentration was higher than nitrate nitrogen concentration during practically all the research, with an average NAR value of 89% without considering the values that were obtained the first days throughout the start-up, until 14th May. Nevertheless, a decrease of the NAR value (74.3%) can be seen on 27th and the reason was that the cycle duration was too long and nitrite was converted to nitrate by the nitratation reaction, as in this day the nitrate nitrogen concentration reached its highest value (3.23 mg/L) after the start-up of the pilot plant, and the concentrations of nitrite and ammonium nitrogen were quiet low (9.32 mg/L and 7.71 respectively) with an ammonium nitrogen conversion of 62%.

Moreover, NAR was 100% on 16th and 23rd May, and 4th June so the inhibition of NOB was perfect these days and nitrate nitrogen concentration was cero in the effluent, i.e. the nitratation reaction didn't happen.

7.2. KINETIC TEST

This test allows to analyse how the concentrations of different nitrogen species vary during the partial nitritation process in the reactor over time; while total nitrogen is kept constant throughout all the test: 19.71 mg/L on 30th May and 55.41 mg/L on 10th June.

In addition, other parameters such as: free ammonia (FA), free nitrous acid (FNA), activity and specific activity of the bacteria, which were in the reactor, were able to be calculated and they are shown below.

Firstly, in the same way that in Fig. 18 it is necessary to remove the nitrogen concentration from the accumulation so as to carry out an isolated study of the partial nitritation reaction. This has a big importance for nitrate nitrogen concentration as it was high in the accumulation tank those days: 3.62 mgN-NO_3^-/L on 30th May and 4.01 mgN-NO_3^-/L on 10th June

$$\rho N - NO_{2REACT}^{-}(mgL^{-1}) = \rho N - NO_{2\ react}^{-} - \rho N - NO_{2\ acc}^{-} \quad \text{(eq. 10)}$$

$$\rho N - NO_{3REACT}^{-}(mgL^{-1}) = \rho N - NO_{3\ react}^{-} - \rho N - NO_{3\ acc}^{-} \quad \text{(eq. 11)}$$

$$\rho N - NH_{4REACT}^{+}(mgL^{-1}) = \rho N - NH_{4\ react}^{+} - \rho N - NH_{4\ acc}^{+} \quad \text{(eq. 12)}$$

Where:

ρN-$NO_2^-{}_{react}$: nitrite nitrogen concentration measured in the reactor (mg/L).

ρN-$NO_3^-{}_{react}$: nitrate nitrogen concentration measured in the reactor (mg/L).

ρN-$NH_4^+{}_{react}$: ammonium nitrogen concentration measured in the reactor (mg/L).

ρN-$NO_2^-{}_{REACT}$: operational nitrite nitrogen concentration in the reactor (mg/L).

ρN-$NO_3^-{}_{REACT}$: operational nitrate nitrogen concentration in the reactor (mg/L).

ρN-$NH_4^+{}_{REACT}$: operational ammonium nitrogen concentration in the reactor (mg/L).

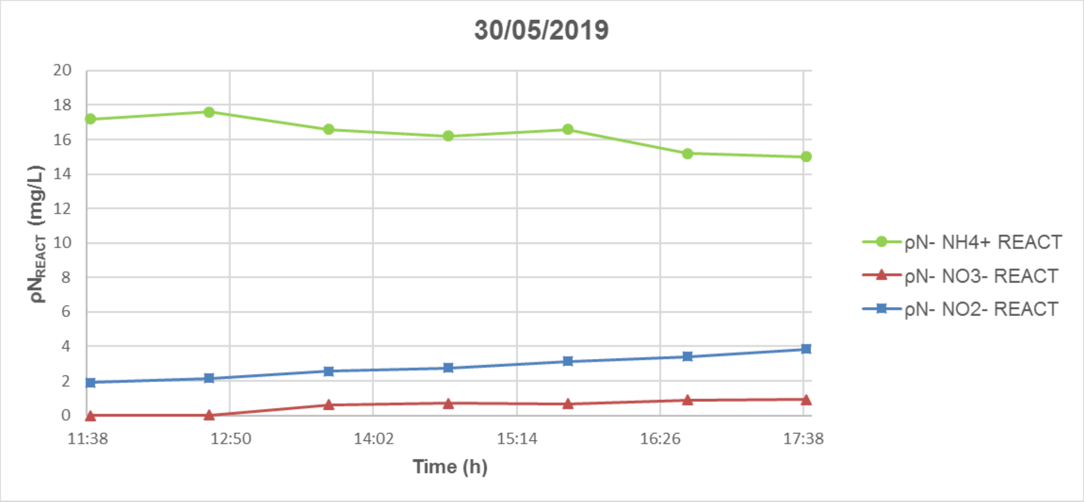


Figure 21. Variation of the nitrogen concentration in different species (N-NH_4^+, N-NO_2^- and N-NO_3^-) every hour in the partial nitritation reactor from the pilot plant on 30th May. Source: author.

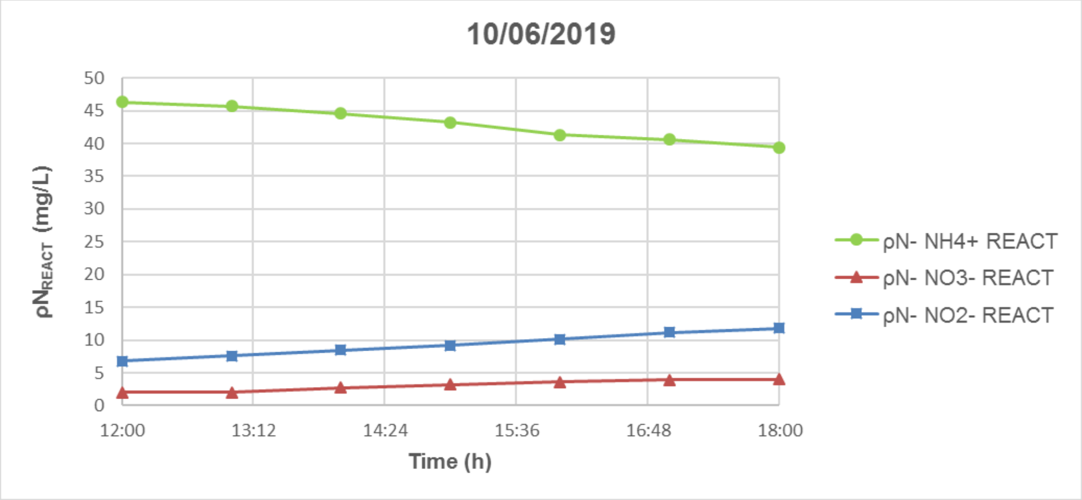


Figure 22. Variation of the nitrogen concentration in different species (N-NH_4^+, N-NO_2^- and N-NO_3^-) every hour in the partial nitritation reactor from the pilot plant on 10th June. Source: author.

Both figures (Fig. 21 and 22) explain how partial nitritation happens. The initial ammonium, that has been fed to the reactor, is oxidized to nitrite by ammonium oxidizing bacteria (AOB) hence ammonium nitrogen concentration (N-NH_4^+) decreases at the same time that nitrite nitrogen concentration increases (N-NO_2^-). This can be observed in Fig. 21 and 22, just like the formation of nitrate through the oxidation of nitrite thanks to nitrite oxidizing bacteria (NOB); since the nitratation reaction was not able to be completely inhibited.

By using the slopes that are obtained with the collected concentrations of ammonium nitrogen and nitrate nitrogen every hour on 30th May and 10th June, the activity of AOB and NOB (measurement that refers to the concentration of ammonium or nitrate nitrogen converted in the reactor per unit time) have been established as it can be seen in Tab. 6 and 7: the slope of ammonium nitrogen concentration is the activity of AOB and the other is the activity of NOB. Besides, with these values and the volatile suspended solids concentration (VSS) around those dates, the specific activity (measurement that refers to the activity of a bacteria (AOB or NOB) per milligram of volatile suspended solids) was calculated (Tab. 6 and 7):

$$ActivityAOB(mgN-NH_4^+/L*d)=\frac{\rho N-NH_{4\ REACT}^+}{\sigma t} \quad \text{(eq. 13)}$$

$$ActivityNOB(mgN-NO_3^-/L*d)=\frac{\rho N-NO_{3\ REACT}^-}{\sigma t} \quad \text{(eq. 14)}$$

$$specific\_activityAOB(mgN-NH_4^+/mgVSS*d)=\frac{activityAOB}{\rho VSS} \quad \text{(eq. 15)}$$

$$specific\_activityNOB(mgN-NO_3^-/mgVSS*d)=\frac{activityNOB}{\rho VSS} \quad \text{(eq. 16)}$$

Where:

σt: temporal variation (d)

ρVSS: concentration of volatile suspended solids (mg/L)

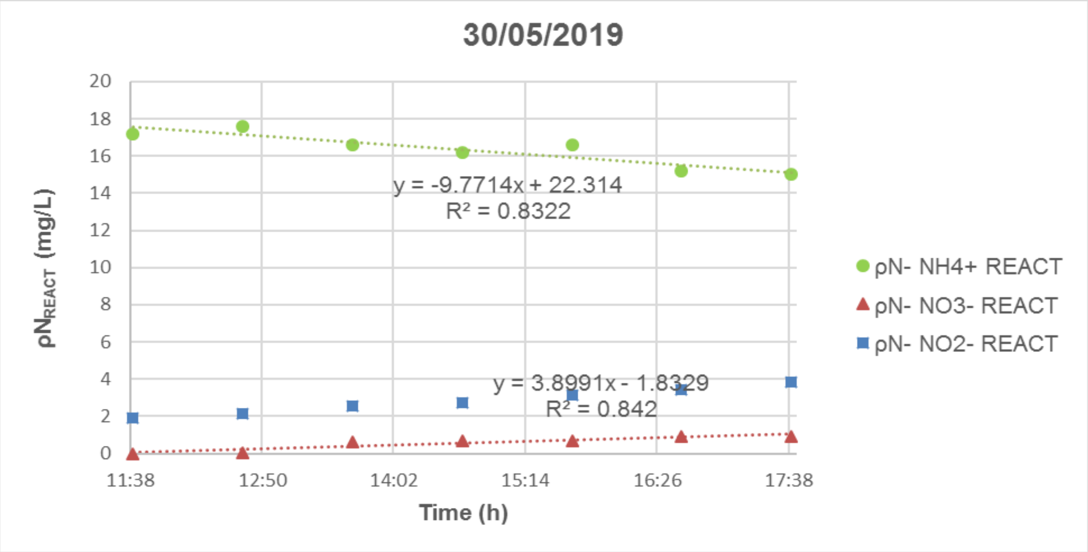


Figure 23. Calculation of the activity of AOB and NOB in the partial nitritation reactor from the pilot plant by using the slopes that are obtained with the collected concentrations of ammonium nitrogen and nitrate nitrogen every hour on 30th May. Source: author.

Table 6. Calculation of the specific activity of AOB and NOB in the partial nitritation reactor from the pilot plant on 30th May by using equations 15 and 16. Source: author.

Activity of AOB (mgN-NH_4^+/L*d)	9.7714
Activity of NOB (mgN-NO_3^-/L*d)	3.8991
ρ_{VSS} (28-may) (mg/L)	20
Specific Activity of AOB (mgN-NH_4^+/mgVSS*d)	0.49
Specific Activity of NOB (mgN-NO_3^-/mgVSS*d)	0.19

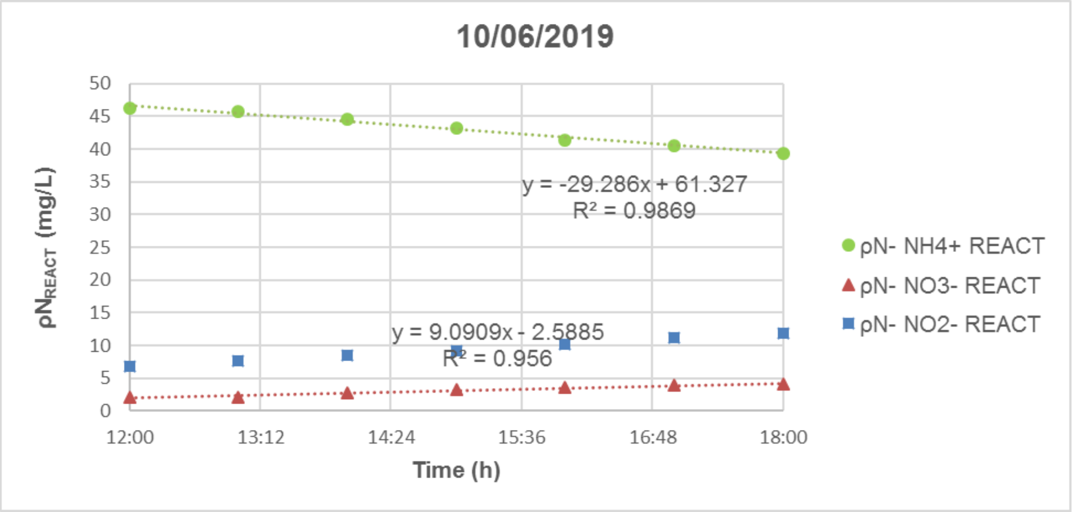


Figure 24. Calculation of the activity of AOB and NOB in the partial nitritation reactor from the pilot plant by using the slopes that are obtained with the collected concentrations of ammonium nitrogen and nitrate nitrogen every hour on 10th May. Source: author.

Table 7. Calculation of the specific activity of AOB and NOB in the partial nitritation reactor from the pilot plant on 10th May by using equations 15 and 16. Source: author.

Activity of AOB (mgN-NH_4^+/L*d)	29.286
Activity of NOB (mgN-NO_3^-/L*d)	9.0909
ρ_{VSS} (12-june) (mg/L)	16
Specific Activity of AOB (mgN-NH_4^+/mgVSS*d)	1.83
Specific Activity of NOB (mgN-NO_3^-/mgVSS*d)	0.57

To calculate specific activities, the value of VSS was obtained the test days of volatile suspended solids in the reactor around 30th May and 10th June, as in neither of those days this analysis was carried out (Fig. 26).

These tables show as ammonium oxidizing bacteria (AOB) activity was higher than nitrite oxidizing bacteria (NOB) activity on both days. Therefore, AOB was the dominating bacteria in nitrifying communities and the partial nitritation reaction prevailed over the nitratation reaction, hence the second step of nitrification process was limited.

Nitrogen loading rate values (NRL) were also calculated by using AOB activity on 30th May and 10th June, and the results were 0.009 kg-N/m^3*d and 0.027 kg-N/m^3*d respectively, with an average NLR of 0.015 kg-N/m^3*d throughout the month of research. Although the amount of nitrogen in the reactor was higher on 10th June due to the excessive addition of ammonium sulfate in the reactor, NLR was too low [53] [54].

Finally, free ammonia (FA) and free nitrous acid (FNA) values were obtained by using equations 1 and 2 and measuring of pH, the ammonium nitrogen concentration and the nitrite nitrogen concentration in the reactor every hour on 10th June. Moreover, the temperature was 20ºC as a consequence of the increase of temperature in Prague at the beginning of June.

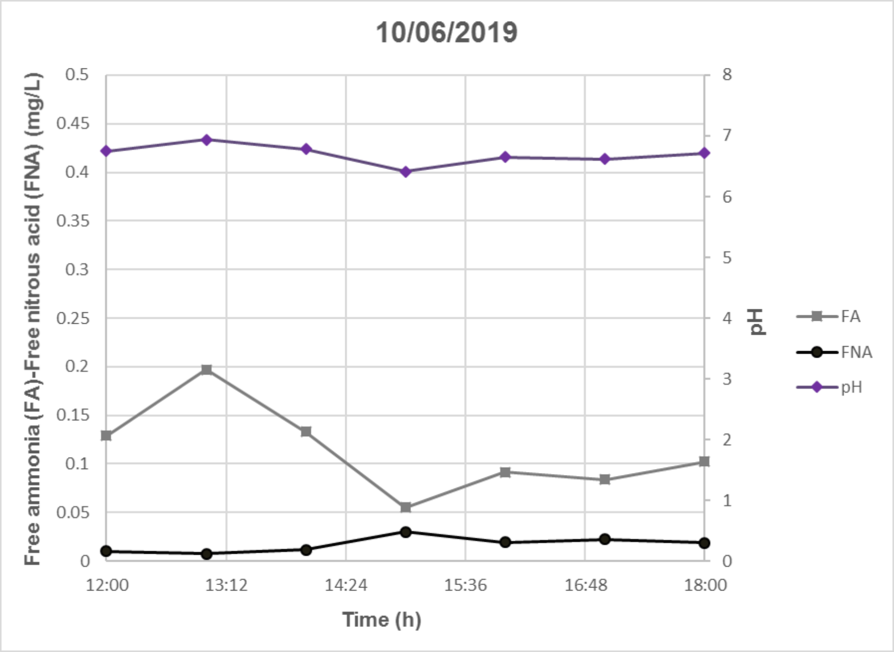


Figure 25. Values of pH, free ammonia (FA) and free nitrous acid (FNA) every hour in the partial nitritation reactor from the pilot plant on 10th June. Source: author.

As Fig. 25 shows, pH was practically constant in the reactor with an average value of 6.7 on 10th June, medium more alkaline than the accumulation due to the addition of sodium bicarbonate ($NaHCO_3$) in feed to favour the growth of AOB. In this date, the values of free ammonia (FA) were between 0.05-0.2 mg/L and 0.007-0.03mg/L for free nitrous acid (FNA), with an average value of 0.11 mg/L and 0.02 mg/L respectively. The FA and FNA values at the end of the cycle were 0.09 mg/L and 0.03 mg/L respectively, and they were calculated by using equations 1 and 2 with the concentrations of ammonium nitrogen and nitrite nitrogen in the effluent on 11th June (Fig. 18), a pH of 6.7 and 20ºC. These results are different to theoretical values of FA and FNA (0.07 mg/L and 0.05 mg/L, respectively) at the end of the cycle, which have been calculated with average pH, 20ºC and half of the total nitrogen as ammonium nitrogen (27.7 mg/L) and the other half as nitrite nitrogen (27.7 mg/L). This difference was occurred due to two reasons: the conversion of 50-57% was not reached ($N\text{-}NH_4^+$ conversion was 39.5%) and part of this ammonium nitrogen was converted to nitrate nitrogen (3.16 mg/L). Thereby, FA was always higher than at the theoretical end of the cycle (>0.07mg/L) and FNA was lower (<0.05 mg/L).

On the other hand, with this figure the relationship between FA-FNA and pH can be confirmed as the decrease in pH at 15:00 h caused the increase in FNA until 0.05 mg/L and also the decreased in FA up to 0.03 mg/L. The reason of this was explained in point 2.4.

7.3. VOLATILE SUSPENDED SOLIDS TEST

In the next figure, the variation of VSS (organic matter and biomass) concentration over time in the reactor from the pilot plant has been presented:

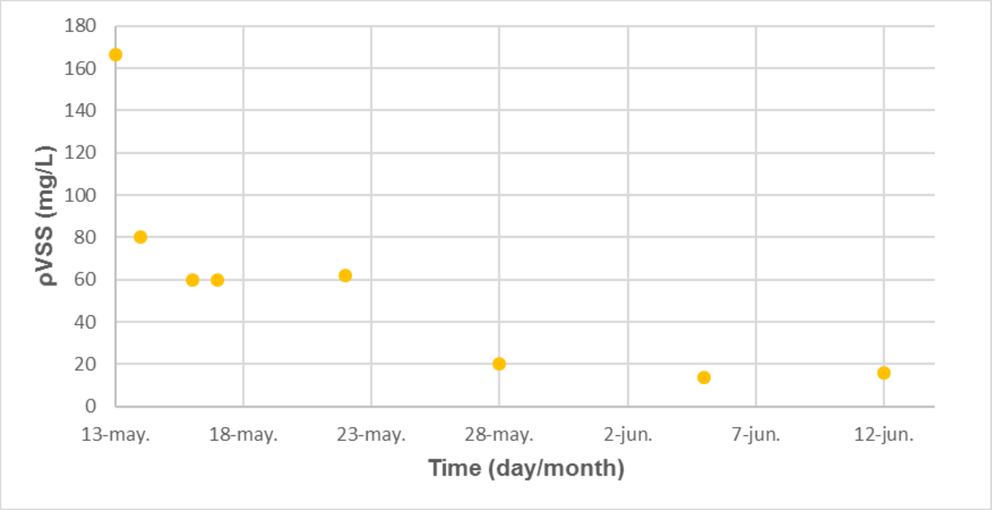


Figure 26. Concentration of volatile suspended solids (biomass and organic matter) in the partial nitritation reactor from pilot plant at UCT during the research time (one month). Source: author.

At the beginning of May, the nitrifying municipal sludge was inoculated in the reactor so the highest value of VSS concentration was reached (166.7 mg/L). After this, the volatile suspended solid concentration started to decrease due to excess sludge washout in effluent in order to control the solids retention time (SRT) in the system. Since 28th May, the value of VSS has been stabilized with an average value of 16.7 mg/L.

The increase of sludge in the reactor is caused by the autotrophic bacteria (for instance: ammonium oxidizing bacteria (AOB) or nitrite-oxidizing bacteria (NOB)) that carry out the partial nitritation reaction and produce the increase of organic matter and the bacterial growth in the reactor by using inorganic matter (for example: sodium bicarbonate, $NaHCO_3$) and the presence of oxygen. For this reason, it is important the stage of process called “sludge waste” (see point 6.1) to control the bacterial population from pilot plant.

8. DISCUSSION OF RESULTS

As in the previous researches that were carried out about partial nitritation by the Department of Water Technology and Environmental Engineering from the University of Chemistry and Technology, Prague (UCT) [21], [55], [56] or other research groups such as Huosheng Li et at. [57], [58] or Ganigué Ramon et al. [59]–[62], the analysis of this method consists of the study of different nitrogen species ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$) over time in the reactor and effluent, and the calculation and measurement of parameters which let understand better the results that are obtained and they supply important information on the operation, the state of the system and the activity of bacteria (ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB)) in the partial nitritation process at low temperatures. The temperature range was finally between 13-26ºC (ambient temperature between May and June in Prague) at the pilot plant, a little higher than originally intended but even though the current set-up needs some improvements (see point 9), in general this facility had the suitable conditions for AOB and NOB as it can be observed in the next discussion.

In the first place, in Fig. 18 it is observed that the tendency of the nitrogen species over time in the effluent from pilot plant is very similar to the results which was obtained in other researches. During the start-up period, the high concentrations of nitrate and ammonium nitrogen decrease at the same time that the nitrite nitrogen concentration increase [57]. Then, the nitrate nitrogen concentration is kept low (~0 mg/L) and constant while the concentration of ammonium and nitrite nitrogen achieve similar values with an ammonium conversion around 50% [57]–[60]. Nevertheless, although this pilot plant shows clear evidences of following this trend, the production of nitrate could not be completely avoided and it had an average value of 2.57 mg/L, except some periods such as from 16th to 23rd May and on 4th June when the value was close to 0 mg/L. Moreover, the ammonium nitrogen conversion underwent many ups and downs that did not allow to keep a stable $N\text{-}NO_2^-/N\text{-}NH_4^+$ ratio around of the ideal value 1.32 [63], until on 23rd May when the system was adjusted and the ammonium nitrogen conversion was kept between 41-62% (Fig. 19) with a $N\text{-}NO_2^-/N\text{-}NH_4^+$ ratio of 1.20±0.30, with the exception of 3rd June when ammonium nitrogen conversion was 81% due to the fact that the cycle length was too long and ammonium was transformed to nitrite and then to nitrate by nitrification. Thereby, inhibiting the production of nitrate in the reactor would be necessary in the future because the optimization ammonium oxidizing bacteria (AOB) conditions was not enough to limit the nitrite oxidizing bacteria (NOB) activity, as it can be seen in next paragraphs. Also it would be very important to increase and maintain the amount of biomass in the reactor (solid retention time, SRT) and configure the cycle length (see point 9) so that the ammonium nitrogen conversion and $N\text{-}NO_2^-/N\text{-}NH_4^+$ ratio are stabilized around 50-57% and 1.32 respectively, and the nitrogen loading rate (NLR) can improve and remain constant, since the maximum value was 0.027 $kg\text{-}N/m^3{*}d$ on 10th June with an ammonium nitrogen conversion of 32% due to the biomass shortage in the reactor (Fig. 26). Furthermore, the average NLR of 0.015 kg-N/m^3*d is too low if it is compared with other studies on SBR systems for PN/A which worked with values between 0.13-0.22 kg-N/m^3*d [55] [64], even 1.23-2 kg-N/m^3*d [65] [66] .

One parameter that can indicate the inhibition of NOB in the reactor is the nitrite accumulation ratio (NAR), which refers the fraction of nitrite nitrogen respect nitrate nitrogen in the effluent. All the bibliography agrees that the key to achieve nitritation is to eliminate or inhibit NOB, through high free ammonia (FA) (>49.7±13.3 mg/L) and low dissolved oxygen (DO) concentrations (0.1–0.6 mg/L) [58], the addition of sulphide (80mg/L) [67], etc (see point 2.4). If the metabolic activity of NOB is just inhibited, nitritation will be unstable and even be destroyed when the conditions favour NOB growth. If NOB is washed out of system, nitritation will be stably performed and not be influenced by the short-term change of operational conditions. The presence of NOB can be determined by specific batch assays outside of the system, or by microbial analysis methods such as FISH or sequencing; whereas the limitation of the activity of NOB, according to the inhibition and washout of these bacteria in the system, is usually demonstrated through a fact that nitrite accumulation ratio (NAR) reaches a high level (>80%) [68].In this research (Fig. 20), although the average NAR value was 89% (without the first data that were obtained during the start-up of the pilot plant), it was not too high if it is compared with the values that were achieved in other researches, such as: 99.8±0.09% [69] or 90% [68] after achieving a stable PN process by adjusting of several parameters. Thereby, on basis of these data and the information that was collected by kinetic tests, it would be important to improve the inhibition of nitrite oxidizing bacteria (NOB) at the pilot plant (see point 9).

The results of the kinetic test on 30th May and 10th June (Fig. 21 and 22, respectively) show a similar trend to the data that were collected by other authors by using this analysis [58], [59]. In contrast to this, there are some differences, such as: the nitrate nitrogen concentration increased over time on both dates, even it reached the value of 4,02 mg/L while nitrite nitrogen concentration was 11.77 mg/L on 10th June; nonetheless, the bibliography indicates that the nitrate nitrogen concentration decreased up to values close to 0 mg/L [58], [59]. Moreover, an ammonium nitrogen conversion of 50~57% was achieved in 4.5h [58] whereas in this pilot plant was accomplished 24% on 30th May and 29% on 10th June in 6h. The reason was that the specific activity of AOB was not high enough (0.49 mgN-NH_4^+/mgVSS*d on 30th May and 1.89 mgN-NH_4^+/mgVSS*d on 10th June, see Tab. 11 and 12), due to the operating temperature (~20ºC) and pH (~6.7) of the system these dates: between 16ºC-20ºC, several researches ([70] and [71]) have demonstrated that the activity and growth of nitrifying bacteria decrease and the nitritation speed is approximately 50% of the optimal reaction speed; furthermore, the inhibition at low temperatures is higher in nitrite oxidizing bacteria (NOB) than ammonium oxidizing bacteria (AOB) [72], thus it contributed to keep the specific activity of NOB low (in this study: 0.19 mgN-NO_3^-/mgVSS*d on 30th May and 0.57 mgN-NO_3^-/mgVSS*d on 10th June, see Tab. 6 and 7). Secondly, the effect of pH on the nitritation speed has been studied [73] for many years and reports [72] claim that a pH between 5-6.7 provokes a low nitritation speed. For these reasons, the cycle length was increased to reach an ammonium nitrogen conversion close to 50-57% under the operation conditions that had been established.

Regarding the values of free ammonia (FA) and free nitrous acid (FNA) during the cycle on 10th (between 0.05-0.2 mg/L and between 0.007-0.03mg/L respectively) were calculated by using the data that was collected every hour on 10th June (Fig. 22); and their values at the end of cycle (0.09 mg/L for FA and 0.03 mg/L for FNA) were calculated with the concentrations of ammonium nitrogen and nitrite nitrogen in the effluent on 11th June. These results show that despite the fact that suspended sludge SBRs are more susceptible to FA/FNA inhibition than biofilm or encapsulated biomass SBRs [55] because suspended sludge enhances contact of AOB with FA compared with the others, in which the FA inhibition of AOB is limited by diffusion [74], neither AOB nor NOB were inhibited in this system by the formation of these compounds, i.e. the equilibrium NO_2^-/HNO_2 did not shift towards free nitrous practically and equilibrium NH_4^+/NH_3 shifted towards free ammonia neither, because as Fig. 22 presents the concentration of nitrite nitrogen and nitrate nitrogen increased during all the time of the same way. The explanation of this is that the values of FA and FNA are outside of inhibition range for these bacteria and only FNA concentration is inside of the inhibition range of NOB at the end of the cycle, hence a clear inhibition of NOB cannot be observed or demonstrated throughout the partial nitritation process according to literature (see point 2.4): FA completely inhibits AOB and NOB at a concentration of 78 mg/L, with FA concentrations between 14 and 17 mg/L only nitrite oxidation is selectively inhibited; FNA inhibits both bacteria when its concentration is between 0.22–2.8 mg/L, and at 0.02–0.04 mg/L it inhibits only NOB [27]. Besides, even though pH (average value of 6.7) was inside of FNA inhibition range from 7.5-6 [27], the free nitrous acid did not increase enough.

Finally, the results of the volatile suspended solids test (VSS) in the partial nitritation reactor for a total ammonium nitrogen concentration (TAN) of 75 mg-TAN/L show that after adding the nitrifying municipal sludge to the reactor with a VSS concentration in the system of 166.7 mg/L, there was a dramatic reduction of 88% in the VSS concentration up to 20 mg/L in 15 days (Fig. 26). According to the literature [55], a previous research on partial nitritation with the same TAN concentration presented a decrease of 69,6% from 230 to 70 mg-VSS/L in nearly 100 days. The comparison of both cases lets check that there was an unrestricted biomass washout in the pilot plant and it would be necessary to add more sludge to the reactor (see point 9) and readjust the process by improving the control of biomass retention (SRT), because its hydraulic retention time (HRT) is short (for example: 0.375 day [55]).

9. SUGGESTED IMPROVEMENTS

The improvements that have been suggested to improve the operation of this pilot plant are:

1) Dosing of sulphide solution before starting up the pilot plant: Although sulphide can negatively affect partial nitritation in certain scenarios and it is reasonable to assume that either sulphide-adapted NOB or sulphide oxidizers will gradually develop, thereby reducing the efficiency of sulphide inhibition, it was recently proven to selectively inhibit undesirable nitrite oxidizing bacteria (NOB) in batch assays at similar or lower sulphide concentrations to 31±41 mg-S/L and at suitable pH [21], [22].

2) Controlling free ammonia (FA) and free nitrous acid (FNA): Another important parameter to inhibit nitrite oxidizing bacteria (NOB) is free ammonia/free nitrous acid because its tolerance is lower than the tolerance of ammonium oxidizing bacteria (AOB) according to literature [27]: with FA concentrations between 14 and 17 mg N-NH_3/dm^3 only nitrite oxidation is selectively inhibited, and in the case of FNA, 0.02–0.04 mgN-HNO_2/dm^3 inhibits only NOB. This control can be carried out through the variation of pH due to its influence on FA and FNA. A pH from 7.5 to 6 increase free ammonia (NH_3) and a pH from 7.5 up to 8.5 free nitrous acid.

3) Adding coagulant and flocculant in the reactor: The biomass washout in the effluent together with the decrease in the activity and the reproduction of nitrifying bacteria in the reactor as a consequence of cold temperatures cause that the nitrification is not effective [72]. The study conditions cannot be changed so one chance to increase the performance of the system is the addition of $FeCl_3$ with FA Calfloc 1502 which enhances settling properties, i.e. it increases solid retention time (SRT).

4) Inoculating sludge in the reactor: The amount of sludge in the reactor is very low as it can be observed in Fig. 26 due to the biomass loss, thus it is necessary to inoculate more fully nitrifying activated sludge from the central municipal wastewater treatment plant (WWTP) in Prague, besides increasing the solid retention time (SRT). In this way, the detailed study of bacteria population by using the fluorescence in situ hybridization (FISH) will be able to carry out.

5) Increasing the duration of the kinetic test: a detailed analysis cannot be carried out with figure 21 and 22 because the cycle length is high and with a duration of 6h this test can only show the tendency of different species in the reactor with an ammonium conversion of 24% on 30th May or 29% on 10th June. It necessary to perform this test longer than the current one so that conclusions are more specific.

6) Controlling temperature: The desired temperature range was 10-15ºC, however the temperature range at the pilot plant was 13-26 °C because the temperature was not controlled and it was mainly given by the ambient temperature of Prague in May and June. Although this increase in temperature did not provoke a huge limitation of this study since it did not have important consequences, it would be suitable to perform a temperature control in the future to carry out a research on partial nitritation more specific around 10-15ºC.

10. CONCLUSION

The present study has shown the success of the partial nitritation (PN) inoculated by nitrifying activated sludge, which was carry out in one reactor with configuration SBR (sequencing batch reactor) of the pilot plant from the University of Chemistry and Technology, Prague (UCT), for the nitrogen removal from effluents of the anaerobic digester with a total ammonium nitrogen (TAN) concentration of 75 mg/L at temperatures between 13-26ºC.In this research, the optimization of the first step of the partial nitritation-anammox (PN/A) has been achieved by using a detailed control of the pilot plant and a continuous monitoring of the different nitrogen species in the accumulation, reactor and effluent, as well as of the amount of biomass and organic matter in the reactor. Thus, the final results have demonstrated that partial nitration can generate an appropriate stream for subsequent anammox process at low temperatures since during most of the research time, the effluent from the pilot plant presented suitable values of the ammonium nitrogen conversion and the $N\text{-}NO_2^-/N\text{-}NH_4^+$ ratio, around 41-62% (Fig. 23) and 1.20±0.30 respectively (Fig. 18), with an average NAR value of 89% (Fig. 19). In spite of some limitations, such as: the loss of biomass from the partial nitritation reactor due to its unrestricted and constant washout in the effluent, and as a consequence the low nitrogen loading rate; the increase in ambient temperature in Prague (Czech Republic) which prevented to work in a temperature range between 10-15ºC and lastly, the exceptional production of nitrate in the reactor with isolated values even of 3.77 mg/L due to the incomplete inhibition of the nitrite oxidizing bacteria (NOB) activity by using the optimization ammonium oxidizing bacteria (AOB) conditions.

To conclude, although it is necessary to carry out some improvements to resolve the problems that appeared at the pilot plant during the research time (see point 9) and to keep researching on partial nitritation process at low temperatures, this study provides valuable information on its operation at temperatures between 13-26ºC as alternative to the nitrification-denitrification (N/DN) process, and it is an important contribution for the next study on anammox at this same university (treatment in two steps). Furthermore, this research constitutes a huge background for other similar projects on a larger scale.

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12. RELATED RESEARCHES

1) Z. Hu *et al.*, “Nitrogen Removal by a Nitritation-Anammox Bioreactor at Low Temperature,” 2013.

2) V. Kouba *et al.*, “How biomass growth mode affects ammonium oxidation start-up and NOB inhibition in the partial nitritation of cold and diluted reject water,” *Environ. Technol.*, vol. 40, no. 6, pp. 673–682, Mar. 2019.

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APPENDIX

Table A. 1. Tap water quality in Prague (Czech Republic) water distribution network in May 2019, with the important values of this research: nitrite, nitrate and ammonium concentration. Source: PVK a.s.: https://www.pvk.cz/res/archive/1828/226756.pdf?seek=1560841520

Parameter	Unit	Aver.		Parameter	Unit	Aver.		Parameter	Unit	Aver.
Clostridium perfringens	CFU/100ml	0		Lead	mg/L	0.0005		Sulfates	mg/L	52.1
Enterococci	CFU/100ml	0		Scent	°	2		Sodium	mg/L	13.0
Escherichia coli	CFU/100ml	0		Aldrin	ng/L	1.5		Silver	mg/L	0.0005
Coliform bacteria	CFU/100ml	0		Dieldrin	ng/L	1.5		1,1,2,2-tetrachloroethene	µg/L	0.03
Abioseston	%	1		Metolachlor (izomers)	ng/L	5.0		Trihalomethanes	µg/L	13.149
Nr of organisms	Individuals/ml	0		Heptachlor	ng/L	1.5		1,1,2-trichloroethane	µg/L	0.03
Nr of living organisms	Individuals/ml	0		Prometryn	ng/L	5.0		Chloroform	µg/L	6.1
Nr of colonies at 22 °C	CFU/ml	16		Acetochlor	ng/L	5.0		Calcium	mg/L	43.3
Nr of colonies at 36 °C	CFU/ml	3		Dichlobenil	ng/L	5.0		Calcium and magnesium	mmol/L	1.35
1,2-dichloroethane	µg/L	0.03		Propachlor	ng/L	5.0		Turbidity	NTU	0.30
Ammonium ions	mg/L	0.02		Diazinon	ng/L	5.0		Iron	mg/L	0.04
Antimony	mg/L	0.0005		Heptachlorepoxid	ng/L	1.5		Temperature	°C	11.3
Arsenic	mg/L	0.0005		Hexachlorbenzen	ng/L	1.5		Alkalinity	mmol/L	1.96
Color	mg/L Pt	2		p,p'-DDE	ng/L	1.5		1,1-dichloroethene	µg/L	0.02
Benzene	µg/L	0.03		p,p'-DDT	ng/L	1.5		1,2-dichloroethene	µg/L	0

Benzo[a]pyrene	ng/L	0.4		lindan	ng/L	1.5		dichloromethane	µg/L	0.25
Beryllium	µg/L	0.05		methoxychlor	ng/L	2.5		ethylbenzene	µg/L	0.03
Boron	mg/L	0.025		atrazine	ng/L	5.0		chlorobenzene	µg/L	0.02
Bromates	µg/L	0.90		atrazine-desethyl	ng/L	5.0		o+m+p-xylen	µg/L	0
TOC – total organic carbon	mg/L	2.29		simazine	ng/L	5.0		styrene	µg/L	0.03
nitrates	mg/L	18.5		propazine	ng/L	5.0		tetrachloromethane	µg/L	0.03
nitrites	mg/L	0.01		tetrbuthylazine	ng/L	5.3		toluene	µg/L	0.03
Fluorides	mg/L	0.12		tetrbuthylazin-desethyl	ng/L	9.0		fluoranten	ng/L	1.2
Aluminum	mg/L	0.025		cyanazine	ng/L	5.0		glyphosphate (N-(phosphonomethyl)glycine)	ng/L	25.0
Magnesium	mg/L	6.7		hexazinon	ng/L	5.0		AMPA (Aminomethylphosphonic acid)	ng/L	25.0
COD_{Mn}	mg/L	0.61		alachlor	ng/L	5.0		chloridazone	ng/L	5
Free chlorine	mg/L	0.04		metazachlor	ng/L	5.6		chloridazone-sum of metabolites	ng/L	307.7
Chlorides	mg/L	25.7		dimethoate	ng/L	10.0		alachlor ESA	ng/L	32.70
Chromium	mg/L	0.0005		desmetryn	ng/L	5.0		metolachlor ESA	ng/L	29.7
Taste	°	2		tetrbutryn	ng/L	5.0		metazachlor ESA	ng/L	56.5
Cadmium	mg/L	0.0001		chlorfenvinphos	ng/L	5.0		metazachlor OA	ng/L	10
Conductivity	mS/m	39.6		sum of pesticides	ng/L	8.204		benzotriazol	ng/L	61.5
Total cyanides	mg/L	0.001		pH		7.65		benzotriazol methyl	ng/L	10
Manganese	mg/L	0.003		sum of PAH	ng/L	0				
Copper	mg/L	0.003		mercury	mg/L	0.0001				
Nickel	mg/L	0.0007		Selenium	mg/L	0.0005				