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OPTIMISATION OF BIOLOGICAL NITROGEN REMOVAL
PROCESSES TO TREAT REJECT WATER FROM ANAEROBIC
DIGESTION OF SEWAGE SLUDGE

Doctoral Thesis directed by Joan Mata Álvarez

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1. INTRODUCTION

1.1 GENERAL VIEW

Nowadays the environmental problems are having more importance in a society that is constantly changing and developing. The population increase during the recent years has evolved an increase of necessities and consequently more residues are produced in the Earth. Therefore, environmental concern is every day much more necessary because the different emissions are polluting air, water and ground (Figure 1.1).
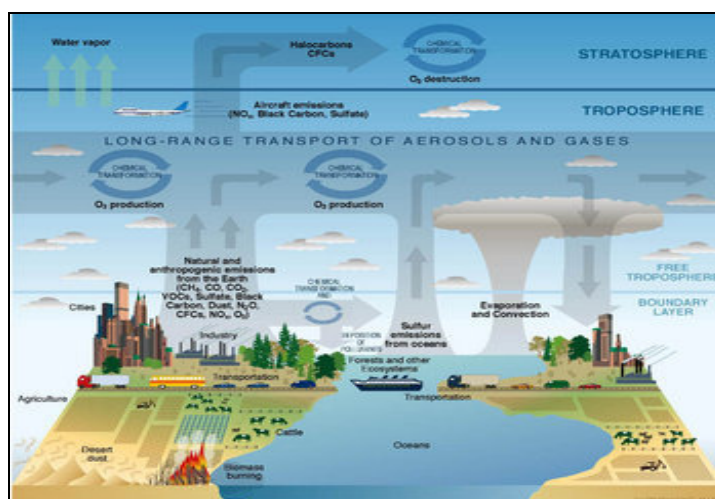

Figure 1.1: Types of contamination

The water as it is stated above is one of the main destinations of world pollutants. Until the last century, microorganisms present in rivers treated the wastewater themselves. However, the huge quantity of contaminants generated, even from urban or industrial origin, lead to the implementation of wastewater treatments plants (WWTPs) in order to return the water to the environment in sustainable conditions. WWTPs with an integration of a series of processes accelerate the biological treatment done in the rivers,

reducing the organic and inorganic contaminants present in the wastewater. There is a recent tendency to use biological processes to treat urban and industrial wastewater (Figure 1.2) because they are the most feasible and do not use chemical reactants (Teichgraber and Stein, 1994; Siegrist, 1996).
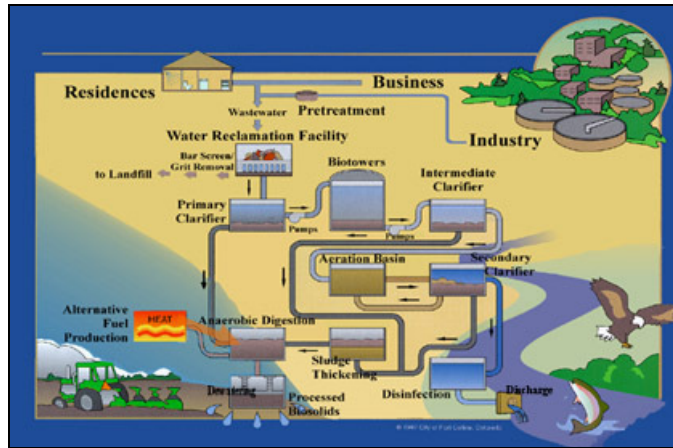

Figure 1.2: Wastewater treatment

The structure of a WWTP is complex and it is important to differentiate between the several contaminants that can be found. The need to treat different kind of contaminants requires the following treatment sequence:

- Pre-treatments and primary treatments: They are processes to remove large solids, suspended materials and fatty materials, physically or through chemical products.
- Secondary treatments: They are the biological treatments, whether aerobic or anaerobic, in order to reduce the organic biodegradable materials.
- Tertiary treatments: They have the purpose to remove the remaining contaminants in the effluent after the secondary treatment.

The latter treatments are the ones that have been more studied and improved recently in order to obtain better effluent requirements and to cope with legislation (Directive 91/271/EEC).

1.2 THE PROBLEM OF NITROGEN

The fact that legislation is every day stricter makes the working treatments expiry in short time. This means the requirement of new measures of treatment. Since last few years, Spanish WWTP were forced to do primary and secondary treatments and the tertiary ones were done or not depending on the plant location. Nutrient treatments (nitrogen and phosphorus) are an example of these because they can cause problems if they are evacuated to the environment at moderate concentrations. WWTP placed in sensitive areas must do tertiary treatments for nutrients removal before evacuating them to the environment in accordance with the 91/271/EEC Directive.

There are many processes for the treatment of nitrogen, normally in NH_4^+-N form, including physic-chemical and biological as it is explained in the following lines.

1.2.1 Chemical processes

1.2.1.1 MAP process

This process consists of the precipitation of the ion $MgNH_4PO_4$ (MAP) through the addition of phosphoric acid and magnesium oxide. It is necessary to control pH between 8.5 and 10 in order to control the precipitation of crystals. The efficiency of the process to remove ammonium is around 90% (Siegrist, 1996).

1.2.1.2 Air stripping process

This process recovers the NH_4^+-N in form of free ammonia (NH_3) with a desorption between the ammonium solution and a gas phase (air). First of all, the pH of the system has to be around 10 in order to convert all the ammonium to free ammonia. At the end, the NH_3 obtained is absorbed with a solution of sulphuric acid to form $(NH_4)_2SO_4$. The process efficiency, working at temperatures between 10 and 22 ºC, is above 97% (Siegrist, 1996).

1.2.1.2 Steam stripping process

In this process the desorption is made with water steam instead of air in order to recover ammonium with a condensation (Teichgraber and Stein, 1994; Siegrist, 1996).

1.2.2 Biological processes

The biological nitrogen cycle is complex and plays an important role in the environment. It has been under study during the last century and recently new reactions, like Anammox, have been discovered enlarging the cycle as it can be seen in Figure 1.3.
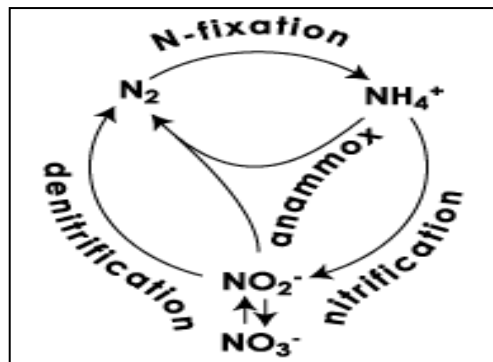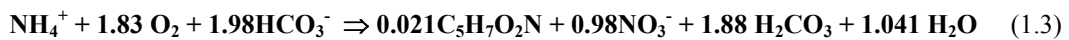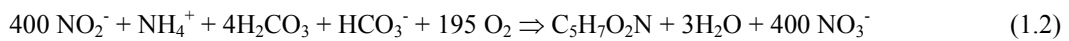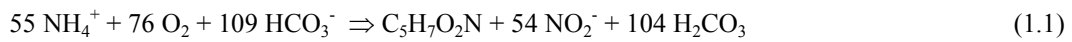

Figure 1.3: Nitrogen cycle

1.2.2.1 Nitrification

Biological nitrification is the oxidation of ammonium nitrogen (NH_4^+-N) to nitrite and nitrate through the action of autotrophic bacteria belonging to the *nitro-bacteria* family. The process is done in two stages: the first called nitritation consists of the oxidation of ammonium to nitrite; the second is called nitratation and converts nitrite to nitrate (Medcalf and Eddy, 1991). Both stages are aerobic and are developed by autotrophic microorganisms that need an inorganic carbon source to grow.

Considering the biomass growth and the bicarbonate used as carbon source, the equations of the different stages (1.1 and 1.2) and the global equation (1.3) are expressed in the following lines:

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$$55 \, NH_4^+ + 76 \, O_2 + 109 \, HCO_3^- \Rightarrow C_5H_7O_2N + 54 \, NO_2^- + 104 \, H_2CO_3 \tag{1.1}$$

$$400 \, NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195 \, O_2 \Rightarrow C_5H_7O_2N + 3H_2O + 400 \, NO_3^- \tag{1.2}$$

$$\mathbf{NH_4^+ + 1.83 \, O_2 + 1.98 HCO_3^- \Rightarrow 0.021 C_5H_7O_2N + 0.98 NO_3^- + 1.88 \, H_2CO_3 + 1.041 \, H_2O} \tag{1.3}$$

Considering equation 1.3, the main aspects that characterise nitrification process are explained below:

- Nitrification needs, according to stoichiometry, 4.18 g O_2 for each g of ammonium oxidised.
- Stoichiometry shows an average efficiency for nitrifying microorganisms of 0.15-0.17 g VSS g^{-1} NH_4^+-N oxidised, where the bacteria composition is expressed as $C_5H_7O_2N$.
- According to alkalinity, 8.62 g of bicarbonate are needed for each g of ammonium. The quantity of inorganic carbon could be a limiting factor during nitrification.
- pH of the system tends to decrease due to alkalinity removal.

Moreover, the efficiency of nitrification process depends on different factors that are presented in the following lines (Medcalf and Eddy, 1991):

- Ammonium and oxygen concentration: The concentration of ammonium and dissolved oxygen has to be enough for the activity of autotrophic bacteria.
- Temperature: Temperature plays and important role in nitrification because it affects bacteria growth during all the process. The optimal temperature for nitrifying bacteria is 30-35 ºC.
- pH and alkalinity: The optimal pH for the oxidation of ammonium through nitrifying bacteria is placed around 7.2 and 8.5.
- Organic load: The presence of organic material (COD) leads to the development of heterotrophic bacteria that uses oxygen in a similar way as nitrifying microorganisms.

These heterotrophic bacteria inhibit nitrification when oxygen is in limiting conditions because their growth rate is 5 times higher than the nitrifying one.

1.2.2.2 Denitrification

Denitrification process is the reduction of nitrate and nitrite to nitrogen gas in absence of oxygen. Nitrite and nitrate act as an electron acceptor. There are two types of denitrification: The autotrophic, done by microorganisms that use an inorganic source like HS^- as electron donor and an inorganic carbon for growth; and the heterotrophic, which uses an organic carbon source as electron donor and for cell growth (Medcalf and Eddy, 1991). According to the latter statement, the removal of nitrate and nitrite can be done simultaneously with the oxidation of organic materials that are in wastewater in order to save the addition of external carbon. When there is any organic carbon source available in the wastewater, this has to be added externally to develop the heterotrophic process. The heterotrophic denitrification is the most common and the one used in the present work. Its stoichiometry varies depending on the organic carbon source used. When methanol is used (considering biomass growth) the reaction is the following:

$$7.4\ NO_3 + 10\ CH_3OH \Rightarrow C_5H_7O_2N + 3.2\ N_2 + 5\ CO_2 + 12.8\ H_2O + OH^- \qquad (1.4)$$
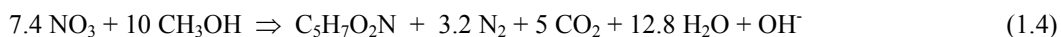
where 2.47 g CH_3OH g^{-1} NO_3^--N removed are needed and 0.45 g VSS g^{-1} NO_3^--N removed are formed.

Similarly to nitrification, there are various factors that affect the efficiency of denitrification process (Medcalf and Eddy, 1991):

- Dissolved oxygen: Oxygen is an inhibitory element for denitrification because its presence favours the aerobic removal of organic carbon source added to denitrify.
- External organic carbon source and carbon/nitrogen ratio (C/N): Depending on the organic carbon source used, the kinetics of the process could vary. The ratio C/N

depends on the source used. In the following lines the C/N ratios for methanol (equation 1.5) and acetic acid (equation 1.6) are shown.

$$NO_3 + 5/6 \ CH_3OH \quad \Rightarrow \quad 0.5 \ N_2 + 5/6 \ CO_2 + 7/6 \ H_2O + OH^- \tag{1.5}$$
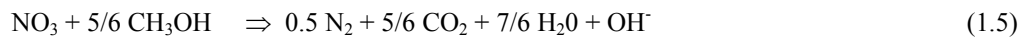
The ratio C/N is 1.90 g Methanol g^{-1} N without considering the biomass cell growth.

$$8 \ NO_3 + 5 \ CH_3COOH \quad \Rightarrow \quad 4 \ N_2 + 10 \ CO_2 + 6 \ H_2O + 8OH^- \tag{1.6}$$
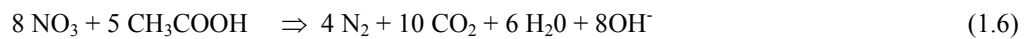
The ratio C/N is 2.67 g Acetic acid g^{-1} N without considering the biomass cell growth.

- pH: The optimum pH range for denitrification is normally between 7 and 8.5. According to alkalinity recovery, during denitrification there is an increase of pH.
- Temperature: The temperature effect in denitrification is lower than in nitrification working correctly at temperatures from 10-40 ºC.
- Inhibitory compounds: There are different compounds that could inhibit denitrification process, but the most important is the oxygen as it has been stated above.

1.2.2.3 Nitrogen Fixation, Ammonification, Assimilation

Nitrogen fixation corresponds to the reduction reaction of N_2 to NH_3 that will be used in the synthesis of organic compounds. In ammonification the organic nitrogen is mineralisated to ammonium, nitrite and nitrate. Finally, assimilation is the process where nitrogen compounds such as NH_4^+ are incorporated as a nutrient into the microorganisms' cell for its growth (Medcalf and Eddy, 1991).

1.2.2.4 ANAMMOX

ANaerobic AMMonium OXidation (ANAMMOX) process consists of another way to produce nitrogen gas. Ammonia is oxidised under anaerobic conditions with autotrophic

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microorganisms from the group of *planctomycetes* (Mulder et al., 1995; van de Graaf et al., 1995; Strous et al., 1999). The process converts ammonium to nitrogen gas with NO_2^- as electron acceptor under anaerobic conditions, and with no organic carbon in the media.
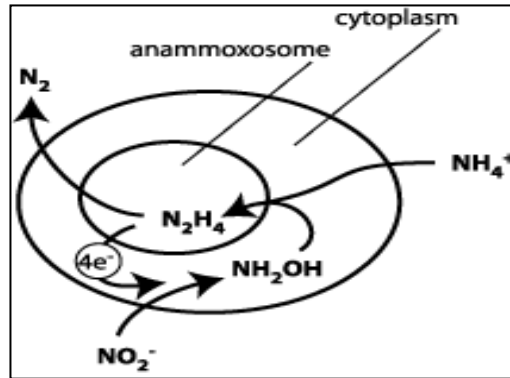

Figure 1.4: Anammox metabolic reaction

The reaction path of Anammox is shown in Figure 1.4, where it can be observed that hydrazine (N_2H_4) and hydroxylamine (NH_2OH) act as intermediates of the process. Similarly to nitrification and denitrification, there are various factors that affect the efficiency of Anammox process:

- Dissolved oxygen: The process is inhibited for oxygen concentrations below 0.5 % air saturation (Strous et al., 1997). For low oxygen concentrations the inhibition is reversible, whereas if the oxygen concentration is too high the process is irreversible. (Egli et al., 2001).

- pH: Anammox working pH range is between 6.7 and 8.3 (Strous et al., 1997)

- Temperature: Anammox bacteria are active between 6 and 43 ºC (Dalsgaard et al., 2002) but the optimal value is found at 37 ºC (Egli et al., 2001).

- Inhibitory compounds: Ammonium and nitrate do not inhibit the process, but the nitrite could be an inhibitory compound itself. The Anammox process stops

completely at nitrite concentrations higher than 20 mmol. The addition of hydrazine and hydroxylamine allows sometimes recovering Anammox capacity (Strous et al., 1999).

1.2.2.5 Denitrification by nitrifiers, heterotrophic nitrification

These new processes are important because they are the responsible of the nitrogen losses in WWTPs.

Denitrification nitrifiers can act in two situations. Under oxygen limiting conditions NH_2OH combined with NO_2^- can produce N_2O (aerobic deammonification). Moreover, under anoxic conditions the ammonia combined with NO_2 leads to NO formation (van Loosdrecht and Salem, 2005).

On the other hand, there is the heterotrophic nitrification where under aerobic conditions and high COD/N ratios (>10) there is a part of N that is oxidised by heterotrophic bacteria. However, the latter is difficult to separate from the part of N that is assimilated by the cell (van Loosdrecht and Jetten, 1998).

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1.3   SUPERNATANT FROM ANAEROBIC SLUDGE DIGESTION (reject water)

1.3.1   Anaerobic sludge digestion

The anaerobic sludge digestion (biometanisation) is a process where, in absence of oxygen, the organic compounds of the withdrawn sludge coming from the different reactors of a WWTP are transformed to biogas (mixture 65% CH_4 and 35% CO_2) through a biological process (Medcalf and Eddy, 1991). The final biogas produced is kept and used to co-generate energy in the WWTP. In the whole reaction, 35% of the treated sludge is reduced.

The conversion process is a complex system of 9 consecutive reactions with four phases (Hydrolytic, Acidogenic, Acetogenic, and Metanogenic) that takes place until biogas is obtained (Medcalf and Eddy, 1991). The temperature at which the process is performed (Psychrophilic: 10-15 ºC; Mesophilic: 35-37 ºC; Termophilic: 50-60 ºC) defines the development of a specific group of microorganisms.

Apart from the main gas products as methane and CO_2, other compounds such as NH_4^+ from the assimilation process gets out of the reactor with the water stream. It is the generation of that ion the cause of the high contamination of NH_4^+-N in the supernatant of anaerobic sludge digester (reject water).

1.3.2 Reject water problems

As it is shown in Figure 1.5, the treatment of reject water would be placed after the centrifuge. But in absence of this treatment, reject water is directly recirculated to the plant head. The latter fact is due to the considerable concentration of nitrogen (1000 mg NH_4^+-N L^{-1}), which represents the 15-25 % of the total N discharged in the plant, and organic non-biodegradable (refractory) compounds (Janus and van der Roest 1997; Mossakowska et al., 1997; Wett et al., 1998; Ghyoot et al., 1999; Rostron et al., 2001; Arnold et al., 2000; Fux et al., 2003). Moreover, the bicarbonate to ammonium ratio (molar basis) is normally low and around 1 (Hellinga et al., 1999; Vandaele et al., 2000).

These are the main contaminants of reject water and when it is recirculated to the head plant it can cause the following problems:

- Overloading of N and water in a WWTP that works near its limit capacity (Grulois et al, 1993). The recirculation must be done in periods of low flow-rate (during night).
- When recirculating the reject water to the head plant, it is necessary to increase the recirculation of the biomass in the main reactor of the WWTP to maintain the microorganisms' population stable (Grulois et al, 1993).
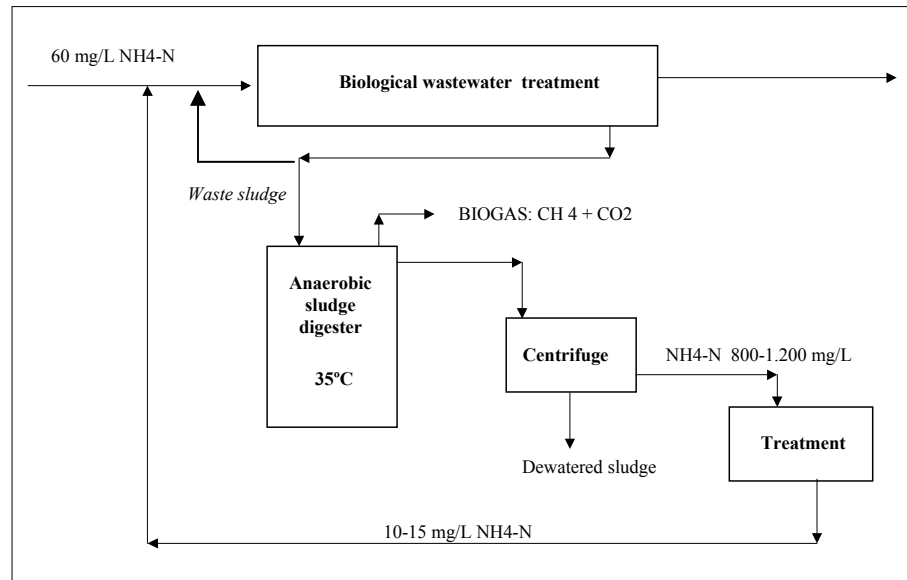

Figure 1.5: WWTP with anaerobic sludge digester and the possible reject water treatment

On balance, although reject water represents a small percentage of the overall wastewater flow-rate in the WWTP, it affects negatively the concentration level of these contaminants in the WWTP outlet stream. Any increase in these concentrations may lead to legal emission limits being exceeded. Therefore, the treatment of reject water might be a very positive solution in WWTPs located in areas with restrictive legislation. The literature suggests that biological treatments are preferable to chemical ones to treat reject water. Moreover, they do not consume chemical products and they produce a better quality sludge that can be used for other applications (Teichgraber and Stein, 1994; Siegrist, 1996).

1.4 THE NOVEL BIOLOGICAL SIDE STREAM ALTERNATIVES

The classical nitrification/denitrification (N/DN) process is the easiest way to treat reject water through a biological process. However this process can be modified in order to obtain economical benefits as it is explained below.

1.4.1 Nitrification/Denitrification via nitrite

From an economic point of view it is better to develop N/DN via nitrite. In this way the cost reduction in terms of oxygen supply and COD (denitrification electron donor) addition is recently under study because the nitrite route would suppose, according to stoichiometry, the saving of 25% of oxygen in nitrification and 40% of COD during denitrification. Different ways to develop partial nitrification are known. Nitritation predominates over nitratation at temperatures over 20ºC, but the total wash-out of nitrite oxidisers can only be achieved working in a narrow range of solid retention time (SRT), namely 1-2 days (Hellinga et al., 1999; Van Dongen et al., 2001). The pH range is also, as it is shown in Figure 1.6, an important parameter for nitratation inhibition (Abeling and Seyfried, 1992; Grunditz and Dalhammar, 2001). If there is dissolved ammonia in the system at a concentration between 1-150 mg L^{-1}, NO$_3^-$-N generation begins to be inhibited (Anthonisen et al., 1976). This free ammonia concentration is achieved when working at pH range of 7.5-9 with high NH$_4^+$-N concentration.
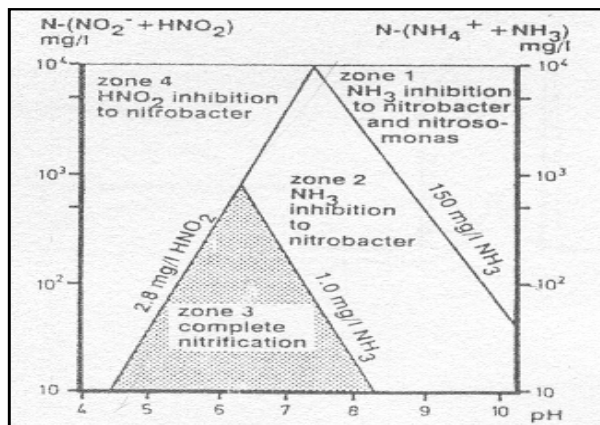

Figure 1.6: Nitrification inhibitory regions

The dissolved oxygen (DO) is the other important parameter to achieve the nitrite route. If there is an oxygen concentration inside the reactor lower than 1.5 mg L^{-1}, as demonstrated in Figure 1.7, there is no total nitrate formation because ammonia oxidation rate is favoured in front of nitrite oxidation kinetics (Piciorneau et al., 1997; Grunditz C. and Dalhammar G. 2001; Pollice et al. 2002; Ruiz et al., 2003). But nitritation at a low DO will only be stable (with no partial nitrate formation) if properly coupled with denitrification (van Loosdrecht and Salem 2005).
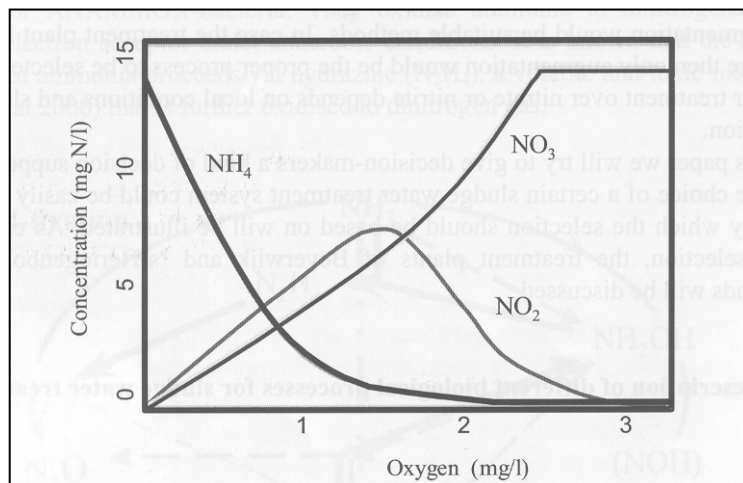
Figure 1.7: Stationary states for different DO

1.4.2 SHARON process

The Single reactor High activity Ammonium Removal Over Nitrite (SHARON) is a patented process were nitrification via nitrite is developed in a continuous chemostat reactor without sludge retention. The high temperatures (> 30ºC) and the low sludge age (< 2 days) are the basis of continuous chemostat SHARON process where it is possible to wash-out the nitrite oxidising bacteria (NOB) due to the better kinetic of ammonium oxidising bacteria (AOB) in the mentioned operation conditions (Figure 1.8). If the aeration is stopped, the process can be combined with denitrification to develop a complete biological nitrogen removal or simply to control nitrification pH (Hellinga,

1998). The process is recently implemented at real scale to treat reject water with very satisfactory results (van Kempen et al., 2001).
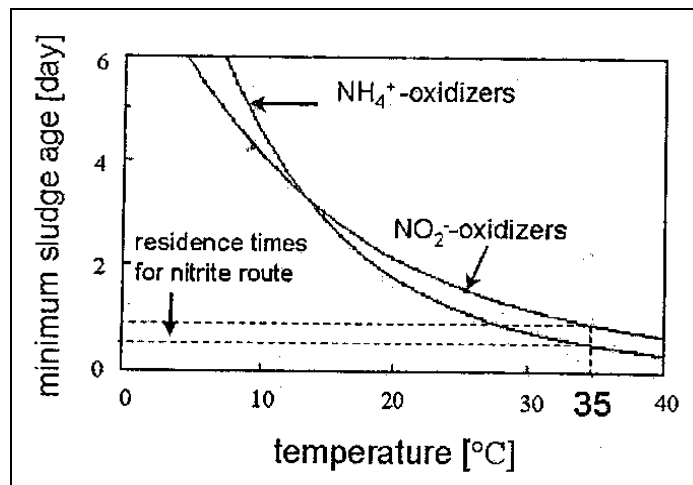

Figure 1.8: NH_4^+ and NO_2^- oxidisers minimum sludge age at different T

1.4.3 ANAMMOX process

Anammox process, as it has stated above (section 1.2.2.4) is one of the nitrogen paths discovered recently. It is an equivalent reaction to denitrification, but it is developed by autotrophic microorganisms that use ammonium as electron donor to reduce NO_2^- to N_2 instead of COD. The process cannot work with nitrate, and NH_4^+-N:NO_2^--N ratio must be around 1:1 (Mulder et al., 1995; Strous et al., 1997; Jetten et al., 1999; Fux et al., 2002). The autotrophic Anammox microorganisms have a very low growth kinetics which leads to a very low sludge production (van de Graff, 1995; Jetten et al., 1999).

The fact that the process must work with nitrite and needs ammonium as electron donor implies nitrification via nitrite of 50% of the ammonium-rich wastewater. Consequently, Anammox could not be developed directly and a previous nitrification reactor to provide an influent with ammonium and nitrite at 50%, like SHARON process, must be operated (Figure 1.9).

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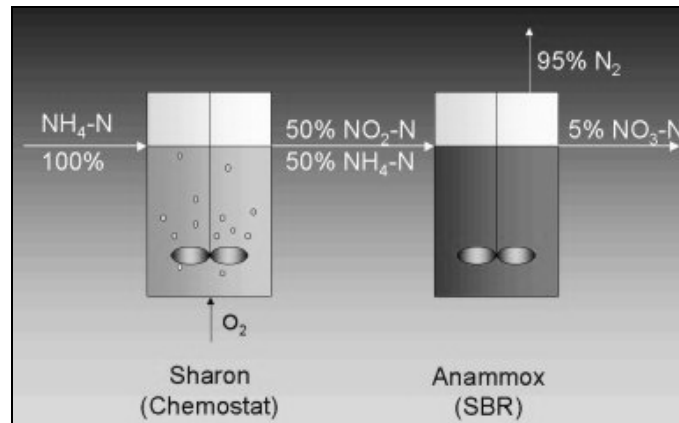
Figure 1.9: SHARON/Anammox system

1.4.4 CANON alternative

Completely Nitrogen removal Over Nitrite (CANON) is a process where the biofilm and granular technology is developed to simplify SHARON-ANAMMOX process in a single reactor (Sliekers et al., 1998). The idea is that in a permanent low aerated reactor, the external part of the biomass develops nitrification and in the interior of the flock or biofilm, Anammox takes place. The main differences respect to SHARON-ANAMMOX is that oxygen is the limiting factor that inhibits and washes out NOB.
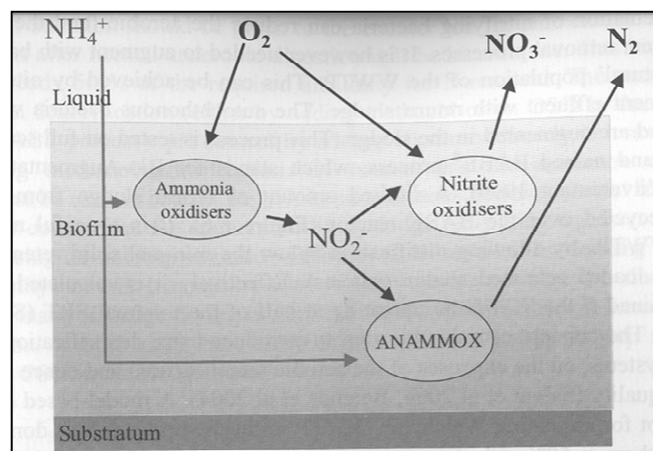
Figure 1.10: Bacteria competition for O_2 and NO_2^- in a CANON reactor

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The key, as it is shown in Figure 1.10, is that NOB competes with AOB for oxygen, and with Anammox bacteria for nitrite. Therefore, at low oxygen concentrations nitrite oxidisers are washed out (van Loosdrecht and Salem, 2005).

1.4.4 BABE process

Bio-Augmentation Batch Enhanced process (BABE) is a side-stream process to help N/DN in the main line of a WWTP. The problem in the main line is that nitrification capacity is very low due to the lower sludge age of the secondary biological reactor (2-3 days), which only allows nitrification sometimes in summer. Doing a nitrification step of reject water and then recirculating the withdrawn sludge to the main line could be a solution, but nitrifiers would be removed by the protozoa (van Loosdrecht and Salem, 2005). BABE proposes a solution to avoid the latter fact (Figure 1.11). If reject water nitrification treatment is joined with recirculated sludge from the biological secondary reactor in a ratio 5:1, the nitrifying bacteria would grown in flocks all together with the secondary biomass. This would prevent the nitrifiers' depravation by protozoa and their wash-out due to low SRT when the sludge is recirculated again to the main line of the WWTP (Salem et al., 2002, 2003)
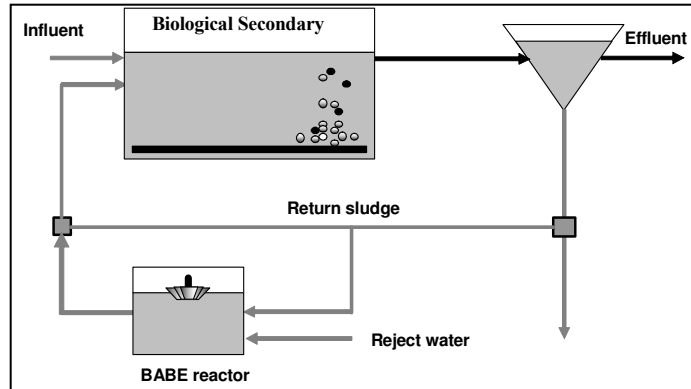
Figure 1.11: Implementation of BABE process

As it can be observed this is not a strict side-stream treatment because it also implies nitrification in the main line. The system is good for WWTPs with no nutrient removal step that must be enlarged because BABE provides a 50% reduction of space and equipments compared with the classical nitrification/denitrification process (Salem et al., 2002, 2004)

1.4.5 SBR Technology

Sequencing batch reactors (SBR) belong to the group of filling-withdrawing reactors and their main features are flexibility, which allows working with a wide range of concentration and streams, and compactness that allows the coexistence of the different sequences (Figure 1.12) in the same tank (Irvine et al., 1997; Ketchum, 1997; Artan et al., 2001; Mace and Mata 2002). Furthermore, the phases of filling and reaction could be done in static, aeration or stirring conditions. According to this, an SBR is able to work in aerobic, anoxic or anaerobic conditions depending on the way of removing contaminants. Therefore, the treatment of a side stream like reject water in an SBR would suppose the settler elimination leading to a cheaper installation.
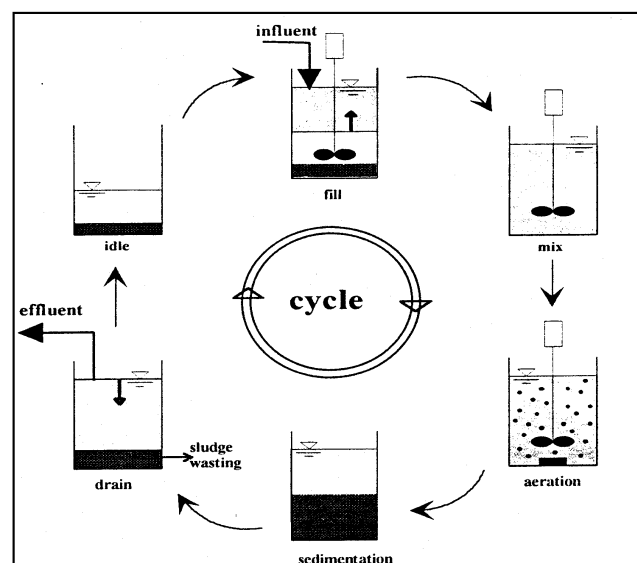
Figure 1.12: SBR stages

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1.5 MODELLING APROACH

International Water Association (IWA) activated sludge models (Henze et al., 2000) represent the most widespread and successful approach to characterise the nutrient removal processes for design and control (Copp et al., 2002; Seco et al., 2004).

In 1987 the Activated Sludge Model No1 (ASM1), which considered two types of microorganisms (autotrophic and heterotrophic) and eight associated processes, was developed by Henze et al. As extensions of this model the dephosphatation is considered in model ASM2d (Henze et al., 1999) and the accumulation of organic material in the cell is considered in model ASM3 (Gujer et al., 1999). However, the model ASM1 is still being used due to its simplicity to explain the processes that take place in biological reactions, and it is the model used in the present work for the modelling of the WWTP under study.

1.5.1 Growth rate kinetics

Bacteria kinetic growth is defined by a Monod approximation in equation 1.8 (Ohron and Artan, 1994).

$$r_X = \mu \cdot X \tag{1.7}$$

$$\mu = \mu_{max} \cdot \left(\frac{S}{S + K_S} \right) \tag{1.8}$$

Where:

r_X : Bacteria kinetic growth. (M (V t)$^{-1}$)

μ : Specific bacteria kinetic growth (t^{-1})

X : Microorganisms concentration (M V^{-1})

μ_{max} : Maximum specific bacteria kinetic growth (t^{-1})

S : Substrate concentration. (M V^{-1})

K_S : Half-saturation constant (M V^{-1}); It corresponds to the substrate concentration that is the half of the maximum kinetic (M V^{-1})

Substrate concentration is related to biomass growth with the reaction yield (Y = g biomass formed g^{-1} substrate consumed). Moreover, in the biological modelling it is usually used the interactive model where there are as many switch Monod functions (S/(K$_S$+S)) as many compounds are necessary for a process. A switch function (Figure 1.13) modifies the maximum kinetic growth. For substrate (S) concentration higher than K$_s$ the function is 1 and when there is no substrate the function comes 0.
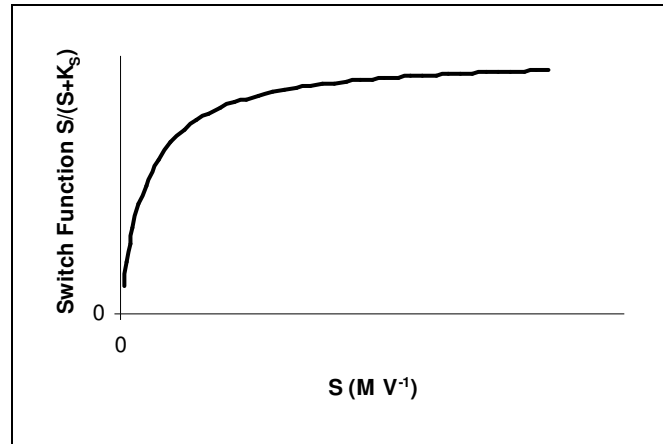
Figure 1.13: S*witch* function representation

Considering the latter in a process influenced by three substrates (S$_1$, S$_2$ and S$_3$), the growth rate expressions of the bacteria would be:

$$r_x = \mu_{max} \cdot \left(\frac{S_1}{S_1 + K_{S,1}} \right) \cdot \left(\frac{S_2}{S_2 + K_{S,2}} \right) \cdot \left(\frac{S_3}{S_3 + K_{S,3}} \right) \cdot X \qquad (1.9)$$

1.5.2 Decay kinetics: decay-regeneration or lineal decay

The concentration of biomass present in the system decreases due to its decay or endogenous respiration, and this decay is proportional to biomass concentration (equation 1.10). The lineal constant k$_d$ is known as decay rate (Ohron and Artan, 1994).

$$r_{decay} = k_d \, X \qquad (1.10)$$

Where:

r$_{decay}$ = decay rate (M (V t)$^{-1}$) and X= microorganisms concentration (M V^{-1})

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The latter is the lineal decay but there are some models like ASM1 that use the concept decay-regeneration (Dold et al., 1980) because they consider that when the cell dies, a part is transformed into inert fraction and the other part in slowly biodegradable COD which is a new substrate for the microorganisms.

1.5.3 Model ASM1 components

ASM1 model is characterised by two types of components: *soluble* (S with the respective component sub-index) and *particulate* (X with the respective component sub-index). In the following lines there are described the components of the model:

- Soluble compounds.

S_s (g_{COD} m^{-3}): Readily biodegradable COD that can directly be removed by bacteria.

S_{ALK} (mol HCO_3^- L^{-3}): Alkalinity in form of bicarbonate.

S_I (g_{COD} m^{-3}): Inert soluble COD, which is considered non-biodegradable.

S_{ND} (g_N m^{-3}): Organic nitrogen.

S_{NH} (g_N m^{-3}): Ammonia nitrogen considering ammonium and ammonia.

S_{NO} (g_N m^{-3}): Nitrate plus nitrite. Stoichiometry is done supposing that all is nitrate.

S_O (g_{O2} m^{-3}): Dissolved oxygen.

-Particulate compounds

X_{BA} (g_{COD} m^{-3}): Autotrophic biomass. They are the bacteria responsible of nitrification and can only work under aerobic conditions oxidising ammonium to nitrate.

X_{BH} (g_{COD} m^{-3}): Heterotrophic biomass. They can grow in aerobic conditions and even in anoxic conditions. They are also the responsible of COD hydrolysis.

X_I (g_{COD} m^{-3}): Inert particulate COD that cannot be degraded.

X_S (g_{COD} m^{-3}): Slowly biodegradable COD.

X_p (g_{COD} m^{-3}): Particulate products formed in the decay process of biomass.

X_{ND} (g_N m^{-3}): Particulate biodegradable organic nitrogen.

1.5.4 Model ASM1 processes

The Biological process and kinetic equations of ASM1 model are shown in Table 1.1.

Table 1.1: Kinetic of the processes in ASM1

(j)	Biological reaction	Kinetic $(\rho_j > 0)$
(1)	*Aerobic growth of heterotrophic*	$\mu_{mH} \dfrac{S_S}{K_S + S_S} \dfrac{S_O}{K_{OH} + S_O} X_{BH}$
(2)	*Anoxic growth of heterotrophic*	$\eta_{NO_3} \mu_{mH} \dfrac{S_S}{K_S + S_S} \dfrac{K_{OH}}{K_{OH} + S_O} \dfrac{S_{NO}}{K_{NO} + S_{NO}} X_{BH}$
(3)	*Aerobic growth of autotrophic*	$\mu_{mA} \dfrac{S_{NH}}{K_{NH} + S_{NH}} \dfrac{S_O}{K_{OA} + S_O} X_{BA}$
(4)	*Decay of heterotrophic*	$b_H\, X_{BH}$
(5)	*Decay of autotrophic*	$b_A X_{BA}$
(6)	*Ammonification*	$k_a S_{ND} X_{BH}$
(7)	*Hydrolysis of organics*	$k_h \dfrac{X_S/X_{BH}}{K_X + X_S/X_{BH}} \left[\dfrac{S_O}{K_{OH} + S_O} + \eta_h \dfrac{K_{OH}}{K_{OH} + S_O} \dfrac{S_{NO}}{K_{NO} + S_{NO}} \right] X_{BH}$
(8)	*Hydrolysis of nitrogen*	$k_h \dfrac{X_{ND}/X_{BH}}{K_X + X_S/X_{BH}} \left[\dfrac{S_O}{K_{OH} + S_O} + \eta_h \dfrac{K_{OH}}{K_{OH} + S_O} \dfrac{S_{NO}}{K_{NO} + S_{NO}} \right] X_{BH}$

According to the process that can develop every type of microorganism the following items have been considered:

✓ Heterotrophic bacteria can grow in aerobic and anoxic conditions (The model introduces a corrector factor, η_{no3}).

- ✓ The model considers that autotrophic microorganisms can only grow in aerobic conditions.
- ✓ In ammonification process the organic nitrogen is transformed into ammonia.
- ✓ In hydrolysis processes the particulate substrates are transformed to lower molecular weight compounds that are finally dissolved and became readily biodegradable substrate. The hydrolysis is developed in aerobic and anoxic conditions in ASM1.
- ✓ Decay process (lysis) represents the process related to the biomass removal.

1.5.5 Petersen Matrix

For simplification purposes, the models are normally presented using a matrix structure. The Petersen Matrix is the normal way to express the ASM and other models because it facilitates the data interpretation and the calculations. The biological reactions are placed in matrix rows (growth, decay etc…) and the state variables are placed in matrix columns (biomass, substrate, dissolved oxygen). In the first column there are specified the reaction names, while the kinetic for each of the considered reactions are placed in the last column. Finally, inside the matrix there are the stoichiometric coefficients ($v_{i,j}$) that indicate how the compound i varies in the biological reaction j. In this way, the horizontal direction of the Petersen Matrix corresponds to the biochemical reactions. In each of these rows it must be applied the continuity equation (see equation 1.11).

$$\sum_{i=1}^{n} v_{i,j} \cdot i_{c,i} = 0 \qquad (1.11)$$

Where:

$v_{i,j}$ = stoichiometric coefficient for compound i in reaction j

$i_{c,i}$ = conversion factor that allows to pass from component i to units of material c to which the continuity equation is applied

The conversion factors are written in a composition matrix (Henze et al., 2000) but in ASM1 they are normally placed directly to Petersen matrix (Table 1.2)

Table 1.2: Process kinetics and stoichiometry for carbon oxidation, nitrification, and dentrification

j / Process ↓ \ Component → i	1 S_I Soluble inert organic matter $[M(COD)L^{-3}]$	2 S_S Readily biodegradable substrate $[M(COD)L^{-3}]$	3 X_I Particulate inert organic matter $[M(COD)L^{-3}]$	4 X_S Slowly biodegradable substrate $[M(COD)L^{-3}]$	5 $X_{B,H}$ Active heterotrophic biomass $[M(COD)L^{-3}]$	6 $X_{B,A}$ Active autotrophic biomass $[M(COD)L^{-3}]$	7 X_P Particulate products arising from biomass decay $[M(COD)L^{-3}]$	8 S_O Oxygen (negative COD) $[M(-COD)L^{-3}]$	9 S_{NO} Nitrate and nitrite nitrogen $[M(N)L^{-3}]$	10 S_{NH} $NH_4^+ + NH_3$ nitrogen $[M(N)L^{-3}]$	11 S_{ND} Soluble biodegradable organic nitrogen $[M(N)L^{-3}]$	12 X_{ND} Particulate biodegradable organic nitrogen $[M(N)L^{-3}]$	13 S_{ALK} Alkalinity – Molar units	Process Rate, ρ_j $[ML^{-3}T^{-1}]$
1 Aerobic growth of heterotrophs		$-\dfrac{1}{Y_H}$			1			$-\dfrac{1-Y_H}{Y_H}$		$-i_{XB}$			$-\dfrac{i_{XB}}{14}$	$\hat{\mu}_H\left(\dfrac{S_S}{K_S+S_S}\right)\left(\dfrac{S_O}{K_{O,H}+S_O}\right)X_{B,H}$
2 Anoxic growth of heterotrophs		$-\dfrac{1}{Y_H}$			1				$-\dfrac{1-Y_H}{2.86\,Y_H}$	$-i_{XB}$			$\dfrac{1-Y_H}{14\cdot2.86\,Y_H}-\dfrac{i_{XB}}{14}$	$\hat{\mu}_H\left(\dfrac{S_S}{K_S+S_S}\right)\left(\dfrac{K_{O,H}}{K_{O,H}+S_O}\right)\times\left(\dfrac{S_{NO}}{K_{NO}+S_{NO}}\right)\eta_g X_{B,H}$
3 Aerobic growth of autotrophs						1		$-\dfrac{4.57-Y_A}{Y_A}$	$\dfrac{1}{Y_A}$	$-i_{XB}-\dfrac{1}{Y_A}$			$-\dfrac{i_{XB}}{14}-\dfrac{1}{7Y_A}$	$\hat{\mu}_A\left(\dfrac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\dfrac{S_O}{K_{O,A}+S_O}\right)X_{B,A}$
4 'Decay' of heterotrophs				$1-f_P$	-1		f_P					$i_{XB}-f_P i_{XP}$		$b_H X_{B,H}$
5 'Decay' of autotrophs				$1-f_P$		-1	f_P					$i_{XB}-f_P i_{XP}$		$b_A X_{B,A}$
6 Ammonification of soluble organic nitrogen										1	-1		$\dfrac{1}{14}$	$k_a S_{ND} X_{B,H}$
7 'Hydrolysis' of entrapped organics		1		-1										$k_h\dfrac{X_S/X_{B,H}}{K_X+(X_S/X_{B,H})}\left[\left(\dfrac{S_O}{K_{O,H}+S_O}\right)+\eta_h\left(\dfrac{K_{O,H}}{K_{O,H}+S_O}\right)\left(\dfrac{S_{NO}}{K_{NO}+S_{NO}}\right)\right]X_{B,H}$
8 'Hydrolysis' of entrapped organic nitrogen											1	-1		$\rho_7(X_{ND}/X_S)$
Observed Conversion Rates $[ML^{-3}T^{-1}]$														$r_i=\sum_j \nu_{ij}\rho_j$

Stoichiometric Parameters:
Heterotrophic yield: Y_H
Autotrophic yield: Y_A
Fraction of biomass yielding particulate products: f_P
Mass N/Mass COD in biomass: i_{XB}
Mass N/Mass COD in products from biomass: i_{XP}

Kinetic Parameters:
Heterotrophic growth and decay: $\hat{\mu}_H$, K_S, $K_{O,H}$, K_{NO}, b_H
Autotrophic growth and decay: $\hat{\mu}_A$, K_{NH}, $K_{O,A}$, b_A
Correction factor for anoxic growth of heterotrophs: η_g
Ammonification: k_a
Hydrolysis: k_h, K_X
Correction factor for anoxic hydrolysis: η_h

1.5.6 Kinetic and stoichiometric parameters of ASM1 model

Table 1.3 presents the kinetic and stoichiometric default parameters used in ASM1 model. All the stoichiometric parameters are temperature independent, but some of the kinetics parameters vary with temperature.

Table 1.3: ASM1 kinetic and stoichiometric parameters

Symbol		Units	Value (at 20 ºC)
Stoichiometric Parameters			
Y_A	Autotrophic yield	mg cell COD mg^{-1} NH$_4^+$-N consumed	0.24
Y_H	Heterotrophic yield	mg cell COD mg^{-1} COD consumed	0.67
f_p	Biomass to particulates	-	0.08
i_{XB}	N in biomass	mg N mg^{-1} ·COD in biomass	0.086
i_{XP}	N in biomass products	mg N mg^{-1} COD in endogenous biomass	0.06
Kinetic parameters			
μ_{mH}	Heterotrophic max. rate	day^{-1}	6 * (θ =0.0981)
K_S	Heterotrophic HS	mg COD L^{-1}	20
K_{OH}	O$_2$ heterotrophic HS	mg O$_2$ L^{-1}	0.20
K_{NO}	Heterotrophic HS (DN)	mg NO$_2^-$-N L^{-1}	0.50
b_H	Decay heterotrophic rate	day^{-1}	0.62* (θ =0.1132)
η_{no3}	Corrector factor for DN	-	0.8
η_h	Hydrolysis corrector factor	-	0.4
k_h	Max. hydrolysis rate	mg slowly biod. COD mg^{-1} cell COD day^{-1}	3.0* (θ =0.1098)
K_x	Hydrolysis HS	mg slowly biod. COD mg^{-1} cell COD	0.03* (θ =0.1098)
μ_{mA}	Autotrophic max. rate	day^{-1}	0.80* (θ =0.0981)
K_{NH}	Autotrophic HS	mg N-NH$_4$ L^{-1}	1.0
K_{OA}	O$_2$ autotrophic HS	mg O$_2$ L^{-1}	0.4
b_A	Decay Autotrophic rate	day^{-1}	0.15* (θ =0.0981)
k_a	Ammonification rate	L mg^{-1} COD day^{-1}	0.08* (θ =0.0693)

(* This parameter varies with T; HS = Half saturation coefficient; biod. = biodegradable)

In Table 1.3 there are the default values at 20 ºC; the parameters values at other temperatures are found applying the Arrenhius equation expressed below, where P is the parameter in considered, T is the operation temperature and θ is the Arrenhius T factor:

$$P = P (20 ºC) e^{(\theta (20-T))} \tag{1.12}$$

1.5.7 Estimation of different COD and N compounds

Once the analysis of wastewater is done, it is necessary to calculate the different fractions of each parameter under study. In order to calculate the COD and N fractions the protocols proposed by Hulsbeek et al. (2002) and Roeleveld and van Loosdrecht (2002) have been followed. They consist of the following relationships:

$$COD_{total,inf} = COD_{particulate,inf} + COD_{soluble,inf} \tag{1.13}$$

$$COD_{soluble,inf} = S_s + S_I \tag{1.14}$$
$$COD_{particulate,inf} = X_I + X_S \tag{1.15}$$

$$S_I = 0.9 * COD_{soluble,eff} - 1.5 \, BOD_{5,eff} \tag{1.17}$$
$$S_s = COD_{solublet,inf} - S_I \tag{1.16}$$

$$X_S = \{[BOD_t/(1-e^{-kt})]/(1 - Y_{BOD})\} - S_S] \text{ (where t=5, k = -0.3, Y = 0.2)} \tag{1.18}$$
$$X_I = COD_{particulate,inf} - X_S \tag{1.19}$$

$$S_{NH4} = N_{Kj} - \Sigma(i_N X + i_N S) \tag{1.20}$$

As it can be observed, it is possible to know all the COD components (S_S, S_I, X_S, X_I) by knowing the $COD_{soluble}$, $COD_{particulate}$ and BOD_5 of the influent and effluent. The Soluble ammonia is calculated by the difference between the total Kjeldahl nitrogen and the different fractions of particulate N compounds using the i_N proposed by Roeleveld and van Loosdrecht (2002).

1.6 SLUDGE RESPIROMETRY

The biological nature of wastewater treatment processes implies that their model parameters must be determined (model calibration) according to the local situation (Vanrolleghem et al., 1999). Respirometry is the most popular tool used for model calibration, and it consists of the measurement and analysis of the biological oxygen consumption under well defined experimental conditions (Rozich and Gaudy Jr, 1992; Spanjers et al., 1998). It is defined as the measurement and interpretation of the Oxygen Uptake Rate (OUR) of an activated sludge (Spanjers and Vanrolleghem, 1995) and it is developed in respirometers, chambers that allow analysing the DO variation in the gas or liquid phase. Spanjers et al. (1998) proposed a classification of the different type of respirometers. The OUR of an activated sludge is mainly composed by two different parts:

- Exogenous OUR: It is the oxygen demand to biodegrade a substrate.
- Endogenous OUR: It is the oxygen demand when there is no substrate present in the media. It is related to the decay rate of bacteria.

The performance of the respirometry in controlled conditions allows relating the OUR with one or more processes described in the models in order to evaluate and finally calculate the following aspects:

- Kinetic parameters
- Stoichiometric parameters
- Compound concentrations

In section 3.1.2 of Chapter 3, there are described the working conditions of a sequential closed respirometer with the DO and OUR profiles obtained.

2. OBJECTIVES AND THESIS STRUCTURE

2.1 OBJECTIVES

The fact that the UE legislation (Directive 91/271/EEC and 2000/60/EEC) will have to be applied in all the country members leads to strength the application and control of new treatments. According to the latter, nitrogen is one of the contaminants that will be more controlled. The treatment of high polluted flow-rates is a good compact solution when considering the economical reasons. In that point, as it has been stated in Chapter 1 the problem of supernatant from anaerobic sludge digesters (reject water) can be included in that kind of situations. Considering its treatment it would be very positive, and it could lead to observe the legislation. Therefore, the scope of the present work will be the study of the biological treatment of real reject water, and is structured in the following objectives:

Reject water characterisation

First of all, the characterisation of a real supernatant from anaerobic digestion of sewage sludge is required in order to know which its pollutants are. The analytical tests of the main pollutants and different respirometric assays are done in order to have the whole system characterised.

Start-up and optimisation of an SBR

Once the characterisation of reject water is performed, its biological treatment with an SBR reactor is proposed from the start-up of the process to its optimisation modifying different operational parameters.

SBR operating with an internal organic carbon sources of the WWTP

Considering the reject water characteristics, it is normally necessary to add an external organic carbon source in the denitrification step. The substitution of that external carbon source for an internal organic carbon source of the own WWTP would be a good solution because it would lead to an important cost reduction. In that way, a study of the different available organic carbon sources of the WWTP is considered.

Comparison SBR vs. SHARON chemostat

When developing tertiary treatments, it is important to minimise their cost. The optimisation of real reject water treatment from a WWTP with SBR and chemostat reactor via nitrite will be done in order to compare its treatment from the operational, kinetic and design point of view to find a feasible and economical treatment.

WWTP modelling

The modelling of the WWTP under study is also proposed in order to determine if the effluent requirements can be well predicted by the ASM1 biological model. Moreover, the enlargement of the WWTP to nitrogen removal step is simulated in order to realise how much extra volume would be necessary. In these simulations, the treatment of reject water, which represents around 25% of total nitrogen discharged in the head plant, is taken into account in the final decision.

2.2 STRUCTURE OF THE THESIS

After the introduction, the objectives and the material and methods used, the thesis results are structured in 6 chapters were the problem of reject water is studied. The structure of the Thesis is represented in Figure 2.1.

In Chapter 4, a general characterisation of the wastewater and the adaptation of microorganisms to nitrification/denitrification process are done to perform the start-up of a SBR. Then in Chapter 5, the operational conditions of the SBR are optimised to obtain the best nutrient removal. Finally, in order to reduce the operation cost, in Chapter 6 the operation of the SBR with hydrolysed primary sludge for denitrification instead of methanol is studied.

In the following chapters, the treatment of reject water is focused from the point of view of comparing its treatment with two types of biological reactors. In Chapter 7, a detailed analysis in terms of operational conditions, kinetic, design and cost is done to treat reject water with a SBR and a SHARON/denitrification process to select the best option. After that, in Chapter 8, the future treatment of reject water with the new developed system Anammox is taken into account. In that way, the partial nitrification is developed in a SBR and in a SHARON chemostat reactor to choose between two feasible ways to obtain the desired influent for the Anammox process.

Finally, in Chapter 9 the modelling of the WWTP under study is done in order to verify the actual effluent pollutants level. Moreover, the plant enlargement to nitrogen removal is also proposed.

With all this information, the general conclusions for reject water treatment are reached.
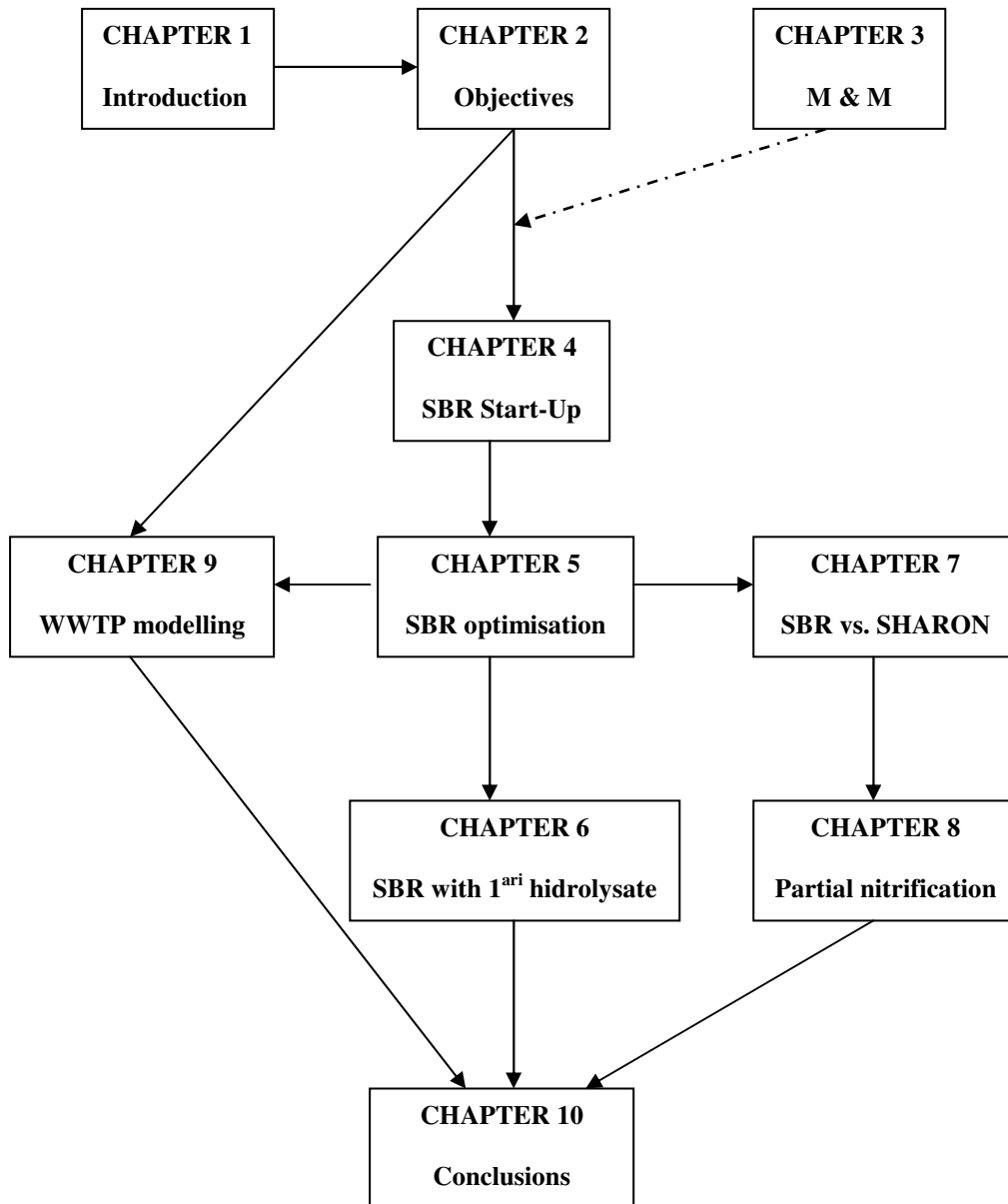
Figure 2.1: Thesis structure

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3. MATERIALS AND METHODS

3.1 EXPERIMENTAL DEVICES

Treatment of reject water was carried out at lab scale, where N/DN was developed in one SBR of 3 L (Figure 3.1a) and one chemostat of 4 L (Figure 3.1b). 5 pumps (3 Cole-Parmer Instrument 7553-85 and 2 EYELA Micro Tube Pump MP-3), 2 oxygen valves and 2 mechanical stirrers were necessary to operate both systems. Two SBR of 1 L were also used for acclimation tests of microorganisms. Moreover, the experimental devices were controlled and monitored by a computer with an acquisition data card (PCL-812PG), a control box and an inter-phase card (PCL-743/745) connecting both systems (Figure 3.1c). The computer worked with *Bioexpert version 1.1 x* Software. Temperature was maintained at T ± 0.5 ºC by means of a thermostatic bath (RM6 Lauda) and pH was measured with an electrode (Crison Rocon 18). Temperature and pH profiles were monitored and these data were then exported and represented in each cycle.

(a)

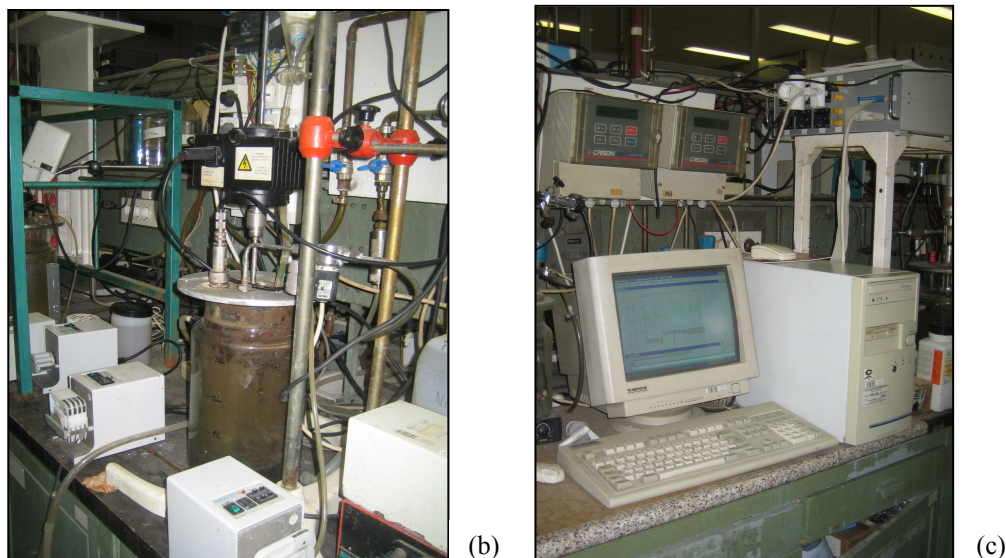**Figure 3.1:** Experimental devices (a) SBR (b) Chemostat reactor (c) Control computer system

Moreover, a closed intermittent-flow respirometer (similar to the ones used by Marsilli-Libelli and Tabani, 2002; Gutiérrez, 2003) was used to characterise the system (Figure 3.2a and b) and calculate the kinetic and stoichiometric parameters (Spanjers et al., 1995). This device consisted of an aeration vessel (3 L) and a stirred watertight closed respiration chamber (0.250 L). A heating system (Polystat, Bioblock Scientific) was used to maintain the temperature at $T \pm 0.5$ ºC in the whole system. The respiration chamber was equipped with a dissolved oxygen electrode (Oxi 340i, WTW) and the pH in the aeration vessel was measured with a Crison pH 28 electrode. When the oxygen level dropped below 2 mg O_2 L^{-1} or the measuring period lasted more than 100 s, the mixed liquor in the measurement vessel was replaced by pumping aerated mixed liquor from the aeration vessel into the measuring chamber for 75 s, time enough to renew for three times the volume of the respiration chamber. The 4-20 mA signals of both oxygen and pH probes were collected and logged on a PC equipped with Advantech Genie software package and a combined A/D I/O Modules (Adam 4050/ Adam 4520/ Adam 4018). pH was controlled within a narrow pH set-point. When the measured pH value did not lie

inside the desired region, acid (HCl, 0.2 N) or base (NaOH, 0.2 N) was added by opening an electromagnetic valve for a very short period of time to adjust the pH.

(a)

(b)

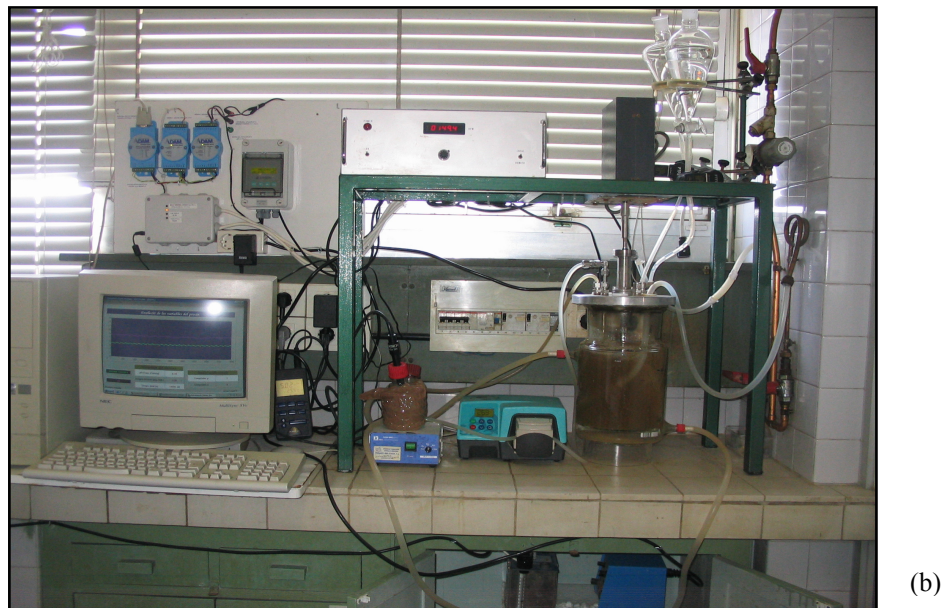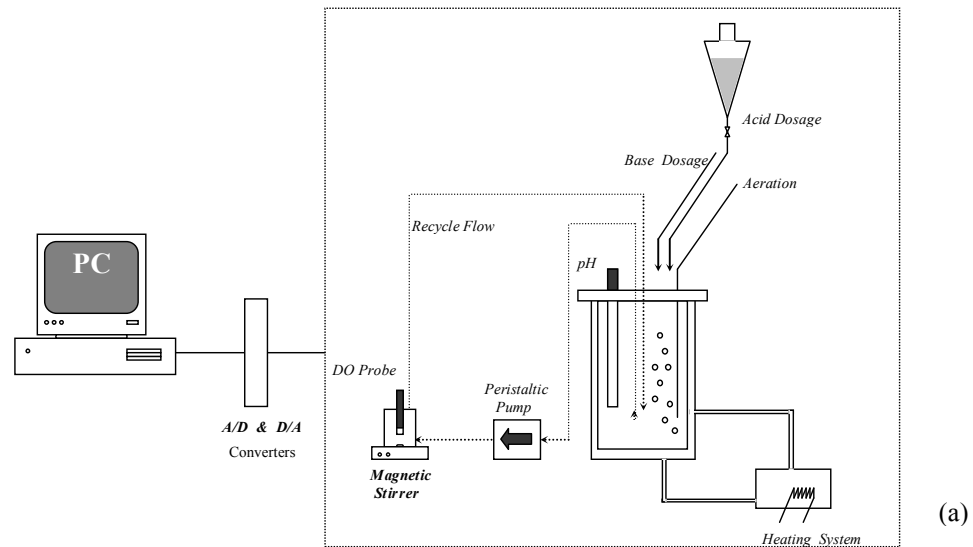**Figure 3.2:** Set-up of the closed intermittent-flow respirometer (a) sketch (b) experimental lab-scale respirometer

With the respirometer kit explained above, the variation of the DO with time was obtained, as it can be seen in Figure 3.3 a. Then with the different DO slopes the OUR was extracted and represented, as shown in Figure 3.3 b.

(a)

(b)
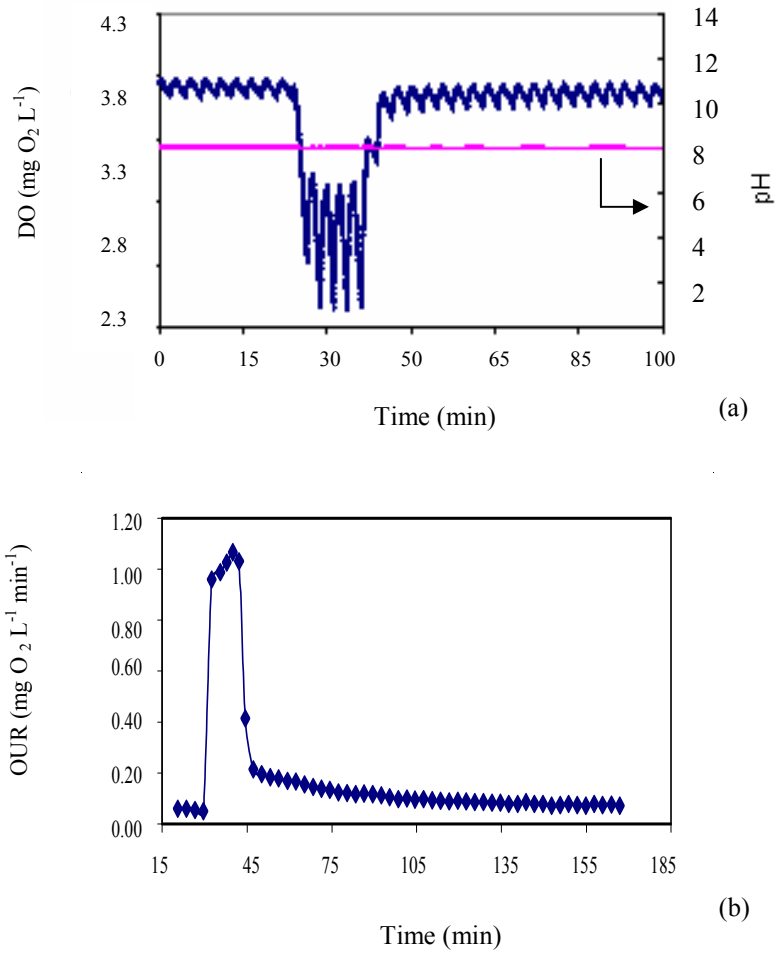
Figure 3.3: Profiles of (a) DO variation (b) OUR variation

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3.2 ANALYTICAL METHODS

Analyses of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD$_5$), alkalinity, nitrogen compounds, volatile fatty acids (VFA), total solids (TS), total volatile solids (TVS), suspended solids (TSS) volatile suspended solids (VSS) and sedimentation tests were performed according to the Standard Methods for the examination of Water and Wastewater (APHA, 1998).

- Ammonium (NH$_4^+$-N) and Alkalinity: Ammonium was determined (Figure 3.4) by an ammonia-specific electrode (Crison, model pH 2002). The samples have to be alkalinised to pH 10-12 with NaOH 10 N in order to achieve the entire free N in form of ammonia. The electrode gives a mV measure that have to be contrasted with the calibration curve done with patrons of known concentration (Log (NH$_4^+$-N) = a · (mV) + b).

  Alkalinity was done through an automatic titration (Schott-TA20plus) with a solution of chloride acid 0.1 N (Figure 3.5).
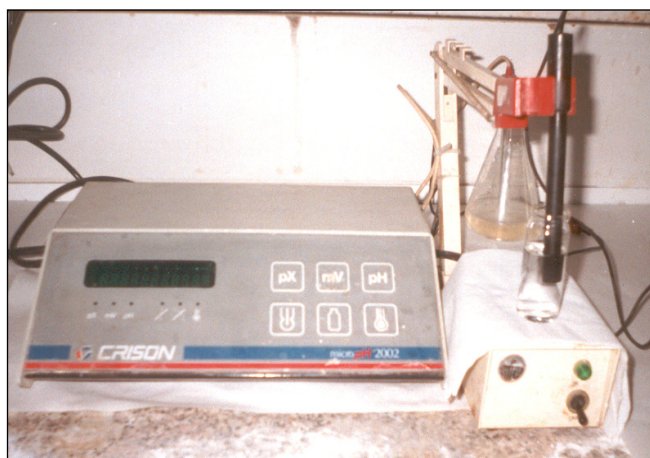
Figure 3.4: Specific NH$_4^+$ electrode

Figure 3.5: Titration device

- <u>Nitrites, nitrates and VFA:</u> Nitrates and nitrites (Figure 3.6) were analysed with capillary electrophoresis (Hewlett Packard 3D) and VFA were analysed (Figure 3.7) with a gas chromatography (HP 5800). Once the samples were withdrawn of the reactor, they were immediately centrifuged at 10000 rpm for 10 min and filtered through 0.45 µm cellulose paper filters.

Figure 3.6: Capillary electrophoresis

Figure 3.7: Gas chromatography

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- COD: Organic materials were oxidised with a mixture of sulphuric acid and potassium dichromate during 2 hours in a digester (Figure 3.8a) at 150 ºC (Velp ECO 25). Silver sulphate was used as a catalyst in order to remove the interference of chlorides. After the digestion the samples were analysed in a spectrophotometer (Shimadzu UV-1203) at 620 nm (Figure 3.8b) where the absorbance was measured. Then it has to be contrasted with known concentration patrons (between 0 to 1000 mg COD L^{-1}) through a lineal calibration line.
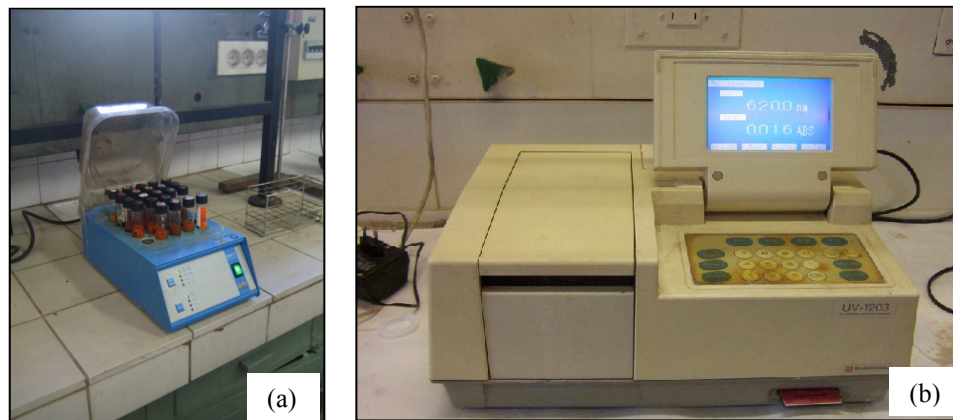
Figure 3.8: Experimental COD device (a) digester (b) spectrophotometer

- BOD_5 : It is related to the amount of biodegradable organic matter in a water sample. During oxidative degradation of organic matter, the aerobic microorganisms consume the dissolved oxygen present in water. BOD_5 was expressed as weight of consumed oxygen per unit volume of water during 5 days. For the evaluation of BOD_5 the WTW OxiTop measuring system (Weilheim, Germany) thermostated at 20ºC was used.

- Solids: The different types of solid analysis are explained below.
  - *Total solids (TS):* A sample was evaporated in a ceramic capsule that was weighted and then dried in an oven at 103-105 ºC. The weight increase respect to the empty capsule represents the total solids.

- Total volatile solids (TVS): The residue obtained from the last method was incinerated at 550 ºC. The solids evaporated represent the volatile solids and were found by difference of the final weight of the capsule with the one obtained in the last method.

- Total suspended solids (TSS): A sample was filtered through a standard filter of 0.45 μm. The remained residue was dried at 103-105 ºC. The weight increase respect to the empty filter represents the total suspended solids.

- Volatile suspended solids (VSS): The residue obtained from the last method was incinerated at 550 ºC. The solids evaporated represent the volatile solids and they were found by difference of the final weight of the filter with the one obtained in the last method.

- Sedimentation experiments: In these experiments the solid volumetric index (SVI), the sludge volume at 30 minutes (V30) and the sedimentation kinetic (Vs) were obtained. The sedimentation test was done in a 1 L Imhoff cone or in a calibrated 1 L test tube, where the evolution of the sludge with time was measured following the inter-phase liquid-solid during a period between 30-120 minutes. In Figure 3.9 an example of the graphic that can be extracted for a test like the one proposed is shown.
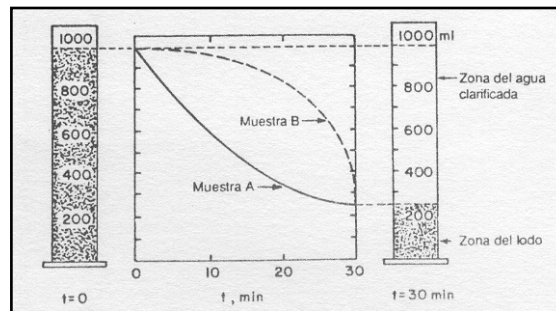
Figure 3.9: Sedimentation test

3.3 SUBSTRATE AND INOCULUM

Reject water was obtained from a mesophilic anaerobic sludge digester of a WWTP situated in the Barcelona metropolitan area. This effluent was centrifuged to remove suspended solids before its recirculation to the plant head. Supernatant was used as a substrate for the experiments and it was collected and kept at 4ºC in the laboratory until its treatment.

The inoculum (microorganisms) to be acclimated to nitrification/denitrification process was taken from the WWTP secondary biological aerobic reactor. This reactor was working with hydraulic retention time of 10 hours, solid retention time of 3-4 days and a biomass concentration around 1000 mg VSS L^{-1}. Once the inoculum was acclimated (Chapter-4) it was used in all the experiments developed in the laboratory in the different reactors.

The hydrolysed primary sludge and the other possible internal organic C-sources for denitrification (Chapter-6) were taken from the same WWTP and kept in the same conditions as reject water.

3.4 RESPIROMETRIC PARAMETERS

In order to characterise the biological degradation process in the optimum working cycles, the most relevant kinetic and stoichiometric parameters (Table 3.1) involved in both organic carbon and nitrogen removal were determined. Respirometry was the tool used to calibrate these model parameters (Vanrolleghem et al., 1999). Each parameter was experimentally determined at least three times and, concomitantly with the OUR monitoring, analysis of NH_4^+-N, NO_2^--N and NO_3^--N were also performed, in order to obtain more reliable experimental values.

Table 3.1: Kinetic and stoichiometric parameters studied

Parameter	Description	Units
Heterotrophic biomass		
Y_H	Yield	mg cell COD mg^{-1} COD consumed
μ_{mH}	Max. growth rate	day^{-1}
k_d	Lineal decay rate	day^{-1}
K_S	Substrate HS	mg COD L^{-1}
K_{OH}	Oxygen HS	mg O_2 L^{-1}
η_{no2}	DN corrector factor	-
Autotrophic biomass		
Y_{AOB}	AOB Yield	mg cell COD mg^{-1} NH_4^+-N consumed
μ_{mAOB}	AOB max. growth rate	day^{-1}
K_{NH}	Ammonia HS	mg NH_4^+-N L^{-1}
K_{OA}	Nitrification oxygen HS	mg O_2 L^{-1}

3.4.1. Maximum growth rate assessment and correction factor

There are two methodologies proposed in the literature to obtain the maximum growth coefficient. The one proposed by Kappeler and Gujer (1992) consists of adding a quantity of readily biodegradable substrate to endogenous biomass in a ratio $S_{to}/X_{to}= 4$. OUR increases until it is stabilised and then it begins to decrease. The maximum rate is obtained from the slope of the logarithmic OUR increase minus the decay rate of the microorganisms. This process is very sensitive to active biomass concentration inside the reactor, which sometimes could produce variations in the final maximum growth coefficient if one modifies the S_{to}/X_{to} ratio, as it was studied by Novak el al. (1994). The other methodology to obtain the maximum growth of biomass consists of the combination of parameters $\mu_{mH}X_{BH}$ by applying the so-called $S_{to}/X_{to}=1/200$ experiment for heterotrophic biomass (Spanjers and Vanrolleghem, 1995). This experiment was run at controlled temperature ($30ºC \pm 0.5$) and pH (8.0 ± 0.1). Average value of $\mu_{mH}X_{BH}$ in mg cell COD g^{-1} VSS h^{-1} was obtained knowing the yield and the decay of microorganisms. In the same way, the combined parameters $\mu_{mAOB}X_{BA}$ for the evaluation of maximum autotrophic growth rate for AOB were determined in which OUR, NH_4^+-N, NO_2^--N and NO_3^--N profiles were analysed.

Heterotrophic growth rate under anoxic conditions (denitrification) was evaluated considering the same Monod Kinetic expressions as used for aerobic conditions multiplied by a correction factor (η_{no2}, see Table 3.1). Correction factor is used because heterotrophic biomass shows a reduced substrate oxidation rate during anoxic conditions with respect to aerobic conditions, since only a fraction of the heterotrophic biomass is able to use nitrite or nitrate as electron acceptor under anoxic conditions (Orhon and Artan, 1994). This kinetic parameter was evaluated experimentally with the simultaneous operation of two batch reactors. One reactor was aerated and the other worked under absence of oxygen, but with nitrite as the final electron acceptor with an excess amount of external carbon source (methanol) (Figure 3.10). The correction factor (equation 3.3) was evaluated by comparison of the OUR and Nitrite Uptake Rate (NUR) values obtained through a mass balance (equations 3.1 and 3.2).

$$OUR_{EXOGEN} = -\frac{dS_O}{dt} = \frac{1-Y_H}{Y_H}\mu_{mH}X_{BH} \qquad (3.1)$$

$$\eta_{no2} = \frac{1.71 NUR_{NO_2 \to N_2}}{OUR} \qquad (3.3)$$

$$NUR_{NO_2 \to N_2} = -\frac{dS_{NO_2}}{dt} = \frac{1-Y_H}{1.71 Y_H}\mu_{mH}\eta_{no2}X_{BH} \qquad (3.2)$$
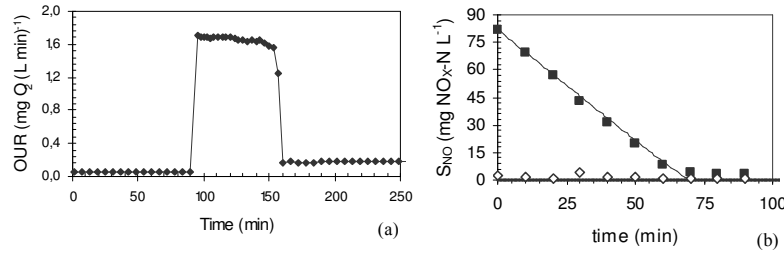
Figure 3.10: Experimental determination of anoxic correction factor for heterotrophic growth. Respirogram in (a) aerobic reactor and (b) N-NO$_X$ profiles inside anoxic reactor using nitrite as final electron acceptor: OUR ($\bullet$), NO$_2^-$-N ($\blacksquare$), NO$_3^-$-N ($\circ$)

3.4.2. Yield parameters assessment

The actual heterotrophic yield coefficient, Y$_H$ was evaluated through a respirometric batch experiment in which four different pulses of a completely biodegradable organic substrate were added to an endogenous activated sludge sample (Brands et al., 1994; Dircks et al., 1999; Vanrolleghem et al., 1999). Methanol was used in this experiment since it was the external COD added during the anoxic periods of the studied case. The plot of the cumulative respiration rate (oxygen consumed, OC) versus the added biodegradable organic substrate concentration, enabled the calculation of (1-Y$_H$) as the slope (see Figure 3.11a). This experiment was run at 30ºC and a pH value of 8.0 $\pm$ 0.1 with a low concentration of VSS, which provided a suitable low OUR that improved the heterotrophic yield assessment. Similarly, the actual autotrophic yield coefficient for

AOB (Y_{AOB}) was estimated from a respirometric batch experiment (30ºC, pH 8.0 ± 0.1, 200 mg VSS L^{-1}) in which three different pulses of ammonia were added to the endogenous activated sludge sample. Concentrations of ammonium, nitrite and nitrate were determined experimentally during the respirometric test. Consequently, the plot of OC versus added NH_4^+-N concentration yielded a straight line with (3.43- Y_{AOB}) as slope (Figure 3.11b). Y_H and Y_{AOB} can also be calculated with one big concentration pulse following the substrate reduction and calculating the OC with the areas of the OUR respirogram.

3.4.3. Decay coefficient assessment

The decay coefficient for heterotrophic biomass with lineal death, k_d, was determined following the protocol established by Marais and Ekama (1976). Sludge withdrawn from the lab-scale reactor was put into an aerated non-fed batch reactor. The endogenous OUR was measured over a period of several days. As shown in Figure 3.11c, the logarithm of the endogenous respiration rate versus time representation provides a straight line with the decay coefficient for heterotrophic biomass (lineal death), k_d, as slope (Sollfrank and Gujer, 1991).

3.4.4. Affinity constants for substrate and oxygen assessment

Oxygen affinity constants for heterotrophic biomass (K_{OH}) and ammonium oxidizers (K_{OA}) were also determined through a respirometric test in which the DO drop in a respiration chamber was monitored after the injection of substrate (as described by Guisasola et al., 2005). During the experiment, the system worked without neither external aeration nor substrate limitations. Oxygen affinity constants were estimated by fitting the experimental DO profiles to an oxygen Monod expression (see Figures 3.11d and e). On the other hand, half-saturation constants for organic substrate (K_S) and ammonium (K_{NH}) were determined by applying the method described by Cech et al. (1984) as shown in Figure 3.11 (f and g). This experiment consisted of the analysis of oxygen consumption rate at different substrate concentrations.

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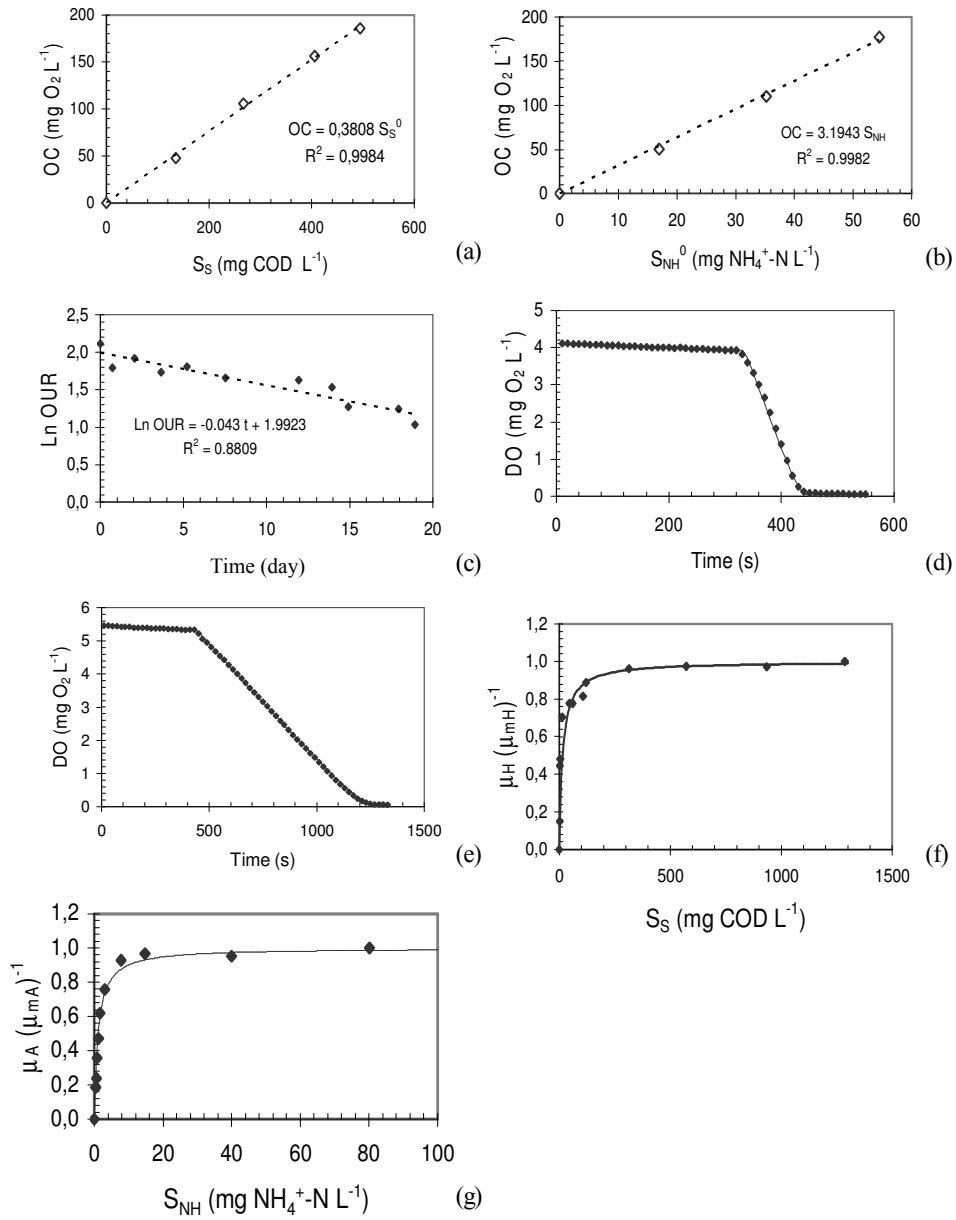
Figure 3.11: Experimental evaluation of model parameters, Y_H (a), Y_{AOB} (b), k_d (c), K_{OH} (d), K_{OA} (e), K_S (f) and K_{NH} (g).

4. START-UP OF A BIOLOGICAL SEQUENCING BATCH REACTOR

Scope

Considering the problem of reject water explained in Chapter 1, the starting up procedure of a SBR to develop biological nitrification/denitrification process is presented. The methodology would begin from the sludge adaptation to the SBR steady state operation for real reject water treatment. Once the acclimation of microorganisms is done, the development of the biological process will be carried out in a lab-scale SBR operating with 3 cycles a day. In every 8 hour cycle the anoxic denitrification phase (2 h) is placed after the aeration nitrification phase (5 h) using stoichiometric methanol as a readily biodegradable carbon source considering the non-biodegradable character of the COD of reject water. The volume exchange and sludge age during a cycle will be tested to reach a good steady state in the SBR to remove nitrogen.

4.1 INTRODUCTION

In recent years much effort has been put into developing tertiary treatments for the internal flows from WWTPs. It is usual for a WWTP to have several internal flows that may be highly polluted with different pollutants because a specific treatment has not been developed. An example of this is the supernatant from anaerobic sludge digesters in biological WWTPs as it has been stated in Chapter 1. This stream is recirculated to the head plant due its high ammonium concentration (Janus and van der Roest; Mossakowska et al., 1997; Wett et al., 1998; Ghyoot et al., 1999; Rostron et al., 2001; Arnold et al., 2000). Although it represents a small percentage of the overall wastewater flow rate in the WWTP, it affects negatively the concentration level of these pollutants in the plant outlet stream. Any increase in these concentrations may lead to legal emission limits being exceeded. Therefore, the treatment of reject water might be a very positive solution in WWTPs located in areas with more restrictive legislation.

As reject water flow rate is very low, a SBR may be an optimal reactor for treating this kind of wastewater. The main features of SBRs are their flexibility, which allows widely different ranges of concentration and streams to be worked with, and their compaction, which allows different sequences of treatment being done in the same tank (Ketchum, 1997; Artan et al., 2001, Macé and Mata-Álvarez, 2002).

4.2 RESULTS AND DISCUSSION

4.2.1 Nitrogen flow in the studied biological WWTP

An urban wastewater biological treatment plant of 200000 inhabitants' equivalent, with an anaerobic sludge digester will be considered. Figure 4.1 shows a scheme of this WWTP. There is a classically activated sludge system without nitrogen removal step (between flows Q6 and Q11) and an anaerobic sludge digester for the sludge withdrawals (between flows Q20 and Q21). The flow rates of each WWTP line (Q: m^3 day^{-1}) are also shown in Figure 4.1. The total flow rate in the plant is 49000 m^3 day^{-1} (Q3), and the reject water flow is 275 m^3 day^{-1} (Q21), which represents 0.6 % of the total water flow. The

incoming wastewater (Q0) has a NH_4^+-N influent concentration around 50-60 mg L^{-1}, which represents an average NH_4^+-N mass loading between 2300 ± 250 kg N day^{-1}.

Line	(m^3 day^{-1})	Line	(m^3 day^{-1})	Line	(m^3 day^{-1})	Line	(m^3 day^{-1})	Line	(m^3 day^{-1})
Q0	46300	Q6	47200	Q12	840	Q18	235	Q24	-
Q1	46300	Q7	64800	Q13	275	Q19	60	Q25	-
Q2	46300	Q8	46300	Q14	1107	Q20	295	Q26	-
Q3	49000	Q9	17600	Q15	2672	Q21	300	Q27	-
Q4	49000	Q10	16700	Q16	1565	Q22	-	Q28	-
Q5	49000	Q11	900	Q17	1800	Q23	33	Q29	-
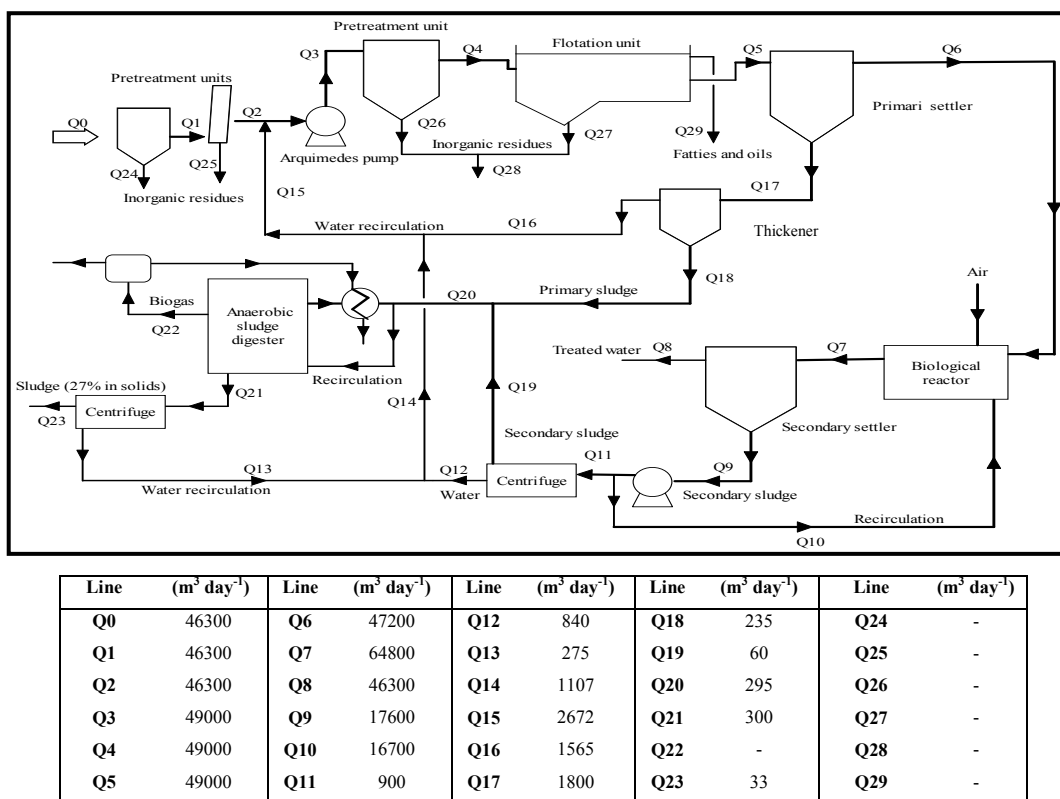
Figure 4.1: WWTP flux diagram and flow-rates (average values of 2004)

As stated above, one of the main contaminants of reject water is NH_4^+-N (800-1200 mg L^{-1}), which accounts around 15-25% of the total N loaded in the plant head. Due to this high concentration, reject water is recirculated to the plant head with other flows (Q15) contributing to increase N concentration in the plant effluent (Q8) because the WWTP does not have a nitrogen removal unit. Treatment of reject water could prove very positive because the supernatant flow-rate is very small and highly contaminated, which means that little equipment would be required. In addition, its rather high temperature -it comes from a digester operated at 35 ºC- will favour the kinetics of N removal. Treatment

of reject water generated in the anaerobic sludge digester would take place immediately after the centrifuge that is placed between flows Q21 and Q13 in Figure 4.1.

4.2.2 Wastewater characterisation

Table 4.1 shows the composition on an average basis of the reject water used in the experiments. The main contaminants are COD, TSS and NH_4^+-N. The bicarbonate to ammonium ratio (molar basis) was very low, which is in concordance with other reported values of reject water characterisation (Hellinga et al., 1999).

Table 4.1: Year average composition of the centrifuged sludge effluent (2004)

Component	Unit	Value	Component	Unit	Value
TSS	mg L^{-1}	700 ± 25	HCO_3^-	mg L^{-1}	3500 ± 500
COD	mg L^{-1}	1700 ± 300	HCO_3^-/N ratio	mol mol^{-1}	1 ± 0.1
NH_4^+-N	mg L^{-1}	850 ± 50	pH	-	8 ± 0.1
P-total	mg L^{-1}	19 ± 1	Temperature	ºC	35 ± 0.5

In order to assess the biodegradability of the reject water COD, two respirograms were evaluated (Figure 4.2). The first one was run at 30ºC and a pH range of 8.0 ± 0.1 and the second one at the same experimental conditions but with 12 mg L^{-1} of Allyl-Thiourea (ATU) as a nitrification inhibitor. In these experiments 150 mL of wastewater were added to 2.85 L of endogenous mixed liquor with 620 mg VSS L^{-1}. The quantity of biomass used for the experiments, coming from the acclimated lab-scale SBR, was relatively low in order to assess the best response of the activated sludge to the addition of wastewater. In Figure 4.2 it can be seen that wastewater includes three clear component fractions, a small readily biodegradable substrate fraction, the ammonium substrate fraction and a slowly biodegradable substrate fraction. As expected, its Biological Oxygen Demand at short time (BOD_{ST}) is very low and thus the COD of wastewater is not appropriate to denitrify.
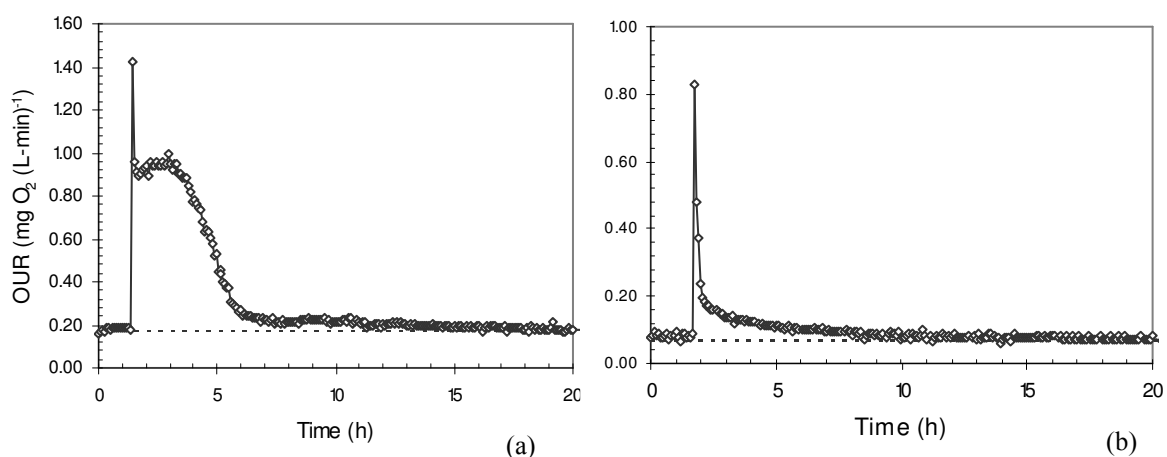
Figure 4.2: Reject water respirograms from Barcelona metropolitan area WWTP (a) without ATU
(b) with ATU addition for nitrification inhibition: Experimental data (-o-o-o-), endogenous OUR (--)

(Data used to do the respirograms can be found in the Annexes I.1 and I.2)

4.2.3 Start up

Biomass adaptation. The first step is the enrichment of biomass from the
WWTP secondary biological reactor in nitrifying/denitrifying organisms. In other words,
the inoculum used was mostly composed by heterotrophic bacteria, which remove
organic carbon aerobically, and a small part by autotrophic bacteria, which remove
ammonium in presence of inorganic carbon (Metcalf and Eddy, 1991). Even though
heterotrophic bacteria can also work under anoxic conditions, the change of electron
acceptor (NO_x^--N) is an important factor to consider an adaptation period. Considering
that the anaerobic digester works at 35 ºC, the SBR acclimation process and the following
treatment has been developed at 28 ºC in order to consider the probable temperature
loses.

pH influence on adaptation. The average percentage of nitrifying organisms
normally lies around 0.5-1 % in a mixed culture from a secondary activated sludge
reactor (Metcalf and Eddy, 1991) and, consequently, optimum environmental conditions
must be fixed in order to enhance their proliferation in the shortest possible period of

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time. According to this and considering that nitrifiers are very sensitive to pH fluctuations, different batch tests of 6 hour length, with an initial concentration of 90 mg NH_4^+-N L^{-1} in diluted reject water, were run. The pH influence was studied between 6 and 9.5 to determine the maximum nitrification activity of the inoculum (see Figure 4.3) with a tightly pH variation of $\pm$ 0.1 at 28 ºC. Even though the specific Ammonium Uptake Rate (sAUR) for the inoculum was always quite low (below 2 mg NH_4^+-N g^{-1} VSS h^{-1}) comparing to good acclimated nitrifying bacteria (Mossakowska et al., 1997), there was a high fluctuation between pH 6 and 9.5 placing its maximum at pH 8. Therefore, the acclimation of nitrifiers was carried out at a controlled pH (8.0 $\pm$ 0.1).
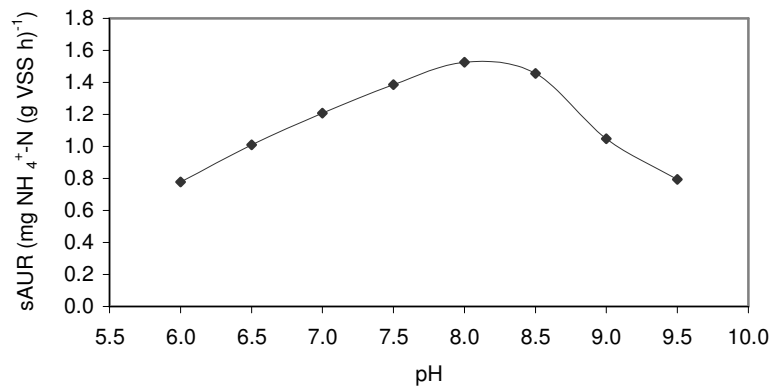
Figure 4.3: Activity of autotrophic bacteria with pH (All data can be found in Annexe I.3)

Since there is not an appreciable pH influence in the activity of heterotrophic bacteria if pH is maintained near neutrality (Orhon and Artan, 1994), no experiments were carried out to find the optimum pH value for developing the growth of denitrifying bacteria. Furthermore, kinetics for heterotrophic bacteria are higher than for autotrophic bacteria (Henze et al., 2000) and the seed used for the start-up was mainly composed by heterotrophic bacteria. Consequently, the acclimation of denitrifiers was not time limiting and pH was controlled within a range near neutrality (7-8.5).

Acclimation of nitrifying microorganisms. In order to develop the nitrifying microbial population, the inoculum was fed with increasing amounts of diluted reject water (150 mg NH_4^+-N L^{-1} as initial concentration, which did not represent substrate limitation) in the own 3L SBR. The system worked daily with two 12 hour cycle during one month at controlled pH value (8.0 ± 0.1), DO higher than 3 mg L^{-1} and with no sludge withdrawal. Nitrite and nitrate accumulation was prevented with the high exchanged volume in every cycle leading to a hydraulic retention time (HRT) of 0.75 days.
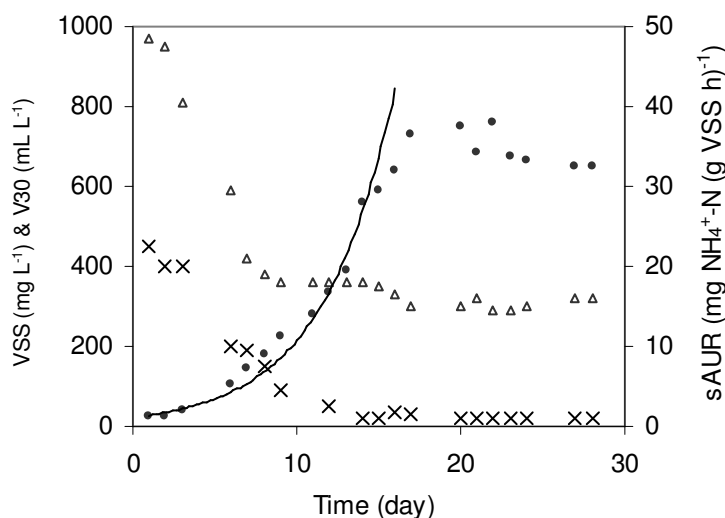
Figure 4.4: Nitrification acclimation: VSS (Δ), sAUR ($\bullet$), V30 ($\times$), Exponential growth (—)

The growth of nitrifiers can be seen in Figure 4.4 where the evolution of NH_4^+-N removal (sAUR) follows an exponential profile until it reaches a maximum value of 30-32 mg NH_4^+-N g^{-1} VSS h^{-1} around the days 17-20. Then, kinetics are stabilised as also did biomass concentration (expressed in units of VSS concentration). The product of nitrification was a mixture of NO_2^--N and NO_3^--N due to the fixed pH and the high ammonium concentrations used (Anthonisen et al., 1976). Two other important facts can be extracted from Figure 4.4: the first is that there is a 65% of biomass reduction showing the low presence of autotrophic bacteria. The second is that, although there is a presence over 300 mg VSS L^{-1}, the sedimentability is quite good with a V30 between 20 and 50

mL sludge L^{-1}, which is a characteristic fact of autotrophic microorganisms due to the lack of filamentous bacteria (Metcalf and Eddy, 1991).

Acclimation of denitrifying microorganisms. The inoculum for each denitrification test was taken from the WWTP like in the nitrification acclimation. In the case of denitrification, the acclimation experiment lasted 15 days and it was done for nitrite and nitrate separately in 2 SBR of 1 L.

Figure 4.5: Denitrification acclimation using (a) NO_2^--N and (b) NO_3^--N as electron acceptor: VSS (Δ), sNUR ($\bullet$), V30 ($\times$), Exponential growth (—)

(Data and calculations used for acclimation processes can be found in Annexes I.4 and I.5)

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The initial concentration of NO_2^--N and NO_3^--N in every daily experiment was 100 mg NO_x^--N L^{-1} and it was mixed with 100 mL of reject water to provide nutrients to the system. Two 12-hour cycles were followed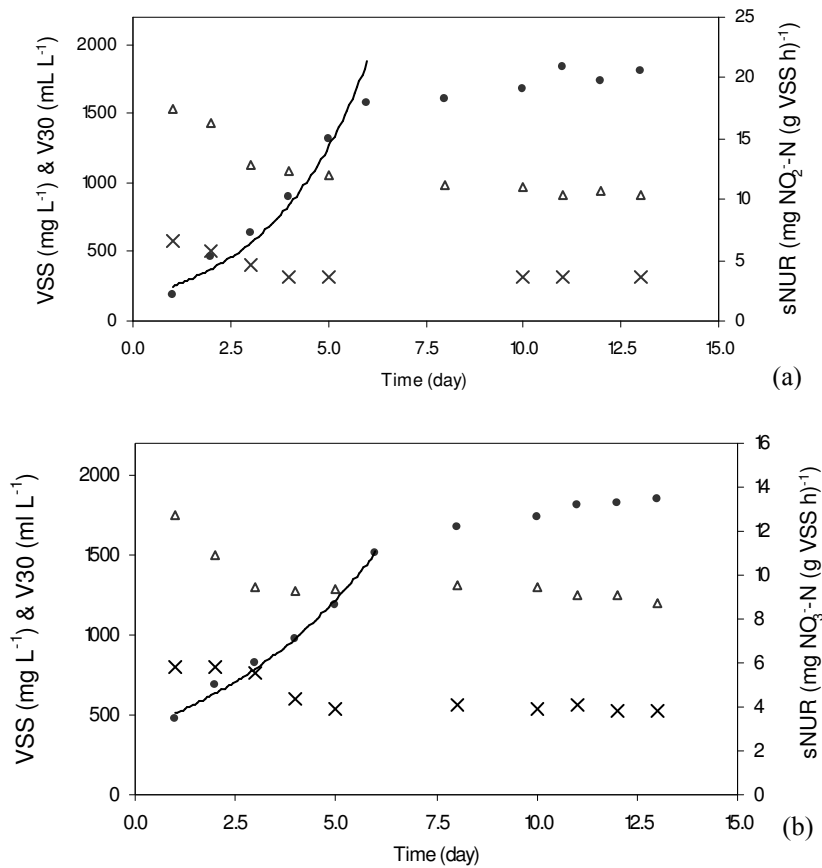 controlling the pH in range 7-8.5. Methanol was added in excess (twice the stoichiometric ratio) as an electron donor to avoid substrate limitations. Figures 4.5a and 4.5b show that the acclimation profiles for both compounds follows an exponential increasing activity during 6-7 days until the stationary stage is reached. However, the final value reached using nitrite as electron donor (20 mg NO_2^--N g^{-1} VSS h^{-1}) is larger than when NO_3^--N is used (14 NO_3^--N g^{-1} VSS h^{-1}). This could be explained because in denitrification process, the slower reaction is the first step (Abeling and Seyfried., 1992). This fact is corroborated as no NO_2^--N accumulation was found when denitrification with NO_3^--N was done.

Considering the sedimentability evolution, it can be seen from Figures 5a and 5b a value of V30 placed around 400 mL sludge L^{-1} for both, showing that the heterotrophic organisms do not have such good sedimentability properties as the autotrophic ones. The losing of biomass in both cases is around 35% which means that not all the heterotrophic bacteria can work in a facultative way.

4.2.4 SBR operation

Once the nitrification and denitrification microorganisms of the three tests were acclimated, they were centrifuged, mixed and introduced in the 3L SBR to start the biological process giving an initial VSS concentration of 1100 mg VSS L^{-1} for the treatment of reject water considering the following starting parameters:

- Considering previous test reported in the literature (Obaja et al., 2003) the SBR was operated with consecutive 8 hour cycle. Each cycle consisted of five stages: aerobic fill (0.25 h), aerobic (5 h), anoxic (2 h), settle (0.5 h) and draw (0.25 h). The N/DN time ratio was set around 2.5 because the nitrifying growth coefficient is nearly 2-3 times smaller than the denitrifying ones (Metcalf and Eddy, 1991). At the beginning of the anoxic period, the stoichiometric amount of external organic carbon (methanol) was added in order denitrification to take place.

- According to the wastewater characterisation, nitrification kinetics and previous experiments, HRT was fixed around 1.3 days. Lower values did not achieve complete N removal in 3 cycles per day.
- Some authors have reported the optimal SRT for a system like this to be between 11 and 30 days (Bortone et al., 1992, 1994) and, consequently, the operating SRT was fixed around 15 days.
- Temperature was kept at a constant level, namely, 28 ºC and pH was controlled inside a pH range of 7-8 by means of an alkalinity dosage system connected to the pH electrode.

4.2.5 Steady state cycle

Table 4.2 shows the operational conditions in the 3 L SBR reactor when the steady state was reached after 1 month.

Table 4.2: Steady state operational parameters and cycle stages

Parameter	Units	Value
Number of cycles a day	-	3
Cycle time	min	480
Internal cycle times		
Nitrification	min	300
Denitrification	min	120
Sedimentation	min	30
Pump operating	min	30
HRT	day	1.3
SRT	day	15
Temperature	ºC	28
pH range operation	-	7-8

Reject water N concentration fed to the SBR reactor was around 900 mg NH_4^+-N L^{-1}. Thus, considering the working parameters explained above, every time the cycle started, 0.75 L of effluent wastewater was withdrawn from the reactor and the same volume of reject water was filled. Therefore, the initial concentration inside the SBR was around 250 mg NH_4^+-N L^{-1}. Figure 4.6 presents the N profiles in the SBR during a cycle under

the working conditions of Table 4.2. It can be seen that there is a complete removal of NH_4^+-N during the 5 hour of aeration in which nitrification takes place with a biomass concentration of 3500 mg VSS L^{-1}. However, this NH_4^+-N removal is not due to a total NO_3^--N formation, as most of the ammonium is converted into NO_2^--N giving and a sAUR around 14 mg NH_4^+-N g^{-1} VSS h^{-1}. Moreover, a small part of the removed NH_4^+-N is used to produce new cellular material.
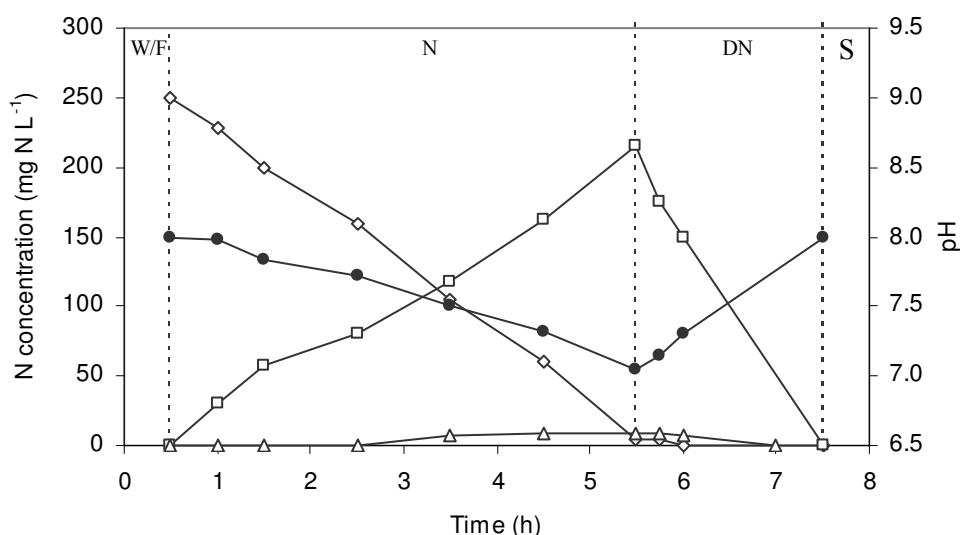
Figure 4.6: Nitrogen and pH profiles during one 8 hour cycle: NH_4^+-N (-◊-), NO_2^--N (-□-), NO_3^--N (-Δ-), pH (-●-), Exponential growth (—), Stage Delimitation (---); (W/F: withdraw and feed stage, N: Nitrification, DN: Denitrification, S: Sedimentation)

There are two reasons that could explain the inhibition of the second step of nitrification: temperature and pH combined with high ammonium concentrations. The second stage of nitrification takes place completely at temperatures below 20ºC, and if temperature increases, nitrate formation begins to be inhibited but this is only achieved with SRT below 2 days (Hellinga et al., 1999; van Dongen et al., 2001). Considering that the studied system works with a SRT of 15 days, pH is the key variable, because if there is unionised ammonia (NH_3) dissolved in the system at a concentration above 1 mg L^{-1},

the NO_3^--N formation begins to be inhibited (Anthonisen et al., 1976; Abeling and Seyfried, 1992). As it has been pointed out above, the working pH range of the process is between 7 and 8.5, and at this pH, ammonia is very likely to be present at concentrations up to 1 mg L^{-1} due to the high ammonium concentrations.

Denitrification process was also carried out correctly and all the NO_x^--N formed (approximately 225 mg L^{-1}), mainly NO_2^--N, was removed and transformed into nitrogen gas (N_2) giving a specific Nitrite Uptake Rate (sNUR) around 30 mg NO_2^--N g^{-1} VSS h^{-1}. The nitrate inhibition has two important economic benefits: to save the 25% of oxygen consumption and to save the 40% of organic carbon source addition for denitrification considering the reaction stoichiometry. Finally, Figure 4.6 also shows pH evolution during the same cycle where pH decreased during nitrification because the reaction is alkalinity consuming. At the end of the aerobic stage, an external addition of HCO_3^- was needed. In contrast, during denitrification, pH increased because denitrification produces alkalinity.

Once the start up was done, a sedimentation test similar to the ones done in the acclimation periods was done as it is shown in Figure 4.7. Three important parameters can be extracted from the graphic. The sedimentation velocity is obtained from the slope of the first period (0.6 m h^{-1}). Moreover the sludge volume at 30 minutes (V30) is 300 mL and the solid volatile index (SVI) is obtained dividing V30 by the biomass concentration inside the reactor (3.5 g L^{-1}) giving a final SVI of 78 mL g^{-1}.

With these values one can realise that the sedimentation properties of the biomass are good and the system is not going to present settling problems. At this point the SBR reactor was already operating with a good steady state but it was not working with its optimal conditions. In other to achieve the best operational conditions modification of HRT, SRT and internal pH control would be needed. Moreover the possible substitution of methanol by an internal carbon source of the own WWTP to avoid the external COD addition would be a feasible solution for further research (explained in Chapter 6). The influent to the WWTP with 850 mg COD L^{-1} (Q1 in Figure 4.1) and the hydrolysed primary sludge from the thickener with 3000 $COD_{soluble}$ L^{-1} (Q18 in Figure 4.1) would be the ones more feasible to be considered.
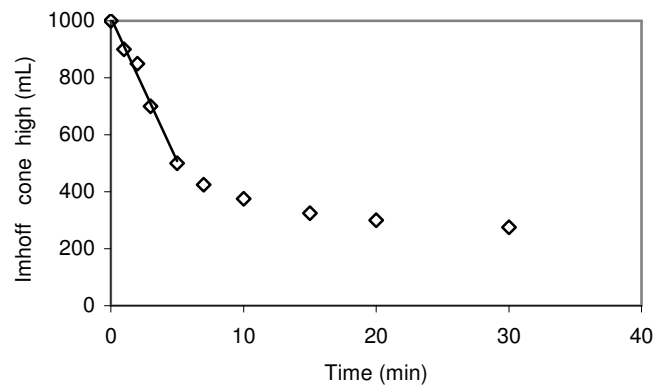
Figure 4.7: Settling experiment of the mixed liquor biomass

4.3 CONCLUSIONS

- ✓ A start up of a SBR was studied from the sludge acclimation to the steady state treating real reject water that included mainly high ammonium concentration and refractory COD.

- ✓ Nitrification and denitrification had different behaviours in acclimation period. The first had an exponential phase that lasted 20 days with a sAUR of 30-32 mg NH_4^+-N g^{-1} VSS h^{-1}. The second was quick lasting 6-7 days with a kinetic at the stationary stage of 20 mg NO_2^--N g^{-1} VSS h^{-1} and 14 mg NO_3^--N g^{-1} VSS h^{-1} showing that the denitrification of nitrate is the bottle neck of the anoxic process.

- ✓ The nitrification/denitrification process in the SBR studied gave efficiencies of nitrogen removal over 95%. Conversion rates of 14 mg NH_4^+-N g^{-1} VSS h^{-1} in nitrification and 30 mg NO_2^--N g^{-1} VSS h^{-1} in denitrification were achieved.

- ✓ Consequently, such a treatment could be very satisfactory for meeting local discharge requirements. Although the laboratory experiments were performed with methanol, the estimations advice to use an internal organic carbon source from the WWTP in order to reduce the operational costs in an industrial SBR.

5. OPTIMISATION OF N/DN PROCESS IN A SBR FOR REJECT WATER TREATMENT

Scope

Considering the results obtained in the acclimation period in Chapter 4, the aim is to perform a study in order to optimise the working conditions inside the SBR to develop biological nitrogen removal via nitrite.

With this purpose, different sequences of treatment are tested to reach the optimal removal of pollutants inside the SBR, controlling parameters such as cycle length, temperature, pH, DO, sludge age and hydraulic retention time. Different external organic carbon sources are tested as readily biodegradable sources for denitrification

5.1 INTRODUCTION

Nitrification/denitrification process has been presented as one effective solution to treat supernatant effluents from anaerobic sludge digesters (reject water) in biological wastewater treatment plants in order to remove its high nitrogen content (Janus and van der Roest, 1997; Wett et al., 1998; Arnold et al., 2000; Rostron et al., 2001) as it has been also shown in Chapter 4.

SBRs are one of the main reactors to treat low flow-rates with high concentration. Treatment efficiency varies depending on the operational conditions (temperature, DO, pH and alkalinity availability) and the strategy implemented in the SBR for N/DN. Working with short sequences implies a more compacted installation, more sedimentation periods and less concentration inside the reactor. On the other hand, a very large sequence implies less sedimentation periods, big installation and high concentration of contaminants at the beginning of each cycle.

As it has stated in Chapter 1, from an economic point of view it is better to develop biological nitrogen removal (BNR) via nitrite since the inhibition of nitrate formation has two important benefits: the saving of 25 % of oxygen consumption and 40% of organic carbon source in denitrification. DO, pH, and temperature are the key parameters that favour one of the two nitrification reactions to predominate over the other (Hellinga et al, 1998; Pollice et al, 2002; van Dongen et al, 2001).

Finally water alkalinity is another important parameter in nitrification. Reject water is characterized by a low bicarbonate to ammonium ratio (Hellinga et al., 1999). Since denitrification supposes a partial recovery of the alkalinity consumed during nitrification, alternation of aerobic/anoxic sub-cycles could represent a good solution to avoid the use of external alkalinity addition.

5.2 RESULTS AND DISCUSSION

5.2.1 Reject water characterisation

Table 4.1 (Chapter 4) showed the composition on an average basis of the reject water used in the experiments where the main contaminants were refractory COD, TSS and NH_4^+-N. Moreover the bicarbonate to ammonium ratio was very low, which is in concordance with other reported values of reject water characterisation (Hellinga et al., 1999).

5.2.2 pH and temperature effect

The influence of temperature and pH was studied for autotrophic and heterotrophic microorganisms. Aliquots (250 mL) of endogenous nitrifying/denitrifying biomass were adjusted at the desired pH set-point and the working temperature. Once the withdrawals of mixed liquor from the steady state SBR described in Chapter 4 were inserted into the respiration chamber (Figure 3.2), the DO concentration slowly decreased giving the endogenous OUR (OUR_{END}). After several minutes, 5mL of a previously pH and temperature controlled substrate solution (ammonium chloride for nitrifying biomass or sodium acetate for heterotrophic biomass) were injected to the mixed liquor and a subsequent sudden drop in the DO profile was observed due to microbial substrate consumption. Thus, exogenous OUR (OUR_{EX}) could be determined. Since OUR_{EX} is proportional to the maximum growth rate, relative biomass activity can be calculated as the ratio between OUR_{EX} for every studied condition and the maximum OUR_{EX} of the experiment. Therefore, for the optimum pH condition, relative biomass activity is 1. When the optimum pH value was evaluated, the same procedure was carried out at this pH condition but changing the temperature set point. These experiments were run with 450 mg VSS L^{-1}, which provided a suitable low OUR that improved the OUR_{EX} profile assessment, since the pH did not change significantly due to relatively low substrate consumption. Substrate concentration inside the respiration chamber was sufficiently high (75 mg NH_4^+-N L^{-1} or 200 mg COD L^{-1}) to avoid substrate limiting growth rates.

pH. Two representative relative microbial activity profiles for heterotrophic and autotrophic biomass versus pH are presented in Figure 5.1(a) and (b) respectively, which are in concordance with other reported values (Grunditz and Dalhammar, 2001; Orton and Arhan, 1994). The experimental profile clearly shows that nitrifying biomass activity is more affected by pH than heterotrophic biomass and both maximum growth rates are reached at a pH region near 8.0.

(a)

(b)
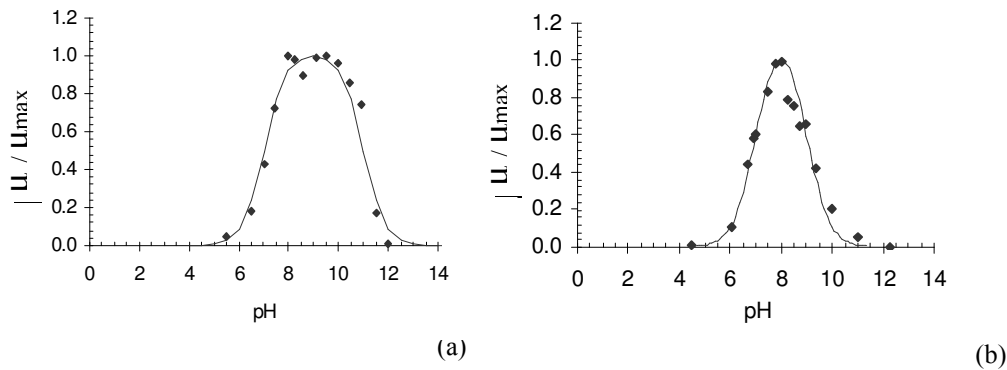
Figure 5.1: Effect of pH (a) in heterotrophic and (b) autotrophic biomass (Dosta et al., 2005)

Temperature. Respiration rates of autotrophic and heterotrophic biomass between 10 and 50 ºC were tested in the respirometric device to analyse the effect of the temperature upon the process kinetics. pH was maintained at 8.0 ± 0.1.

(a)

(b)
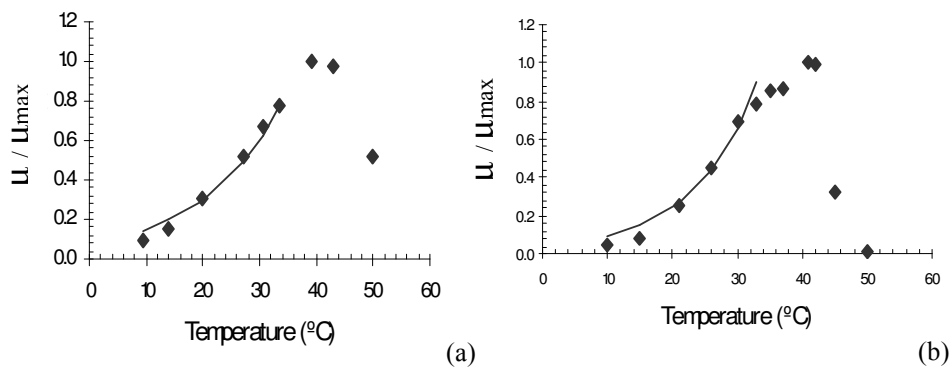
Figure 5.2: Temperature effect (a) in heterotrophic (b) autotrophic biomass (Dosta et al., 2005)
(Data to calculate Figures 5.1 and 5.2 can be found in Annexes II.1 and II.2)

Figure 5.2 shows the autotrophic and heterotrophic activity ratio for all the tested temperatures showing that the best removal efficiencies are placed between 30-40 ºC. Considering that the mesophilic anaerobic sludge digester works at 35 ºC, the proposed working temperature is 32 ºC, thus avoiding a heating system.

5.2.3 Starting up of the SBR strategy

A batch test with acclimated biomass (Chapter 4) was carried out in order to verify the N/DN kinetics. Nitrification was concluded to be around 2-3 times slower than denitrification when using a readily biodegradable COD source. This study corroborates the experimental kinetics reported in literature (Metcalf and Eddy, 2001).

The HRT initially chosen to start the process was around 1 day. The SRT mainly used during this study was reduced to 11 days in order to achieve younger bacteria that will lead to better kinetics. Temperature was kept at a constant value of 32 ºC, biomass concentration was 2500 ± 250 mg VSS L^{-1}, pH remained between 7.5 and 8.5 and DO during aerobic phases was maintained below 1 mg L^{-1} in order to assure N/DN via nitrite process. At the beginning of denitrification phase, an external biodegradable organic carbon source (namely, acetate, acetic acid or methanol) was added due to the lack of readily biodegradable COD in the reject water (Figure 4.2).

5.2.4 Optimising the operational cycle via nitrite

In order to find the best cycle sequence to optimise the process, SBR cycles of 2, 4, 6, 8 and 12 hours were tested until the N/DN steady state was reached with the acclimated biomass of Chapter 4.

Each cycle sequence lasted more than 2 months and sodium acetate was used for denitrification. Nitrification kinetic is compared in Figure 5.3 for every studied cycle length in terms of sAUR. The best efficiencies were obtained in 8 and 12 h cycle with sAUR of 20-21 mg NH_4^+-N g^{-1} VSS h^{-1}. Below 8 hour cycle, biomass activity increases with cycle length rising, and over 8 hour cycle biomass activity is less increased showing a free ammonia inhibition.
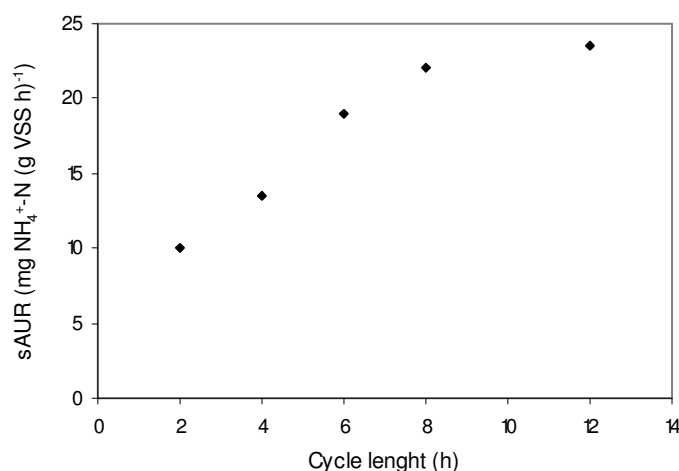
Figure 5.3: Variation of sAUR with cycle length in the SBR (Galí, 2004)

Presence of free ammonia is related to two factors (Anthonisen et al., 1976): pH and NH_4^+-N. When the length of the cycle is increased, higher pH and $N-NH_4^+$ concentration inside the SBR are present, which leads to more free ammonia in the medium that eventually can inhibit the process performance. In shorter cycles it is shown that the dilution effect of pollutants inside the reactor affects the kinetics of the process, obtaining less removal efficiency. Another disadvantage of working with short cycles is that the overall settling time increase with respect to larger periods. Thus, as a safe operating a cycle length of 8h was selected (Further information for this experiment can be found in Galí, 2004).

As the wastewater had not enough alkalinity to develop the overall nitrification reaction without external control, a good SBR strategy could avoid the addition of external chemicals to control the alkalinity level. Three different 8 hour cycle strategies were tested (5 h nitrification; 2.25 h denitrification) varying the number of internal aerobic/anoxic sub-cycles and using sodium acetate as external carbon source. Each cycle sequence was operated during approximately 1-2 months and the average sAUR obtained once steady state was achieved is presented in Table 5.1. The use of three internal sub-

cycles made the control of pH unnecessary, due to the partial alkalinity recovery during denitrification. Moreover, this strategy prevents nitrite accumulation inside the SBR which could lead to a nitrification rate inhibition and improves 15 % the nitrification efficiency.

Table 5.1: Cycle sequences with different sub-cycles using acetate for denitrification

	sAUR	pH control
	mg NH_4^+-N g^{-1} VSS h^{-1}	
1 sub-cycle	17 ± 1	Yes
2 sub-cycles	18 ± 1	Yes
3 sub-cycles	22 ± 1	No

(Profiles of these experiments can be found in Annexe II.3)

In that point different organic carbon sources (acetate, acetic acid and methanol) were tested in the 8 hour N/DN cycle with the 3 aerobic/anoxic sub-cycles. The three carbon sources tested obtained similar efficiencies in nitrification but acetate was little better than the other two in denitrification step as it is presented in Table 5.2.

Table 5.2: Cycle sequences working with different organic sources in denitrification stage

	C/N	sAUR	sNUR	Cost
	g C g^{-1} NO_2^--N	mg NH_4^+-N g^{-1} VSS h^{-1}	mg NO_2^--N g^{-1} VSS h^{-1}	€ kg^{-1}
Acetate	3.20	17 ± 1	50 ± 2	0.2
Acetic acid	2.40	18 ± 1	42 ± 2	0.075
Methanol	1.67	21 ± 1	42 ± 2	0.18

(Further information for this experiment can be found in Galí, 2004)

Therefore, the economical reason would be the best selection criteria. Methanol is the one that has lower C/N ratio but acetic acid is the cheapest. Considering this it seems that the latter would be the selected but if acetic acid is used it would imply extra alkalinity addition to buffer the solution. Therefore and considering the economical factors it was concluded that methanol would be more useful for a full scale reactor to treat reject water. In addition it prevents a sudden and eventual pH increase.

Table 5.3: Steady state operational parameters and cycle stages

Parameter	Units	Value
Number of cycles a day	-	3
Cycle time	min	480
Internal cycle times		
Nitrification	min	300
Denitrification	min	135
Sedimentation	min	30
Pump operating	min	30
HRT	day	1
SRT	day	11
Temperature	°C	32 ± 0.5
VSS	mg L^{-1}	2500 ± 250
N° sub-cycles	-	3
pH range operation	-	7-8.5
pH control	-	no
DN C-source	-	methanol

The 8 hour optimised cycle using methanol in each denitrification step is shown in Figure 5.4. Nearly 300 mg L^{-1} of NH_4^+-N were removed and transformed to NO_2^--N giving and HRT around 1 day under the operational conditions of Table 5.3.
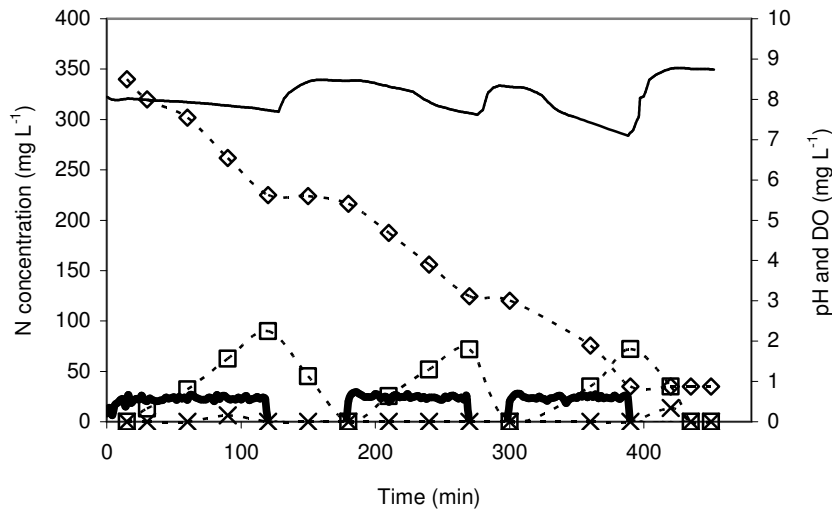
Figure 5.4: Experimental concentration and pH profiles inside SBR: NH_4^+-N (--◊--), NO_2^--N (--□--), NO_3^--N (--x--), pH (solid line) and DO (bold solid line)

Controlling the pH range within 7.5-8.5 with sub-cycles and maintaining the DO below 1 mg L^{-1} during aerobic periods was demonstrated to be a good way to develop N/DN via nitrite. Moreover, this strategy implies working conditions with low nitrite concentrations inside the reactor which avoids toxicity inhibition of nitrite. With these conditions the reached average total nitrogen removal efficiency is around 0.8-0.9 kg N day^{-1} m^{-3}.

Another important aspect to consider is the COD evolution inside the SBR in order to see if there is methanol accumulation.
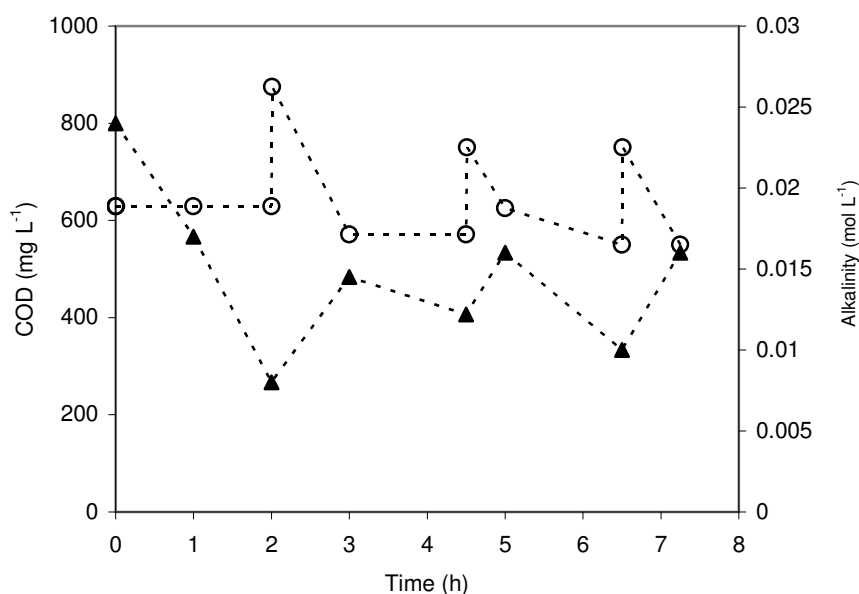
Figure 5.5: Experimental COD (--o--) and alkalinity (--▲--) profiles inside SBR

Looking at Figure 5.5 one can see that there was a complete removal of methanol in the denitrification steps. Moreover, considering the wastewater characterisation there was more than 50 % of slowly biodegradable COD removal inside the reactor due to long aeration and dilution effect. In Figure 5.5 it is also shown the alkalinity profile of the 8 hour cycle where there was a good alkalinity recovery in denitrification steps.

5.3 CONCLUSIONS

- ✓ Reject water treatment with SBR appeared to be a very satisfactory alternative for meeting local discharge requirements.

- ✓ Since sAUR was assessed for every studied case, it was demonstrated that the kinetics increase with temperature until 37ºC. The influence of the cycle length was also studied providing an optimum efficiency for 8 hour time cycle.

- ✓ In order to avoid the use of external additives to control the pH in an optimum interval (7.5-8.5), the best strategy consisted of alternating different aerobic-anoxic sub-cycles during the operational cycle with methanol as a carbon source.

- ✓ The low nitrite concentration formed due to the sub-cycles strategy contributed to improve the SBR performance due to the lack of toxicity inside the reactor.

- ✓ The no nitrate formation was achieved correctly combining the pH range and the low dissolved oxygen concentration inside the reactor.

6. USE OF HYDROLYSED PRIMARY SLUDGE FOR DENITRIFICATION IN A SBR

Scope

It has been seen in the previous chapters that running a biological process to treat real reject water needs an external addition of organic carbon. Considering that the treatment would be placed in the own WWTP it is interesting to find an internal carbon source in order to substitute methanol in the denitrification step. According to this, the aim of this Chapter is to study the denitrification capacity of several organic internal C-sources (including hydrolysed primary sludge) from a real WWTP with anaerobic sludge digester. Then, the biological treatment of real reject water in a SBR working with the selected C-source in the denitrification step is tested considering the operational optimised conditions of Chapter 5.

6.1 INTRODUCTION

The treatment of reject water has been presented in the previous chapters as a successful solution to reduce nitrogen discharge in the plant exit. The addition of an organic carbon source in denitrification leads to a partial recovery of the alkalinity consumed during nitrification. This fact makes possible the development of N/DN properly considering that reject water is characterized by low bicarbonate to ammonium ratio (Hellinga et al., 1999). Normally, methanol or acetate are the organic carbon sources used to denitrify (Chapter 5) when there is a lack of available organic carbon. But the main problem of this addition resides in its cost. However, in a WWTP it would be possible to find a useful carbon source to substitute the chemicals. Different studies have been done to find out a useful C-source to denitrify the main line of a WWTP (Esoy and Odegaard, 1994; Canziani et al., 1995) showing the hydrolysed primary sludge as the most appropriate. Although there is not a specific work focused on the direct application of these sources for reject water treatment, their conclusions can be taken into account.

The VFA found in the soluble COD of that hydrolysed primary sludge is the powerful fraction to be used for denitrification (Esoy et al., 1998). Different methods to hydrolyse the primary sludge are widely known like the thermal hydrolysis (Barlindhaug and Odegaard, 1996), biological hydrolysis (Esoy and Odegaard, 1994; Canziani et al., 1995) or chemical hydrolysis with acid and alkali (Aravinthan et al., 2001). In biological hydrolysis the fraction of readily biodegradable COD is higher than in the other two, but the degree of solubilization is lower (Esoy and Odegaard, 1994). The characteristics of the hidrolysate can vary considerably but in mixed chemical/biological sludge from a WWTP the degree of solubilization of biological hydrolysed sludge is around 11% (Esoy et al., 1994) where the fraction of VFA of the soluble COD is between 60-70 % (Esoy and Odegaard, 1994; Canziani et al., 1995). Using the hidrolysate of the primary sludge in denitrification is also positive when there is an anaerobic zone for phosphorus removal in the reactor (Esoy et al, 1998). But one has also to consider that the hydrolysed sludge contains a slightly high concentration of ammonium (Esoy and Odegaard, 1994; Canziani et al., 1995) which would be discharged in the reactor. If the WWTP has a two phase

anaerobic sludge digester another option would be the use of the supernatant from the acid phase digester (Elefsiniotis et al., 2004).

6.2 RESULTS AND DISCUSSION

6.2.1 Internal organic C-sources selection

Figure 4.1 showed a scheme of the biological WWTP under study. First of all, the water is driven to a primary treatment (Q3 to Q6) followed by a conventional activated sludge system without nitrogen removal step (between flows Q6 and Q11). Moreover, the WWTP has a one phase anaerobic sludge digester for the treatment of primary and secondary sludge (between flows Q20 and Q21). The flow rates (Q: m^3 day^{-1}) of each WWTP stream are also detailed in Figure 4.1. The total flow rate in the plant is 49000 m^3 day^{-1} (Q3), and the supernatant of the anaerobic sludge digester (reject water) flow is 275 m^3 day^{-1} (Q13), which represents a 0.6 % of the total water flow. Due to this high ammonia concentration, reject water is recirculated to the plant head with other flows (Q15) and this contributes to an increase of nitrogen concentration in the plant effluent (Q8) because the WWTP does not have a nitrogen removal unit.

Considering the diagram and flow rates shown in Figure 4.1 there are different organic carbon sources that could be used for denitrification step in reject water treatment apart from the hydrolysed primary sludge of the thickener (Q18). First of all, it has to be verified if the own reject water (Q13) could have some biodegradable COD that has not been treated in the anaerobic digestion. The wastewater that enters to the WWTP (Q1) and the influent to the secondary biological reactor (Q6) have a considerable part of biodegradable COD but it should be evaluated if their use would be feasible. Finally, the biological hydrolysis of the sludge that takes place at the bottom of the secondary clarifier (Q11) could also be useful to denitrify.

6.2.2 C-sources characterisation

Table 6.1 shows the composition on an average basis of all the organic sources tested where the main contaminants analysed are COD, BOD$_5$, VFA, N and the solids.

Table 6.1: Characterisation of different C-sources (average values January-April 2006)

Parameter	Units	Reject water	Influent WWTP	Influent Biological Reactor	Primary hidrolysate	Secondary hidrolysate
Line	-	Q13	Q1	Q6	Q18	Q11
Flow-rate	$m^3 \, day^{-1}$	275	46300	47200	235	900
COD_{total}	$mg \, L^{-1}$	1300 ± 100	900 ± 100	450 ± 50	60000 ± 1000	1900 ± 150
$COD_{soluble}$	$mg \, L^{-1}$	300 ± 50	240 ± 50	75 ± 20	3000 ± 200	55 ± 5
VFA (COD)	$mg \, L^{-1}$	0	0	0	2000 ± 200	0
BOD_5	$mg \, L^{-1}$	120 ± 20	400 ± 20	250 ± 20	-	-
$N\text{-}NH_4^+\text{-}N$	$mg \, L^{-1}$	650 ± 50	60 ± 10	50 ± 10	180 ± 10	35 ± 5
$N\text{-}NO_2^-\text{-}N$	$mg \, L^{-1}$	< 5	< 5	< 5	30 ± 3	< 5
$N\text{-}NO_3^-\text{-}N$	$mg \, L^{-1}$	< 5	8 ± 2	8 ± 2	20 ± 2	10 ± 1
TS	$mg \, L^{-1}$	2750 ± 50	2350 ± 50	1800 ± 50	35000 ± 1000	3500 ± 50
TVS	$mg \, L^{-1}$	930 ± 50	750 ± 50	325 ± 50	30000 ± 1000	2200 ± 50
TSS	$mg \, L^{-1}$	600 ± 50	575 ± 50	120 ± 20	35000 ± 1000	2200 ± 50
VSS	$mg \, L^{-1}$	600 ± 50	575 ± 50	120 ± 20	28000 ± 1000	2000 ± 50
pH	-	8.3 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	6.2 ± 0.1	7.3 ± 0.1

Reject water (Q13). Usually, reject water contains refractory COD but considering the quantity of COD shown in Table 6.1 it has to be verified that is not useful for denitrification because it would be the most economical organic carbon source in the WWTP. However, the low values of BOD_5/COD and BOD_5/N that could be calculated from Table 6.1 demonstrated a low quantity of biodegradable COD which leads to a low denitrification capacity (5-6%).

Influent flow-rate (Q1) and secondary biological reactor influent (Q6).
Considering, as reported in Table 6.1, the amount of BOD_5 in the plant influent (Q1) and also in the influent to the biological reactor (Q6) it is clear that both streams would be good for denitrification. The main problem does not lay in the quality of the wastewater COD but in the quantity of this water that would have to be added to the reactor treating reject water. From stoichiometric balances it is deduced that it would be necessary to add around 430 kg biodegradable COD per day for denitrification step of reject water via

nitrite in the SBR. Considering the BOD_5 values of Table 6.1, this would lead to by-pass 950 m^3 day^{-1} of Q1 or 1700 m^3 day^{-1} of Q6. The latter fact would imply to discard this option from the economic point of view because it would mean a reactor to treat reject water 4-6 times larger than the SBR operating with methanol. Moreover, when by-passing such a quantity of wastewater the treatment temperature will drop affecting the process kinetic. Finally, the capacity to denitrify the main line of the WWTP would also be reduced. Consequently, these two flow rates are not considered to be used.

Hydrolysed primary sludge (Q18). Considering the concentrations presented in Table 6.1, the hydrolysed primary sludge of the thickener is the source that owns more quantity of readily biodegradable COD according to the high quantity of VFA. The primary hydrolysed sludge contains a large percentage (3-3.5 %) of total and volatile solids and it would be centrifuged at 7500 rpm when doing the different tests because most part of COD in the solids is slowly biodegradable or refractory. Moreover, around the 60% of the soluble COD of the hidrolysate is composed by VFA which is in concordance with the experiments done by Esoy and Odegaar (1994).
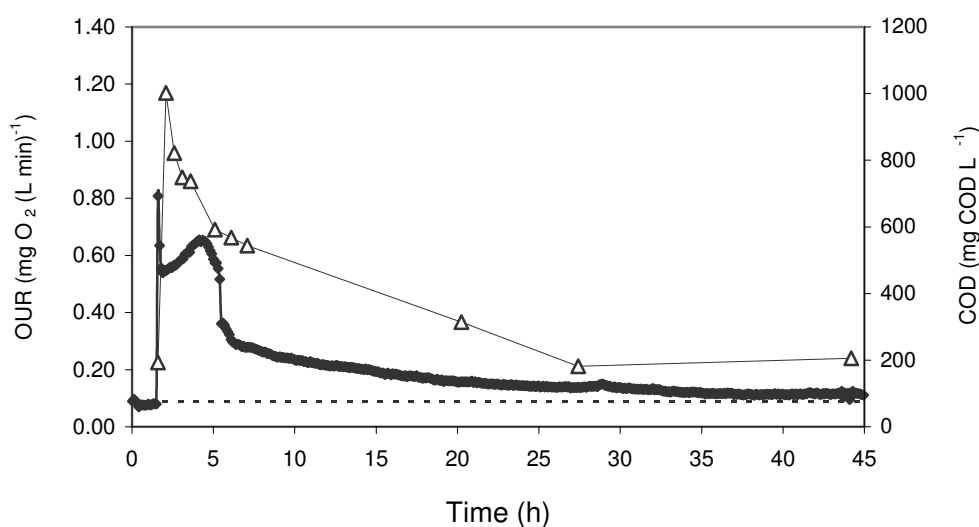
Figure 6.1: Respirogram of the primary thicker sludge: COD (--Δ--) and OUR (--●--)

(Data used to calculate the respirogram can be found in Annexe III.1)

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In order to assess the biodegradability of the hydrolysed sludge, one respirometric assay was done at 30ºC and a pH range of 8.0 ± 0.1 using 12 mg L^{-1} of ATU as a nitrification inhibitor due to its considerable ammonium concentration. In these experiments, 200 mL of centrifuged primary sludge were added to 2.5 L of endogenous mixed liquor acclimated to N/DN process with 670 mg VSS L^{-1}. This respirogram and the COD removal profile are presented in Figure 6.1 where it can be seen that wastewater includes three clear component fractions, a readily biodegradable substrate fraction, a large biodegradable substrate fraction and a slowly biodegradable substrate fraction. Looking at the respirogram and at the COD profile in the same experiment it is clear that most part (80%) of the centrifuged primary hydrolysed sludge is biodegradable. Considering the previous results, the hydrolysed primary sludge seems a good source to be operated instead of methanol. In order to verify its potential, a batch test was done. In the SBR vessel of 3 L, an initial volume of 2500 mL with a concentration of 100 mg NO_2^- -N L^{-1} was mixed with 500 mL of centrifuged hydrolysed primary sludge.

Figure 6.2: Batch test of the primary and secondary hidrolysate: NO_2^--N (--□--), pH (solid line)

Moreover, 3700 mg VSS from a N/DN SBR treating reject water via nitrite with methanol, which had an sNUR of 42 mg NO_2^--N g^{-1} VSS h^{-1} (Chapter 5), were also

added, leading to a final biomass of 1500 ± 100 mg VSS L^{-1}. In Figure 6.2 it can be seen that this experiment with primary hidrolysate gave a sNUR of 25 mg NO_2^--N g^{-1} VSS h^{-1} consi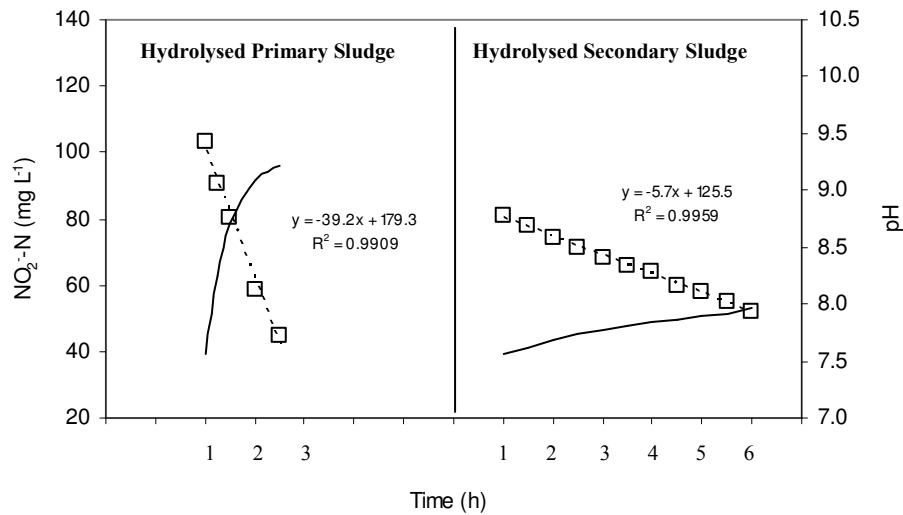dering the graphic slope and the VSS. This specific efficiency is a good value taking into consideration that the sludge is not used to operate with the proposed new carbon source.

Hydrolysed secondary sludge (Q11). In the bottom of the secondary clarifier the activated sludge is hydrolysed and it could be used as an organic carbon source for denitrification. Looking at the values of soluble COD and VFA in Table 6.1 it would not be as powerful as the hydrolysed of the primary thickener. However, this source was tested because it could be useful to consider reject water denitrification with secondary sludge in biological BABE process to maintain the pH. This process consists, as it is stated in Chapter 1, of by-passing a part of the recycled sludge from the secondary biological reactor to a nitrification reject water process. After passing through the nitrification treatment, the withdrawals from secondary sludge combined with the nitrification one are recycled again to the main line. This fact leads to increase the nitrification capacity of the biological secondary reactor at lower SRT due to the nitrifiers incorporation mixed with the recycled sludge (Salem et al., 2002, 2003, 2004).

In order to know the influence in the denitrification kinetics, a batch test was run. The nitrification of an amount of nitrogen from reject water produced 80 mg NO_2^--N L^{-1} in a SBR with biomass of the same characteristics than in the previous experiments at 3000 mg VSS L^{-1} in a mixed liquor of 2.4 L. After that, 600 mL of secondary hydrolysed sludge (2300 ± 100 mg VSS L^{-1}) were added to the SBR to follow the ratio (5 reject water: 1 recycled sludge) proposed by Salem et al. (2002) and with anoxic conditions the denitrification began to take place following the profile of Figure 6.2. The kinetics of denitrification are low (NUR=5.7 mg NO_2^--N L^{-1} h^{-1}; sNUR= 2 mg NO_2^--N g^{-1} VSS h^{-1}) when considering this source for the denitrification but it seems a good solution to buffer the control of reject water pH if the BABE process is developed in a WWTP.

Considering all the experiments done, the only internal C-source available for denitrification is the hidrolysate from the primary thickener which confirms the results

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found in the literature. In the following lines the operation and optimisation of a SBR using this source for denitrification is going to be evaluated.

6.2.3 SBR Operation

The 8 hour SBR nitrification/denitrification via nitrite optimised cycle for reject water treatment (800-900 mg NH_4^+-N L^{-1}) using methanol as a carbon source for denitrification (Chapter 5) is taken as an example to start the process. In that optimised cycle: aerobic fill (0.25 h), aerobic (1.75 h), anoxic (0.75 h), aerobic (1.50 h), anoxic (0.50 h), aerobic (1.5h), anoxic (0.75 h), settle (0.50 h) and draw (0.25 h), nearly 300 mg NH_4^+-N L^{-1} were removed and transformed to NO_2^--N with a HRT around 1 day, SRT of 11 days, with three internal aerobic/anoxic sub-cycles to control the pH range within 7.5-8.5 and maintaining the DO below 1 mg L^{-1} in a 3L SBR. At these conditions the average total nitrogen removal reached an efficiency of 0.8-0.9 kg N day^{-1} m^{-3} and the specific efficiencies for nitrification and denitrification were 22 mg NH_4^+-N g^{-1} VSS h^{-1} and 42 mg NO_2^--N g^{-1} VSS h^{-1} respectively.

The new proposed cycle would consist of substituting the methanol for the primary hydrolysed sludge maintaining the other operational conditions. Considering the values of VFA of Table 6.1 it would be needed to add inside the reactor around 1.1-1.2 L of centrifuged hydrolysed primary sludge to have enough COD to remove the nitrite formed with similar conversions and efficiencies like the optimised cycle proposed in Chapter 5. This quantity of external source leaded to do some modifications in the system. Instead of a 3 L reactor, it was needed to operate with a 4 L SBR where in each cycle 2.3 L of effluent were withdrawn at the end of the cycle and then the SBR was filled with 1.1 L of reject water to begin the cycle. The remaining volume until 4 L was filled with 1.1-1.2 L of centrifuged primary hidrolysate, containing 5000 mg COD L^{-1} and 700 mg VSS L^{-1}. This addition was done, as it can be seen in Figure 6.3, in 3 separated portions of 550, 200 and 400 mL at the beginning of first, second and third denitrification phase respectively. At these conditions, the reactor was operated during 2 months obtaining the profiles shown in Figure 6.3 where the profiles of pH, NH_4^+-N and NO_2^--N inside the reactor were very similar of those using methanol.
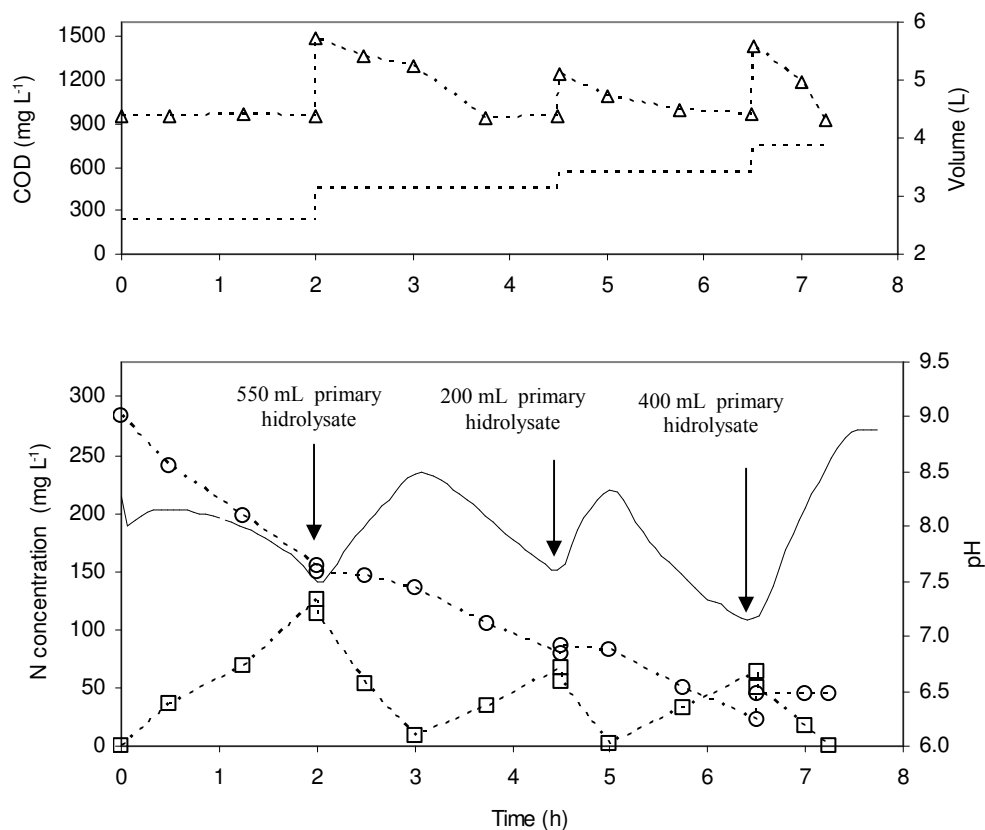
Figure 6.3: Experimental concentration and pH profiles inside SBR: NH_4^+-N (--o--), NO_2^--N (--□--), COD (--Δ--), pH (solid line) and volume (point solid line)

The COD profiles showed a good utilisation of the VFA from the hydrolysed primary sludge added in each denitrification phase remaining only a little part of COD that was removed in the aeration periods. However, the non-biodegradable SBR effluent COD was higher when using the centrifuged primary hidrolysate than when using methanol (Table 6.2). The latter could be explained because primary hidrolysate has an important part of particulate and non-biodegradable COD compared with methanol that has not any of them. The other important difference resides in the 30 % volume variation during the process. The new volume addition in the denitrification steps provokes the dilution of VSS and NO_2^--N but increase the concentration of NH_4^+-N considering the ammonium values of Table 6.1. This extra amount of ammonia means more nitrogen to be removed

and justifies why the charge of reject water in this experiment is lower than when using methanol and implies the increase of residual ammonium left at the end of the cycle. Table 6.2 shows the main differences of the system being operated with methanol and with primary hydrolysed sludge. The effluent characteristics are in the same range for both reactors, the total nitrogen discharge per day is nearly the same but the conversion per volume of reactor is higher when using methanol due to the lower reactor volume and the average specific efficiencies are slightly better when using methanol due to the effect of biomass dilution. Although the operation with hydrolysed primary sludge would mean a bigger reactor construction (25%), the fact of avoiding the addition of methanol would represent a cost reduction of 0.2-0.3 € kg $^{-1}$ N removed from a total average cost of 0.9-1.4 € kg $^{-1}$ N (van Loosdrecht and Salem, 2005).

Table 6.2: Denitrification in SBR with methanol and centrifuged primary hidrolysate

Parameters	Units	Methanol	Primary Sludge
Operational conditions			
Initial volume	L	3	2.7
Final volume	L	3	4
Initial VSS	mg L^{-1}	2500 ± 250	3400 ± 250
Final VSS	mg L^{-1}	2500 ± 250	2700 ± 250
Efficiency			
N removal efficiency	%	> 95	> 95
N discharged	kg N day^{-1}	2.7	2.5
Daily N treated	kg N day^{-1} m^{-3}	0.80 ± 0.05	0.7 ± 0.05
NH$_4^+$-N effluent	mg N L^{-1}	35 ± 5	50 ± 5
NO$_2^-$-N effluent	mg N L^{-1}	0	0
COD effluent	mg N L^{-1}	600 ± 50	900 ± 50
average sAUR	mg NH$_4^+$-N g^{-1} VSS h^{-1}	22 ± 1	17 ± 1
average sNUR	mg NO$_2^-$-N g^{-1} VSS h^{-1}	47 ± 2	38 ± 2

6.2.4 Evolution of primary hydrolysed sludge with time

Considering the fact that the operation with hydrolysed primary sludge would suppose a 25% more of reactor volume, it was considered how it could be reduced. One solution would be testing other methods to hydrolyse the primary sludge like the thermal treatments or the use of chemicals but this is out of the scope of the work.

The primary hydrolysed sludge from the WWTP has a residence time in the thickener lower than half a day. Therefore it would be interesting to know how the VFA of the primary sludge would increase in a stirred tank at psychrophilic (20º) conditions. In order to verify the hydrolysed primary sludge evolution a six day experiment was done where the amount of VFA, $COD_{soluble}$ and NH_4^+-N was analysed (Figure 6.4). At the end of the first day the quantity of VFA increase around 40% and from day 1 to day 5 it was an average VFA increasing of 18%.
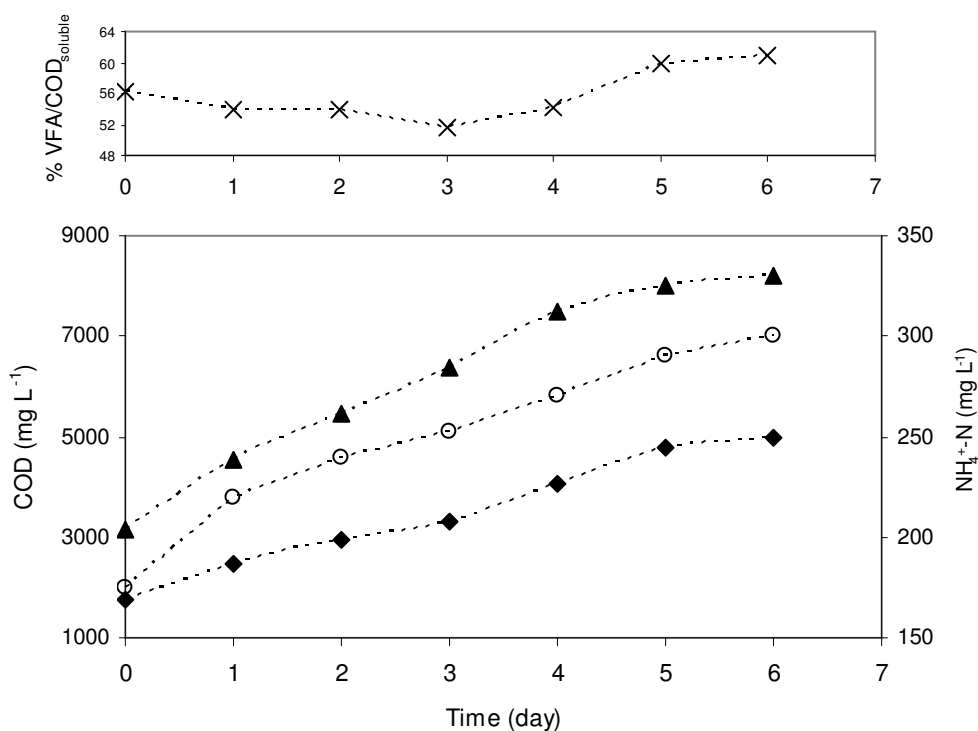
Figure 6.4: Six day experiment: NH_4^+-N (--o--), VFA (--♦--), $COD_{soluble}$ (--▲--)

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During the whole experiment VFA/COD$_{soluble}$ was maintained between 55-60% which indicates that most part of the COD generated became from the hydrolysis process. Considering the VFA it can be seen that the more days pass the more VFA are in the system and, consequently, less quantity of them would be necessary to add to the SBR which would reduce the volume of the reactor. However, at the same time that the VFA increase, it was accompanied by an increase of the quantity of ammonia due to the hydrolysis effect. Considering this fact the first day the quantity of NH$_4^+$-N would increase 25% and then a 7% every day. This nitrogen would be discharged to the reactor and will have to be treated, which would reduce the nitrification capacity of the SBR. Considering the characteristics of the WWTP, its space and emplacement this more hydrolysed sludge could be considered to be used.

6.3 CONCLUSIONS

- ✓ Different internal organic carbon sources were tested in a WWTP finding the hydrolysed primary sludge as the only feasible to be used.
- ✓ The operation of a SBR with hydrolysed primary sludge was satisfactory obtaining similar efficiencies like when using methanol
- ✓ From the point of view of costs the use of VFA from primary sludge would suppose a 25% bigger reactor construction but it would lead to save during the process operation 0.2-0.3 € kg^{-1} N removed.
- ✓ The extra hydrolysis of primary sludge in a separate reactor was considered, as more VFA are produced. However, the parallel increase of NH$_4^+$-N poses some questions that should further be investigated.

7. BIOLOGICAL NITROGEN REMOVAL WITH A SBR AND A CHEMOSTAT

Scope

Up to this point, the SBR has been used as the only reactor to treat reject water with nitrification/denitrification via nitrite. Considering the literature, it is also possible to achieve the nitrite route in a chemostat reactor.

Therefore, in this Chapter it is carried out a study to treat real reject water in order to compare N/DN via nitrite in an SBR reactor and in a chemostat SHARON/denitrification process in terms of operational conditions, kinetic, design and cost. Different sequences of treatment are tested to reach the optimal removal of pollutants via nitrite controlling temperature, pH, DO, SRT and the HRT in a chemostat and in a SBR. Methanol is used as a readily biodegradable carbon source for denitrification in both cases.

7.1 INTRODUCTION

The economic aspect is one of the main points when treating reject water. Nitrification via nitrite can be achieved working at temperatures over 20ºC and sludge age below 2 days (Hellinga et al., 1999; Van Dongen et al., 2001), maintaining the pH between 7.5-9 combined with the presence of high ammonium concentrations (Anthonisen et al., 1976; Hellinga et al., 1999) or working with DO below 1 mg L^{-1} (Pollice et al., 2002; Ruiz et al., 2003). It is important to take into account that the option of nitrite accumulation by pH control could present extra costs since extra alkalinity is needed and not all alkalinity in the wastewater can be used for pH control. On the other hand nitritation at a low DO will only be stable (with no partial nitrate formation) if properly coupled with denitrification (van Loosdrecht and Salem 2005).

SBRs and chemostat continuous reactors are two of the most extended reactors to develop N/DN for low flow rates. The main features of the SBRs are their flexibility and compactness (Ketchum, 1997; Artan et al., 2001; Macé and Mata-Álvarez, 2002) and N/DN via nitrite can be achieved combining low DO and controlled pH range (Chapter 5). Chemostat reactors allows working without sludge retention and the nitrate step is stopped working around 37ºC with autotrophic SRT below 2 days (Hellinga et al., 1998; van Dongen et al., 2001; van Kempen et al., 2001). The latest is known as SHARON process.

7.2 RESULTS AND DISCUSSION

Table 7.1 shows the composition on an average basis of the reject water used in the experiments where COD, TSS and NH_4^+-N were the main contaminants. The bicarbonate to ammonium ratio (molar basis) was nearly 1, which is in accordance with other reported values of reject water characterisation (Hellinga et al., 1999; Vandaele et al., 2000). Considering the value of BOD_5 it was again observed that wastewater includes a very small fraction of readily biodegradable substrate which is useless to denitrify.

Table 7.1: Year average composition of the centrifuged sludge effluent (2005)

Component	Unit	Value	Component	Unit	Value
TSS	mg L^{-1}	700 ± 25	P-total	mg L^{-1}	20 ± 2
VSS	mg L^{-1}	600 ± 25	HCO$_3^-$	mg L^{-1}	3500 ± 500
COD	mg L^{-1}	1700 ± 300	HCO$_3^-$/N ratio	mol mol^{-1}	1 ± 0.1
BOD$_5$	mg L^{-1}	120 ± 20	pH	-	8 ± 0.1
NH$_4^+$-N	mg L^{-1}	850 ± 50	Temperature	ºC	35 ± 0.5

7.2.1 SBR cycle of 8 hours

Previous experiments demonstrated that working with 8 hour cycle with 3 internal aerobic/anoxic phases and methanol for denitrification was an optimal way to treat reject water with N/DN via nitrite (Chapter 5). Therefore, 3 daily 8 hour cycle were operated during 6 months following an internal three aerobic/anoxic sub-cycle strategy to control nitrite accumulation and alkalinity limitations, as it is shown in Figure 7.1. Each cycle consisted of nine stages: aerobic fill (0.25 h), aerobic (1.75 h), anoxic (0.75 h), aerobic (1.50 h), anoxic (0.50 h), aerobic (1.5h), anoxic (0.75 h), settle (0.5 h) and draw (0.25 h). Table 7.2 shows the average operational conditions.

Table 7.2: Operational conditions of the different processes tested

	Reactor	NH$_4^+$-N $_{inf}$	VSS	SRT	HRT	pH	T	DO
	L	mg L^{-1}	mg L^{-1}	day	day	-	ºC	mg L^{-1}
SBR	3	800 ± 100	2500 ± 250	11	1	7.3-8.1	32 ± 0.5	<1
Chemostat	4	700 ± 100	1200 ± 100	2	2	6.8-8	33 ± 0.5	>3

(inf= influent)

To assure the nitrite route, pH was nearly all the time maintained between 7.3 and 8.1 by the internal aerobic/anoxic stages and the air flow was regulated during aerobic periods maintaining a DO level below 1 mg L^{-1}. At the beginning of each denitrification phase a quantity of methanol to assure complete denitrification was added as electron acceptor. In each cycle, 275 mg NH$_4^+$-N L^{-1} were properly nitrified to NO$_2^-$-N and then denitrified obtaining an effluent with 5 mg L^{-1} of NH$_4^+$-N and 20 mg L^{-1} NO$_2^-$-N which gave a total nitrogen efficiency of 0.8 kg N day^{-1} m^{-3} (Figure 7.1). The specific rates were

22 mg NH_4^+-N g^{-1} VSS h^{-1} for nitrification and 47 mg NO_2^--N g^{-1} VSS h^{-1} for denitrification.
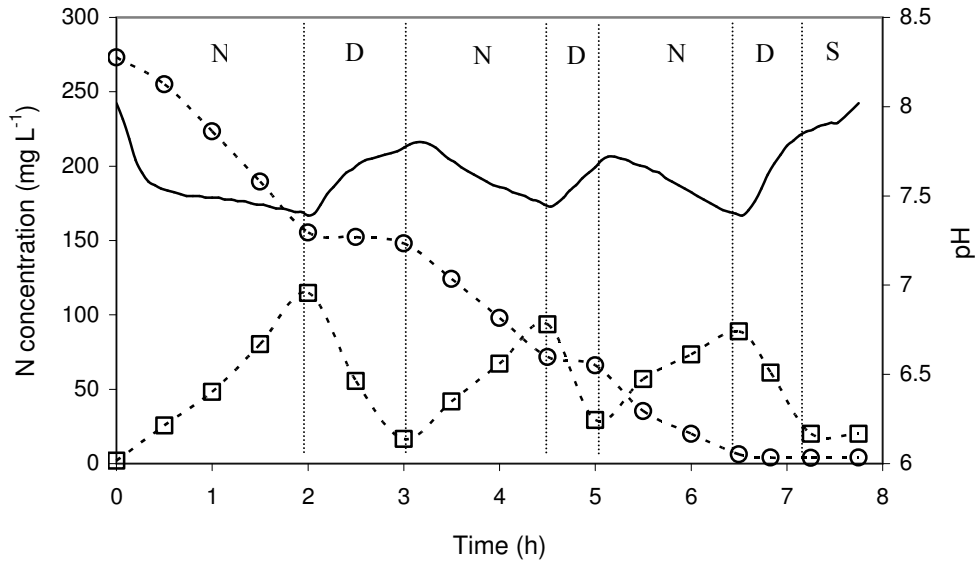
Figure 7.1: Experimental concentration and pH profiles inside SBR in a 8 hour cycle with 3 aerobic/anoxic sub-cycles: NH_4^+-N (--o--), NO_2^--N (--□--), pH (solid line); (N: nitrification, D: denitrification, S: sedimentation)

7.2.2 SHARON/DN chemostat reactor

The process was developed in a chemostat reactor during a period of 6 months working with Table 7.2 conditions to avoid nitrate formation (Hellinga et al., 1998). SHARON process was combined with denitrification varying the total HRT between 2-4 days using intermittent N/DN periods of 1 hour for complete BNR via nitrite. The dosage of methanol for denitrification was done during the first 30 minutes of each denitrification phase to prevent its accumulation and degradation in the subsequent aerobic stage. Previous studies demonstrated that the nitrite route in SHARON process with the conditions mentioned above could be reached in a range of temperature between 30-37 ºC (Hellinga et al., 1999). Below this range the formation of nitrates might occur more

easily. Therefore, considering the characteristics and origin of the reject water the working temperature was fixed at 33 ºC.

Five different phases where the reactor was working in different conditions can be distinguished in Figure 7.2. The oxygen concentration was always maintained above 3 mg L^{-1} in nitrification periods and the obtained pH profile in all the situations was always within the range 6.8-8 without control, due to the correct alkalinity recovery in denitrification. The start up of the process was done in phase-1 (days 0-15) with a total HRT of 4 days and excess addition of methanol for denitrification. The total nitrogen concentration in the effluent was nearly zero. After start-up, phase-2 (days 15-75) conditions were fixed and the HRT was reduced to 3 days. This made NH_4^+-N concentration increase until it stabilised at 30-40 mg NH_4^+-N L^{-1}. Due to the excess of methanol in denitrification, there were neither nitrites nor nitrates. Consequently, it is not possible to assure that the system had nitritation behaviour without nitrate formation. Therefore, in phase 3 (days 75-92) methanol was added under NO_2^--N denitrification stoichiometric ratio and, as it can be seen in Figure 7.2, most part of the product of nitrification was NO_3^--N. Although the aerobic HRT was 1.5 hour the system was not working with only nitritation. Therefore, the flow-rate (phase 4: days 100-125) was increased to decrease the total HRT until 2 days (1 day aerobic HRT) maintaining the limitations of methanol addition. Nitrate concentrations in the effluent began to disappear and when NO_2^--N was the main product, methanol was added in a stoichiometric ratio for nitrite denitrification during phase 5 (days 125-200).

The final obtained effluent had 10 mg NH_4^+-N L^{-1} and 25 mg NO_2^--N L^{-1} which means a total average nitrogen efficiency of 0.4 kg N day^{-1} m^{-3}. The specific efficiencies are 27 mg NH_4^+-N g^{-1} VSS h^{-1} for nitrification and 27 mg NO_2^--N g^{-1} VSS h^{-1} for denitrification. Moreover, the process showed a good capacity to sustain the fluctuations in the NH_4^+-N concentrations during the whole treatment.
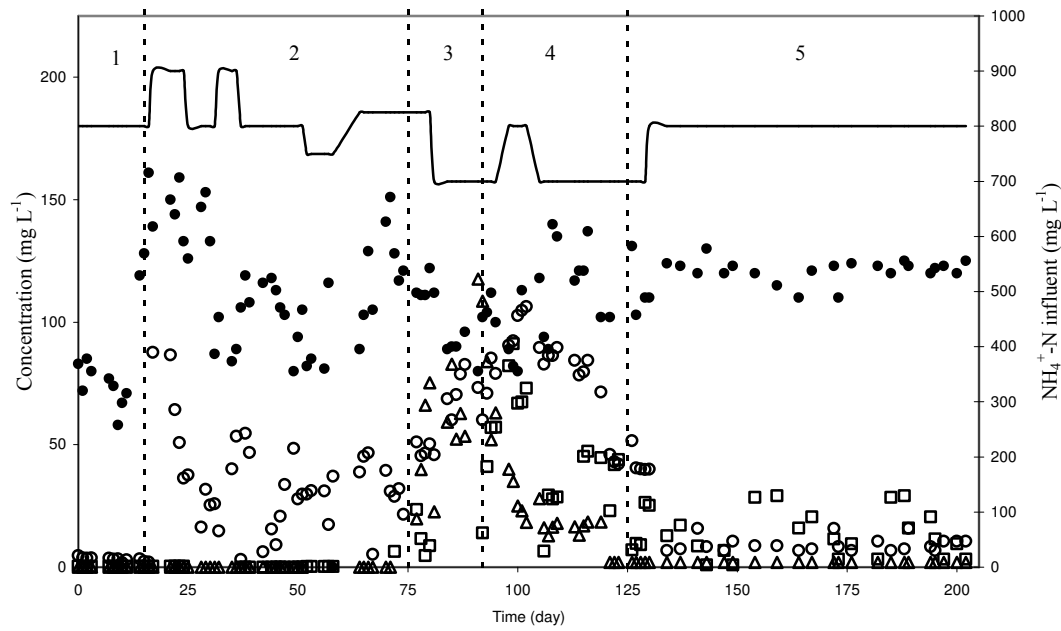
Figure 7.2: Reactor effluent daily average profiles during 6 months operation: NH_4^+-N (-- o--), NO_2^--N (--□--), NO_3^--N (--Δ-), VSS·10 (--●--), influent NH_4^+-N (solid line)

7.2.3 Comparison of the different processes

In the following lines a comparison of both systems is done from the point of view of reactor operation and efficiency (Table 7.3), kinetic and stoichiometric parameters (Table 7.4) and design aspects and operation costs (Table 7.5).

Reactor operation and efficiencies. Temperature and pH range in both reactors were very similar, whereas the DO control around 1 mg L^{-1}, to provide the nitrite route in SBR, would represent lower oxygen supply. Figure 7.3 shows the pH and DO profiles of 3 consecutive operational days for the (a) SBR and (b) SHARON/DN process which demonstrate the good steady state achieved for both reactors.

From Table 7.3 it can be extracted that both systems will manage correctly flow-rate fluctuations, but the SHARON/DN system shows a better behaviour when there are fluctuations in ammonium influent concentrations and no flow-rate modifications are

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needed. In contrast, the SBR needs an HRT modification to maintain the stationary state which made the SBR to be less stable than the chemostat operation. Comparing the efficiencies of the processes, it could be appreciated that in both reactors nearly complete BNR via nitrite (> 95% N removal) is achieved and the system would provide good effluent requirements. However, when considering the absolute removal efficiency, the SBR is better (0.8 in front of 0.4 kg N day^{-1} m^{-3}) due to the lower HRT achieved in the process. If one looks at the specific efficiencies, sAUR is a little better for the SHARON/DN whereas sNUR is higher for the SBR due to the half time given for denitrification. In addition the chemostat can directly work without sludge retention which would save the sedimentation step and would provide more time for reaction.
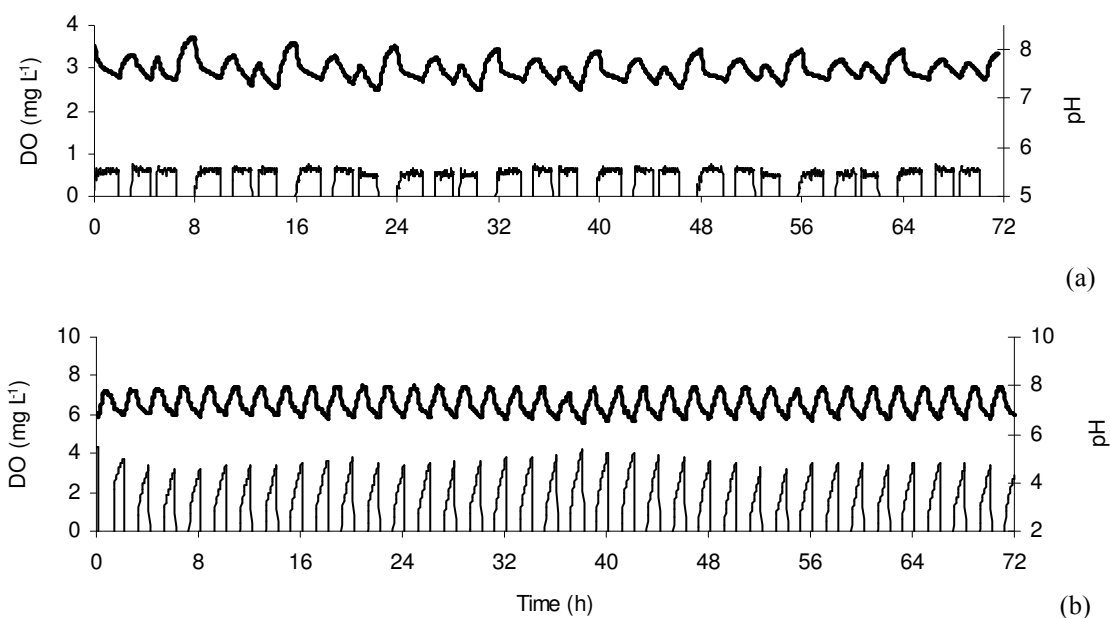

Figure 7.3: pH (bold solid line) and DO (solid line) profiles of (a) the SBR (b) the SHARON/DN reactor during 3 consecutives days

Table 7.3: Comparison of SBR and SHARON/DN performances

Parameter	Units	SBR	SHARON / DN
Operational conditions			
Heating requirements	°C	32 ± 0.5	33 ± 0.5
pH control	-	no	no
DO control	mg L^{-1}	<1	>3
Efficiency			
N removal efficiency	%	> 95	> 95
Daily N treated	kg N day^{-1} m^3	0.8 ± 0.05	0.4 ± 0.05
NH_4^+-N effluent	mg N L^{-1}	< 5	10 ± 2
NO_2^--N effluent	mg N L^{-1}	20 ± 5	25 ± 5
sAUR	mg NH_4^+-N g^{-1} VSS h^{-1}	22 ± 1	27 ± 1
sNUR	mg NO_2^--N g^{-1} VSS h^{-1}	47 ± 2	27 ± 2
Sludge retention requirement	-	yes	no
Reactor operation *			
Flow rate fluctuations	-	=	=
N fluctuations	-	-	+
Stability	-	-	+

*(+ better; = equal; - worse)

Kinetic and stoichiometric parameters. The main stoichiometric and kinetic parameters of the IWA Activated Sludge Models (Henze et al., 2000) were assessed by respirometric batch tests (Chapter 3; section 3.4) where the effect of every studied parameter was highly pronounced under the tested experimental conditions (Vanrolleghem et al., 1999). Maximum growth rate constant for heterotrophic (μ_{mH}) and autotrophic biomass (μ_{mAOB}) were determined using the procedure of Novak et al. (1994) in the SBR and with a mass balance in the chemostat. The half-saturation constants for ammonium (K_{NH}) and (Ks) were determined by applying the method described by Cech et al. (1984). Oxygen affinity constants for ammonium oxidizers (K_{OA}), and COD (K_{OH}) were assessed with a batch test where DO drop was monitored in a respiration chamber without aeration after the substrate injection (as described by van Hulle et al., 2004).

Table 7.4: Average values of model parameters for the studied cases (pH= 8 and T = 30 ºC)

Parameter	SBR	SHARON / DN	Units
Heterotrophic biomass			
Y_H	0.6 ± 0.01	0.6 ± 0.01	mg cell COD mg^{-1} COD consumed
μ_{mH}	4.3 ± 0.3	3.7 ± 0.3	day^{-1}
K_S	15 ± 8	9 ± 3	mg COD L^{-1}
K_{OH}	0.2 ± 0.05	0.15 ± 0.05	mg O$_2$ L^{-1}
η_{no2}	0.85 ± 0.02	0.85 ± 0.02	(-)
k_d	0.2 ± 0.05	0.2 ± 0.05	day^{-1}
Denitrifiers	60	38	%VSS
Autotrophic biomass			
Y_{AOB}	0.2 ± 0.01	0.2 ± 0.01	mg cell COD mg^{-1} NH$_4^+$-N consumed
μ_{mAOB}	1.3 ± 0.2	2.4 ± 0.3	day^{-1}
K_{NH}	1.3 ± 0.4	2.15 ± 0.7	mg NH$_4^+$-N L^{-1}
K_{OA}	0.15 ± 0.07	0.46 ± 0.1	mg O$_2$ L^{-1}
Nitrifiers	9	5	%VSS

(Data to calculate the parameters of Table 7.4 can be found in Annexe IV.1)

Table 7.4 shows the main stoichiometric and kinetic parameters studied for both processes. These parameters give a new perspective of what is happening inside the system, and corroborates what has been explained above. The autotrophic yield (Y_{AOB}) is the same in both systems. The K_{NH} and the population of nitrifiers are a bit higher in the SBR than in the chemostat. However, the main differences lie in the maximum autotrophic growth constant for the SHARON/DN sludge which is higher than in the SBR sludge due to its reduced SRT and in the half-saturation constant of oxygen for ammonia oxidisers (K_{OA}), which is 3 times lower for the SBR. This value combined with the half-saturation coefficient for nitrite oxidisers (K_{NO}) would give the necessary information to establish the working conditions to stop the second step of nitrification in the SBR process. The denitrification heterotrophic parameters are improved for the SBR system which is not strange looking at the shorter and therefore better denitrification periods and the higher proportion of heterotrophic population as it can be seen in Table

7.3. The corrector factor for denitrification and the decay rate were determined all together for both biomasses considering that they will be in a very similar range.

Design and cost estimation. The WWTP under study has an average influent flow-rate of 49000 m^3 day^{-1} with a reject water flow-rate of 275 m^3 day^{-1}. It has been considered the nitrogen concentrations of Table 7.2 and the removal efficiencies of Table 7.3 to determine the design and economical aspects of Table 7.5. There it is shown that the volume requirements for the SHARON/DN reactor are higher than for the SBR due to the operating HRTs. In contrast, the SBR would need a buffer tank of 100 m^3 due to its batch operation. For the investment of the reactors, the design criterion of the WWTP was considered. This would lead, considering a depreciation of 20 years at an interest rate of 5%, to a cost of 39000 € $year^{-1}$ for the SBR and 59000 € $year^{-1}$ for the SHARON/DN chemostat. The 50% of the cost corresponds to the civil engineering and the rest is for the equipment and mechanical costs. The dosage of methanol was 1.7 g^{-1} methanol g NO_2^--N (0.18 € methanol kg^{-1}) and the sludge production was calculated considering the observed yield (Y_{obs}) from Table 7.5. The oxygen consumption was obtained through a mass balance over the gas and liquid phase considering the oxygen demand, the reactor volume and reactor height of Table 7.5 (see Annexe IV.2). For air cost calculation it was considered an efficiency of 70% for turbines with an electricity price of 0.09 € (kw h)$^{-1}$. The disposal of the produced sludge would suppose an extra cost of 20 € $tone^{-1}$ residue produced. With these values, the obtained costs per kg N removed are shown in Table 7.5. The main difference between both systems is the investment cost, which made the SHARON/DN process to be a bit more expensive due to its larger dimensions.

The operation costs are exactly the same for both systems because they are consuming the same quantity of COD in denitrification, the oxygen requirements are very similar and the sludge production is little higher in SHARON/DN due to the higher $Y_{obs.}$ The operation with the SBR would suppose a total cost of 1.01 € kg^{-1} N while when the process is operated with a chemostat SHARON/DN the cost would be a little higher (1.28 € kg^{-1} N). These results are in the range (0.9-1.4 € kg^{-1} N) reported by van Loosdrecht and Salem (2005) and are more economical than the ones proposed by Fux et al. (2003),

namely 1.4 € kg $^{-1}$ N for an SBR and 1.63 € kg $^{-1}$ N for a SHARON reactor. But it is important to consider that it is all rather marginal differences and a significant cost fraction comes from local situation.

Table 7.5: Design parameters and operation cost estimation operation

Parameter	Units	SBR	SHARON / DN
Design aspects			
N-load	kg N day^{-1}	240	240
Y_{obs}	g COD g^{-1} COD removed	0.35	0.47
Reactor height	m	5	6
Reactor volume	m^3	300	600
Extra tank volume	m^3	100	0
Oxygen Demand	kg O$_2$ day^{-1}	775	775
Air demand	m^3 day^{-1}	33500	34500
Cost			
Investment	€ kg $^{-1}$ N	0.45	0.67
Oxygen	€ kg $^{-1}$ N	0.20	0.23
Maintenance	€ kg $^{-1}$ N	0.04	0.05
Methanol	€ kg $^{-1}$ N	0.3	0.3
Sludge disposal	€ kg $^{-1}$ N	0.02	0.03
TOTAL	€ kg $^{-1}$ N	1.01	1.28

7.3   CONCLUSIONS

✓ Two different ways of developing nitrification via nitrite were tested with good performance and pollutants removal in both cases.

✓ From the point of view of nitrogen conversion, it seemed that the SBR was better than the chemostat SHARON/denitrification due to the lower HRT.

✓ On the other hand the chemostat process performance was better when there were modifications in the influent flow-rate and nitrogen concentration which lead to a more stable process.

- ✓ The chemostat would be slightly expensive due to the larger reactor necessities.
- ✓ Therefore, the selection of one or other system would have similar results in terms of N-removal and would depend on the emplacement of the WWTP and its available space.

8. TWO WAYS TO ACHIEVE A REAL ANAMMOX INFLUENT

Scope

Normally the biological nitrogen removal process is done with nitrification/denitrification. But recently the Anammox process is found to be a new path of nitrogen cycle that could substitute the denitrification step. Anammox bacteria oxidise nitrite to nitrogen gas using ammonium as electron donor, instead of the COD used in denitrification, with a 1:1 ammonium/nitrite ratio. Therefore, the aim of this Chapter is to carry out a study to compare side by side partial nitrification in a SBR reactor and in a chemostat SHARON process to achieve a properly Anammox influent from real reject water. In order to reach a good partial nitrification via nitrite, different sequences of treatment are tested controlling temperature, pH, DO, SRT and HRT.

8.1 INTRODUCTION

Anammox is a new process (Mulder et al.1995) developed in the last 10 years. It is equivalent to the classical denitrification but using ammonium as electron donor to reduce nitrite instead of using organic compounds. The nitrite form is the oxidised compound available for the process in an ammonium/nitrite ratio of 50% because nitrate cannot be used as an electron acceptor (van de Graaf et al., 1995).

As stated above, the good development of this process needs an appropriate influent of NH_4^+-N and NO_2^--N at 50%. But it is important to know which would be the possibilities to transform high contaminated ammonia streams into the correct Anammox influent flow rate, which means having NH_4^+-N, NO_2^--N and avoiding NO_3^--N formation.

Partial nitrification of ammonium to nitrite is presented as a possible way to achieve the required Anammox influent. Nitrification to nitrite only can be achieved working at temperatures over 20 ºC and SRT below 2 days (Hellinga et al., 1999; Van Dongen et al., 2001) and working with high ammonium concentrations and pH 7.5-8.5 (Chapter 5).

The key to obtain partial nitrification resides in the alkalinity/ammonium ratio. The molar stoichiometric relationship for complete ammonium oxidation must be 2 mol HCO_3^-/mol NH_4^+. Therefore, to provide a 50% ammonium oxidation, ammonium and bicarbonate should be present in a molar ratio of 1:1. If the wastewater has not this relationship, the pH would have to be controlled in the process.

SBRs and chemostat continuous reactors are the generally preferred reactors to develop the classical nitrification/denitrification process and could be chosen to develop a partial nitrification via nitrite. In SBRs the nitrification via nitrite could be achieved working with high ammonium concentration and an appropriate pH range (Anthonisen et al., 1976; Chapter 5). In a chemostat reactor this nitrification can be reached when working around 37 ºC with autotrophic SRT below 2 days (Chapter 7). This process is known as SHARON process (Hellinga et al., 1998; Van Dongen et al., 2001).

Reject water is a good example of high concentrated ammonium stream (800-1000 mg NH_4^+-N L^{-1}) to which the partial nitrification via nitrite could be achieved due to its low characteristic ammonium to bicarbonate ratio.

8.2. RESULTS AND DISCUSSION

8.2.1. Reject water characterisation

Table 7.1 showed the average composition of the reject water used in the experiments. The main contaminants were COD, TSS and NH_4^+-N. But given the scope of this Chapter the important parameter is the bicarbonate to ammonium ratio (molar basis). This alkalinity to ammonium ratio of the tested reject water was nearly 1 resulting in an appropriate alkalinity to nitrify 50% of the ammonium. This value is in concordance with theoretical and other reported values of reject water characterisation (Hellinga et al., 1999) and shows that partial nitrification can be feasible without pH control.

8.2.2 SBR operation

Considering the NH_4^+-N concentration and the pH of reject water, it was decided to work with 6 cycles a day in order to prevent the inhibition of the process by free ammonia concentration (Anthonisen et al., 1976). A 4 hour SBR cycle was operated during 5 months to study the partial nitrification via nitrite.

Table 8.1: Comparison of SBR and SHARON operational conditions

Operational conditions	Units	SBR	SHARON
Reactor volume	L	1	4
Temperature	ºC	30 ± 0.5	35 ± 0.5
pH range	-	6.5-8	6.5-6.7
DO	mg L^{-1}	3	3
NH_4^+-N influent	mg L^{-1}	800 ± 50	700 ± 50
VSS	mg L^{-1}	1200 ± 100	400 ± 50
SRT	day	5	1
HRT	day	0.35	1

Each cycle consisted of four stages: aerobic fill (5 minutes), aerobic (210 minutes), settle (20 min) and draw (5 minutes). The exchange volume was 50% resulting in a hydraulic retention time of 8 hours (0.35 days), SRT was controlled at 5 days by wasting excess sludge at the end of aerobic phase, temperature was controlled at 30 ± 0.5ºC,

biomass concentration was 1200 ± 100 mg VSS L^{-1}, pH fluctuated between 6.5 and 8 without external control and the air flow was regulated during aerobic periods maintaining a DO level above 3 mg L^{-1} (Table 8.1). As it can be seen in Figure 8.1 under the operating conditions described in Table 8.1 and the wastewater characteristics, at the beginning of each cycle there are 600 mg NH_4^+-N L^{-1} und 200 mg L^{-1} NO_2^--N inside the SBR. Nearly 200 mg NH_4^+-N L^{-1} were oxidised to NO_2^--N obtaining an average effluent with approximately, 400 mg NH_4^+-N L^{-1} and 400 mg NO_2^--N L^{-1}. It is important to notice that the system worked until being limited by its alkalinity and this fact affected a little the NH_4^+-N removal during the last hour. In this point, the alkalinity was not enough to maintain a favourable pH and the oxygen concentration began to increase until reaching the value of 6 mg L^{-1} at the end of the cycle (Figure 8.1). This fact is clearly shown when pH began to fall below 7 and it is observed in the average ammonium uptake rate presented in Table 8.2 with 40% inhibition at pH 6.3 (sample 6).
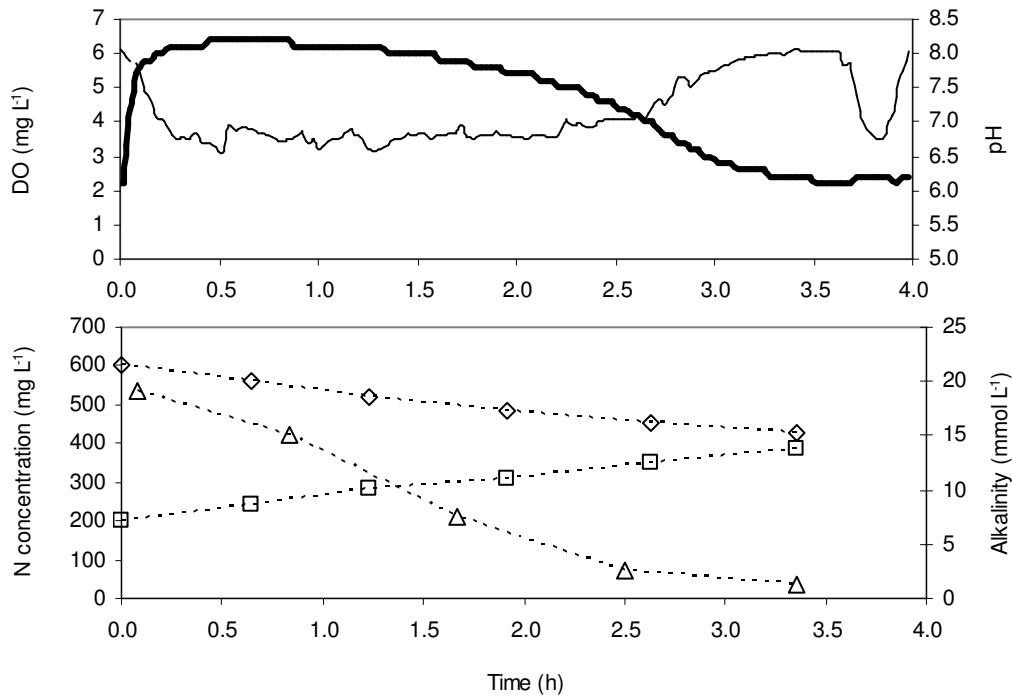

Figure 8.1: Experimental concentration and pH profiles in a 4 hour SBR cycle: NH_4^+-N (--◇--), NO_2^--N (--□--), alkalinity (--Δ--), DO (solid line), pH (bold solid line)

Table 8.2: Representative cycle characteristics for the SBR reactor performing partial nitritation

Sample	Time	NH_4^+-N	NO_2^--N	pH	Partial sAUR
-	h	mg L^{-1}	mg L^{-1}	-	mg NH_4^+-N g^{-1} VSS h^{-1}
1	0	602 ± 75	200 ± 25	8.1	
2	0.6	559 ± 75	242 ± 25	8.1	52 ± 2
3	1.2	521 ± 75	282 ± 25	7.9	51 ± 2
4	1.9	486 ± 75	320 ± 25	7.5	40 ± 2
5	2.6	452 ± 75	352 ± 25	6.7	37 ± 2
6	3.4	425 ± 75	385 ± 25	6.3	29 ± 2

Furthermore, a representation of the relative activity in front of pH was performed from the representative DO profiles of the 4 hour cycle. OUR can be calculated from DO profile during the operational cycle calculating the oxygen gas-liquid mass transfer. Since the dissolved oxygen and the ammonium concentration were not-limiting, the OUR was proportional to the maximum autotrophic growth rate at the studied pH.
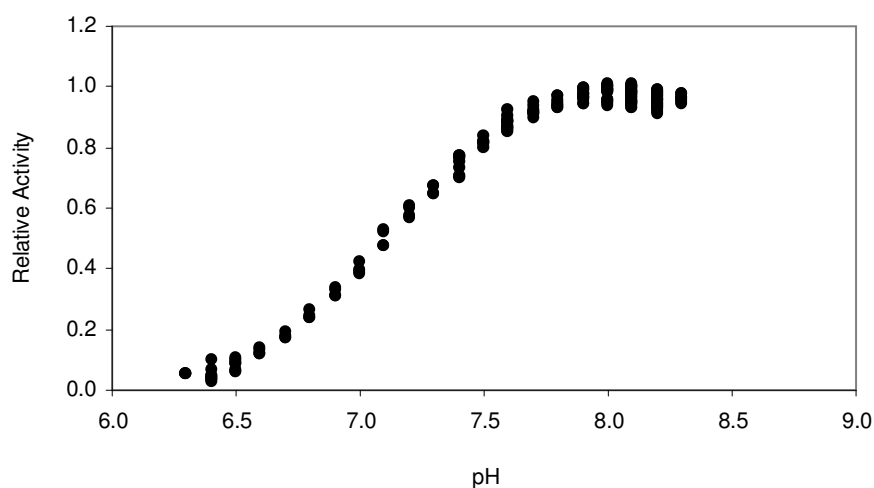

Figure 8.2: Relative activity of ammonium oxidizers in front of pH
(Data to develop this analysis can be found in Annexe V.1)

Therefore, the ratio between the OUR at every studied pH and the OUR at the optimum pH value (OUR_{max}) provides the relative activity of autotrophic biomass. Figure

8.2 presents these values where it is clearly stated that the nitrification rate has its maximum value between pH 7.5-8 and below this optimal range it starts to decrease reaching the minimum at 6.3. Since the ammonium and nitrite concentrations only change slightly, it can be concluded that the decrease in the rate is mainly due to a pH effect. The volumetric rate of ammonium removal for the process was 1.1 kg N day^{-1} m^{-3} and the specific activity of nitrification was 42 mg NH$_4^+$-N g^{-1} VSS h^{-1}. It should be noted that a large part of the VSS is non-nitrifying. When there were fluctuations in the influent nitrogen concentration due to modifications in anaerobic sludge digester conditions, the system worked correctly but it was necessary to modify the HRT in order to achieve a good conversion.

8.2.3 Chemostat reactor

A chemostat reactor was operated during a period of 6 months under the working conditions specified in Table 8.1. In order to avoid nitrate formation, SRT was varied between 1-2 days (Hellinga et al., 1999). Since no sludge immobilisation was applied, SRT was the same as the HRT. Temperature was controlled at 35 ± 0.5 ºC, biomass concentration was 400 ± 50 mg VSS L^{-1}, DO was always maintained above 2 mg L^{-1} and the pH range was uncontrolled but was very stable at a value around 6.7. The total biomass content indicated that there was a significant (25%) oxidation of the VSS present in the influent.

In Figure 8.3 the concentrations in the SHARON reactor during the 6 months of operation are shown. There were clearly 3 different stages in the operation. The start up of the process occurred in phase-1 (days 0-20) with an HRT of 1.8 days obtaining an effluent concentration composed by 50% of ammonium and nitrites that reached a stable value around 350-400 mg N L^{-1} at day 12-14. No nitrate presence was detected at this relative long SRT (Hellinga et al., 1999; Van Dongen et al., 2001) and the pH was maintained around 7. Other start up experiments of SHARON reactors performed at HRT higher than 2 days led to formation of NOB.

At day 20, HRT was changed from 1.8 to 1.4 (phase 2: days 20-95). The system behaviour remained as expected, obtaining the desired effluent composition for an

Anammox process. The system also managed well when there were operational variations in influent NH_4^+-N and the resulting effluent was always a mixture of NH_4^+-N and NO_2^--N in a 1:1 ratio without any modification in HRT.

Finally, in phase 3 (days 95-180) the HRT was fixed at 1 day to improve the quantity of nitrogen treated obtaining the same results than in the previous step, but treating nearly 50% more nitrogen than in phase 2. The final average effluent obtained had 350 mg NH_4^+-N L^{-1} and 350 mg NO_2^--N L^{-1} which means a total average nitrogen conversion rate of 0.35 kg N day^{-1} m^{-3} related to NH_4^+-N and a sAUR of 39 mg NH_4^+-N g^{-1} VSS h^{-1}.
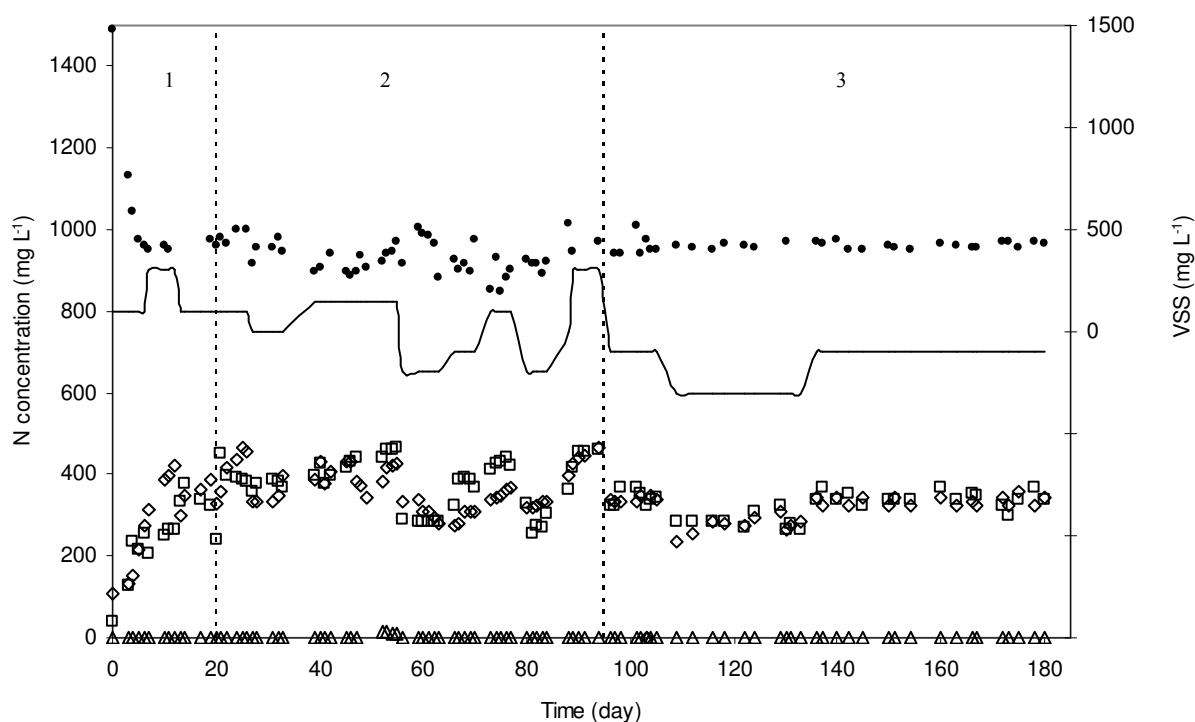
Figure 8.3: Reactor profiles during 6 months operation: NH_4^+-N (--◊--), NO_2^--N (--□--), NO_3^--N (--Δ-), VSS (--●--) , influent NH_4^+-N (solid line)

8.2.4 Kinetic and stoichiometric parameters

The main stoichiometric and kinetic parameters of the IWA Activated Sludge Models (Henze et al., 2000) were assessed by respirometric batch tests (Chapter 3, section 3.4) The combined parameters $\mu_{mAOB}\ X_{BA}$ for the evaluation of maximum autotrophic growth rate for AOB were determined through a $S_{to}/X_{to}=1/200$ respirometric experiment for autotrophic biomass (Spanjers and Vanrolleghem, 1995).

$$X_{BA} = \frac{1}{\left(i_{XB}+\dfrac{1}{Y_{AOB}}\right)}\frac{\left(S_{NH,i}-S_{NH,f}\right)_{DAILY}}{\left(1+b_A SRT\right)}SRT \tag{8.1}$$

The maximum specific growth rate for ammonium oxidizers (μ_{mAOB}) was subsequently assessed calculating X_{BA} by means of a COD balance (equation 8.1) considering the autotrophic yield coefficient for ammonium oxidizers (Y_{AOB}) determined by respirometry. A default value of 0.2 days^{-1} at 20 ºC (Henze et al., 2000) was established for the autotrophic decay coefficient. Half-saturation constant for ammonium (K_{NH}) was determined by applying the method described by Cech et al. (1984). Oxygen affinity constant for ammonium oxidizers (K_{OA}) was assessed through a batch test in which the DO drop was monitored in a respiration chamber without aeration after the injection of substrate (as described by van Hulle et al., 2004). In Table 8.3 the main stoichiometric and kinetic parameters studied for both processes are presented where, the maximum autotrophic growth rate for SHARON process is 2.00 day^{-1}, which is in concordance with the results obtained by Van Hulle et al. (2004). This value was higher than the obtained for partial nitrification in the SBR (1.00 day^{-1}) because the SHARON process favours high microbial specific growth rates (Hellinga et al., 1999). On the other hand the percentage of nitrifiers is higher in the SBR than in the chemostat due to the higher SRT. Moreover, the oxygen affinity constant for ammonium oxidizers in the SHARON process is slightly higher when compared with the results obtained in the SBR operating with partial nitrification.

Table 8.3: Average values of autotrophic biomass parameters (pH = 8)

Parameter	SBR *	SHARON **	Units
Y_{AOB}	0.20 ± 0.01	0.20 ± 0.01	mg cell COD mg^{-1} NH$_4^+$-N consumed
$\mu_{mAOB} X_{BA}$	13.1 ± 0.3	4.7 ± 1	mg cell COD L^{-1} h^{-1}
$\mu_{mAOB} X_{BA}$ / X_{VSS}	10.1	13.4	mg cell COD g^{-1} VSS h^{-1}
μ_{mAOB}	1.00	2.00	day^{-1}
K_{NH}	5.1 ± 0.4	5.1 ± 0.4	mg NH$_4^+$-N L^{-1}
K_{OA}	0.34 ± 0.07	0.56 ± 0.06	mg O$_2$ L^{-1}
nitrifiers	19	10	% VSS

(* Values for 30 ºC; ** Values for 35 ºC);   (Parameters calculation in Annexe V.2)

Since both systems operated under high ammonium concentrations, the biomass developed was not accustomed to work under limiting substrate conditions and, therefore, half-saturation constant for ammonium concentration were very similar for both digesters and slightly higher than those proposed in literature (Van Hulle et al., 2004; Hellinga et al., 1999).

8.2.5 Comparison of the different processes

Figure 8.4 shows pH and DO (mg L^{-1}) of one operation day in the (a) SHARON continuous process and in the (b) SBR with 4 hour cycle. The main differences are the pH and DO fluctuations in the SBR due to the batch operation mode, whereas in the SHARON process the profiles are always stable and constant. However, both systems were working correctly with nearly complete alkalinity removal resulting in similar pH values under than 7.
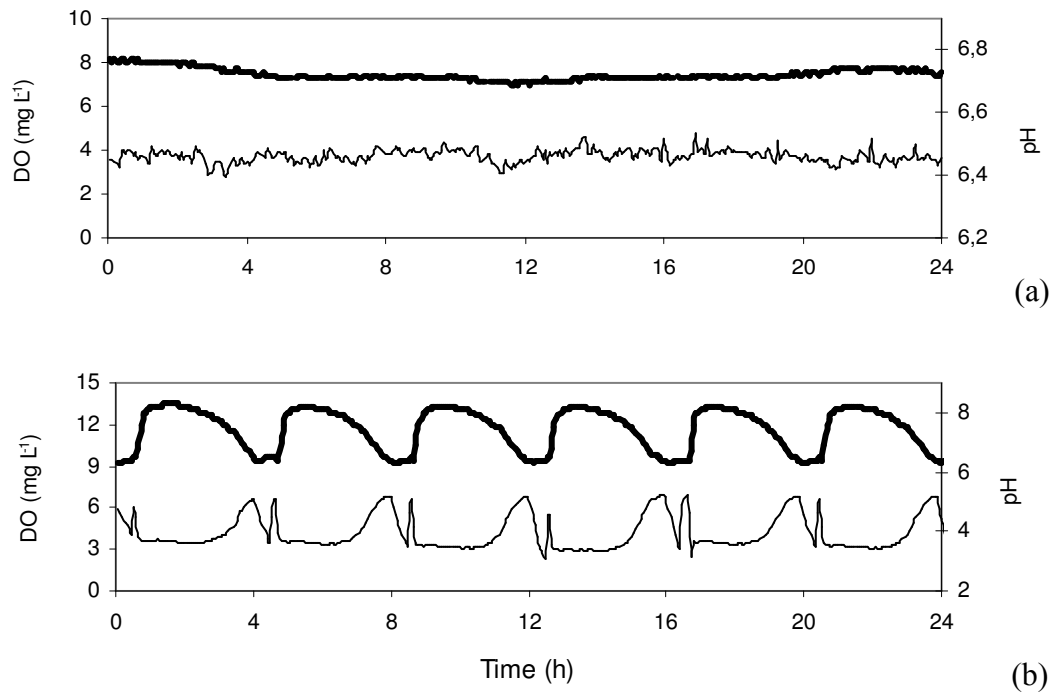
Figure 8.4: pH (bold solid line) and DO (solid line) profiles of (a) SHARON chemostat (b) SBR during 1 day

In Table 8.4 there are shown the efficiencies of both processes. Comparing the partial nitrification via nitrite in the SBR and in the chemostat, it can be appreciated that both systems manage to carry out the process correctly with a very similar sAUR. Whereas, considering the conversion rates, the SBR is better (1.1 front 0.35 kg N day^{-1} m^{-3}) due to the higher nitrifying biomass achieved in the process. But from the stability point of view, SHARON process demonstrated to be better when there were fluctuations in NH$_4^+$-N concentrations. The latter is an important factor to consider when the feasibility and good performance of a system is required.

Table 8.4: Comparison of SBR and SHARON efficiencies

Efficiencies	Units	SBR	SHARON
Removal NH_4^+ efficiency	%	50	50
NH_4^+-N effluent	mg L^{-1}	425 ± 50	350 ± 50
NO_2^--N effluent	mg L^{-1}	385 ± 50	350 ± 50
sAUR	mg NH_4^+-N g^{-1} VSS h^{-1}	42 ± 2	39 ± 2
Removal rate	kg NH_4^+-N day^{-1} m^{-3}	1.1 ± 0.1	0.35 ± 0.05

8.2.6 Starvation conditions

In order to know how the systems behave under starvation conditions, for instance due to temporary maintenance of dewatering units, biomasses of both reactors were aerated without addition of influent (endogenous conditions) during five consecutive days. Both reactors were then started again with the steady state operating conditions explained above. Only the SHARON reactor was able to recover its complete activity in less than two days. The SBR could not reach steady state conditions until after 3-4 days, showing that the lower maximum ammonium growth rate and high steady state SRT has a large influence on the system when its operating conditions are modified. In addition, lower SRT, like in SHARON process, support better the changes. Figure 8.5 shows the behaviour of the SHARON process in starvation conditions. At the beginning of the experiment, the influent, composed by 600 mg NH_4^+-N L^{-1} was stopped to be fed (0.25 day) and pH began to drop below 6 until alkalinity was completely consumed. This made the ammonium concentration to drop below the normal effluent characteristics (225 mg L^{-1} instead of 300 mg L^{-1}). Simultaneously, the nitrite began to increase up to 380 mg L^{-1}. Although the unfavourable HRT conditions, there were no nitrates in the media showing that the wash-out of nitrite oxidisers was done properly during the 6 month operation. At day 5, when the influent was fed again to the system, ammonium concentration began to increase and nitrite decreased until it reached its usual value in less than two days. The pH profile increased during the first 10 hours after restarting the feed until a value of 7.5 due to the acid/alkali equilibrium and then recovered its normal value of 6.7 in less than two days. This last experiment showed the reliability and stability of the SHARON process in front of the SBR process.
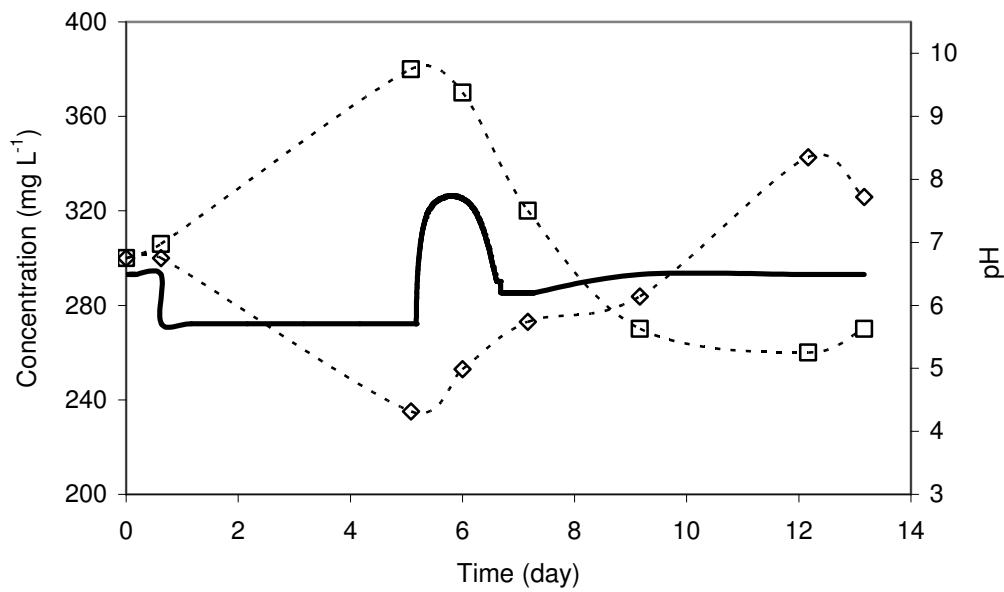
Figure 8.5: Chemostat profiles during starvation experiment: NH_4^+-N (--◊--), NO_2^--N (--□--), pH (bold solid line)

8.3 CONCLUSIONS

✓ The partial nitrification via nitrite was correctly achieved to treat real reject water in a 4 hour cycle SBR reactor and a SHARON continuous process.

✓ Two different ways to stop nitrate route were successfully tested and provided good performance and pollutants removal.

✓ The specific efficiencies and the kinetic and stoichiometric parameters were quite similar for both reactors having the SHARON process high ammonium growth rate.

✓ The total daily nitrogen removal was higher in the SBR due to the lower HRT achieved which would mean a smaller volume reactor.

✓ According to the stability of the system, the SHARON reactor presented a better capacity to assume ammonium concentration fluctuations and starvation periods.

9. WASTEWATER TREATMENT PLANT MODELLING

Scope

Modelling of a WWTP allows assessing if the plant is working at the right conditions and can also provide information about any change to be done. Therefore, in this Chapter the WWTP under study is modelled with the ASM1 model in order to see if the actual pollutant profile fits the model predictions. Moreover, modelling for enlarging the plant with a nitrification/denitrification step is done. AQUASIM 2.0 is the software used to perform the simulation tests.

9.1 INTRODUCTION

Activated sludge models (Henze et al., 2000) represent the most widespread and successful approach to simulate the nutrient removal processes. In this way, the modelling of a WWTP can be very positive in order to verify if the effluent requirements are well predicted. As it is stated in Chapter 1, the biological nature of wastewater treatment processes implies that their model parameters must be determined (model calibration) according to the local situation (Vanrolleghem et al., 1999). Initially, however, the model is applied with the default values proposed. As it is presented (Chapter 1), ASM1 is still one of the most used models for the simulation of biological processes.

AQUASIM 2.0 (Swiss Federal Institute for Environmental, Switzerland 1998) is the registered software used to model the different biological processes. It is necessary to define the variables of interest, as well as the most important parameters such as the initial conditions, the biochemical reactions, the number and volume of tanks, and the kinetic and stoichiometric parameters. In addition the software works in a matrix structure, and the stoichiometry of the different reactions must be listed in a matrix form (see AQUASIM 2.0 Software screen capture in Annexe VI.1).

9.2. RESULTS AND DISCUSSION

9.2.1. WWTP view

The urban wastewater biological treatment plant under study is treating the wastewater from an area of 200000 inhabitants' equivalent. As it is stated in Figure 4.1 the head of the WWTP has a primary treatment (Q3 to Q6) composed by a filtration unit, a coagulation-flocculation step and a primary settler. Then, the wastewater flows to a conventional activated sludge system without nitrogen removal step (between flows Q6 and Q11). Moreover, the WWTP has a one phase anaerobic sludge digester for the treatment of primary and secondary sludge (between flows Q20 and Q21). The average total flow rate in the plant is 49000 m^3 day^{-1} (Q3).

9.2.2 The activated sludge system

Considering the plant configuration, the important section from the biological point of view is the secondary activated sludge treatment. In that way, the modelling of the biological reactor and the settler with ASM1 is developed.

First of all, it is necessary to characterise the wastewater under study. In Table 9.1 the average concentration and characterisation of the main pollutants from the secondary reactor wastewater (influent and effluent) are shown. It is important to notice that the main pollutants are COD, BOD_5, suspended solids and nitrogen, obtaining very good removal efficiencies for the first three, and having only a bit reduction of nitrogen by assimilation due to the lack of nitrification/denitrification treatment in the plant.

Table 9.1: Reactor influent and effluent characteristics (average values of 2005)

Component	Unit	Influent	Effluent
TSS	mg L^{-1}	120 ± 10	26 ± 5
COD	mg L^{-1}	450 ± 100	90 ± 10
CODs	mg L^{-1}	75 ± 20	60 ± 10
BOD_5	mg L^{-1}	250 ± 50	14 ± 2
N_{total}	mg L^{-1}	70 ± 10	45 ± 5
NH_4^+-N	mg L^{-1}	50 ± 10	45 ± 5
NO_3^--N	mg L^{-1}	0	< 4
P-total	mg L^{-1}	< 10	< 4
Alkalinity	mol m^3	7 ± 0.5	6 ± 0.5
pH	-	7.5 ± 0.1	7.5 ± 0.1
Temperature	ºC	10-20	10-20

The operational characteristics of the biological reactors under operation are also important to know when doing the simulation tests. According to that, in Table 2 there are the main points of the operational conditions where it can be seen that the actual operating volume is around 18000 m^3.

Table 9.2: Activated sludge operational characteristics

Parameter	Units	Value
Number reactors	-	8
Volume 1 reactor	m^3	3000
Operating reactors	-	6
HRT	hour	9-10
SRT	day	3 [s] - 4 [w]
TSS	mg TSS L^{-1}	1000 [s] - 2000 [w]
Recirculation (Q_{rec})	% $Q_{influent}$	100
$Q_{influent}$	m^3 day^{-1}	46000
DO	mg L^{-1}	1
Temperature	ºC	10 [w] - 20 [s]
pH range operation	-	7-7.5

(s = summer conditions; w =winter conditions)

9.2.3 Modelling approach

In order to start the modelling of the biological secondary treatment, it is important to calculate all the soluble (S) and particulate (X) parameters that are needed in the ASM1 model. The latter components, related to COD and nitrogen, are calculated following the protocol explained in Chapter 1 (section 1.5.7). In Table 9.3 there are the main relationships extracted from calculations of COD and nitrogen mass balances that are introduced in the modelling process.

Table 9.3: Fraction of particulate and soluble compounds

Parameter	Value
Fraction of biodegradable COD on total COD	0.5
Fraction of dissolved inert COD on total inert COD	0.7
Fraction of dissolved biodegradable COD on total biodegradable COD	0.12
Fraction of dissolved nitrogen on Kjeldahl nitrogen	0.7
Fraction of NH_4^+ on dissolved Kjeldahl nitrogen	0.64

Considering that the modelling parameters in the process are COD and nitrogen, it is important to know their daily fluctuations. In addition, in Figure 9.1 there are presented the fluctuations values of COD, N_{total} and flow-rate related to their average values (Table 9.1 and 9.2). The variations are also going to be introduced in the model.
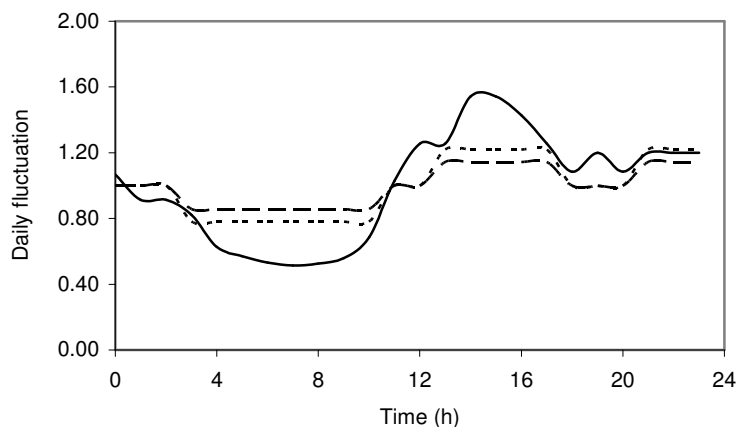

Figure 9.1: Secondary treatment flow-rate (solid line), COD (----) and N ($——$) daily fluctuation

The stoichiometric and kinetic parameters for the process modelling are introduced according to the default constant values and the default variation values with temperature reported in Table 1.3 (Chapter 1).

9.2.4 Modelling the secondary treatment with ASM1

Once all the needed parameters are known and considerations are done, the modelling experiments can be started. It is important to know that the WWTP behaviour is not the same in summer that in winter conditions. In this way, the modelling is going to be done in these extreme conditions reached in the wastewater under study (10-12 ºC in winter and 20-22 ºC in summer). The ASM1 model parameters, the secondary treatment influent characteristics and operational conditions explained above were used. Although the WWTP is operating with 6 reactors of 3000 m^3, in the simulation one continuous stirred tank of 18000 m^3 was considered to simplify the simulation test. Moreover, for the settler a volume of 1 m^3 with total solid separation in the effluent was selected.

WWTP in winter conditions. With the influent and operational characteristics mentioned, the simulation test started. Figure 9.2 shows the daily profiles of (a) soluble components and Xs and (b) particulate components of the effluent from the secondary reactor before entering the settle
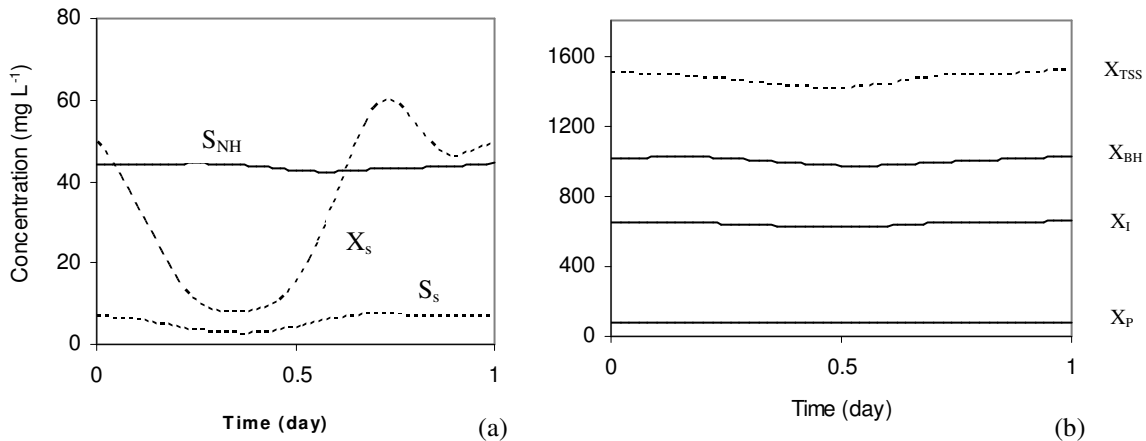

Figure 9.2: Secondary reactor winter daily profiles (a) S_{NH}, S_S and X_S (b) Particulates fractions ($X_{BA} = 0$ mg COD L^{-1}) (The simulated values can be found in Annexe VI.2)

The COD effluent concentration and the nitrogen concentration are nearly the same than the real values. It is important to remark that with the actual conditions, there is only aerobic COD removal without nitrification. The biomass concentration predicted by the model is lower than the measured in the WWTP reactors (Figure 9.2 b).

WWTP in summer conditions. In this period only the temperature and the sludge age were changed according to Table 9.2. Figure 9.3 shows the daily simulated data for (a) soluble compounds and Xs and (b) particulate compounds. In this situation the simulation results are a bit different compared with what happens in the real situation. It is normal that in summer some nitrification takes place due to the temperature effect. But in this case the model predicted nearly 70% nitrification when normally there is no

more than 20% (WWTP personal communication). The biomass concentration (X_{TSS} = 1000 mg TSS L^{-1}) is well predicted by the model.
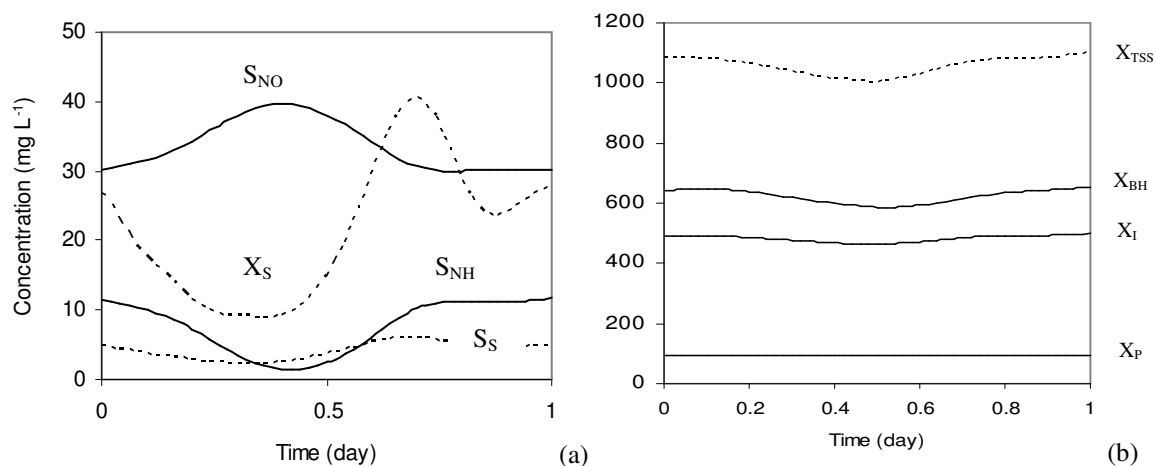

Figure 9.3: Secondary reactor summer daily profiles (a) S_{NH}, S_{NO}, S_S and X_S (b) Particulates fractions (X_{BA} = 50 mg COD L^{-1}) (The simulated values can be found in Annexe VI.3)

There are different reasons that could explain what is happening. The first is that the WWTP uses superficial turbines for the aeration process which lead to have the bottom regions nearly unaerated. This fact affects nitrification because in those regions there are low oxygen concentrations. The available oxygen is consumed by the heterotrophic microorganisms which have more affinity and the nitrification kinetic is reduced. The second fact is that in the real WWTP there is a small part of particulate compounds that leave the settler with the treated wastewater as it can be seen in Table 9.1. This fact makes reduce the sludge age and consequently affects nitrification. Finally, it could be that the actual sludge age was lower than the calculated by the WWTP which would imply lower nitrification.

In both situations, winter and summer, if the reject water treatment was carried out the total nitrogen effluent in the secondary clarifier would be reduced around 20-25%, which would suppose a better effluent quality.

9.2.5 Enlarging the WWTP to nitrification/denitrification

Considering the results reported in the previous section it can be extracted that the enlargement of the WWTP with a nitrogen removal step would be very positive taking into account the law requirements. The actual wastewater BOD_5/N ratio is around 3.5. In that way the implementation of the nitrification/denitrification step is considered placing the denitrification (R1) before the nitrification (R2) to use the biodegradable COD of the wastewater in the anoxic process (Figure 9.4). It would be also needed to increase the percentage of total recirculation, with a new recirculation flow-rate (Qrec2), from nitrification to denitrification reactor.
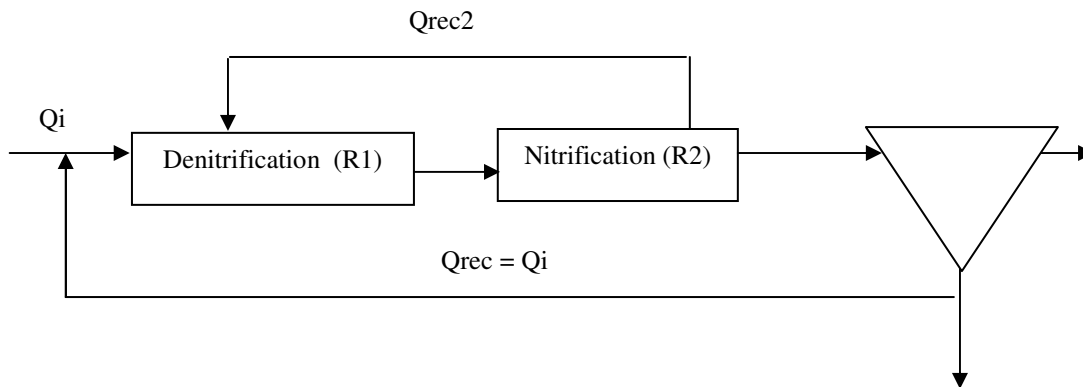
Figure 9.4: WWTP proposed modification

Two kinds of situations were considered. In the first one the nitrogen removal step was implanted considering the actual available volumes to find the maximum removal efficiency. In the second situation, it was studied how much extra volume construction would be needed to achieve nitrogen removal under law limits (Directive 91/271/EEC). In both situations, it was chosen to work with an average temperature of 15 ºC and with a new sludge age of 9 days in order to achieve correctly the nitrification in winter and summer. Moreover, the oxygen concentration proposed was 2 mg L^{-1} instead of 1 in order to work with better kinetics avoiding low oxygen concentrations in the bottom regions.

Modification with the actual volumes. Considering the proposed modification shown above, the enlargement of the WWTP to nitrogen removal step was considered working with the total available volume of the WWTP (24000 m³). In that way, different flow-rate recirculation percentages and size volumes for nitrification and denitrification were tested and modelled (see Annexe VI.4). The best treatment sequence was achieved when working with a denitrification tank of 7000 m³, a nitrification tank of 17000 m³ and a total recirculation percentage (Qrect = Qrec + Qrec2) of 200% of the initial flow-rate Qi. Figure 9.5 shows the daily effluent concentration profiles for (a) denitrification reactor and (b) nitrification reactor working with a total biomass concentration around 5000 mg TSS L⁻¹.
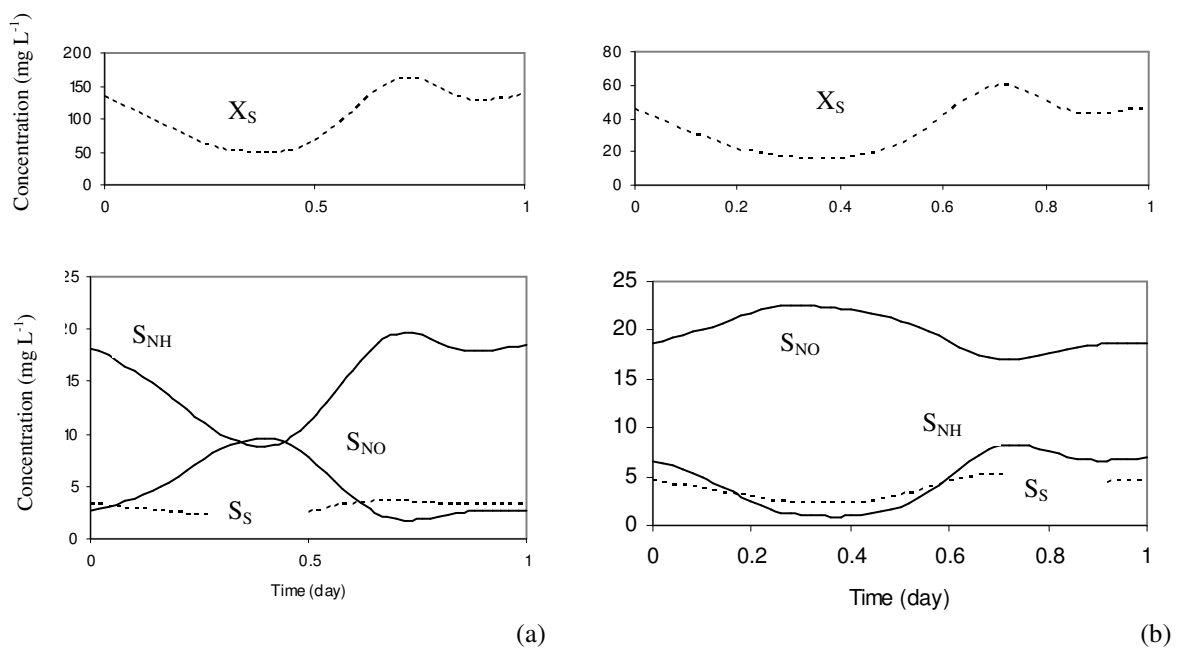

Figure 9.5: Nitrogen removal step daily profiles (a) denitrification reactor (b) nitrification reactor

(The simulated values for enlarging the WWTP to N/DN can be found in Annexe VI.5)

As shown in Figure 9.5a, the final COD obtained (Ss and Xs) is low, meaning that most part of it is used for denitrification. The nitrogen concentration is composed by ammonia (< 8 mg L^{-1}) and nitrates (20 mg L^{-1}). These values are better than when no nitrification step was proposed but they are still over the limits proposed by the 91/271/EEC directive (N_{total} < 10 mg L^{-1} for WWTP > 100000 inhabitants equivalent).

Modification with new tanks. With the wastewater characteristics it is not possible to achieve complete nitrification/denitrification. Extra recirculation did not improve denitrification if and external carbon source is not added because there is a lack of COD to perform denitrification. The construction of extra volume would only reduce a little the nitrogen effluent concentrations which would not compensate the cost (see Annexe VI.6).
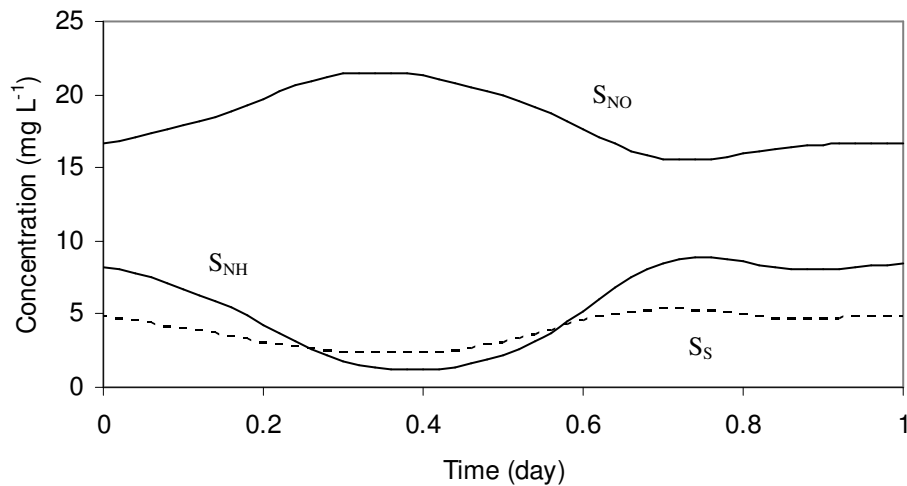

Figure 9.6: Clarifier effluent after denitrification/nitrification reactors

Figure 9.6 shows the clarifier effluent nitrogen pollutants with the volume and recirculation rates proposed in Figure 9.5. As it can be seen, there is nearly no ammonia concentration (< 8 mg L^{-1}) in the daily clarifier effluent and nitrate concentration is

around 15-17 mg L^{-1}. If further denitrification is required, an external organic carbon source must be added. But in this situation, the reject water treatment as proposed between Chapters 4-8, could be very positive. It would suppose the reduction of 25% of total nitrogen entering the secondary treatment. The total nitrogen concentration in the effluent of the WWTP could be reduced between 6-8 mg L^{-1} obtaining a total nitrogen effluent concentration around 15-17 mg N L^{-1}. This value is still over the law limits but would suppose lower external carbon source addition.

Therefore, the addition of an external organic carbon source or an internal organic carbon from the own WWTP coupled with reject water treatment would be the best election to reduce costs.

9.3 CONCLUSIONS

- ✓ The WWTP simulation fitted correctly with the real profiles in winter, but the model predicted more nitrification than the occurred in summer.
- ✓ The enlargement of the WWTP with a nitrification/denitrification step would result in nitrogen concentration over the law limits with the actual wastewater characteristics.
- ✓ An extra addition of organic carbon to complete the denitrification in the main line combined with reject water treatment would be the best economical election to operate and to observe the law.

10. CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Reject water treatment appears to be a very satisfactory alternative for meeting local discharge requirements. In that way, sequencing batch reactors and chemostat reactors were studied to treat reject water where the removal of nitrogen contaminants was over 95%. On balance, the techniques used in the laboratory were feasible with the operational conditions worked. In the following lines there are the general conclusions that can be extracted.

Reject water characterisation

Reject-water composition includes mainly high ammonium and COD concentrations. The latter has a very small fraction of BOD_{ST} which is useless for denitrification.

Start-up and optimisation of an SBR

A start up of a SBR to treat reject water was studied from sludge acclimation to steady state. In that acclimation period nitrification and denitrification had different behaviours. The first had an exponential phase that lasted 20 days with a sAUR of 30-32 mg NH_4^+-N g^{-1} VSS h^{-1}, and was very pH dependent placing the optimum value for inoculum adaptation at 8. The second was quick, lasting 6-7 days with a kinetic at the stationary stage of 20 mg NO_2^--N g^{-1} VSS h^{-1} and 14 mg NO_3^--N g^{-1} VSS h^{-1} showing that denitrification of nitrate is the bottleneck of the anoxic process. Nitrification/denitrification in the SBR studied gave reasonable pollutants removal. Conversion rates of 14 mg NH_4^+-N g^{-1} VSS h^{-1} and 30 mg NO_2^--N g^{-1} VSS h^{-1} were good,

and a significant reduction of N in the plant effluent can be achieved (25% of the 2500 ± 250 kg N day^{-1} evacuated by the WWTP). Consequently, such a treatment proves to be very satisfactory for meeting local discharge requirements.

However, when the system is optimised, better values are achieved. The sAUR was assessed for every studied case and it was demonstrated that the kinetics increase with temperature until 37ºC. Moreover the influence of the cycle length was also studied providing an optimum efficiency for 8 hour time cycle. In order to avoid the use of external chemicals to control the pH in an optimum interval (7.5-8.5), the best strategy consisted of alternating different aerobic/anoxic sub-cycles during the operational cycle with methanol as a carbon source for denitrification. The low nitrite concentration formed due to the sub-cycles strategy contributes to improve the SBR performance due to the lack of toxicity inside the reactor.

In that optimised SBR the nitrification via nitrite was achieved correctly combining the pH range and the low dissolved oxygen concentration inside the reactor. This fact allows saving costs in terms of oxygen supply in nitrification (25%) and COD addition in denitrification (40%).

SBR operating with hydrolysed primary sludge

Although laboratory experiments were performed with methanol, the estimations suggest the use an internal organic carbon source from the WWTP in order to reduce the operational costs in an industrial SBR. In that direction, different internal organic carbon sources from the WWTP under study were tested. The hydrolysed primary sludge was found as the only feasible source to be used.

The operation of a SBR with hydrolysed primary sludge was satisfactory obtaining similar efficiencies like when using methanol. From the economic point of view the use of VFA from primary sludge would suppose a 25% bigger reactor construction, but it would lead to a saving of 0.2-0.3 € kg $^{-1}$ N removed during the process operation.

Comparison SBR vs. SHARON chemostat

Two different ways of developing nitrification via nitrite were tested with good performance and pollutants removal in both cases. Considering the point of view of nitrogen conversion, the SBR appeared to be better than the chemostat SHARON/denitrification due to the lower HRT. Moreover the SBR would become a cheaper process. However, the chemostat process performance was better when there were modifications in the influent flow-rate and nitrogen concentrations which mean a more stable process. Therefore, the selection of one or other system would have similar results in terms of N-removal and would depend on the emplacement of the WWTP and its space.

The SBR and the chemostat were also compared to achieve an influent ready for Anammox process. The two different ways to stop the nitrate route were successfully tested and provided good performance and pollutants removal. The obtained specific efficiencies and the kinetic and stoichiometric constants were quite similar for the SBR and for the SHARON process. The total daily nitrogen removal was higher in the SBR due to the lower HRT achieved, which would imply a smaller volume reactor.

WWTP modelling

The modelling process showed that the WWTP data fitted correctly during winter periods, but the model predicted more nitrification in summer than the real values obtained. This could be explained by the existence of unaerated bottom regions that affect nitrification kinetics and the difference between the real and supposed sludge age. Moreover, the enlarging of the WWTP to nitrogen removal step with the actual volumes would imply the treatment of reject water separately combined with a little addition of an external carbon source or an internal carbon source from the own WWTP to complete the denitrification in the main line. With this situation, the WWTP would be able to achieve nitrification/denitrification with law requirements.

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NEW PROPOSALS AND RECOMMENDATIONS

Laboratory operation

Laboratory experiments were done obtaining satisfactory results. However, there are different items that can be modified in order to obtain better results.

One of the most important aspects is the economic point of view, as it has been seen in Chapter 6 when using primary hidrolysate to denitrify. Therefore, considering the results obtained in this experiment it would be positive to consider the operation of the chemostat SHARON/denitrification reactor with primary hidrolysate in order to see how this system is affected. Another option to avoid the methanol addition would be trying the autotrophic denitrification using HS^-. The latter would be feasible because the anaerobic digestion produces H_2S, which must be removed.

Moreover, the alternative of using partial nitrification combined with Anammox process would be also a very good solution. According to that, the development of an Anammox reactor filled with the influent proposed in Chapter 8 would be very important in order to observe its behaviour.

Modelling

The modelling of the WWTP has shown satisfactory results, but there are different options that could be tested. First of all, the application of BABE process in the WWTP could be considered in order to see the savings in space and cost. Secondly, the WWTP is structured with 4 biological lines with two reactors in each one, but the modelling has been considered as only one big reactor. According to that, the modelling considering the different flow-rate percentages that goes to each line would be more relevant.

Finally, the calculation of the real SRT through a phosphorous mass balance and a real integral sample collecting should be done in order to verify if one of the default parameters proposed by the ASM1 model must be changed.

11. NOMENCLATURE

SYMBOL	UNITS	DESCRIPTION
ANAMMOX	(-)	ANaerobic AMMonium OXidation
AOB	(-)	Ammonia Oxidant Bacteria
ASM	(-)	Activated Sludge Model
ATU	(-)	Allyl-Thiourea
AUR	$(mg\ NH_4^+\text{-N}\ L^{-1}\ h^{-1})$	Ammonium Uptake Rate
b_A	(day^{-1})	Autotrophic decay
b_H	(day^{-1})	Heterotrophic decay
BNR	(-)	Biological Nitrogen Removal
BABE	(-)	Bio-Augmentation Batch Enhanced process
BOD_{ST}	$(mg\ BOD\ L^{-1})$	Biological Oxygen Demand at short time
BOD_5	$(mg\ BOD\ L^{-1})$	Biological Oxygen Demand at 5 days
CANON	(-)	Completely Autotrophic Nitrogen removal Over Nitrite
COD	$(mg\ COD\ L^{-1})$	Chemical Oxygen Demand
DN	(-)	Denitrification
DO	$(mg\ O_2\ L^{-1})$	Dissolved oxygen concentration
eff	(-)	Effluent
f_p	(-)	Particulate fraction
HRT	(day)	Hydraulic retention time
HS	(-)	Half saturation coefficient
g	(-)	Gram
h	(-)	Hours
inf	(-)	Influent
i_N	$(mg\ N\ mg^{-1}\ COD)$	Proportion of nitrogen
i_{XB}	$(mg\ N\ mg^{-1}\ COD)$	Proportion of nitrogen in biomass
i_{xp}	$(mg\ N\ mg^{-1}\ COD)$	Proportion of nitrogen in particulates

SYMBOL	UNITS	DESCRIPTION
IWA	(-)	International Water Association
Kj	(-)	Kjeldahl
k_d	(-)	Lineal decay rate coefficient
k_h	(day^{-1})	Hydrolysis rate
K_x	$(mg\ I\ L^{-1})$	HS hydrolysis
K_{NH}	$(mg\ NH_4^+\text{-}N\ L^{-1})$	Ammonia HS in nitrification
K_{NO}	$(mg\ N\text{-}NO_3^-\text{-}N\ L^{-1})$	Nitrate HS in DN
k_a	$(L\ mg^{-1}\ COD\ day^{-1})$	Ammonification rate
K_{OA}	$(mg\ O_2\ L^{-1})$	Oxygen HS for nitrification
K_{OH}	$(mg\ O_2\ L^{-1})$	Oxygen HS for DN bacteria
K_S	$(mg\ COD\ L^{-1})$	Oxygen HS for heterotrophic bacteria
L	(-)	Litre
M	(kg)	Weight
μ	(day^{-1})	Specific kinetic growth
μ_{max}	(day^{-1})	Max. specific kinetic growth
μ_{mA}	(day^{-1})	Max. specific autotrophic kinetic growth
μ_{mAOB}	(day^{-1})	Max. specific AOB kinetic growth
μ_{mH}	(day^{-1})	Max. specific heterotrophic kinetic growth
$\nu_{i,j}$	(-)	Stoichiometric coefficient for i component in process j
M & M	(-)	Materials and Methods
min	(-)	Minutes
N/DN	(-)	Nitrification/Denitrification
η_{no2}	(-)	Corrector factor in nitrite denitrification
η_{no3}	(-)	Corrector factor in nitrate denitrification

SYMBOL	UNITS	DESCRIPTION
ŋ_h	(-)	Corrector factor in hydrolysis
N	(-)	Nitrogen
NOB	(-)	Nitrite Oxidant Bacteria
NUR	(mg NO_X^--N L^{-1} h^{-1})	Nitrate/nitrite Uptake Rate.
OUR	(mg O_2 L^{-1} min^{-1})	Oxygen Uptake Rate
OC	(-)	Oxygen consumption
Qrec	($m3$ day^{-1})	Recirculation flow-rate
r_x	(mg cellular COD L^{-1} h^{-1})	Kinetic of bacterial growth
r_{deacy}	(mg cellular COD L^{-1} h^{-1})	Kinetic of decay rate
rpm	(-)	Revolutions per minute
S_{ALK}	(mol HCO_3^- L^{-1})	Alkalinity
sAUR	(mg NH_4^+-N g^{-1} VSS h^{-1})	Specific Ammonium Uptake Rate
S	(mg COD L^{-1})	Substrate concentration
S_I	(mg COD L^{-1})	Soluble inert COD
S_{ND}	(mg N L^{-1})	Soluble organic nitrogen
S_{NH}	(mg NH_4^+-N L^{-1})	NH_4^+ concentration
S_{NO}	(mg NO_3^--N L^{-1})	NO_3^- concentration
sNUR	(mg NO_X^--N g^{-1} VSS h^{-1})	Specific Nitrate/Nitrite Uptake Rate
S_O	(mg O_2 L^{-1})	Dissolved oxygen concentration
S_S	(mg COD L^{-1})	Readily soluble COD
SBR	(-)	Sequencing Batch Reactor
SHARON	(-)	Single-reactor High activity Ammonia Removal Over Nitrite
SRT	(day)	Solid Retention Time
SVI	(mL g^{-1})	Solid Volumetric Index
S_{TO}/X_{TO}	(-)	Food/biomass ratio
s	(-)	Seconds

SYMBOL	UNITS	DESCRIPTION
t	(day)	Time
T	(ºC)	Temperature
TS	(mg TS L^{-1})	Total solids
TSS	(mg TSS L^{-1})	Total suspended Solids
TVS	(mg TVS L^{-1})	Total volatile solids
X	(mg cellular COD L^{-1})	Biomass concentration
X_{BA}	(mg cellular COD L^{-1})	Autotrophic biomass concentration
X_{BH}	(mg cellular COD L^{-1})	Heterotrophic biomass concentration
X_I	(mg COD L^{-1})	Inert particulate COD
X_{ND}	(mg N L^{-1})	Particulate organic nitrogen
X_S	(mg COD L^{-1})	Particulate biodegradable COD
X_P	(mg COD L^{-1})	Particulate inert COD products
X_{TSS}	(mg TSS L^{-1})	Total suspended solids concentration
Y	(mg COD mg COD)$^{-1}$	Yield
Y_H	(g COD mg COD)$^{-1}$	Heterotrophic yield
Y_A	(mg COD N-NH_4^+-N)$^{-1}$	Autotrophic yield
Y_{AOB}	(mg COD N-NH_4^+-N)$^{-1}$	AOB autotrophic yield
V	(L)	Volume
VFA	(-)	Volatile Fatty Acids
Vs	(m h^{-1})	Sedimentation speed
V30	(mL)	Sedimentability at 30 minutes
VSS	(mg VSS L^{-1})	Volatile suspended solids
WWTP	(-)	Wastewater Treatment Plant

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