



UNIVERSITA' POLITECNICA DELLE MARCHE
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Master degree in environmental engineering

**MODELLING AND PARAMETERS CALIBRATION OF A BIOLOGICAL
TREATMENT FOR NUTRIENTS REMOVAL AFTER DIRECT MEMBRANE
FILTRATION OF URBAN WASTEWATER**

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Abstract

At present, wastewater is addressed like a dirty stream to treat with the most cost-effective technologies in order to stay within the effluent limits imposed by law. This approach leads current centralised wastewater treatment plants to be not environmentally sustainable and to waste many resources. In particular, in the conventional activated sludge process, energy potential is wasted through the aerobic conversion of the organic matter into carbon dioxide through an energy intensive system. A promising solution consists in applying direct membrane filtration to raw wastewater, in order to redirect the carbon mass flow to anaerobic digestion for its energetic valorisation, and treat the permeated flow with low energy footprint technologies. This thesis is focused on the evaluation, through modelling, of the feasibility of a biological treatment for the permeate of direct ultrafiltration deriving from a pilot plant set in Alboraya, Valencia. Because of the low COD/N ratios characteristic of the permeate stream, the biological methods suitable for its treatment are those based on partial nitrification, in which nitrite is accumulated to be directly denitrified. In the first part of this work a dynamic calibration is carried out in the model BNRM2. The value of anoxic heterotrophic growth yield is assessed together with the biomass distribution, furthermore deviations in permeate quality data are adjusted. In the second part of the work the implementation of a partial nitrification process to treat the permeate is evaluated, performing different sets of simulations. The final configuration, selected as optimal to set up the process in the sequencing batch reactor, is a single stage configuration operated with a dissolved oxygen concentration of 0.7 mgO₂/l and a sludge retention time of 8 days. The influence of the addition of an external inhibitor to speedup the start-up of the process is also studied.

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Chapter 1. Introduction

1.1 Current situation of wastewater treatment

In European countries the current standard method for urban wastewater treatment consists in sending the drain water, through sewer systems, to **centralised wastewater treatment plants (WWTP)**, except for few isolated dwellings where small decentralised plants are present. In the plant, pollutants are removed from the stream through a combination of physical, chemical and biological processes, before the discharge in a water body. The treatment train for centralised plants is typically composed of:

- Pre-treatment: physical unit processes for the removal of coarse solids, small particles, grease, oil and every element which could damage the subsequent treatment equipment;
- Primary treatment: physical-chemical units aimed at removing suspended solids, through sedimentation and with an eventual addition of chemicals;
- Secondary treatment: biological unit processes for the removal of organic and inorganic dissolved substances. Primary target compounds are the chemical oxygen demand (COD) and nutrients (nitrogen and phosphorous). The activated sludge used for pollutants degradation is removed from the stream through a physical separation unit.
- Tertiary treatment: additional units for disinfection and removal of residual suspended solids.

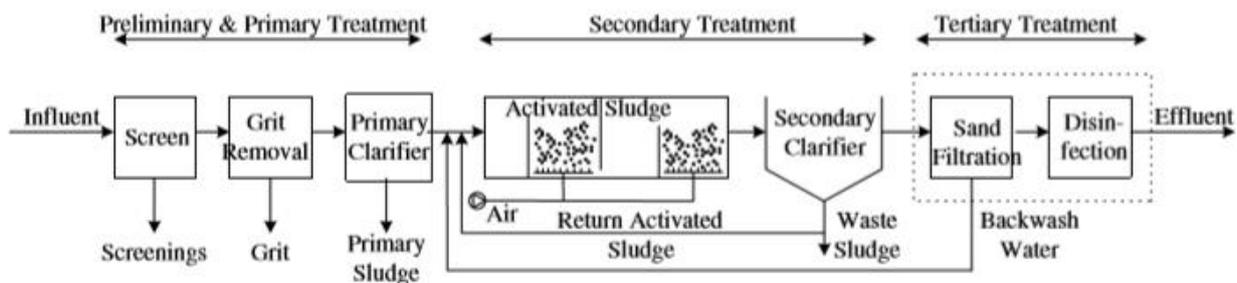


Figure 1.1.1 Typical flow scheme of a conventional activated sludge process. Adapted from (Metcalf & Eddy, 2003).

The material removed in the different units described above, is collected to be disposed in landfill, except for the primary and the waste activated sludge, which are sent to another section of the plant, the sludge line. The purpose of this compartment is to stabilize and reduce the volume of the sludge before its disposal and can be more or less complex according to the plant dimension. In medium/large plants, designed for a population equivalent over 50.000, sludge stabilization occurs through **anaerobic digestion**. In this process, a large fraction of the organic matter is broken down by bacteria into carbon dioxide (CO₂) and methane (CH₄) under anaerobic conditions. The biogas is then used for biofuel, heat and electricity production. Thus, the digester has a huge potential as source of clean energy for WWTPs, that in the European Union account for more than 1% of the total electricity consumption (European Union, 2017). The aim of the succession of the different treatment units is to decrease pollutants concentrations under limits imposed by law. At

European level the targets to achieve are enclosed in the **Council Directive of 21 May 1991** concerning urban wastewater treatment (91/271). In table 1 there are the limits for discharge of conventional pollutants and in table 2 the target values for nutrients discharged in areas identified as sensitive for eutrophication risk.

Table 1. Requirements for discharges of conventional pollutants from urban wastewater treatment plants (European Decree 91/271)

Parameters	Concentration	Minimum percentage of reduction (%)	Reference method of measurement
Biochemical oxygen demand (BOD5 at 20 °C) without nitrification (%)	25 mg/l O ₂	70-90 40 under Article 4 (2)	Homogenized, unfiltered, undecanted sample. Determination of dissolved oxygen before and after five-day incubation at 20 °C ± 1 °C, in complete darkness. Addition of a nitrification inhibitor
Chemical oxygen demand (COD)	125 mg/l O ₂	75	Homogenized, unfiltered, undecanted sample Potassium dichromate
Total suspended solids	35 mg/l (%) 35 under Article 4 (2) (more than 10 000 p.e.) 60 under Article 4 (2) (2 000-10 000 p.e.)	90 (%) 90 under Article 4 (2) (more than 10 000 p.e.) 70 under Article 4 (2) (2 000-10 000 p.e.)	— Filtering of a representative sample through a 0,45 µm filter membrane. Drying at 105 °C and weighing — Centrifuging of a representative sample (for at least five mins with mean acceleration of 2 800 to 3 200 g), drying at 105 °C and weighing

Table 2. Requirements for discharges of nutrients in sensitive areas (European Decree 91/271)

Parameters	Concentration	Minimum percentage of reduction (%)	Reference method of measurement
Total phosphorus	2 mg/l P (10 000 - 100 000 p.e.) 1 mg/l P (more than 100 000 p.e.)	80	Molecular absorption spectrophotometry
Total nitrogen (%)	15 mg/l N (10 000 - 100 000 p.e.) 10 mg/l N (more than 100 000 p.e.) ⁽¹⁾	70-80	Molecular absorption spectrophotometry

At national level, the European Directive has been transposed by specific decrees, for Spain it is the Royal Decree 509/1996. From an analysis emerges that current **centralized WWTPs are highly efficient and cost effective to satisfy the present discharge limits, but are far from being environmentally sustainable** (Wim

H. Rulkens, 2015). The main issue related to the conventional treatment approach is the **waste of valuable resources**. Wastewater contains useful organic and inorganic compounds: first of all **nitrogen** and **phosphorous** that are known to be good fertilizers, but also **other materials** such as cellulose fibers, biopolymers, bioplastics and protein are being studied to be recovered (Jan Peter van der Hoek, 2016). In a World where resources are depleting fast, reuse and recovery of these substances has a great potential. **Water** itself is an important resource that, if treated to reach a certain level of purity, can be used for irrigation and other purposes. In a period where always more countries are suffering for water scarcity, also water recovery is of increasing interest. Lastly, a lot of **energy** potential is wasted in the conventional activated sludge (CAS) process, where the COD is aerobically degraded by heterotrophic bacteria, or used as substrate by autotrophic microorganisms for nitrogen removal. An optimization of this process is important to save energy on two fronts: to preserve COD for energy production in anaerobic digestion and to avoid energy consumption due to oxygen delivery to the reactor. Moreover, the biological aerobic degradation contributes to the **environmental footprint** of the plant turning the organic matter into CO₂, a strong greenhouse gas that is released in the atmosphere. At the beginning of their development WWTPs were thought for protecting downstream users from health risks, afterwards protecting nature from nutrient pollution also became a goal. Today, the new environmental challenges like climate change, water scarcity and resources depletion, are bringing attention to a more circular and clean management of WWTPs. In Europe, this trend is encouraged by the **Water Framework Directive** (WFD, 2000/60/EC), which commits member states to reach by 2050 a good status of waters, identifying new emerging target pollutants like pesticides, heavy metals, hormone disruptors and medicinal substances and promoting water reuse. It's clear that the next step in the evolution of WWTPs, is to switch to systems that pay attention to every aspect of environmental sustainability, namely **Water Resource Recovery Facilities (WRRF)**. This concept implies designing and redesigning treatment plants with technologies that are less energy-intensive and that can recover energy, water, fertilizers and other products from wastewater. The first step toward this goal, is to implement secondary treatments that involve anaerobic biological processes rather than aerobic ones, since aeration usually consumes from 40% to 60% of the electricity used by the plant (Hui Gong, 2018). An example is the anammox, anaerobic ammonium oxidation process, which converts ammonia into nitrogen gas using autotrophic bacteria, according to the following stoichiometry: $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}$. Secondly, the highest amount of COD possible should be separated and sent to anaerobic digestion, instead of being degraded and turned into useless CO₂. This goal can be achieved using membrane filtration to retain the organic material. Finally, nutrients can be recovered through different physical-chemical systems, such as precipitation in form of struvite or ion exchange columns. The present work contributes in studying how to treat the stream, saving aeration costs, in a process of carbon redirection, therefore falls within the field of WRRFs.

1.2 New concepts on carbon redirection in wastewater

Carbon redirection consists in catching the organic load present in wastewater to send it to anaerobic digestion for its energetic valorisation. It implies a reduction of the carbon mass flow destined to be consumed in energy-intensive aeration processes, to redirect it to anaerobic energy recovery systems. This is a key concept in Water Resource Recovery Facilities, that push toward **energy-neutral** or **energy-positive** plants. The conventional activated sludge (CAS) scheme of treatment oxidize 30% of the inlet chemical oxygen demand (COD) into carbon dioxide (CO₂), by means of biological processes that require extensive aeration. It is estimated that 20-30 kWh_e · PE⁻¹ · y⁻¹ are consumed to turn organic carbon into a useless greenhouse gas to reach the discharge limits. The remaining 70% of COD is transformed into waste activated sludge (WAS), that must be treated and disposed. In medium/large size plants, this sludge goes to anaerobic digestion, together with primary sludge, where anaerobic stabilization of carbon produces methane for energy generation plus digested residual material.

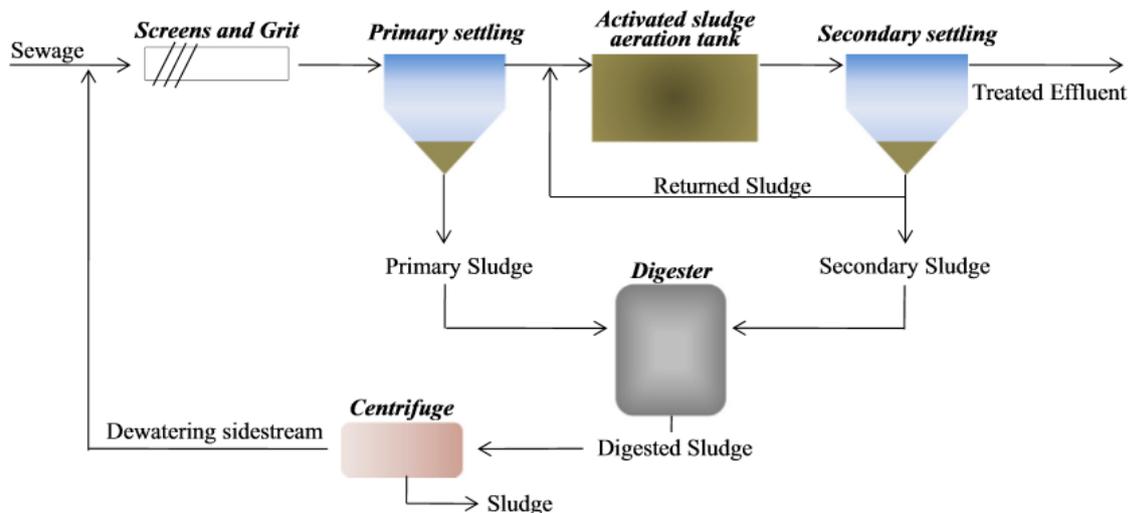


Figure 1.2.1 scheme of a conventional activated sludge (CAS) system.

This system is not enough efficient to ensure energy neutrality, for different reasons. The first is the **amount** of COD directed to the digester, that must be increased since the higher the amount of organics the higher the energy production (Hui Gong, 2018). The second is the COD **concentration**: it has been demonstrated that low organic concentrations and low temperatures are a barrier for the AD process, it's needed a level above 5 gCOD · L⁻¹ for the biogas produced to cover all the heat input costs (Perry L. McCarty, 2011). Lastly, COD **quality** is important: it's more difficult to recover energy from COD in the form of active sludge than in a raw state. This is because WAS consists of aggregated bacterial cells and extracellular polymeric substances that are more difficult to hydrolyse into small soluble compounds utilizable by microorganisms, than raw wastewater carbon in form of simple proteins or polysaccharides (Beraki Bahre Mehari, 2018) (Hui Gong, 2018). Carbon redirection technologies act like a **pre-concentration treatment** that accumulates particulate,

colloidal and soluble organic matter before it is biologically degraded by secondary treatments. A promising pre-concentration system is **direct membrane filtration**. Applying this technology to raw wastewater, suspended solids and suspended COD can be separated from the soluble fraction, in which valuable compounds such as nutrients (NH₄ and PO₄, mainly) are dissolved and can be subsequently recovered if there is an adequate treatment. Furthermore, pathogens can also be retained in the filtration process. The pore size of the material constituting the membrane, also called membrane cut-off, determine the components that are separated, called “**retentate**” and consequently the level of purity of the stream that crosses the membrane, the “**permeate**”.

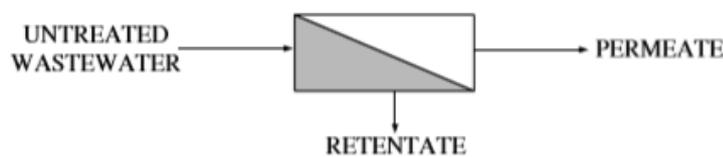


Figure 1.2.2 Schematic representation of Direct Membrane Filtration (Ravazzini, 2008)

Decreasing the membrane pore size, the quality of the **permeate** increases and can reach strict requirements. Microfiltration (MF), and Ultrafiltration (UF) are typically used due to their ability to retain suspended solids and leave an effluent with low turbidity, yet rich in dissolved nutrients. Therefore, the effluent is optimal to be reused for irrigation purposes, decreasing the need for chemical fertilizers. On the other hand, if the effluent will be discharged in a water body, the permeate must undergo a biological treatment for nutrients removal e.g. anammox. The **retentate**, with high organic content, is sent to anaerobic digestion, where it is valorised energetically, and its level of pathogens is reduced through high temperature. Thanks to the high concentration of elements in the retentate it is possible to recover **phosphate** in the form of struvite. **Nitrogen** can be isolated as well through struvite and other mechanisms like stripping or transmembrane chemisorption (Janus M.H., 1997). If **heavy metals** are present, they can be removed from the concentrate through precipitation, and **toxic organics** can be destructed by chemical oxidation (Wim H. Rulkens, 2015). About the sludge solid residue, studies are being conducted for its use in soil applications (S. Babel, 2009). This scheme of treatment, summarized in figure 4, has been demonstrated technically feasible at pilot scale, but still present some difficulties. The biggest limit for membrane technology is **fouling**, that consists in the accumulation of material on the membrane surface or into pores. This problem is solved through backwash cycles and air scouring, that increase the energy consumption, therefore further researches to optimize the process at a larger scale are required. Obviously, this scheme of treatment is economically feasible only if enough benefits can be obtained from the recovered nutrients and the produced effluent. This means to promote and regularise reuse through policies and legislation, raise public awareness and improve the marketability of the recovered material (Sina Shaddel, 2019). A first step could be the establishment of

stricter standards for discharge. In conclusion, for further developments of WRRFs it is important to conduct more research on technical aspects, but most of all the support of all the stakeholders of the sector.

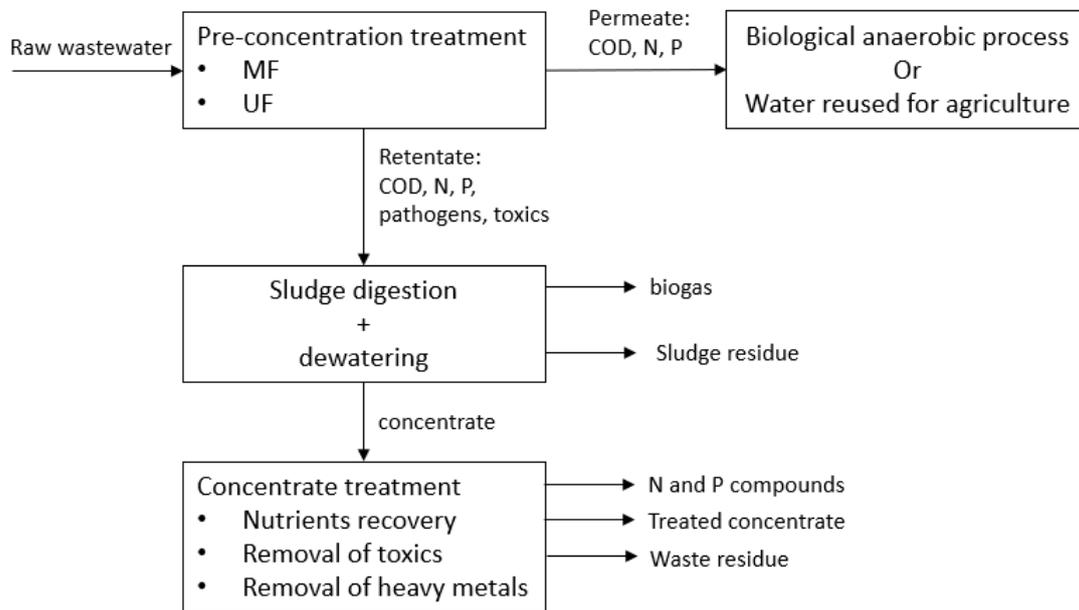


Figure 1.2.3 scheme of a WRRF based on membrane filtration

1.3 Permeate quality and treatability

In the field of carbon redirection via membrane, studies focused on permeate quality and treatability are still a few. The reason is that this branch is relatively new, and so far researches have been concentrated mainly on the energetic potential of the retentate and optimization of the membrane process. This thesis covers this gap, addressing the feasibility of two different biologic permeate post-treatment processes, through the simulation of said processes. The identification of a proper treatment starts firstly from the stream characterization, in fact the suitability of a process depends on the nature and quantity of the present pollutants. Therefore, quality and treatability of the stream are closely related.

1.3.1 Membrane retention

The first factor to define permeate quality in a direct filtration process is the **membrane pore size**, as a matter of fact all the substances larger than the pores are fully removed. Microfiltration (cut-off of 0.1-10 micron) and ultrafiltration (cut-off of 0.1-0.01 micron) can retain suspended solids and many microorganisms, but both cannot remove dissolved substances, unless they are first adsorbed or coagulated (ULTRAFILTRATION, NANOFILTRATION AND REVERSE OSMOSIS, s.d.). As a general principle, MF/UF membranes constitute a barrier for particles, while they are permeable to dissolved compounds. The result is a permeate **free from suspended solids** and with **low turbidity** but containing **soluble nutrients and organic matter** (A.M. Ravazzini, 2005). According to Scott et al. (Scott, 1995) municipal wastewater is composed by 35% of easily

settleable solids ($>100\ \mu\text{m}$), 35% of colloids ($0.001\text{-}100\ \mu\text{m}$) and 30% of soluble solids ($<0.001\ \mu\text{m}$). Membranes can remove the first two categories, for this reason they decrease the turbidity, that is the loss of transparency due to the effect of suspended particles and colloidal material (Turbidity in waste water, s.d.). Consequently, out of the total **COD** present in wastewater, the particulate and colloidal fractions are retained, while the soluble fraction passes through the medium. Also for **nitrogen and phosphorous** the particulate fractions are removed from the stream, but they usually represent a small part of the total concentration, as it can be noted from table 1.3.2, in which the average retained nitrogen is reported to be the 30% of the total. According to a study by Ravazzini et al. (A.M. Ravazzini, 2005), the total nitrogen concentration in the permeate corresponds mostly to the ammonia concentration in the influent and the total phosphorous to the orthophosphates in the influent. The removal of solids is effective if the medium permeability is controlled, because colloids, together with other solutes, often cause irreversible fouling of the medium, affecting the permeate quality.

1.3.2 Factors affecting permeate quality

As already said in the previous paragraph, fouling can affect the performance of the membrane, for this reason, to avoid this phenomenon, different methods are generally put in place, that in turn have an influence on the permeate composition. One fouling control strategy is **coagulation/adsorption** (Hui Gong Z. J., 2015), that consists in adding flocculants, adsorbents or both (hybrid process). The organic foulants are aggregated by coagulation or are adsorbed on porous adsorbents by adsorption, such that the produced particles can be physically retained by the membrane (J. W. Lee, 2005). Foulants are retained because of their increased size, but also for the induced adsorbent cake layer, that performs as a pre-filter preventing their deposition on the membrane surface (Hui Gong Z. J., 2017). In this way also part of the soluble COD can be accumulated inside the dense cake layer instead of passing through the membrane (Zhengyu Jin H. G., 2016). The coagulation/adsorption process was demonstrated to increase the retained COD by several studies, reaching removal efficiencies over 80% as shown in table 1.3.1.

Another mechanism commonly employed in membrane systems for fouling control is agitation of the filter medium, that can be realised through **air bubbles** or **mechanical vibration**. In the first case, when air is supplied, the biological degradation of organic matter is promoted if the SRT is sufficiently high (G. Mezohegyi, 2012). The consequence is a decrease of the soluble COD of the permeate. On the other hand, the mechanical agitation of the membrane module must be controlled: a low agitation can cause an excessive development of the cake layer, a high agitation can solubilize the colloidal organic matter, increasing the COD concentration in the permeate. Mezohegyi et al. (G. Mezohegyi, 2012) compared the biodegradation of COD with time under different conditions, the outcomes are shown in figure 1.3.1. From the represented graph it is evident that at real-scale operations there is always a fraction of particulate matter that gets lost through mineralization, also in non-aerated control tank.

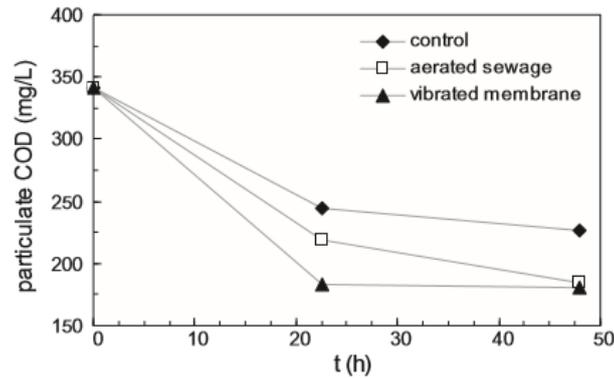


Figure 1.3.1 particulate COD degradation under different conditions (G. Mezohegyi, 2012).

Rulkens et al. (Wim H. Rulkens, 2015) reported that during a filtration experiment of 8 days the 50% of the initial amount of COD had disappeared for unintentional biodegradation. Faust et al. (L. Faust, 2014) minimized the COD loss to 1-2% by setting the SRT at 0.125-0.25d. As shown in the figure, this degraded fraction can increase of around 25 mg/l in aerated tank and of 75 mg/l in vibrating membrane modules where the oxygen, supplied or captured from the liquid surface because of the vibration, enhance the aerobic bacteria metabolism. Jin et al. (Zhengyu Jin, 2016) suggested that mineralized organic matter can decrease of 10% replacing continuous aeration with no aeration or air back-flushing. On the other hand, absence of a good membrane scouring system can increase the amount of COD retained in the cake layer. Gong et al. (Hui Gong Z. J., 2015) found out that on a total 84% of the COD retarded by the membrane, 23.8% was collected in concentrated sewage to send to the digester, while 60.2% was retained in cake layers or remained deposited in the reactor. They claim that organic matter recovery could be dramatically improved by enhanced aeration or other cleaning methods. Figure 1.3.1 also shows how a significant part of the biodegradation takes place in the first 24 hours of the process. In long term study another issue that can produce COD loss is the sedimentation of the particulate fraction in the feed tank, to avoid this problem stirring should always be present.

Another method for fouling control is **chemically enhanced backwash**. Lateef et al. (Shaik Khaja Lateef, 2013) tested how the use of different chemicals affect permeate quality. The use of sodium hydroxide or citric acid resulted to have no effect on COD concentration. Instead the use of sodium hypochlorite (NaOCl) rose the amount of COD dissolved in the permeate, because of its oxidative action on particulate and colloidal organic matter. At the same time, the presence of NaOCl inhibited almost completely the biodegradation of organics, but this effect can be achieved simply eliminating aeration.

In last analysis, the difference in the permeate produced by the treatment of **raw wastewater or primary clarifier effluent** is considered. Results by Ravazzini et al. (A.M. Ravazzini, 2005) show that during filtration of raw wastewater the cake that develops is compact and generates a resistance that can be overcome by high values of transmembrane pressure (TMP). In case of clarified water, the cake produced offers less

resistance to filtration thanks to its higher porosity and can be effectively contrasted by the flux velocity at low TMP, because high TMP promotes the cake layer accumulation instead of its dissolution. By the way this difference doesn't affect permeate quality that was similar in both cases. In the tables below, results of permeate quality after filtration, gathered from different papers, are reported.

Table 1.3.1 COD removal efficiency of membrane filtration by different references

Reference	Coagulation Yes/no	Influent Total COD	Influent Dissolved COD	Permeate COD	% Reduction
(Zhengyu Jin H. G., 2016)	no	345.3	114.2	138	60
(Zhengyu Jin H. G., 2015)	no	341.3	114	115.8	66.1
(Wim H. Rulkens, 2015)	no	450	/	86	81
(A.M. Ravazzini, 2005)	no	218	/	138	37
(Hui Gong Z. J., 2015)	yes	472	/	75.5	84
(Zhengyu Jin H. G., 2016)	yes	345.3	114.2	39.7	89
(Hui Gong Z. J., 2017)	yes	260	/	19	92.9
(Hui Gong Z. J., 2018)	yes	231	/	32.7	85.6
(Zhengyu Jin H. G., 2015)	yes	341.3	114	97	71.5

Table 1.3.2 nitrogen removal efficiency of membrane filtration by different references

Reference	Influent		permeate		% reduction
	TN	NH4-N	TN	NH4-N	
(Wim H. Rulkens, 2015)	/	34.5	/	25.2	27
(A.M. Ravazzini, 2005)	32	/	29	/	10
(Zhengyu Jin H. G., 2016)	55.4	/	35.7	/	36.5
(Hui Gong Z. J., 2017)	/	27.4	/	16.5	40
(Hui Gong Z. J., 2018)	34.4	/	25.7	/	25

1.3.3 Permeate treatability

Different removal efficiencies can be achieved, depending on the soluble COD concentration of influent wastewater. If the membrane is operated efficiently and all the particulate solids are retained, the higher is the soluble COD present in the wastewater the higher will be the COD remaining in the permeate. This is valid unless coagulation/adsorption is employed, in this case also part of soluble COD is retained, and the removal efficiency increases. Although this increase of retained organic matter is positive for extracting more energy in the digestion step, it affects the feasibility of a conventional activated sludge process as post-treatment. In fact, during denitrification bacteria exploit the COD as substrate to remove nitrogen, therefore a stoichiometric ratio **COD/N** of at least **4.2 mg COD/mg N** is required (Metcalf & Eddy, 2003). Due to the membrane capacity of retaining better COD respect to nutrients, that are mostly present in a soluble form, the relation COD/N is usually low, around **3 mg COD/mg N** (A.M. Ravazzini, 2005) (A. F. van Nieuwenhuijzen, 2000). It is evident that a conventional nitrification-denitrification treatment is not suitable for the permeate produced by direct membrane filtration and that alternative methods must be developed. Different techniques have been advanced for the treatment of this stream, but to date their study has been mostly theoretical, and just for some specific cases pilot plant and laboratory tests have been carried out. A first option to take into account is the use of the produced permeate for **irrigation purposes**, exploiting the fertilizing properties of dissolved nutrients. If this is not possible alternative biological processes must be employed.

A first method is **partial nitrification-denitrification**. This process is particularly suitable for permeate treatment because the COD requirement in the anoxic step is lower than in nitrification-denitrification, up to 40% (Yongzhen Peng, 2006). Partial nitrification is based on the accumulation of nitrites that are subsequently

oxidized by other processes, in this case denitrification. Nitrification is a sequencing biological oxidation process, that takes place in two steps: in the first step ammonia is turned into nitrites by ammonia-oxidizing bacteria (AOB), in the second step nitrites are oxidized into nitrates by nitrite-oxidizing bacteria (NOB). To have a partial nitrification it is necessary to establish the conditions for which AOB development and NOB inhibition are promoted, such that nitrate production is suppressed and nitrite is directly denitrified.

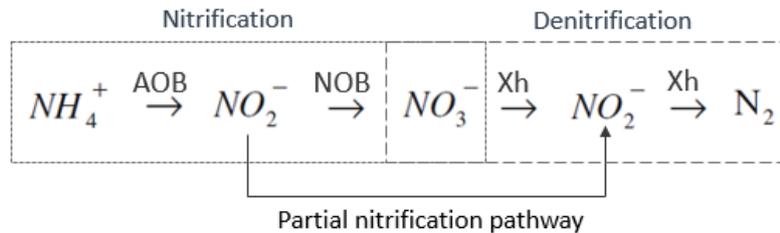


Figure 1.3.2 scheme of partial nitrification-denitrification process

In normal conditions, nitrite is rarely accumulated because ammonia oxidation is the velocity-limiting step, while nitrite oxidation is rapid (Yongzhen Peng, 2006). In order to reach this goal, operating parameters and factors must be properly regulated, for example: pH, dissolved oxygen (DO), temperature, free ammonia (FA) and nitrous acid concentrations, inhibitory compounds (Banashri Sinha, 2006). This treatment is technically feasible and economically advantageous for wastewater with high ammonium concentrations and/or low C/N ratios (Yongzhen Peng, 2006).

Another possible method is **partial nitrification-Anammox (PNA)**. This process involves ammonium oxidizing bacteria (AOB) converting approximately half of the ammonium to nitrite, followed by the remaining ammonium and nitrite being converted to N₂ by anammox bacteria (Yuanyuan Miao, 2018).

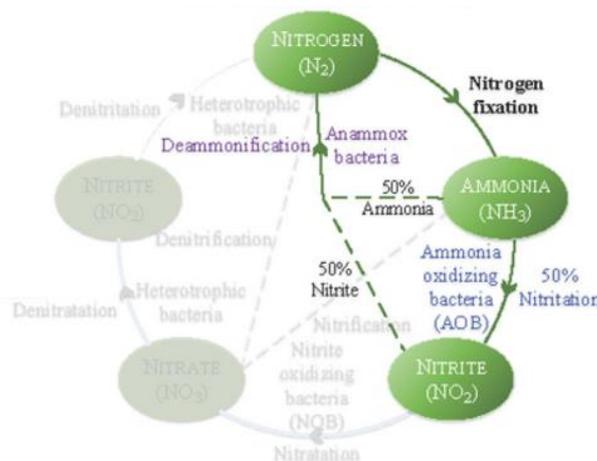


Figure 1.3.3 Scheme of the PNA process (Moomen Soliman, 2016).

This alternative is attractive when there is a low COD concentration in the permeate, with C/N ratios lower than 0.5 (Yuanyuan Miao, 2018). compared to classical nitro-denitro process, the need for organic carbon decreases by 100%, aeration requirements by about 60% and sludge production by about 90%. The main disadvantage of anammox process is that anammox bacteria (AnAOB) are characterized by lower growth rates compared to those for AOB and NOB, with doubling time of 15-30 days (Tiago R.V. Akaboci, 2018). The situation is worsen by low temperatures that slow down the growth rates even more and by low nitrogen contents that decrease the net biomass production (Clara Reino, 2017). For this reason until now this treatment has been applied to rejection water of sludge digestion and leachate water of landfill, characterized by high temperature and high ammonia concentration (Yongzhen Peng, 2006). The challenge is to make this process suitable at mainstream line, where the common conditions of low-strength nitrogen concentrations (around $50\text{mg N-NH}_4^+\cdot\text{L}^{-1}$) and low temperature (around 15°C) affect microbial activity (Tiago R.V. Akaboci, 2018). A solution is to use a biomass with dense structure and good settleability, such as granular sludge, that can provide a long biomass retention time favourable for the growth of slow rate anammox bacteria (Yuanyuan Miao, 2018). The PNA can be implemented as single-stage or two-stage process. Anammox bacteria are strictly anaerobic organisms, thus the presence of dissolved oxygen, necessary to carry out the partial nitrification, is inhibitory also in low concentrations (K. Egli, 2001). Nevertheless, with intermittent aeration, experiments show that anammox is reversibly inhibited, making possible the combination of partial nitrification and anammox in one reactor (single stage) (Strous M., 1997). PNA process take place in single-stage in granular biomass systems, where the large sludge particles promotes a favourable inside anoxic environment for the growth of anammox bacteria (Yuanyuan Miao, 2018).

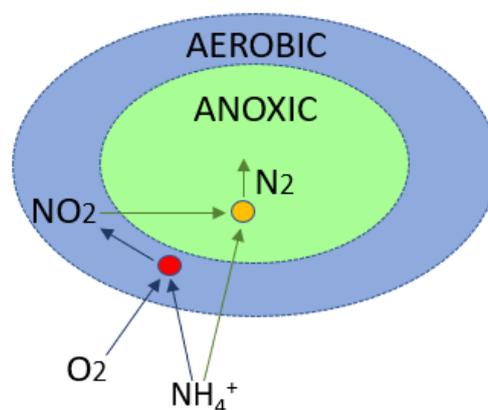


Figure 1.3.4 scheme of a biomass granule

However, this system presents some disadvantages like nitrite competition by NOB and AnAOB, low nitrogen removal rates, destabilization of PN at long term (Clara Reino, 2017). In the two-stage strategy the two

processes take place in different reactors, allowing a more stable performance and control of each single process (E. Isanta, 2015). Since ammonium oxidation is an alkalinity consuming process, when the anammox reactor follows the partial nitrification reactor, a parameter to check is the alkalinity/ammonium ratio that must be kept around 1 to guarantee the substrate to autotrophic AnAOB bacteria (Lei Zhang, 2008).

1.4 Simulation of the biological treatment

Mathematical models for wastewater treatment allow to study the characteristics of a current process and predict how the process will behave if some operational parameters are modified. Modelling and simulation are useful tools in the selection of operational strategies that improve process stability, effluent quality and operational costs (Salima, 2017). Biological processes that take place in wastewater treatment are characterized by high complexity and dynamical behaviour, mathematical models have therefore become essential for their study. Through modelling it is possible to know the quantitative and qualitative influence of each parameter which constitute the model over the global process and detect the interactions between the components. The simulation of the biological process allows to investigate the effectiveness of a conventional treatment over a wastewater with specific characteristics, for example in the present study, a stream treated with direct membrane filtration. At the same time simulation through mathematical models consent to design a process suitable for the stream, predicting the effect of operational changes. Mathematical models are also used to optimize the processes of existing or newly designed plants. In other words, mathematical models are employed for **learning** the process behaviour, for **designing** appropriate treatments and for process **optimization**. Biological models for activated sludge process simulation have substantially improved from their development up to date. At the beginning they considered just organic matter degradation, while today models take into account also the biological degradation of nitrogen and phosphorous. In 1983, the International Association on Water Quality formed a task group to review existing models and implement the capability of simulating nitrification and denitrification processes (Jeppsson, 1996). The result was the development of the Activated Sludge Model No 1 (ASM1). The peculiarity of this model was the introduction of the **matrix format**, still employed in mathematical modelling. In this format each component of the model is associated to a stoichiometric coefficient that represent the variation of the component during a certain process. The value of the coefficient is zero if the component doesn't participate to the process, positive if the component is generated, negative if the component is consumed. In the model soluble components are denoted S and a particulate component are denoted X. To retrieve the value of the coefficients representing the components variation, the model applies continuity or conservation equations together with equations which represent the kinetic of the process. In these equations kinetic and stoichiometric coefficients appear, such as bacterial growth and decay rates, half-saturation coefficients, growth yields. The process during which the coefficients to use in the model are selected is known as **model calibration**. In the case of activated sludge models, the calibration process is traditionally known as the

estimation of the model parameters to fit a certain set of data obtained through controlled experiments at pilot scale or bench scale plants (Jeppsson, 1996). The calibration can be carried out with a static or dynamic procedure. The former uses as target values average data that reproduce the stationary state of the plant, leading to find parameters that best represent the plant behaviour in long periods. The latter uses dynamic profiles of influent and effluent composition, collecting samples with a certain frequency during the process, and allow to describe dynamic and short period situations. In the present study a dynamic calibration is set up. The model employed in the present work is the **Biological Nutrient Removal Model N°2 (BNRM2)**, that maintain the standardization introduced by the ASM1. This model includes the main physical, chemical and biological processes that can occur in a WWTP. Among the physical processes considered there are settling and clarification processes, volatile fatty acids elutriation and gas-liquid transfer. The chemical interactions taken into account are acid-base reactions and precipitation, assuming equilibrium conditions. The biological processes included are organic matter, nitrogen and phosphorous removal for mainstream treatment and acidogenesis, acetogenesis and methanogenesis for the simulation of anaerobic digestion. BNRM2 considers the possible growth of all the bacterial groups present in the model in each operation unit. The environmental conditions (aerobic, anoxic or anaerobic) and the available substrate will determine which bacterial groups can proliferate. This bacterial selection is modelled including switching functions for electron acceptors in the kinetic expressions (R. Barat, 2013).

Chapter 2. Objectives

The first objective of this thesis is to perform a **dynamic calibration** of the Biological Nutrient Removal Model Nº2 (BNRM2) for the sludge of a sequencing batch reactor (SBR) fed with permeate from an ultrafiltration process of raw wastewater. The membrane separation system and the biological treatment are part of a pilot plant, from which samples were collected to obtain reference experimental data. The aim of the calibration is the estimation of the model parameters that fit the set of data obtained experimentally. This step is fundamental to obtain from the model truthful prediction about the effects that subsequently tested treatments have on the stream under exam.

Second objective is to follow up with a **simulation study of the viability of partial nitrification** in mainstream. This part has the aim of proving the feasibility of the process for the analysed stream and finding the best reactor configuration and operational parameters to implement a successful partial nitrification treatment. At the end of this phase it is possible to have an estimation of how long it takes to set up the process under the selected conditions.

The last objective is to study the **effect of the addition of an external inhibitor** to promote the partial nitrification process. Different concentrations and dosing periods can be tested performing multiple simulations. From the outputs the effectiveness of this strategy can be evaluated and the best modality to supply the inhibitor can be elected.

Chapter 3. Partial nitrification: state-of-the-art

3.1 Implementation of the partial nitrification process

As mentioned in the previous chapters, nitrification involves two processes: oxidation of ammonia to nitrite by AOB and oxidation of nitrite to nitrate by NOB. The objective of partial nitrification is to favour nitrification and inhibit nitrification, in order to obtain nitrites accumulation. The generation and maintenance of a PN reactor require that NOB are washed out from the biomass while AOB are accumulated (Banashri Sinha, 2006). To date, researchers have developed many control methods and strategies to achieve this goal acting on operating parameters and factors (Yongzhen Peng, 2006). Among these the most common are: reactor's temperature, pH, dissolved oxygen (DO) concentration, aeration pattern and duration, sludge retention time (SRT), free ammonia (FA) and nitrous acid concentrations, inhibitory compounds (Quing Yang, 2007). The critical point is to suppress nitrite oxidation without excessively delaying the ammonia oxidation rate, since both AOB and NOB are sensitive to the process operating conditions (Banashri Sinha, 2006). Moreover during nitrification, AOB and NOB co-exist and benefit from this close physical association; for example nitrite oxidizers remove nitrite that is toxic to ammonia oxidizers (Yongzhen Peng, 2006). Modern microbiologic equipment allows to identify and investigate individual microbial species present in a sample, in this way the effect of operational changes on each population can be assessed. These tools have made it possible to research in different studies the inhibitory factors that have a selective inhibition on NOB and a minimal effect on AOB, or the competitive factors which don't inhibit NOB but give a competitive advantage to AOB (Banashri Sinha, 2006). A successful implementation of partial nitrification at mainstream conditions implies a stability of the process in the long terms, that means achieving an effective repression of NOB activity (Clara Reino M. E.-O., 2016). The first process involving partial nitrification able to reach stable conditions in full-scale applications has been the SHARON process (Yongzhen Peng, 2006). The Single reactor High Activity Ammonia Removal Over Nitrite, abbreviated as SHARON process, is able to selectively wash out nitrite oxidizers exploiting short residence time (1-1.5 days) and high temperature (30-40°C) (Banashri Sinha, 2006). The necessity of temperatures over the typical range of WWTPs (5-20°C) and the need of high ammonium concentrations has limited the development of this technology. Several authors have been investigating different techniques to obtain a stable PN process in conventional conditions. By now, successful partial nitrification processes recorded are obtained in sequencing operation process, and few are achieved in a continuous-flow process (Yongzhen Peng, 2006). Many parameters have been suggested for influencing nitrite accumulation, either individually or in combination with other factors (Banashri Sinha, 2006).

Influence of pH free ammonia and free nitrous oxide concentrations

Anthonisen Et al. (C. Anthonisen, 1976) studied the inhibitory effect on nitrite accumulation of free ammonia NH_3 (FA) and free nitrous acid HNO_2 (FNA) concentrations. During partial nitrification, AOB use **free ammonia** as substrate for the oxidation, releasing nitrite and other possible products like nitrogen gas and nitric oxides.

Nitrosomonas sps. and Nitrobacter sps. are both sensitive to their own substrate and even more to the substrate of the other (Banashri Sinha, 2006). Although in literature different threshold inhibition concentrations can be found, they all indicate that NOB are more sensitive to FA than AOB. The reported FA inhibition constants range from 7–605 mgN/L for AOB (Yongzhen Peng, 2006) (R. Ganigué, 2007) and 0.1–5 mgN/L for NOB (AC Anthonisen, 1976) (U. Abeling, 1992). By the way, free ammonia has only an inhibitory effect on NOB, doesn't kill them and after a period of cultivation they get acclimated to FA recovering the activity (M Beccari, 1983). The experiments by Villaverde et al. (S Villaverde, 2000) demonstrated how NOB develop the capability to resist high concentrations of free ammonia after exposure for long periods of time to constant FA concentrations. Both AOB and NOB are capable of acclimating to FA levels as high as 40 mg NH₃-N/l (O Turk, 1989). The reversibility of nitrifiers inhibition by FA should be considered when setting up a partial nitrification process in order to select other factors to rely on to maintain the process stable (Fdz-Polanco F, 1994). As far as free **nitrous oxide** is concerned it was detected to act as an inhibitor in nitrification process, affecting both AOB and NOB. The inhibition thresholds show that NOB are more sensitive to FNA than AOB by approximately one order of magnitude. As a result, very high concentrations of FNA may cause the wash out of both AOB and NOB from nitrifying systems, but a lower FNA concentration range is suitable for NOB washout (Yan Zhou, 2011). From studies conducted by Ma et al. (J. Ma, 2010) on activated sludge emerged that FNA in the range 0.01-0.025 mg HNO₂-N/L inhibited nitrification by 40%, FNA concentration of 0.2 mg HNO₂-N/l completely stopped the process. Total ammoniacal nitrogen is divided in free ammonia and ammonium (TAN=NH₃+NH₄⁺), as well as total nitrite concentration is given by the sum of free nitrous acid and nitrite (Nt=HNO₂+NO₂⁻). The equilibrium between free ammonia/ammonium and free nitrous acid/nitrite is determined by the **pH**, thus this parameter can be adjusted to induce inhibition of nitrite oxidizers (Banashri Sinha, 2006). The equilibria are represented in the figure below:

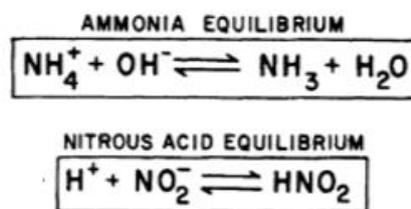


Figure 3.1.1 nitrification equilibria (AC Anthonisen, 1976).

When the pH increases, the concentration of free ammonia increases. The relation between free ammonia and pH is described by the formula:

$$FA = \frac{[TAN] \cdot 10^{pH}}{\frac{K_a}{K_w} + 10^{pH}}$$

Where TAN is the total ammoniacal nitrogen concentration and K_a and K_w are ionization constants of ammonia and water. A pH in the range of 7.5-8.5 is able to inhibit the nitrite-oxidizers through the increase in FA concentration. On the contrary, decrease of pH to 7.0 implies absence of NH_3 and predominance of NH_4^+ (Banashri Sinha, 2006). The raise of the pH may occur naturally or by the addition of a base such as sodium hydroxide (AC Anthonisen, 1976). On the other hand, according to the nitrous acid equilibrium, the concentration of FNA will increase as the pH decreases. The relationship among FNA and pH is:

$$FNA = \frac{[NO_2^- - N]}{K_d \cdot 10^{pH}}$$

Where NO_2^- is the nitrite concentration and K_d the ionization constant for nitrous acid. A reduction in pH level will produce not only a decrease of FA for the adjustment of the ammonia equilibrium, but also a decrease in the total ammonia concentration as it is oxidized to nitrite, relieving the inhibition of the nitrobacters (AC Anthonisen, 1976). Abeling and Seyfried (U. Abeling, 1992) reported that **pH values above 7.5** should be maintained to selectively inhibit the nitrite oxidation and accumulate nitrite in the system. Most of the literature in which pH is used as the key parameter to achieve partial nitrification states the optimal pH in the **range 7.5-8.5** (Banashri Sinha, 2006). Anthonisen et al. plotted FA, FNA and pH values to identify zones where nitrification inhibitions were likely to occur, the results are represented in figure.

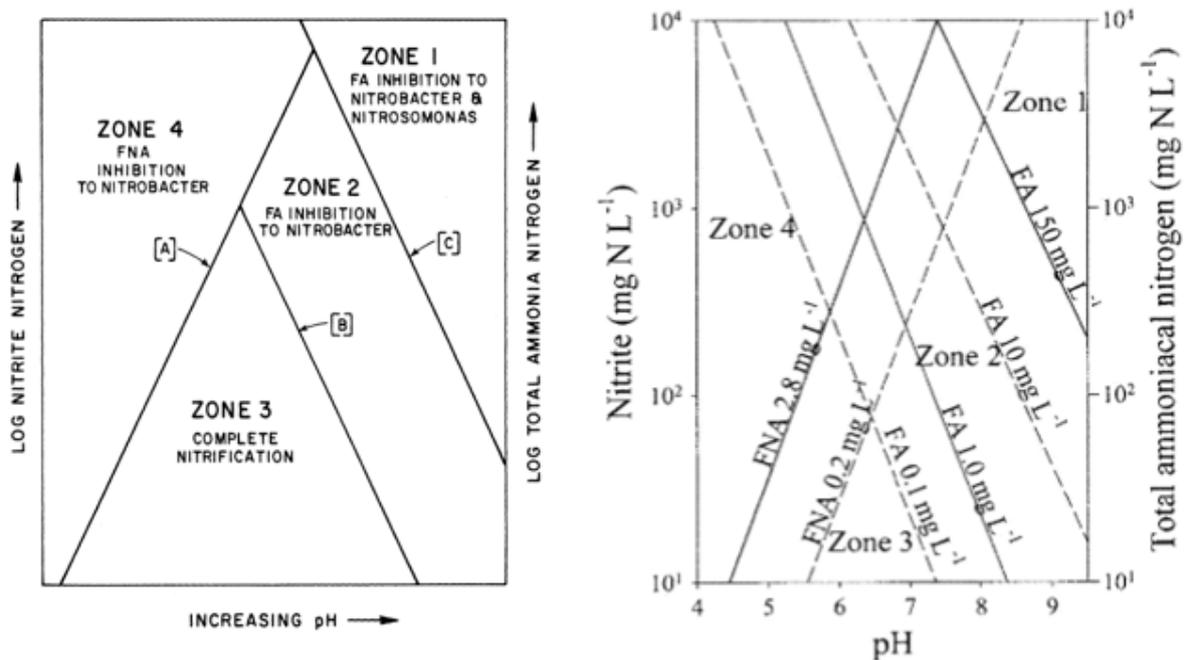


Figure 3.1.3 zones of nitrification inhibition determined by free ammonia and free nitrous oxide concentrations. In the figure on the right the dashed lines mark the lower limit and the solid lines mark the upper limit of the range of boundary conditions of zones of nitrification inhibition (AC Anthonisen, 1976) (Banashri Sinha, 2006).

Influence of temperature

Temperature is another parameter which has an influence on the nitrification process. It has been noted that nitrite accumulates more during the summer period than during the colder seasons (Tonkovic, 1998) (RK Gelda, 1999). The reason is the different sensitivity to temperature changes of the two populations of bacteria implied in the process (C Hellings, 1998). It is thought that the optimum temperature for nitrification is higher than that for nitrification and that at higher temperatures, the growth rate of NOB is lower than that of AOB (JW Mulder, 1997). For **temperatures over 25°C**, ammonium oxidizers effectively outcompete the nitrite oxidizers (M Brouwer, 1996). It has been demonstrated that temperature affects the ratios $\text{NH}_3/\text{NH}_4^+$ and $\text{HNO}_2/\text{NO}_2^-$, in particular an increase in temperature causes an increase in FA concentration (Banashri Sinha, 2006). NOB inhibition due to the higher FA outweighs the activity stimulant of higher temperature, permitting nitrite accumulations of 80% (F. Fdz-Polanco, 1994). Balmelle et al. (B. Balmelle, 1992) declared that for a range of temperature between 10 and 20°C nitrite build-up remained low also with inhibiting concentrations of FA, because the effect of the Nitrobacter activation by temperature prevailed over its inhibition by FA. Normal conditions in WWTPs favour growth of NOB, for this reason there is commonly a complete oxidation of ammonium into nitrate (Banashri Sinha, 2006). Because of its high specific heat, it is not reasonable to raise temperature of wastewater, many authors suggests that keeping **25°C** is enough when reactor system's temperature is the sole parameter considered (C Hellings, 1998) (B. Balmelle, 1992) (AC Anthonisen, 1976). Nevertheless, different observations can be found in literature, for example the experiments by Dongen et al. (van Dongen, 2001) stated that the optimal range is **between 30 and 37°C**, values supported by other authors (C Hellings, 1998) (R van Kempen, 2001). The approach of using high temperatures to reach partial nitrification has already been proven to be by the SHARON process a viable and robust system. By the way, when other factors contribute to the accumulation of nitrite, it is possible to achieve a successful partial nitrification also at lower temperatures typical of wastewater plants. Yang et al. (Quing Yang, 2007) were able to reach nitrite accumulation for a long period at temperatures between 11.9 and 26.5 °C through a real-time control strategy which allowed to regulate aerobic and anoxic durations based on ammonia and COD influent concentrations. In conclusion temperature is an important but not limiting parameter for the success of the process.

Influence of the Sludge Retention Time (SRT)

Because of the different growth rates characterizing AOB and NOB, also the minimum requested sludge ages are different (Yongzhen Peng, 2006). Properly adjusting the **sludge retention time (SRT)** is possible to selectively washout the slower groups of bacteria. In the case of nitrification, the nitrite oxidizers need a higher retention time since their growth is slower than ammonium oxidizers. The adjustment of SRT together with high temperatures is the strategy adopted by the well consolidated SHARON process, in which SRT as low as **1-1.5 days** are used. The relationship between temperature and minimum required residence time for

the two groups of bacteria is shown in figure. The graph represented points out that for temperatures over 14°C it is possible to wash out the NOB, keeping the AOB, using a proper residence time.

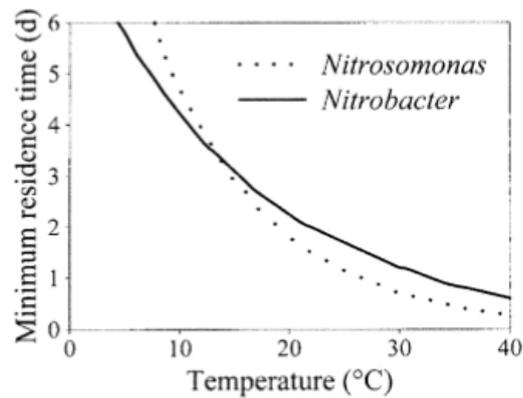


Figure 3.1.4 Effect of temperature on minimum SRT for ammonia and nitrite oxidation (Banashri Sinha, 2006).

Van Kempen et al. (R van Kempen, 2001) from experience on full-scale operations, suggest to maintain SRT between **1 day and 2.5 days**. Nevertheless, many literatures reported successful partial nitrification under long sludge age (Yongzhen Peng, 2006). Pollice et al. (A. Pollice, 2002) found out that under limited oxygen supply, nitrite accumulation was stably obtained independently from the chosen sludge age. Instead for non-limiting DO concentration at a given temperature and pH, the sludge age resulted to be the critical parameter for partial nitrification. In traditional continuous stirred tank reactors (CSTRs) for mainstream wastewater treatment, the low ammonium concentration in the mixed liquor selects for the slow growing AOB (K strategist) characterized by low growth rate, therefore it is difficult to use low SRT for NOB washout (Jun Wu, 2016). In sequencing batch reactor (SBR) the high initial ammonium concentration lead to a predominance of fast growth R strategist and low SRT could be used as a strategy for nitrification inhibition.

Influence of dissolved oxygen (DO)

Dissolved oxygen (DO) and aeration duration are economically feasible control parameters (Yongzhen Peng, 2006). AOB and NOB are both aerobic bacteria, but the ammonia oxidizers seem to be more robust towards low DO than the nitrite oxidizers (Banashri Sinha, 2006). In fact, the dissolved oxygen half-saturation coefficients are different and estimated as 0.3 mg/l for AOB and 1.1 mg/l for NOB (Weismann, 1994). This means that a low DO concentration more significantly affects the activity of NOB than that of AOB, according to the Monod kinetic of growth (HG Leu, 1998). When the reactor is operated at low DO condition, growth rate of ammonia oxidizer can be more than two times faster than nitrite oxidizers (Tokutomi, 2004). By the way, the critical values of DO recorded in literature are different. Garrido et al. (JM Garrido, 1997) obtained maximum ammonium oxidation and nitrite accumulation for DO equal to 1.5 mg/l. In a study by Hanaki et al. (K Hanaki, 1990) with a suspended growth reactor at 25°C, nitrite oxidation was strongly inhibited by DO lower than 0.5 mg/l, and the positive effect on AOB growth yield compensated the reduced ammonia oxidation rate per unit mass of cell. Different values of DO half-saturation coefficients can be found in the

ranges 0.2–0.5 mg/l for AOB and 0.7-2 mg/l for NOB (C Picioreanu, 1997). The dissimilarity in optimal DO concentrations can be related to the dimensions of the sludge flocs. Guo et al. (Jianhua Guo, 2009) investigated the influence of floc size, founding out that large floc diameters promoted partial nitrification. The reason is that the larger the floc the more it is difficult for the oxygen to diffuse inside it, thus low oxygen concentration zones are developed even with higher levels of oxygen supplied. As a consequence, depending on floc characteristics, the NOB half-saturation coefficient may increase, not for an increased bacterial capacity in handling oxygen, but for the interposition of the floc physical resistance. Low DO condition presents some drawbacks to take into account like lower nitrification rate and filamentous bulking sludge. Yongzhen et al. (Yongzhen Peng, 2006) suggest that to obtain nitrite accumulation maintaining a good ammonia oxidation rate, DO concentration should be maintained about **1.0–1.5 mg/L**. Another strategy to obtain nitrite accumulation is regulating the duration for which oxygen is supplied. This can be done through **real-time aeration duration control**, with two methods. The first consists in detecting the “ammonia valley”, the time in which the pH reaches a minimum value, that corresponds to the end of the ammonia oxidation process (figure 3.1.4). From this moment the aeration is shut down in order to avoid further transformation of nitrite into nitrate. Just applying aerobic duration control and without regulating DO concentration or other factors Guo et al. (J.H. Guo, 2009) were able to achieve and maintain a nitrite accumulation ratio of 95% in long term operations, using pH as real-time control parameter.

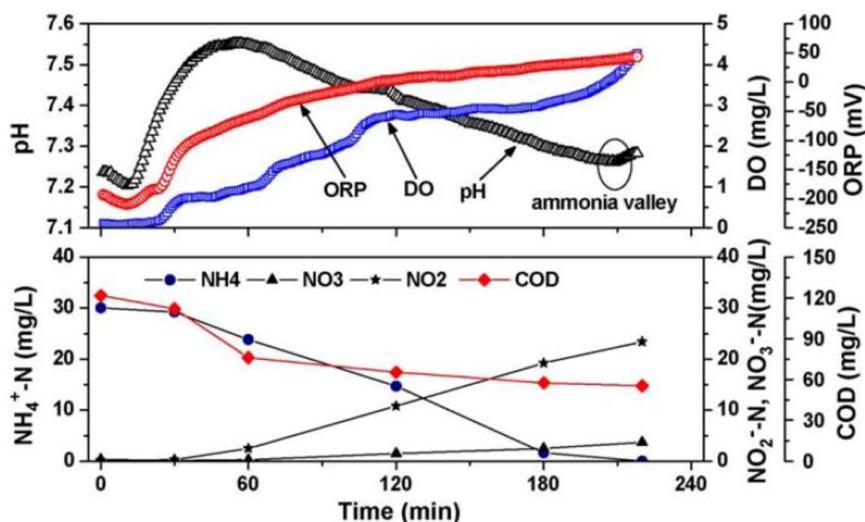


Figure 3.1.5 profiles of control parameters and pollutants concentrations in a nitrification cycle (J.H. Guo, 2009).

The second method is based on the measurement of the oxygen uptake rate (OUR) during the process, to stop the aeration as soon as OUR drops below a threshold value, that indicates the end of the nitrification phase. By the way, this control parameter isn't considered reliable because affected by processes different from ammonia oxidation, like heterotrophic oxidation (Richard Blackburne, 2008).

Influence of Inhibitors

Another possibility to suppress NOB activity is adding inhibitory compounds others than the ones which naturally appear in the process (FA and FNA). Many compounds have been studied in literature for their

inhibition capacity. **Oxidants** such as **chlorite** and **chlorate** were proven to operate a selective inhibition over NOB (Yongzhen Peng, 2006). Belser and Mays (L.W. Belser, 1980) showed through their results that 10mM of ClO_3^- had no effect on AOB activity, whereas NOB were completely inhibited. **Sulfide** was observed to provoke inhibition to both AOB and NOB, but with more significant effects on nitrite oxidation (D.I. Bejarano Ortiz, 2013). The experiments by Bejarano Ortiz et al. (D.I. Bejarano Ortiz, 2013) showed that sulfide concentrations in the range 3.1-112 mg HS^- /l decreased specific rates by 51 to 92% for NH_4^+ oxidation and by 77 to 97% for NO_3^- production. **Volatile fatty acids** like formic acid, acetic acid, propionic acid and n-butyric acid all inhibit nitrite oxidation, without exhibiting substantial effect on ammonia oxidation. The critical concentrations reported by Eilersen et al. (A.M. Eilersen, 1994) to halve the activity of NOB are 2mM for formic acid, 115mM for acetic acid, 33mM for n-butyric acid and 68mM for propionic acid. **Hydroxylamine** exhibited an irreversible inhibition on NOB (Yongzhen Peng, 2006). Partial nitrification was successfully attained dosing hydroxylamine in concentrations of 10 mg/l in aerobic granular reactors (Guangjing Xu, 2012), 5.25 mg/l in biofilm reactors (Satoshi Okabe, 2011) and 2.5-5 mg/l in a submerged filter system (O.J. Hao, 1994). **Heavy metals** have been found with the potential to inhibit both nitrification and nitrification. Chromium, nickel, copper, zinc, lead, and cadmium are the metals identified as inhibiting for nitrification (G. Camilla, 1998). Different inhibition thresholds are reported in literature for the same metal, often differing for more than one order of magnitude (table 3.1.1). The inhibition produced by metals is affected by factors like sludge type, exposure time and temperature (Xuan Li, 2016).

Table 3.1.1 Ranges of metal concentrations of inhibition to nitrification under pure culture (G. Camilla, 1998).

Metal	Concentration ($\mu\text{g/l}$)
Cr	0.7–785
Ni	3–860
Cu	3–5,730
Zn	3–1,000
Pb	0.09–1,680
Cd	0.01–20

Influence of alkalinity

During the biological ammonia conversion into nitrite, protons are generated, causing an alkalinity consumption of 7.14 mg CaCO_3 /mg $\text{NH}_4\text{-N}$ (Adrianus van Haandel, 2007). Alkalinity is one of the most important substrates for AOB, thus influence their activity (Xiaoqing Zhang, 2013). An ammonia to alkalinity ratio (A/a) of 8/1 is generally required to achieve complete nitrification in a biological reactor (Barillo, 2015). Zhang et al. (Xiaoqing Zhang, 2013) tested the effect of different A/a ratios on the performance of a partial nitrification process taking place in a membrane bioreactor. They found out that when alkalinity was sufficient or in excess, ammonia was almost completely oxidized, reaching removal efficiencies near 100%, on the contrary, ammonia concentration in the effluent increased more and more with a gradual decrease

of alkalinity. The nitrite to ammonia ratio in effluent of PN is a key point for ANAMMOX process, in which ammonium and nitrite should be fed in stable and close to equimolar proportions (Borja Valverde Perez, 2012). This means that the removal efficiencies of the partial nitrification process must be limited to around 50%, and this target can be achieved controlling alkalinity.

3.2 Discussion of different control methods

Different factors can contribute to the success of a partial nitrification process. The challenge is to find an effective strategy that overcomes the limitations of the well-known SHARON process: high strength ammonia wastewater, high temperatures, low SRT. Many authors developed stable partial nitrification processes at mainstream conditions, commonly using a combination of different methods, especially during the start-up process. Zeng et al. (Wei Zheng, 2012) reached a partial nitrification-denitrification process at normal temperature of 19°C and long SRT (50–66d) exploiting low DO concentrations (<0.8 mg/l), which favoured AOB dominance in the start-up period, reaching 95% nitrite accumulation after 60 days. Successively, real-time control of aeration duration based on on-line monitoring of pH was implemented to maintain the stability in the performance. Using just DO limitation as control method, under long-term NOB community may shift from *Nitrobacter* to *Nitrospira*, deteriorating the nitrification (Guoqiang Liu, 2013). Isanta et al. (Eduardo Isanta, 2015) managed to maintain partial nitrification at long-term in a granular reactor at low temperature just adjusting the ratio oxygen/ammonium. Nevertheless, they noticed that among NOB, *Nitrobacter* sp was predominant, contrarily to other N-removal systems in which *Nitrospira* sp. is the most present at low temperature. In general combining two or more control strategies is better for nitrification stability and complete NOB washout (Jia Li, 2019). Wang et al. (Dongbo Wang, 2015) achieved stable nitrification in a mainstream reactor at room temperature (22°C) with a SRT of approximately 8 days combining the effect of FNA inhibition and DO limitation (low concentration and control of the aeration length). NOB activity was suppressed because the FNA eliminated the *Nitrospira* sp. and the low DO washed out the *Nitrobacter* sp. Moreover, to provide an ammonium/nitrite ratio suitable for the subsequent anammox treatment, the aeration was switched off after around 50% of influent ammonium was oxidized. Many authors used real-time aerobic duration control as the main parameter to achieve partial nitrification, also without limiting DO concentration. This is the case of Guo et al. (J.H. Guo, 2009) that achieved more than 90% nitrite accumulation in a SBR at medium and low winter temperatures just controlling aeration length. When the aerobic duration control is the sole parameter used for the nitrification selection, Blackburn et al. (Richard Blackburne, 2007) suggest to add an inhibitor to decrease the start-up period, that in their case was of 300 days to get 80% nitrification. The technique employed was to dose formic acid to induce an initial 40% nitrite accumulation, and later stabilizing nitrification at 80% using only aerobic duration control. The SRT was set at 8 days, but poor sludge settling events led to increase the value to recover the biomass. Also Li et al. (Jia Li, 2019) used a combination of hydroxylamine (NH₂OH) addition and real-time aeration control. Dosing

an initial concentration of the inhibitor every 24 hours nitritation was established and the nitrite accumulation ratio increased from 2% to 93% in 19 days. After this period NH_2OH addition was stopped and partial nitrification remained stable with 97% NO_2 accumulation through real-time aeration control. During the process the temperature was maintained at $25 \pm 1^\circ\text{C}$ and the sludge retention time at approximately 20 days.

Chapter 4. Materials and methods

4.1 Dynamic calibration

For the determination of the kinetic parameters to use in the model, a dynamic calibration was carried out based on the experimental data obtained from a pilot plant. The experiments were performed by the CALAGUA research group, formed by personnel from the Research Institute of Water and Environmental Engineering the Polytechnic University of Valencia and the Department of Chemical Engineering of the University of Valencia. In this section the configuration of the pilot plant and the operational parameters set for the biological process are presented. Following, a description of the different samples collected is provided, together with the analytical methods employed for the determination of their characteristics.

4.1.1 Experimental setup and operational parameters

The reference pilot plant is situated in the wastewater treatment plant “EDAR Carraixet”, in Alboraya, Valencia. It is composed by an initial screening, followed by an equalization tank, a compartment for membrane filtration, accumulation tanks and a sequencing batch reactor. A schematic view of the plant is given in figure 4.1.1.

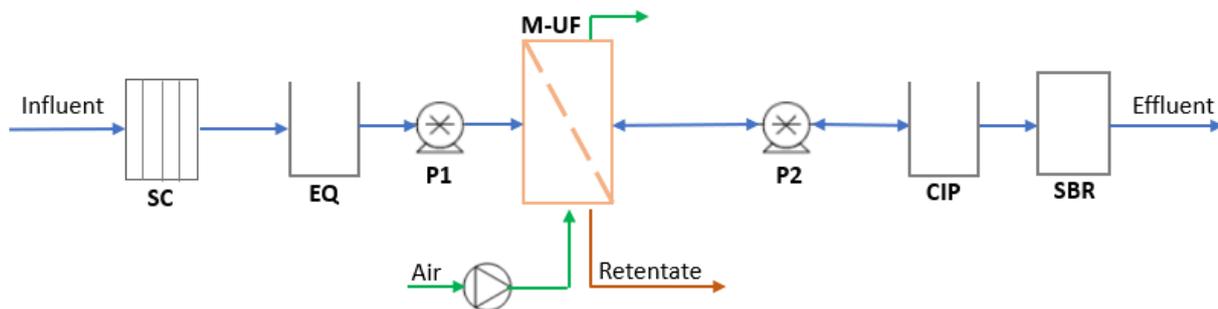


Figure 4.1.1 scheme of the pilot plant: SC=screening, EQ=equalization tank, P1 and P2=pumps, M-UF=membrane ultrafiltration, CIP=clean in place, SBR=sequencing batch reactor

The influent to the pilot plant is raw wastewater, which after the initial **screening** pre-treatment is stored in the **equalization tank** to be successively sent to a direct membrane separation process. This process consists in a **membrane ultrafiltration**, with a cut-off of 0.03 μm , performed by a Hollow Fiber type membrane in PDVF. The permeate is then accumulated in a **clean in place** (CIP) tank at Ambiental temperature, with low hydraulic retention time (30 minutes) and stirring to avoid particle settling. The liquid is consequently pumped to a **sequencing batch reactor (SBR)**, where the biological treatment is put in place. The reactor has a total volume of 18 litres, working volume of 13 litres and is heated to maintain a temperature of 20-23°C. The dissolved oxygen is kept at a concentration between 0.3 and 0.7 mgO_2/l through automatic control. A monitoring system is present, which includes: DO, REDOX, temperature, pH and conductivity probes. Acetic acid is automatically added to supply COD deficiency, in stoichiometric amount based on ammonia influent concentration. The hydraulic retention time and solids retention time are respectively 12.5 hours and 20 days. The reactor was operated as a multiple feed, with the permeate fed in three different steps. For each

step a volume of 1.67 litres was supplied, for a total of 5 litres of permeate treated in each cycle. Each step is composed of an anoxic phase of 23 minutes and an aerobic phase of 57 minutes, to form a cycle of 4 hours and 47 minutes. Each day 5 total cycles take place.

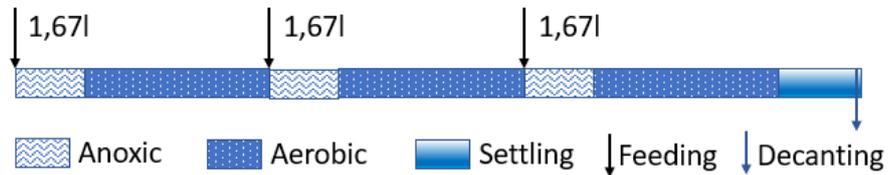


Figure 4.1.6 multiple feed phases.

A scheme of the SBR is provided in the figure below, together with a table that summarizes the operational parameters.

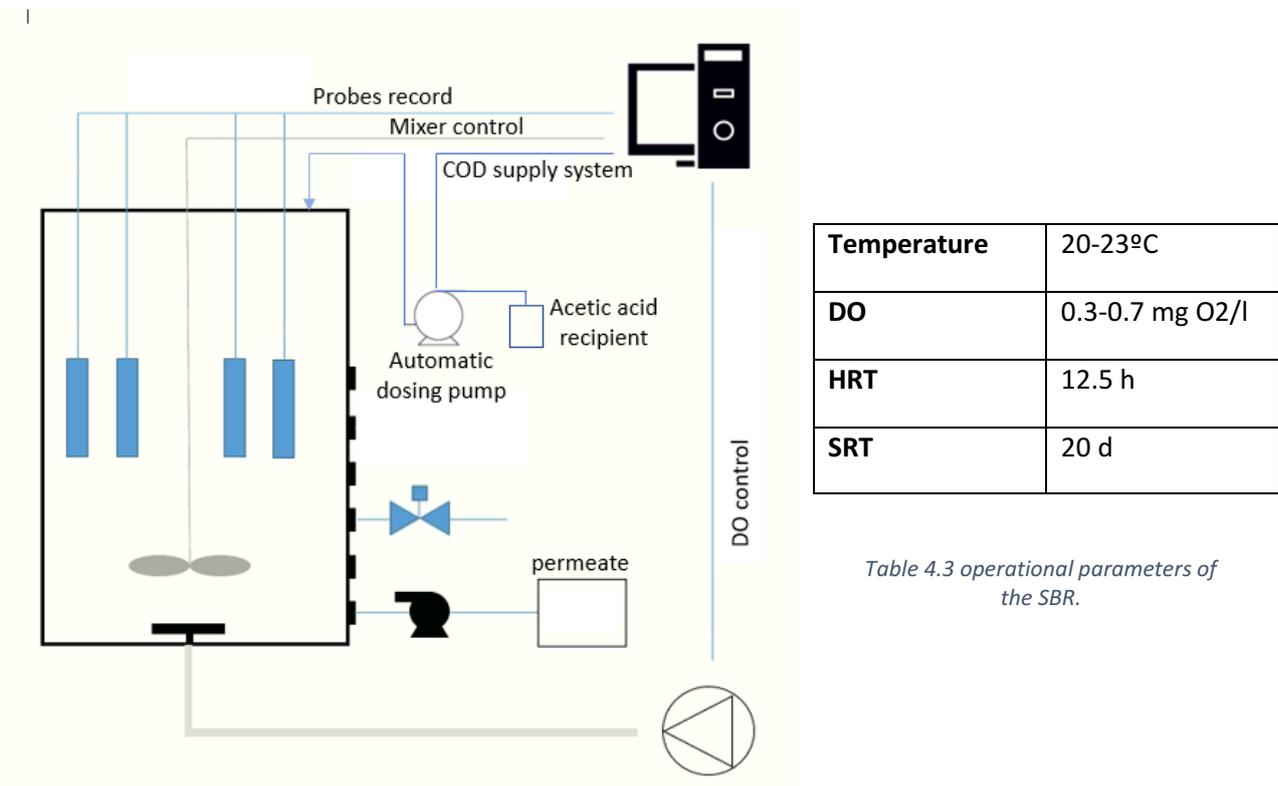


Figure 4.1.7 Sequencing Batch Reactor

This configuration has been used to calibrate the BNRM2 model developed in MATLAB. The operational parameters described above have been transferred to model to be able to reproduce the experimental tests.

4.1.2 Sampling and analytical methods

The study period lasted from 29/01/2020 to 19/02/2020. Samples were collected in different phases along the treatment, in order to track the parameters evolution:

- **S_previousCycle**: taken at conclusion of the reaction phase, after sedimentation, before the following cycle starts;
- **S_initial**: taken at the beginning of the cycle, after feeding step;
- **S_endAnx**: taken at the end of the first anoxic step;
- **S_endAer**: taken at the end of the first aerobic step;
- **S_permeate**: to analyse the characteristics of the permeate before biologic treatment.

For each of the listed samples were conducted laboratory tests to evaluate the chemical oxygen demand (COD), ammonia (NH₄), nitrite (NO₂) and nitrate (NO₃) concentrations. Another **sample** was collected from the SBR, during the reaction phase, to determine the characteristics of the **mixed liquor (S_mixLiq)**: total suspended solids (TSS), total volatile suspended solids (TVSS), chemical oxygen demand (COD) and biodegradable chemical oxygen demand (CODs). Moreover, the **BOD** of the permeate was **punctually measured**. All the samples were filtered in situ, to avoid biological activity during the storage.

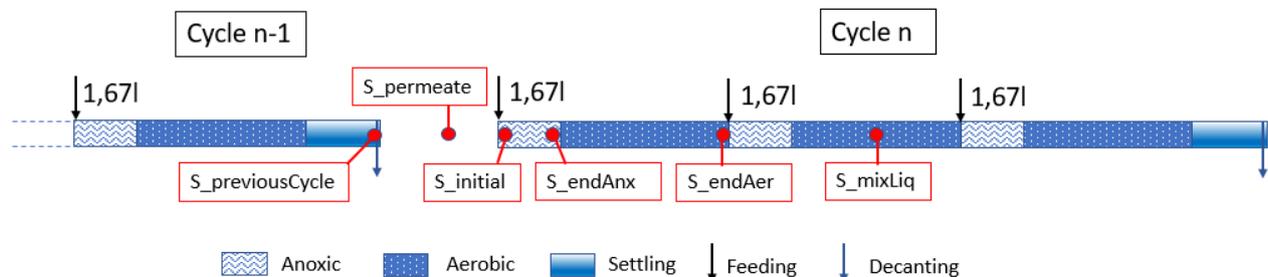


Figure 8.1.4 samples collected during the process

The **analytical methods** employed for the evaluation of the different parameters are described as follows. The concentration of **ammonium** (NH₄⁺), **nitrite** (NO₂⁻) and **nitrate** (NO₃⁻) were determined at the beginning through ion chromatography. The equipment employed was an IC model 761 Compact of the commercial house Metrohm. Also other methods of analysis were employed, suggested by the Standard Methods for the Examination of Water and Wastewater, and equipment like the multiparameter analyser SmartChem200 of the house Metrohm. The test for the determination of **total nitrogen** of the company Merck was performed. Organic and inorganic compounds were turned into nitrates according to the Koroleff method, through decomposition with an oxidant in a thermoreactor. Nitrates, in sulfuric and phosphoric solution, form with the 2,6-dimethylphenol the 4-nitro-2,6-dimethyl-phenol, which is determined photometrically at 338 nm with a spectrophotometer (Spectroquant VEGA 400). The concentration of **total suspended solids** (TSS) was determined passing the sample through a filter with pore size 0.45 μm and weighting the retained material after drying it in an oven at 105°C. The increment in the filter weight represents the quantity of TSS in the sample. The **volatile suspended solids** (VSS) and **non volatile suspended solids** (NVSS) were defined through the calcination of the TSS at 550°C. For the determination of the **chemical oxygen demand** (COD) two analytical methods were put in place, different for the concentration range of COD for which they are employed. The measurement of COD in both cases was achieved indirectly, evaluating the excess of

dichromate ($\text{Cr}_2\text{O}_7^{2-}$) used. The first of the methods employed was the standard open reflux method for the oxidation of the organic matter, designed for a COD range between 50 and 900 mg COD/l. the excess of dichromate, in this case, was determined through a redox titration. The second method was the test by the commercial house Merck, for COD values ranging from 10 and 150 mg COD/l. This method consists in a digestion with closed reflux and evaluation of the excess dichromate through colorimetry.

4.2 Partial nitrification study

After the calibration, the kinetic parameters retrieved were used to run the model for the evaluation of the implementation of a partial nitrification process for the permeate treatment. In this phase the possibility to achieve nitrite accumulation in the mainstream was assessed and the best configuration and operational parameters to reach partial nitrification in the biological reactor were found through different sets of simulations.

4.2.1 Operational parameters

In this phase some operational parameters like **HRT, SRT, DO**, aren't fixed, because different values are tested in order to find the best process arrangement. The main difference with the set-up of the calibration process, is that the **duration of the aerobic phase** is variable and it is a function of the ammonia availability. When the ammonia concentration drops below a selected value (0.05 mg/l) the aerobic process stops and the next step of the cycle starts. In this way, the process is optimized through aeration control and the necessary hydraulic retention time to complete the process can be estimated at the end of the simulation. Also the **reactor configuration** is object of study. Although the number of feeds per cycle are varied to examine different configurations, the relation between the **total volume fed** with respect to the **total volume of the reactor** is maintained (5 total litres fed in 13 litres of reactor). The **permeate** of ultrafiltration fed to the process is still assumed to have the quality described for the calibration phase, since the stream characterization was conducted on the influent data available. The **temperature** is kept at 23°C, because this is the average value of the records obtained during the entire experimental process, and it is thought to be the temperature that would be present in any other scenario. In addition, the model now considers the effect of free ammonia (FA) and free nitrous oxide (FNA), as inhibitory compounds for the partial nitrification process. This means that among the kinetic parameters the **FA and FNA inhibition constants** for the two groups of bacteria involved have been included. Their values were selected from the different available in literature as reported in table.

	KFA (NH ₃)	KFNA (HNO ₂)
AOB	10	0.4
NOB	1	0.01

Table 4.4.1 free ammonia (FA) and free nitrous oxide (FNA) half saturation constants for ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB)

4.2.2 simulation sets

The first set of simulations conducted was aimed at identifying the best **feeding configuration** to operate the SBR reactor to achieve partial nitrification. Two different models to simulate the single-feed and multiple-feed three stages configuration were implemented in MATLAB. The two simulations were run separately with the same input parameters, coming from the reactor operating conditions and from the calibration process. In the second set of simulations the values of **sludge retention time (SRT)** and **dissolved oxygen (DO)** concentration were assessed. The model elected as the best in the first set was simulated with different combinations of SRT and DO to detect the optimal way to operate it. The SRT was set at 20, 10, 8 and 5 days and for each SRT, DO concentrations of 1.2, 0.7 and 0.5 mg O₂/l were tested. For each combination the outputs allowed to evaluate the evolution in pollutant's concentration, but also the way sludge properties and durations of the aerobic phase were affected, in order to have a broad vision for the selection of the best option. In literature the addition of an inhibitor is suggested to shorten the start-up time in the setup of a partial nitrification process. In the third set of simulations the effect of the **dosage of an external inhibitor** in certain concentrations for different periods of time was studied. The tests were performed for efficacy of the inhibitor equal to 0.5, 0.75 and 1, that means for concentrations that provoke 50%, 75% and 100% inhibition only in NOB growth, since it is considered that a specific inhibitor is used for this group of bacteria, such as hydroxylamine, for example, which does not affect the development of AOB. The number of cycles considered to dose the chemical were: 60, 48, 24, 16 and 32.

Chapter 5. Model calibration

5.1 Experimental data

The experimental data, characterizing the biological process carried out in the pilot plant of Alboraya, were available for different days within the period 29 January-19 February 2020, during which the samples were collected. The values gathered in the following tables, resulting from the analysis on the samples described in chapter 4, have been provided by the Calagua research group. The **first table** shows the results of the analysis on the sample S_mixLiq and give the composition of the mixed liquor.

Table 5.1.1

Date	Mixed liquor		
	CODt (mg/l)	TSS (mg/l)	VSS (mg/l)
29-jan	3124	2650	1686
31-jan		2171	1852
03-feb	2181	1984	1438
05-feb		1846	1532
07-feb	2025	1755	1505
10-feb	1763	1519	1291
12-feb		1395	1180
14-feb	1638	1350	977

In the **second table** the values of nitrogen and chemical oxygen demand are reported as resulted from the laboratory test on the sample S_initial. These values are compared with the ones calculated through an initial mass balance. When the two set of values differ, those retrieved from the balance must be taken into account because S_initial could be not homogeneous.

Table 5.1.2

Date	Beginning of the cycle				Initial balance			
	Nh4 (mg N/l)	No3 (mg N/l)	No2 (mg N/l)	DQOs (mg N/l)	Nh4 (mg N/l)	No3 (mg N/l)	No2 (mg N/l)	DQOs (mg N/l)
31-jan	9,3	4,2	0,4	62,0	9,14	9,77	0,2	58,0
03-feb	9,2	9,9	0	54,0	9,14	10,34	0,0	54,5
10-feb	6,65	11,5	2,5	61,0	7,10	10,84	0,0	71,5
14-feb	5,4	8,5	0	56,0	7,28	9,43	0,0	53,0

The **third table** displays the values of nitrogen and organic matter of the samples S_endAnx and S_endAer.

Table 5.1.2

Date	End of anoxic step				End of aerobic step				End of previous cycle			
	Nh4 (mg N/l)	No3 (mg N/l)	No2 (mg N/l)	DQOs (mg /l)	Nh4 (mg N/l)	No3 (mg N/l)	No2 (mg N/l)	DQOs (mg /l)	Nh4 (mg N/l)	No3 (mg N/l)	No2 (mg N/l)	DQOs (mg /l)
31-jan	8,7	3,1	0,15	97	0	11,8	0	72	0,5	8,2	0,1	50
03-feb	9,3	3	0	45	0	12,5	0	41	0,3	10,3	0	45
10-feb	7,2	5,9	2,7	43	0	13,1	0		0,38	18,8	0,1	62
14-feb	7,3	4,1	0,1	34	0	11,4	0	34	0,1	11,4	0	50

The **last table** shows the results obtained from the samples S_previous cycle and S_permeate.

Table 5.1.3

Date	Permeate sample	
	Nh4 (mg/l)	CODs (mg/l)
29-jan	46,1	114
31-jan	53	95
03-feb	53	100
05-feb	46,2	100
07-feb	48,55	80,5
10-feb	41,2	117
12-feb	36	100
14-feb	42,2	68
17-feb	54	80

Due to the wide availability of data, it was possible to represent the time evolution of the reactor inserting in input to the model the influent quality of different periods of time. The dates considered are reported in table 5.1.5 together with their characteristic values in influent. For each day the correspondent cycle was estimated knowing that 5 cycles per day took place since the first day analysed. The biological oxygen demand (BOD) in influent to the reactor (BOD initial) was obtained as the sum of the BOD present in the permeate (BOD permeate) and the BOD externally supplied as acetic acid (BOD supplied).

$$BOD\ initial = BOD\ supplied + BOD\ permeate$$

Since the acetic acid was provided through an automatic dosing system, it was possible, having access to the data stored in the computer of the plant, to calculate the concentration in the permeate of the BOD supplied as:

$$BOD\ supplied = \frac{(W_i - W_f) \cdot C_a}{d_a \cdot V_p}$$

Where:

W_i = initial weight of the solution as stated in the records of the balance (g);

W_f = final weight of the solution as stated in the records of the balance (g);

C_a = concentration of the acetic acid solution (mg/l);

d_a = density of the acetic acid solution (g/l);

V_p = volume of the permeate (l).

On the other hand, the BOD already present in the stream, was estimated from the experimental data subtracting from the total amount of COD dissolved in the permeate, given in table 5.1.4, the fraction of inert COD (S_i).

$$BOD_{permeate} = COD_{permeate} - S_i$$

S_i value is represented by the COD present at the end of the aerobic step, reported in table 5.1.3, considering that all the biodegradable fraction that may arrive at the aerobic stage is completely oxidized by the end of this process, thus the COD left represents the inert non-biodegradable part that entered the reactor. However, it must be considered that this concentration is referred to the volume of the reactor and not to that of the feed, for this reason a dilution coefficient was used. Another parameter, with a dynamic evolution in time required in the inputs, was the ammonia influent concentration (NH_4 initial). This value was taken from the characterization of the permeate reported in table 5.1.4.

Table 5.1.4 Days considered in the simulation and corresponding permeate quality

Date	Cycle	BOD supplied	BOD Permeate	BOD initial	NH_4 initial
31/01/20	10	151,4	34,6	186	53
03/02/20	25	159,7	39,6	199,3	53
05/02/20	35	106,6	40,4	147	46,2
10/02/20	60	79,4	34,4	113,8	41,2
14/02/20	80	90,8	37,2	128	42,2

5.2 kinetic parameters

The major part of kinetic parameters inserted in the model were taken from literature and maintained such. The only kinetic parameter to be calibrated was the anoxic heterotrophic growth yield (Y_h anx). The yield coefficient represents the biomass produced per unit of substrate degraded, so the efficiency of conversion from substrate to cells. Its typical bibliographic value of 0.625 mg COD/mg COD is referred to aerobic degradation. When heterotrophic bacteria work under anoxic conditions, like in the present study, the

degradation process is less efficient and the anoxic yield should be reduced to about 0.42 mg COD/mg COD (A. Muller, 2003). The role of this coefficient in the reactions of aerobic and anoxic growth is represented in the stoichiometric matrixes reported below, where S_s is the organic matter concentration and S_{no3} the nitrate concentration.

Table 5.2.1

	S_s (mg COD/l)	S_{no3} (mg N/l)	X_h (mg COD/l)
Aerobic growth	$-\frac{1}{Y_h}$	0	1
Anoxic growth	$-\frac{1}{Y_{h\ anx}}$	$-\left(\frac{1 - Y_{H\ anx}}{2.86 * Y_{H\ anx}}\right)$	1

The anoxic growth yield determines the amount of substrate needed for cells growth and relates the amount of nitrate denitrified to the amount of COD consumed, therefore it influences the necessary COD/N ratio. As a general rule the more the substrate is biodegradable the more is the biomass produced for unit of substrate consumed, so the higher is the yield. In the case under exam, part of the organic matter is in the form of acetic acid, readily biodegradable COD. Nevertheless, in literature, it was observed that yield coefficients can vary for the same substrate, which can be referred to the relative acclimation to the substrate (Arifur Rahman, 2014). Simulations were carried out initially with a typical value of anoxic heterotrophic growth yield that was later assessed to match the experimental effluent data. The default values of kinetic parameters given in input for the groups of bacteria involved in the simulated processes are summarized in the tables below, the reference literature is provided by Hence et al. and Jiménez Douglas (M. Henze, 1999) (Douglas, 2010).

Table 5.2.2 default kinetic parameters for heterotrophic bacteria

Heterotrophic bacteria		
Growth yield	Y_h (mg COD/mg COD)	0.63
Anoxic Growth yield	$Y_{h\ anx}$ (mg COD/mg COD)	0.42
Maximum growth rate	U_h (d^{-1})	6
Decay rate	B_h (d^{-1})	0.4
Fraction of inert endogenous decay	F_{xi} (-)	0.1
Maximum fermentation rate	Q_{fe} (d^{-1})	3
Reduction factor for denitrification with NO ₂	N_{no2}	0.4
Reduction factor for denitrification with NO ₃	N_{no3}	0.4
Inhibition coefficient for oxygen	K_{o2} (mgO ₂ /l)	0.2
Inhibition coefficient for nitrites	K_{no2} (mgNO ₂ /l)	0.5

Inhibition coefficient for nitrates	Kno3 (mgNO3/l)	0.5
Semi-saturation constant for BOD	Ka (mgBOD/l)	4
Semi-saturation constant for ammonia	Knh4 (mgNH4/l)	0.05
Semi-saturation constant for phosphorous	Kp (mgP/l)	0.01
Semi-saturation constant for alkalinity	Kalk (mgCaCO3/l)	0.1
Thermal correction factor	KT	1.072
pH correction factor	KpH	0.00000023

Table 5.2.3 default kinetic parameters for NOB

Nitrite oxidizing bacteria		
Growth yield	Yamm (mgCOD/mgCOD)	0.07
Maximum growth rate	Uamm (d-1)	0.42
Decay rate	Bamm (d-1)	0.075
Fraction of inert endogenous decay	Fxi	0.1
Inhibition coefficient for oxygen	Ko2 (mgO2/l)	0.67
Semi-saturation constant for ammonia	Knh4 (mgNH4/l)	0.01
Semi-saturation constant for phosphorous	Kp (mgP/l)	0.01
Thermal correction factor	KT	1.06
pH correction factor	KpH	0.0000000001

Table 5.2.4 default kinetic parameters for AOB

Ammonia oxidizing bacteria		
Growth yield	Yamm (mgCOD/mgCOD)	0.17
Maximum growth rate	Uamm (d-1)	0.88
Decay rate	Bamm (d-1)	0.15
Fraction of inert endogenous decay	Fxi	0.1
Inhibition coefficient for oxygen	Ko2 (mgO2/l)	0.52
Semi-saturation constant for ammonia	Knh4 (mgNH4/l)	0.54
Semi-saturation constant for phosphorous	Kp (mgP/l)	0.01
Thermal correction factor	KT	1.12
pH correction factor	KpH	0.0000001

5.3 Results

Before calibrating the anoxic heterotrophic growth yield, it was necessary to assess the biomass distribution in the different groups of bacteria, other input required by the model. In fact, the lack of microbiological data made it necessary to hypothesize an initial biomass composition, and retrieve later more accurate seed values based on the outputs obtained. The first simulation was carried out with the bibliographic value of anoxic Y_h and distributing the suspended solids in the different fractions starting from the results on the mixed liquor sample. The total chemical oxygen demand of the mixed liquor (COD_{tot}) is divided into: autotrophic bacteria ($X_{NOB} + X_{AOB}$), heterotrophic bacteria (X_h) and inert suspended solids (X_i). The soluble suspended fraction (X_s) is neglected since its value is much lower respect to the other contributions. The first simulation was run up to stationary output values, that gave back the following final biomass concentrations:

Table 5.3.1

	<u>COD</u>	<u>%COD</u>
<u>X_h</u>	340	44%
<u>X_i</u>	330	43%
<u>X_{AOB}+X_{NOB}</u>	100	13%

These results allowed calculating the amount of initial COD of autotrophic bacteria as a value close to: $3124 \cdot 0.13 = 416$ mg/l. To distribute this total concentration among ammonia oxidizers and nitrite oxidizers different tests were performed, in which the output NH_4 consumption rate was evaluated and compared to the pH drop. During nitrification, hydrogen ions are produced as ammonia is oxidized, such that there is a progressive decrease in the pH until the end of the process, when all the ammonia has been oxidized and the pH stabilizes to a constant value. The pH evolution during one treatment cycle from the 3rd February is shown below.

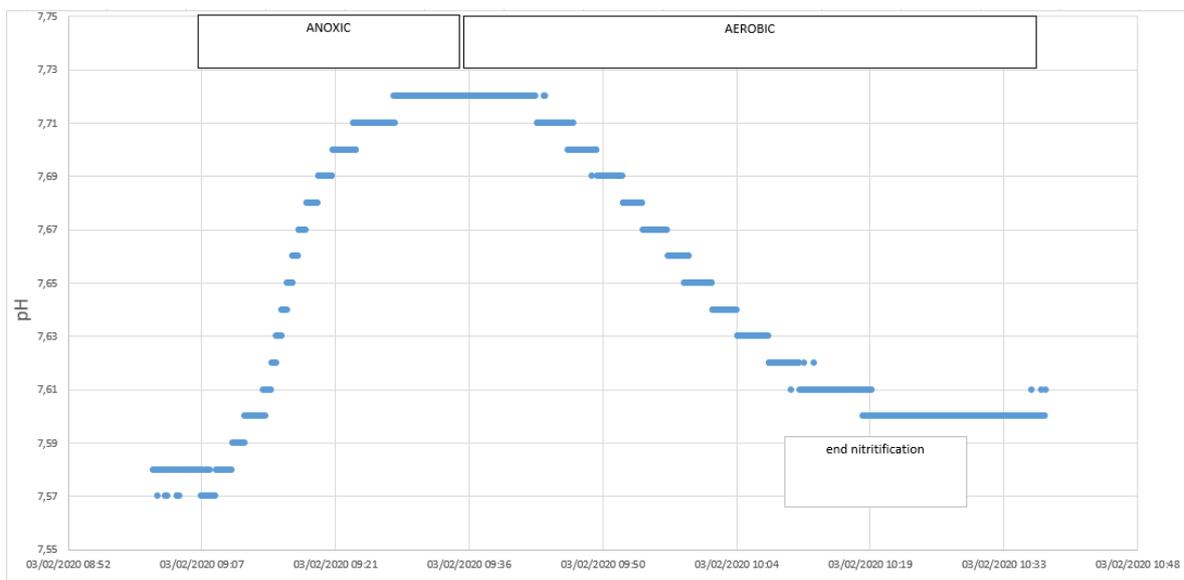


Figure 5.3.9 pH evolution in one cycle from 03/02

From the graph depicted in figure 5.3.1, it is evident that since the beginning of the aerobic phase the pH gradually decreases until reaching, after around 40 minutes, a stationary value. The pH stabilization indicates the moment in which the nitrification process is concluded. The concentrations of AOB and NOB that provided a matching trend of NH₄ and pH drop were verified for 4 study cycles: 10, 25, 60, 80. The results of two simulations performed for the calibration of the 3rd of February (10th cycle) are shown in figure 5.3.2 and 5.3.3 and can be compared to the pH trend of that day represented in figure 5.3.1.

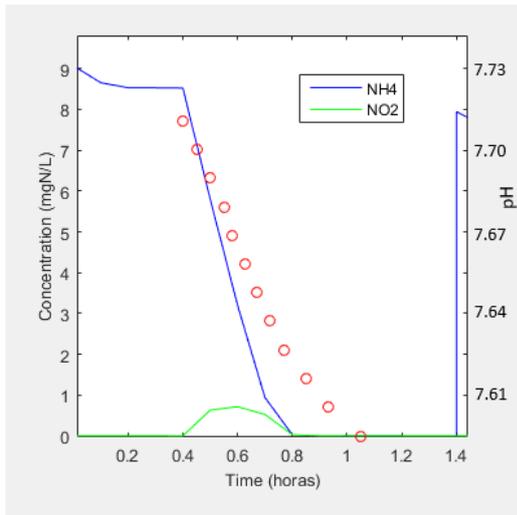


Figure 5.3.2

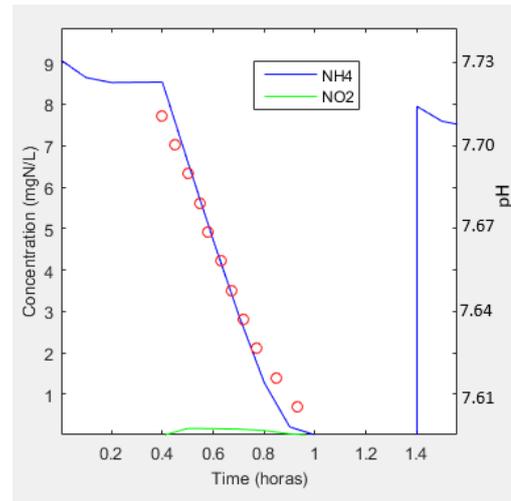


Figure 5.3.3

Analysing the outputs in the first picture, it can be noted that the input concentrations of nitrite oxidizers and ammonia oxidizers used for that simulation, $X_{nit} = 222 \cdot 13/8$ mg/l $X_{amm} = 195 \cdot 13/8$ mg/l, failed in representing the real process. In fact the ammonia decreasing rate is too fast and all the ammonia is depleted in around 20 minutes of aerobic phase, before the nitrification process actually ends. The proportion X_{nit}/X_{amm} was adjusted until arriving to the input concentrations of $X_{nit}=322 \cdot 13/8$ mg/l and $X_{amm}=95 \cdot 13/8$ mg/l, which gave as outcome the fitting NH₄ trend depicted in picture 5.2.3. Besides Once defined the amounts of autotrophic bacteria the remaining COD: $3124 - 416 = 2708$ mg/l must be divided among heterotrophic bacteria (X_h) and inert material (X_i). To adjust the X_h/X_i ratio different values were tested until the outputs approximated well the experimental trends of total chemical oxygen demand (COD_t) and total suspended solids (XTSS). The outcomes obtained simulating the process with a misfit and a fitting proportion of X_h and X_i for the 10th cycle, are shown in figures 5.2.4 and 5.2.5. In the non-matching curve (figure 5.2.4) the X_h/X_i ratio used was too high, for this reason the COD, consumed by the numerous heterotrophs, drops faster than the experimental curve. In the matching curve (figure 5.2.5) the concentration of heterotrophic bacteria was decreased at $1097 \cdot 13/8$ mg/l and the concentration of suspended inert increased at $1600 \cdot 13/8$ mg/l.

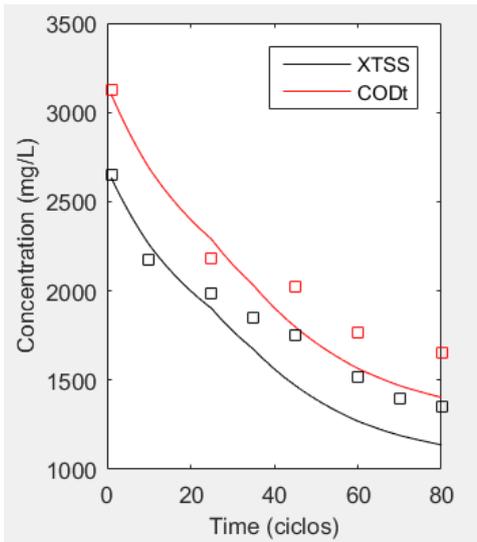


Figure 5.3.4

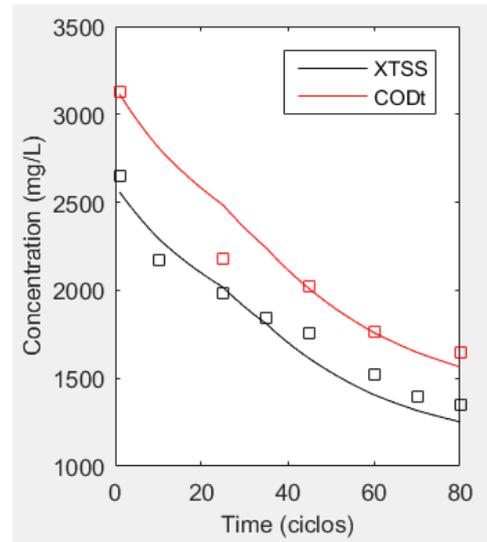


Figure 5.3.5

At this point, in the simulation performed with the calibrated biomass values the difference between the effluent nitrate values of the model and the experimental ones were estimated. This difference is represented graphically from figure 5.3.6.

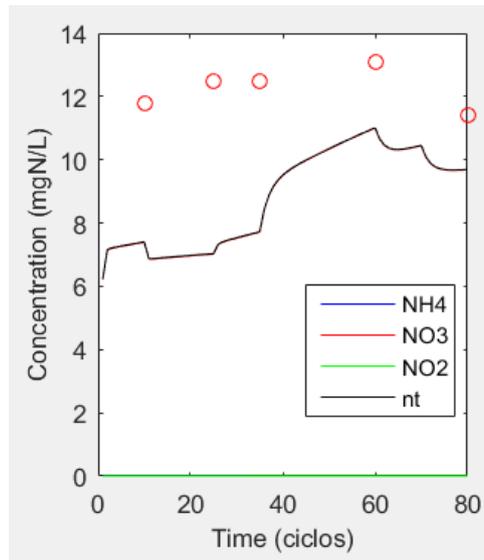


Figure 5.3.6

From here on, the calibration of the anoxic heterotrophic growth yield started, with the aim of finding the value that provided the best representation of the output nitrate levels. Each time the anoxic Y_h value was changed, also the biomass distribution between heterotrophic bacteria and inert fraction was readjusted, since the yield coefficient directly influence the heterotrophic biomass growth rate and therefore the COD drop. Four anoxic yields were tested, the comparison between the experimental and model nitrate effluents for five simulated cycles (10, 25, 35, 60, 80) is represented in table 5.3.2.

Table 5.3.2

cycle	10	25	35	60	80
Experimental NO3 effluent	11,8	12,5	12,5	13,1	11,4
Yh,anx=0,44	7,1	6,86	7,65	11,01	9,82
Yh,anx=0,48	7,95	7,72	9,45	12,83	11,7
Yh,anx=0,52	8,73	8,2	10,85	13,85	12,8
Yh,anx=0,56	9,8	9,5	13,5	15,4	14,8

The difference between the experimental and model outcomes was calculated in absolute value and cumulated for each cycle in order to obtain the final results shown in table 5.3.3.

Table 5.3.3

Yh anx	\sum No3 experimental – NO3 model
0.44	18.9
0.48	11.7
0.52	11.2
0.56	11.7

The Yield that gave the smallest difference between the experimental nitrate effluent and the model nitrate effluent is anoxic Yh = 0.52. This value is the one which best reproduce the real reactor activity, therefore it is assumed as the final calibrated value. This result is in accordance with the ones available in literature. As a matter of fact Rahman et al. (Arifur Rahman, 2014) calculated the anoxic yield with ten different analytical methods and found out that the values fell in the range 0.423±0.014 to 0.512±0.021 mgCOD/mgCOD. Analysing the outputs of the model with the calibrated values of anoxic heterotrophic yield and biomass, it is clear that, even if they present the same trend, they still don't perfectly match the experimental data (figure 5.3.7). The flaw can be attributed to the estimation of the influent BOD. As previously explained this value was retrieved in part from the difference in weight and from the concentration of the acetic solution and in part from the initial experimental value of COD, taking into account its inert fraction. The concentration of acetic acid used for the calculations was the initial one, but considering the biodegradability and volatility of this compound, its percentage in solution is destined to slowly decrease with time. Even if measures were taken to prevent this phenomenon (e.g. the solution was replaced weekly) the working environment of the pilot plant is not aseptic and some variation should be expected. On the other hand, also the analytical procedure followed to determine the COD of the permeate can involve a certain measurement error, since measurements are carried out from a small sample volume. Considered the methods employed for their estimation, the initial ammonia concentration values were considered to be accurate, whereas the initial

BOD values were assumed affected by various possible errors. For this reason the BOD/N ratios in input to the model were adjusted, varying the BOD concentration, to reach a better fit of the experimental effluent data. The results of this procedure are shown below.

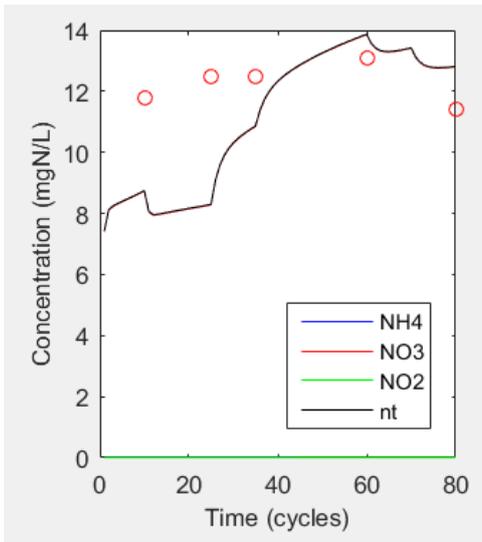


Figure 5.3.7

Cycle	Perm. BOD	Perm. NH4	BOD/N
1-10	186.0	53	3.51
10-25	199.2	53	3.76
25-35	147.0	46.2	3.18
35-60	113.8	41.2	2.76
60-70	121.3	41.7	2.91
70-80	128.0	42.2	3.06

Table 5.3.4

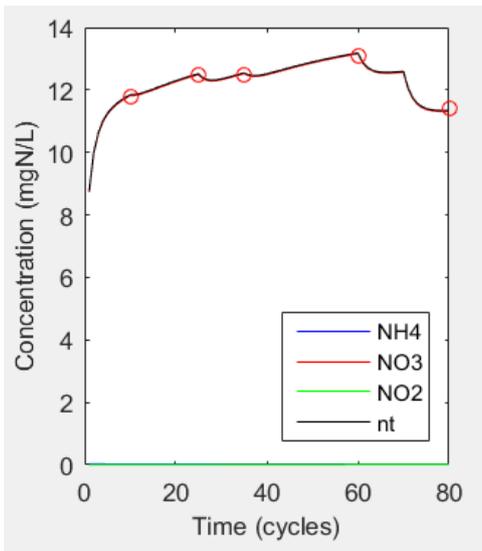


Figure 5.3.8

Cycle	Perm. BOD	Perm. NH4	BOD/N
1-10	175	53	3.3
10-25	176	53	3.32
25-35	145	46.2	3.14
35-60	122	41.2	2.96
60-70	128.5	41.7	3.08
70-80	138	42.2	3.27

Table 5.3.5

Comparing the experimental and the adjusted values of COD, it can be noted that the difference is minimal, so attributable to an experimental error, except for one point (from cycle 10 to 25) for which there is a discard of 23.2 mg/l.

Table 5.3.6

Cycle	Experimental BOD	Ajusted BOD	Difference
1-10	186.0	175	11
10-25	199.2	176	23.2
25-35	147.0	145	2
35-60	113.8	122	-8.2
60-70	121.3	128.5	-7.2
70-80	128.0	138	-10

The graphs returned by the calibrated adjusted model of the evolution of the concentrations during the cycle are reported in the figures below. The main discrepancy that can be detected between the experimental data and the model results is an ammonia consumption of 1 mg N/l during the anoxic phase, that in reality doesn't occur. The results are referred to the first stage of the SBR, the following two stages are expected to show the same response, as the same feed volume is provided.

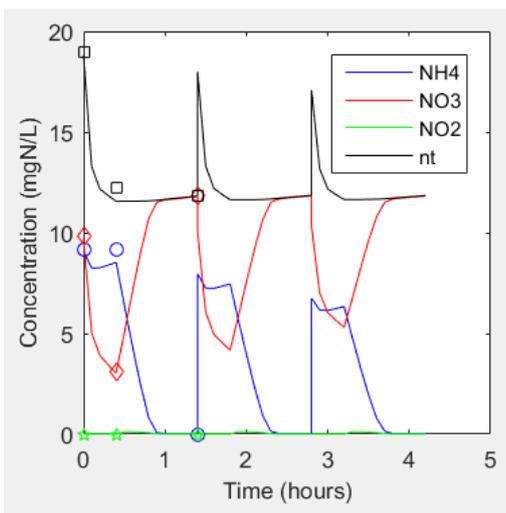


Figure 10.3.9 results of cycle 10

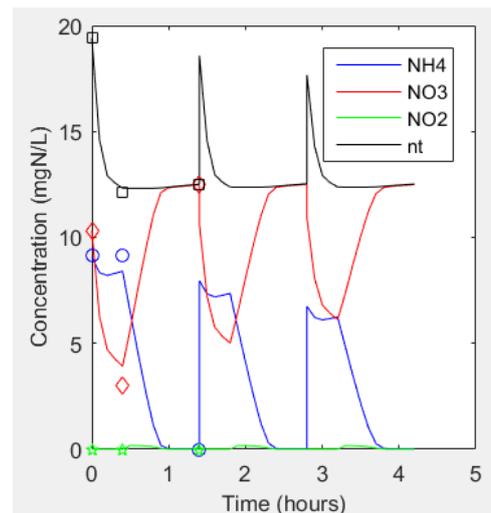


Figure 5.3.10 results of cycle 25

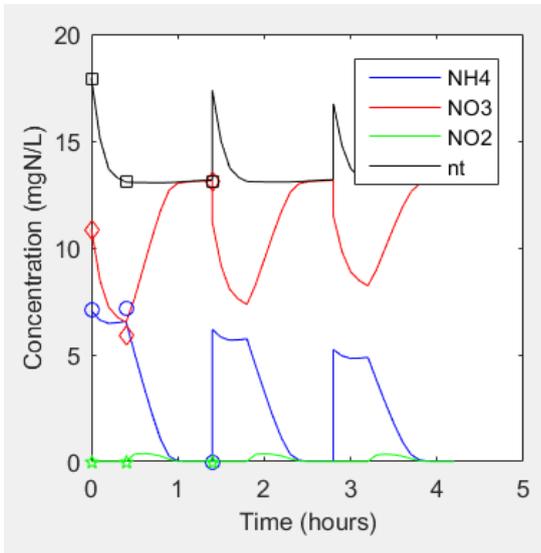


Figure 5.3.11 results of cycle 60

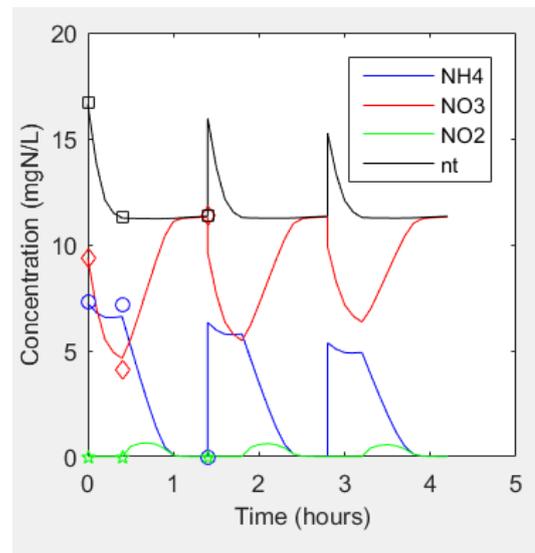


Figure 5.3.12 results of cycle 80

Chapter 6. Partial nitrification results

The adjusted values retrieved through the calibration procedure were used as model inputs for the study of the possible implementation of a partial nitrification process to treat the permeate. The anoxic yield was set at 0.52 mg COD/mg COD, all the other kinetic parameters remained the ones shown in tables 5.2.2, 5.2.3, 5.2.4 and the two inhibition constants for free ammonia and free nitrous oxide for both AOB and NOB were added with the values reported in table 4.2. The biomass was distributed as found in chapter 5: $X_h=1097*13/8$, $X_i=1600*13/8$, $X_{amm}=95*13/8$, $X_{nit}=322*13/8$. The permeate values inserted were the ones referred to the day 7th of February (45th cycle), therefore BOD=122 and NH₄=41.2 as indicated by table 5.2.9 and a Si = 39 experimental value of that day. All the other operational parameters have been already discussed in chapter 4.

6.1 Reactor configuration

The first set of simulations conducted was aimed at identifying the best **feeding configuration** to operate the SBR reactor to achieve partial nitrification. Two different models to simulate the single-feed and multiple-feed three stages configuration were implemented in MATLAB. The two simulations were run separately with the same input parameters. The output graphs representing nitrite and nitrate evolution are shown in figure 6.1.1 for the multiple feed simulation and 6.1.2 for the single feed simulation.

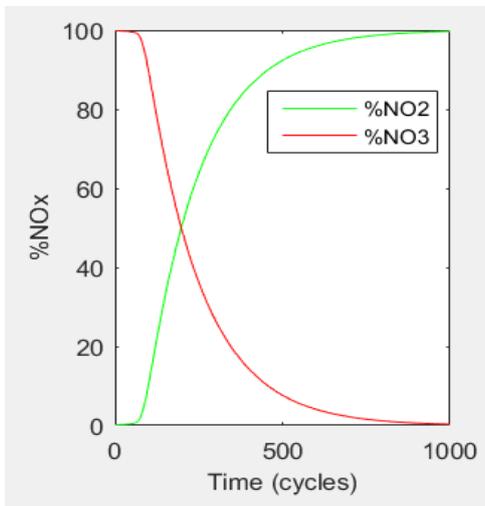


Figure 6.1.1 multiple feed result

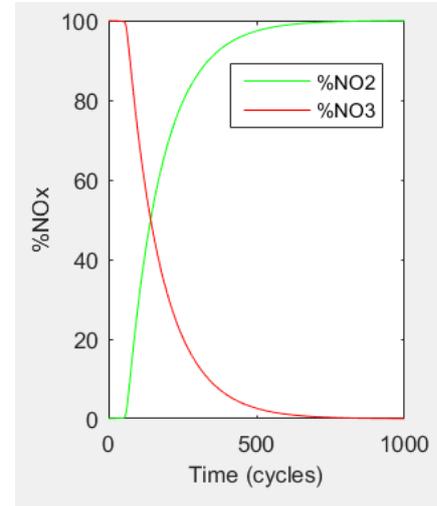


Figure 6.1.2 single feed result

From the figures above it can be deduced that the single stage configuration allows to accumulate nitrite faster. An explanation can be given considering that feeding all the permeate in one step, the initial amount of ammonia is higher than if it is fed in three different steps. The higher the ammonia availability, in limiting substrate conditions, the faster the growth of AOB for the Monod kinetic. In addition to allowing a fast accumulation of ammonia oxidizers, the single feed shows nitrite oxidizers concentrations tend to zero going on with the cycles, so completely flushes NOB and make it more difficult for this group of bacteria to return

in more favourable conditions. This is not true for the multiple stage in which concentrations of nitrite oxidizers decrease slowly maintaining their presence at higher levels.

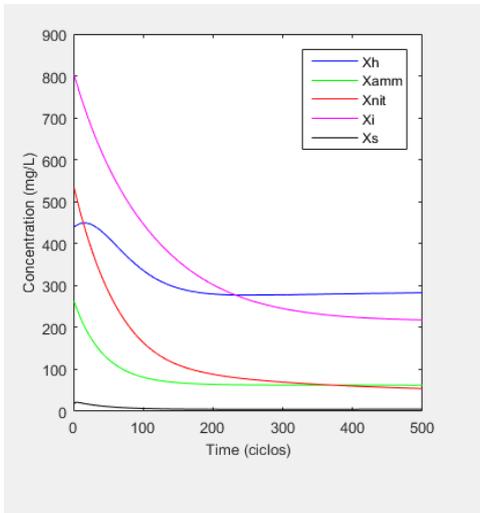


Figure 6.2.3 multiple feed. Biomass evolution

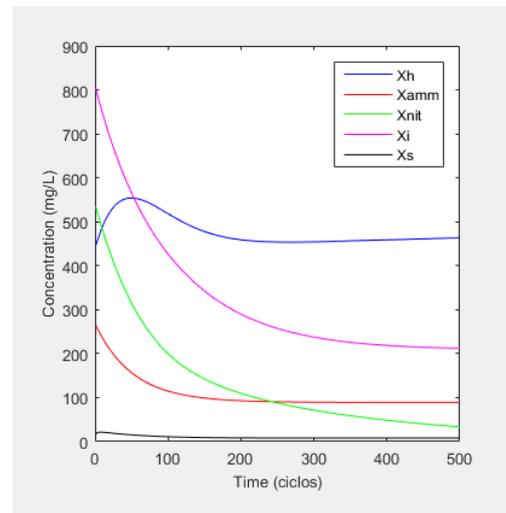


Figure 6.1.4 single feed. Biomass evolution

Higher levels of ammonia also lead to a higher concentration of free ammonia, that is a great inhibitor for nitrite oxidizers. The concentration trends of free ammonia in a cycle for the two models is represented in figure 6.1.5 (multiple feed) and 6.1.6 (single feed). At these FA concentrations, the growth kinetics of AOBs are inhibited by less than 5% at most, while NOBs can inhibit up to 30% of their growth. Due to the high pH, the FNA concentration is not inhibitory for any group of bacteria, nor under any configuration.

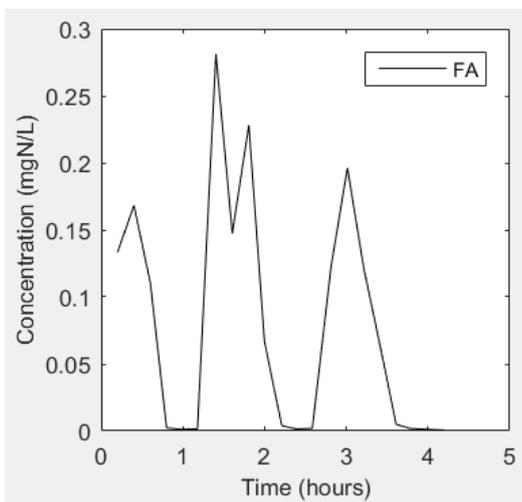


Figure 6.1.5 multiple feed. FA concentration

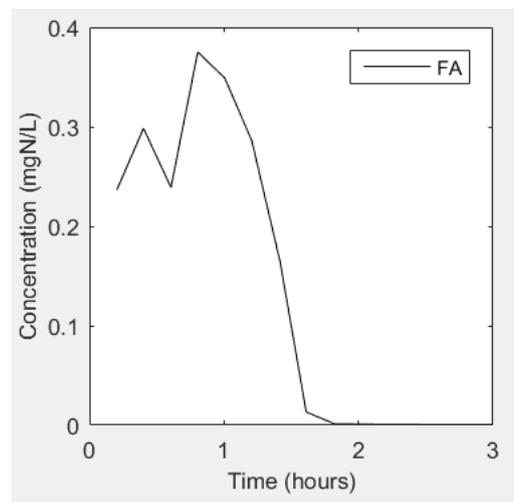


Figure 6.1.6 single feed. FA concentration

Another aspect, that is not considered in the model, is that higher ammonia values promote the growth of r-strategist ammonia oxidizing bacteria, that are a specie with high growth rate. This predominance of fast-growing microorganism over the slowest AOB specie, the k-strategist, speeds up the ammonia consumption, thus the nitrite generation (Wu, He, van Loosdrecht, & Pérez, 2016). These theoretical evidences support the results of the simulation in the election of the **single-feed configuration** as the most appropriate to

implement a partial nitrification process. The following simulations will be applied to the single stage model to detect the optimal parameters for its operation.

6.2 Dissolved oxygen and Sludge retention time

In the second set of simulations the values of sludge retention time (SRT) and the dissolved oxygen (DO) were assessed. The single stage SBR was simulated with different combinations of SRT and DO. The SRT was set at 10, 8 and 5 days and for each SRT, DO concentrations of 1.2, 0.7 and 0.5 mg O₂/l were tested. In line with the theory (R van Kempen, 2001) (Banashri Sinha, 2006), the results show that nitrite accumulation is promoted by lower SRT and lower DO concentrations, conditions that give a competitive advantage to AOB over NOB. Nevertheless, the best values were chosen also considering the effect of the two parameters over the duration of the aerobic phase and the total suspended solids. The SRT selected to operate the partial nitrification process was of 8 days, in order to keep it as low as possible as suggested by literature to outcompete NOB, but at the same time to ensure a better sedimentation than with lower values. In fact the main difference between a solid retention period of 8 days and 5 days is the amount of TSS produced, that differ of around 100 mg/l. In addition, the time of the aerobic phase to complete the process is optimized with a higher SRT that provides higher nitrification rates. As far as the dissolved oxygen is concerned, lower values imply longer aerobic times to complete the process. A higher duration of the aerobic phase increases the final HRT of the process, to which corresponds a loss of treatment capacity of the reactor. A DO of 0.7 mg O₂/l was selected because the duration of the aerobic phase (2.68h) wasn't far from that of the highest DO value (2.24h). Moreover, keeping a DO concentration lower than 1 mgO₂/L improves the competition of AOB over NOB, providing a more robust partial nitrification process if some problem occurs in the operation of the reactor (Li, Zhang, Liu, Lin, & Peng, 2019)(Blackburne, Yuan, & Keller, 2008b). The following table shows the results obtained in this set of simulations. For each pair of dissolved oxygen and SRT values, the number of cycles necessary to achieve a NO₂ accumulation of 5% and 80%, with respect to NO_x, has been taken as a result. The duration of the aerobic phase has also been recorded up to when 5% and 80% NO₂ accumulation was achieved. Finally, the concentration of TSS in the steady state has been considered.

Table 6.5.1

	SRT= 20 d			SRT= 10 d			SRT= 8 d			SRT= 5 d		
DO (mgO ₂ /l)	1,2	0,7	0,5	1,2	0,7	0,5	1,2	0,7	0,5	1,2	0,7	0,5
Number of cycles for 5% NO ₂	67	66	66	52	52	52	48	48	48	39	38	38
Number of cycles for 80% NO ₂	257	246	237	158	154	149	134	131	128	95	93	91
Duration of aerobic phase in 5%NO ₂ (h)	1,7	1,99	2,31	2,06	2,45	2,9	2,24	2,68	3,19	2,78	3,37	4,01
Duration of aerobic phase in 80%NO ₂ (h)	1,61	1,88	2,2	2,1	2,53	3,03	2,32	2,85	3,45	1,21	3,82	4,7
TSS at the end (mgTSS/l)	859,9	792	730	522,3	476,1	435	438	398,3	363,1	296,1	268,3	242,9

Considering the aerobic durations obtained in the selected set-up and the time necessary for the other steps (20 minutes feeding, 20 minutes emptying, 30 minutes of anoxic, 40 minutes of sedimentation) it was possible to calculate the duration of one cycle, thus the number of cycles per day. From here, dividing the

volume of the reactor by the daily flowrate, the hydraulic retention times were estimated as 11.7h for 5% nitrite accumulation and 12.2h for 80% nitrite accumulation. Turning the number of cycles into number of days it emerged that it takes 9 days to reach nitrite accumulation ratio of 5% and 26 days to reach 80% accumulation. The values characterizing the chosen configuration are put together in the table below.

Table 6.2.2

SRT (d)	8
OD (mgO ₂ /l)	0,7
Number of cycles for 5% NO ₂	48
Number of days for 5%	9
Number of cycles for 80% NO ₂	131
Number of days for 80%	26
Duration of aerobic phase in 5%NO ₂ (h)	2,68
HRT 5% (h)	11,7
Duration of aerobic phase in 80%NO ₂ (h)	2,85
HRT 80% (h)	12,2
TSS at the end (mgTSS/l)	398,3

6.3 External inhibitor

In literature it is suggested to add an inhibitor to shorten the start-up time. In the third set of simulations the effect of the dosage of an external inhibitor in certain concentrations for different periods of time was studied. The tests were performed for efficacy of the inhibitor equal to 0.5, 0.75 and 1, that means for concentrations that provoke 50%, 75% and 100% selective inhibition over nitrite oxidizing bacterial growth. The number of cycles considered to dose the chemical were: 48, 24, 16, 32 and 60. It came out that the speedup of the process was significant just when the inhibitor was added for a number of cycles at least equal to that in which the nitrite start appearing, that is for 48 cycles, after which 5% accumulation was detected without inhibitor. Furthermore, the effect improved when the inhibitor was dosed in concentrations that caused higher inhibitory levels on nitrite oxidizing bacteria. In the most favourable simulated case to obtain inhibition, that corresponds to dosing for 60 cycles 100% inhibitory concentration, the cycles required to reach 80% nitrite accumulation were 102. The comparison between the accumulation without and with the external inhibitor is shown in figures 6.3.1, 6.3.2. Converting the cycles obtained to reach 80% nitrite accumulation in number of days for the abovementioned configuration, it results that the addition of inhibitor allowed to speed up the accumulation process from 26 to 20 days. It can be concluded that the addition of an external inhibitor for the case study is not recommended, since to spend money on a chemical for reaching partial nitrification one week earlier is not worth it. The process was built and optimized to reach maximum accumulation of nitrite and is already fast compared to other studies in which the possibility of a partial nitrification was evaluated at existing plant conditions. Nevertheless, it should be considered that the model works under ideal conditions, where all the parameters are set and maintained at certain values. In

reality the process could take longer and in this case the supply of the inhibitor was demonstrated to be a valid solution with the modalities depicted in this last set of simulations.

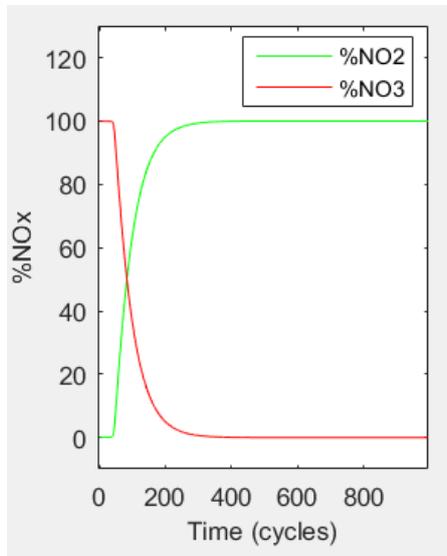


Figure 6.3.12 nitrite accumulation without inhibitor.

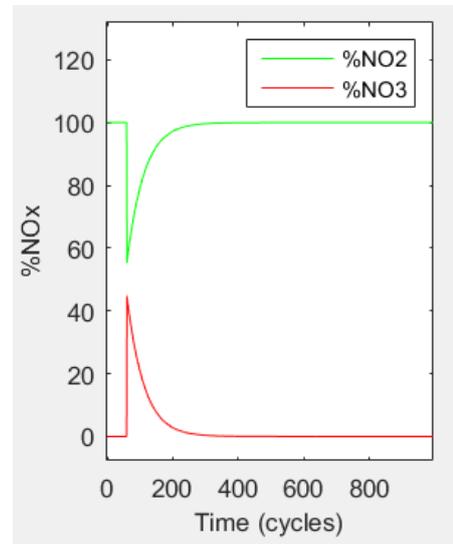


Figure 6.3.11 nitrite accumulation adding the external inhibitor for 60 cycles in 100% inhibitory concentration.

Chapter 7. Conclusions

In the field of research and development of technologies to switch from Wastewater Treatment Plants to Resource Recovery Facilities, a pilot plant was set up in Alboraya, Valencia, operating a direct membrane ultrafiltration of raw wastewater, followed by a biological treatment of the permeate. This work was focused on the biological process. In the first part of the study, a dynamic calibration of the biological sequencing batch reactor was performed using the model BNRM2. The only kinetic parameter to be calibrated was the **anoxic heterotrophic growth yield** ($Y_{h\text{ anox}}$). Its value resulted to be 0.52, higher than the typical bibliographic of 0.42, but in any case falling in the range indicated by literature. During the calibration procedure also the **activated sludge composition** was assessed to overcome the lack of data due to the inaccessibility to the laboratory during the lockdown. The approximated distribution of the mixed liquor in the different fractions is: $X_h=1097 \cdot 10^{-3}$, $X_i=1600 \cdot 10^{-3}$, $X_{amm}=95 \cdot 10^{-3}$, $X_{nit}=322 \cdot 10^{-3}$. Lastly, some deviations in the quality data of the permeate were corrected. The adjusted **BOD/N ratios** for the different days of the survey came out with an average value of 3.2 mgBOD/mgN. The stoichiometric ratio for denitrifying with acetic acid is 3.7, whereas with wastewater is 4.2, thus it was expected a ratio ranging from 3.7 to 4.0. The fact that the real BOD/N ratio is lower than the stoichiometric means that there is a nitrate accumulation in the reactor. In the second part of the work the implementation of a partial nitrification process was object of study. Simulating different conditions, it was found out that:

- The best configuration to obtain nitrite accumulation is the **single stage**;
- The optimal dissolved oxygen-sludge retention time combination to operate the single stage reactor, in order to promote the presence of ammonia oxidizing bacteria over nitrite oxidizing, keeping the reaction time as low as possible and without compromising the sedimentability of the sludge is: **DO = 0.7 mgO₂/l, SRT = 8 days**.
- The addition of **an external inhibitor** accelerates the start-up time of the process just if it is dosed for a number of cycles of at least 48 and has a more significant effect the higher its concentration.

The method employed to obtain nitrite accumulation, was a combination of low dissolved oxygen concentration and aeration duration control strategy. In fact the model was set to stop the aerobic phase as soon as the ammonia oxidation process was considered terminated. The results show that this strategy is effective since using the same sludge and the same operative data, when the duration of the aerobic phase was not controlled, like occurred in the calibration model, nitrite didn't accumulate, because NOB had enough time to develop; on the contrary, when the aerobic duration was adjusted with the ammonia consumption, nitrite started accumulating.

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