TREATMENT OF MATURE URBAN LANDFILL LEACHATES BY ANAMMOX PROCESS

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PhD Thesis

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CERTIFIQUEN

Que aquest treball, titulat "**Treatment of mature urban landfill leachates by anammox process**", que presenta el llicenciat MAEL RUSCALLEDA BEYLIER per a l'obtenció del títol de doctor, ha estat realitzat sota la seva direcció i compleix els requeriments per optar a la Menció Europea.

I perquè en prengueu coneixement i tingui els efectes que correspongui, presentem davant la Facultat de Ciències de la Universitat de Girona l'esmentada Tesi, signant aquesta certificació a

Girona, Octubre de 2011

Mª Dolors Balaguer Condom   Jesús Colprim Galceran

"La manera com veiem el problema és el problema"

Stephen R. Covey

Agraïments/Acknowledgements

Els agraïments d'una tesi són un capítol fonamental: fa que la gent que no aguanti més d'una pàgina d'introducció aprovi el balanç final de quatre anys de feina i marca la lectura dels següents capítols a qui se'ls ha de llegir. Així que val més esmerçar-s'hi, però començar a escriure sempre és difícil, així que he buscat diferents mètodes en bones referències.

El mètode Ganigué (2010) parteix de la premissa que no es pot fer content a tothom i prescriu que les coses cal deixar-les anar tal com raja. Això sí, començant pel començament i això vol dir en Jesús i la Marilós. Els complements perfectes, el yang i el ying en perfecte equilibri. Els he d'agrair l'oportunitat d'haver pogut formar part d'aquest seguit de projectes , des del CLONIC fins al LEAMMOX i els que queden per venir. Vaig començar amb la branca de gestió d'ambientals sota el braç i he acabat amb un bagatge d'experiència impressionant. Si la paciència és la mare de la ciència, ells amb mi n'han tingut molta!

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Table of contents

Agraïments/Acknowledgements

List of Tables

List of Figures

Notation and abbreviations

η: Anoxic reduction factor

μ_{AMX}: Maximum growth rate for anammox bacteria [d^{-1}]

μ_H: Maximum growth rate for heterotrophic bacteria [d^{-1}]

ANAMMOX: ANaerobic AMMonium Oxidation

AOA: Ammonium Oxidizing Archaea

AOB: Ammonium Oxidizing Bacteria

ASM: Activated Sludge Models

b_{AMX}: Decay rate coefficient for anammox [d^{-1}]

b_H: Decay rate coefficient for heterotrophic bacteria [d^{-1}]

BOD: Biochemical Oxygen Demand [$mgO_2\cdot L^{-1}$]

BOD_u: Ultimate Biochemical Oxygen Demand [$mgO_2\cdot L^{-1}$]

CANON: Completely Autotrophic N-removal Over Nitrite

CLSM: Confocal Laser Scanning Microscope

COD: Chemical Oxygen Demand [$mg\ O_2\cdot L^{-1}$]

DEMON: pH controlled DEamMONification system

DO: Dissolved Oxygen [$mg\ O_2\cdot L^{-1}$]

DOC: Dissolved Organic Carbon [$mg\ C\cdot L^{-1}$]

EEM: Excitation-Emission Matrix

EC: Electrical Conductivity [$\mu S\cdot cm^{-1}$]

EfOM: Effluent Organic Matter

FA: Free Ammonia [$mg\ NH_3$-$N\cdot L^{-1}$]

FISH: Fluorescent *in situ* Hybridisation

f_i: Inert fraction of biomass [$g\ COD\cdot g^{-1}COD$]

FNA: Free Nitrous Acid [$mg\ HNO_2$-$N\cdot L^{-1}$]

HCO_3^-: Bicarbonate [$mg\ HCO_3^-$-$C\cdot L^{-1}$]

HRT: Hydraulic Retention Time [d]

IC: Inorganic Carbon [$mgC\cdot L^{-1}$]

$i_{N,BM}$: Nitrogen content of the biomass [$g\ N\cdot g^{-1}$ COD]

K_H: Maximum specific hydrolysis rate [d^{-1}]

K_{NO2}^{AMX}: Nitrite saturation constant for anammox [$g\ NO_2$-$N\cdot m^{-3}$]

K_{NH4}^{AMX}: Substrate saturation constant for anammox [$g\ NH_4$-$N\cdot m^{-3}$]

K_{NO2}^{H}: Nitrite saturation constant for heterotrophs [$g\ NO_2$-$N\cdot m^{-3}$]

K_{NO3}^{H}: Nitrate saturation constant for heterotrophs [$g\ NO_2$-$N\cdot m^{-3}$]

K_I^H*:* Nitrite inhibition constant for heterotrophs [$g\ NO_2$-$N\cdot m^{-3}$]

K_S: Heterotrophic bacteria half-saturation constant for ready biodegradable substrate [$g\ COD\cdot m^{-3}$]

K_x: Half-saturation constant for slowly biodegradable substrate [$g\ COD\cdot g^{-1}$ COD]

MLSS: Mixed Liquor Suspended Solids [$mgSS\cdot L^{-1}$]

NDR: Nitrogen Discharge Rate [$kg\ N\cdot m^{-3}\cdot d^{-1}$]

NLR: Nitrogen Loading Rate [$kgN\cdot m^{-3}\cdot d^{-1}$]

NH_4^+-N: Ammonium [$mg\ NH_4^+$-$N\cdot L^{-1}$]

NO_2^--N: Nitrite [$mg\ NO_2^-$-$N\cdot L^{-1}$]

NO_3^--N: Nitrate [$mg\ NO_3^-$-$N\cdot L^{-1}$]

$NH_4^+{}_{ANAMMOX}$: Ammonium removed via anammox [$mg\ NH_4^+$-$N\cdot L^{-1}$]

$NO_2^-{}_H$: Nitrite removed by heterotrophic denitrification [$mg\ NO_2^-$-$N\cdot L^{-1}$]

$NO_3^-{}_H$: Nitrate removed by heterotrophic denitrification [$mg\ NO_3^-$-$N\cdot L^{-1}$]

NOB: Nitrite Oxidizing Bacteria

NRR: Nitrogen Removal Rate [$kg\ N\cdot m^{-3}\cdot d^{-1}$]

OLAND: Oxygen-Limited Autotrophic Nitrification/Denitrification

OM: Organic Matter

PANAMMOX: Partial nitritation-ANAMMOX in SBR

PARAFAC: PARAllel FACtor analysis

PID: Proportional-Integral-Derivative controller

PN-SBR: Partial Nitritation-Sequencing Batch Reactor

Q_i: Inflow [$L \cdot d^{-1}$]

r^2: Square of the correlation coefficient

RBC: Rotating Biological Contactor

RO: Reverse Osmosis

rDNA: Ribosomic Deoxyribonucleic Acid

SAA: Specific Anammox Activity [$kg\ N \cdot kg^{-1}\ VSS \cdot d^{-1}$]

SMP: Soluble Microbial Products

SBR: Sequencing Batch Reactor

SHARON: Single reactor High activity Ammonia Removal Over Nitrite

S_I: Inert soluble organic matter state variable [$g\ COD \cdot m^{-3}$]

S_{IC}: Total inorganic carbon state variable [$g\ C \cdot m^{-3}$]

S_{N2}: Total nitrogen gas state variable [$g\ N \cdot m^{-3}$]

S_{NH}: Total ammonium and ammonia nitrogen state variable [$g\ N \cdot m^{-3}$]

S_{NO2}: Total nitrite and nitrous acid nitrogen state variable [$g\ N \cdot m^{-3}$]

S_{NO3}: Total nitrate nitrogen state variable [$gN \cdot m^{-3}$]

SND: Simultaneous Nitrification-Denitrification

SRT: Sludge Retention Time [d]

S_S: Ready biodegradable organic matter state variable [$g\ COD \cdot m^{-3}$]

T: Temperature [ºC]

TOC: Total Organic Carbon [$mg\ C \cdot L^{-1}$]

TSS: Total Suspended Solids [$mg\ TSS \cdot L^{-1}$]

VER: Volumetric Exchange Ratio

V_{max}: Maximum water volume [L]

V_{min}: Minimum water volume [L]

VSS: Volatile Suspended Solids [$mgVSS \cdot L^{-1}$]

WWTP: WasteWater Treatment Plant

X_{AMX}: Anaerobic ammonium oxidising bacteria biomass state variable [$g\ COD \cdot m^{-3}$]

X_H: Heterotrophic biomass state variable [$g\ COD \cdot m^{-3}$]

X_I: Inert particulate organic matter state variable [$g\ COD \cdot m^{-3}$]

X_S: Slowly biodegradable substrates state variable [$g\ COD \cdot m^{-3}$]

Y_{AMX}: Yield of anaerobic ammonium oxidation [$g\ COD \cdot gN^{-1}$]

Y_H:Yield of denitrification [$gCOD \cdot g\ COD^{-1}$]

ΔNH_4^+: Ammonium increment

ΔNO_2^-: Nitrite increment

ΔNO_3^-: Nitrate increment

Summary

This thesis is the result of a long collaboration of the LEQUIA-Group with CESPA-GR, a private corporation of environmental services that manages several landfill sites for the disposal of solid municipal wastes. Landfilling is the most used solution for the final treatment of urban solid wastes. These facilities have been highly engineered during the last decades in order to limit biogas emissions and the percolation of landfill leachates to the adjacent water bodies. The management of landfill leachate arises as one of the main environmental concerns in these facilities. These streams are highly contaminated wastewater with a complex mixture of organic and inorganic compounds and characterized by a high ammonium content and low biodegradable organic matter fraction. Leachates are often treated by expensive physicochemical processes dealing with nitrogen recovery, such as ammonia stripping. Conventional biological nitrogen removal with the combined nitrification/denitrification is cheaper but the carbon to nitrogen (C:N) ratio in leachates makes necessary the use of external carbon source addition for complete denitrification. During the last two decades, the feasibility to deal with fully autotrophic nitrogen removal in combined partial nitritation-anammox processes to treat highly nitrogen loaded streams with low C:N has been tested and demonstrated. A wide number of experiences applying fully autotrophic partial nitritation-anammox is focused on the treatment of sludge digestion supernatant, but there are still very few studies reporting the use of anammox process to treat landfill leachate.

Combined partial nitritation-anammox processes in two separated tanks was proposed in our group to treat landfill leachate. A stable partial nitritation process was implemented in a SBR, obtaining a suitable effluent to feed the anammox process. This thesis deals with the implementation of the anammox process in SBR for the treatment of mature urban landfill leachates after a partial nitritation process.

Experiments carried out with lab-scale anammox enrichment were focused on the study of the long term operation of the anammox process in a SBR and the exploration of the anammox biomass characteristics. High nitrogen removal could be sustained over a long period obtaining a good effluent quality containing mainly nitrate. Compact anammox granules showed an average specific anammox activity around 0.7 kg N·kg^{-1} VSS·d^{-1}. Nitrite concentration is the main concern in SBR anammox reactors treating influents with high nitrite levels. Punctual

accumulations of nitrite up to 100 mg NO_2^--N·L^{-1} are not critical and can be solved by enlarging the reaction phase. The specific anammox activity can be partially recovered after severe nitrite inhibition by washing the system to remove nitrite. However, prolonged expositions (several days) to nitrite levels around 30-40 mg NO_2^--N·L^{-1} result on SAA decrease. It was found that, even without a source of organic carbon, active heterotrophic bacteria were present in the anammox enrichment.

The application of the anammox process to treat landfill leachate was first tested in a lab-scale anammox SBR, seeded with granular biomass from the anammox enrichment. Previous tests revealed a negative impact of the leachate matrix on the biomass acclimatized to mineral medium and the lab-scale anammox SBR was fed with diluted leachate (the dilution factor was reduced with the increase of the NLR). Anammox biomass can be acclimatized to the leachate matrix, but an adaptation period is required. However, the tolerance of the biomass to the leachate increase over the time as well as the impact of changes on the leachate characteristics is progressively reduced. High nitrogen removal rates up to 0.78 kg N·m^{-3}·d^{-1} and high removal efficiencies (above 90%) can be obtained in a lab-scale anammox SBR treating leachate.

Urban landfill leachates treated in this study presented high COD values. The readily biodegradable fraction was very low, resulting in low C:N ratio, and was removed in the previous partial nitritation process. However, an important fraction of this COD was constituted by of slowly biodegradable organic matter. The presence of slowly biodegradable substrate concluded in a limited presence of heterotrophic denitrification in the lab-scale anammox reactor, which contributed with anammox to nitrogen removal. However, this heterotrophic process could compete with the anammox process for substrate if enough organic carbon was available and therefore, would need to be monitored. In this sense, a mass balance methodology was used to determine the contribution of the heterotrophic route in the nitrogen removal of the system, and to assess the process performance.

With the experiences acquired with the anammox enrichment and the lab-scale anammox reactor, the start-up of a pilot-scale anammox reactor (0.4 m^3) was carried out. The strategy used for the start-up was based on the dilution of the leachate matrix and the application of different influent nitrite to ammonium molar ratios below 1.32, in order to focus on the effluent nitrite. The aim was to allow a correct biomass acclimatization to landfill leachate and guarantee a stable

operation without nitrite peaks. The reactor was started at a very low nitrogen loading rate because of the low initial mixed liquor volatile suspended sludge content. However, the nitrogen removal capacity increased rapidly and an influent containing 100% landfill leachate coming from the previous partial nitritation process was treated after less than 160 days, with a nitrogen loading rate up to 0.4 kg $N \cdot m^{-3} \cdot d^{-1}$.

Thus, this thesis presents the anammox process integrated in a two-step combined partial nitritation-anammox treatment in SBR (PANAMMOX® process) as a feasible alternative for the biological treatment of mature urban landfill leachates. The work identifies the key parameters for the long term operation of anammox SBR feed with leachate and investigates the effect of the landfill leachate characteristics on the process performance.

Resum

Aquesta tesi és el resultat d'una llarga col·laboració del Grup LEQUIA amb l’empresa CESPA-GR, una empresa dedicada a prestació de serveis ambientals que s'encarrega de la gestió de diversos abocadors de residus sòlids municipals. Els abocadors són la solució més utilitzada per al tractament final de residus sòlids urbans. El disseny i la tecnologia d’aquestes instal·lacions han estat millorats durant les últimes dècades per tal de limitar les emissions de biogàs i la filtració de lixiviats als aqüífers i aigües superficials. La gestió dels lixiviats és una de les principals preocupacions ambientals en aquestes instal·lacions. Els lixiviats són aigües residuals amb un elevat poder contaminant i una composició complexa amb una barreja de compostos orgànics i inorgànics i es caracteritzen per un alt contingut d'amoni i una limitada fracció biodegradable de la matèria orgànica. El tractament dels lixiviats inclou sovint processos fisicoquímics per a l’eliminació del nitrogen, com l’extracció de l'amoníac en fase gas. L’eliminació biològica de nitrogen mitjançant el procés combinat de nitrificació/desnitrificació convencional té un cost més reduït, però requereix l’addició d'una font externa de carboni per la desnitrificació completa. Durant les últimes dues dècades, s’han desenvolupat i provat noves alternatives per tractar corrents amb alta càrrega amoniacal i baixa relació C:N, basades en l’eliminació autotròfica del nitrogen en processos combinats de nitritatió parcial i anammox. La majoria d’experiències en applicacions de nitritació parcial i anammox se centren en el tractament de retorns de digestió de fangs i encara hi ha molt pocs estudis enfocats a l’ús d’aquests processos aplicats al tractament de lixiviats d’abocador.

El procés combinat de nitritació parcial i anammox es pot dur a terme en un únic reactor o en tancs separats. En estudis previs s’havia aconseguit una nitritació estable tractant lixiviats d’abocador en un reactor SBR, obtenint un efluent adequat per alimentar el procés anammox. Aquesta tesi aborda l'aplicació del procés anammox en un reactor SBR per al tractament de lixiviats madurs d’abocadors de residus sòlids urbans.

Els experiments duts a terme amb un reactor a escala de laboratori enriquit amb biomassa anammox es centren en l'estudi de l'operació a llarg termini del procés de anammox en un SBR i l'exploració de les característiques de la biomassa anammox. S’ha pogut tractar aigua residual amb alta càrrega nitrogenada, obtenint elevades eficiències d’eliminació de nitrogen, fins i tot amb llargs períodes d’operació. S’han obtingut grànuls compactes amb una activitat específica d'uns 0.7 kg N·kg^{-1} SSV·d^{-1}. La concentració de nitrit és un paràmetre clau en els reactors SBR anammox que tracten afluents amb nivells de nitrit elevats. Acumulacions puntuals de nitrit de fins a 100 mg NO_2^-- N· L^{-1} poden ser resoltes mitjançant l'ampliació de la fase de reacció. L'activitat específic anammox pot ser recuperat parcialment després d'un episodi sever d'inhibició per nitrit mitjançant rentat del sistema. No obstant, exposicions prolongades (diversos dies) a nivells de nitrit del voltant de 30-40 mg NO_2^-- N· L^{-1} conclouen en una disminució de l'activitat específica de la biomassa anammox. Es va trobar que, fins i tot sense una font de carboni orgànic a l'afluent, hi havia presència de desnitrificants heteròtrofs actius en l'enriquiment amb anammox.

L'aplicació del procés anammox per al tractament de lixiviats d'abocador es va provar per primera vegada en un anammox SBR a escala de laboratori, inoculat amb biomassa granular de l'enriquiment d'anammox. Les proves prèvies van revelar un impacte negatiu de la matriu de lixiviats en la biomassa aclimatada a medi mineral sintètic i l'SBR a escala de laboratori s'alimentava amb lixiviat diluït (el factor de dilució es va reduir amb l'augment de la NLR). La biomassa anammox pot aclimatar-se a la matriu del lixiviat, però necessita un període d'adaptació. No obstant això, la tolerància de la biomassa cap als lixiviats augmenta amb el temps i l'impacte dels canvis de les característiques del lixiviat sobre la biomassa es redueix progressivament. Els resultats demostren que es poden tractar altes càrregues de nitrogen (fins a 0.78 kg N·m^{-3}·d^{-1}) assolint eficiències d'eliminació de nitrogen per sobre del 90% en un reactor anammox SBR tractant lixiviats d'abocador.

Els lixiviats d'abocador tractats en aquest estudi van presentar valors alts de DQO. La fracció de matèria orgànica fàcilment biodegradable va ser molt baixa, el que resulta en baixa relació C: N, i era eliminada en el procés de nitritació parcial anterior. No obstant això, una fracció important d'aquesta DQO estava constituïda per matèria orgànica lentament biodegradable. La presència de substrat lentament biodegradable va permetre la presència limitada de desnitrificants heterotròfics en el reactor anammox a escala de laboratori, que van contribuir amb juntament amb la biomassa anammox en l'eliminació de nitrogen. La baixa biodegradabilitat de la matèria orgànica en els lixiviats fa difícil la quantificació de la font de carboni disponible per a la desnitrificació. En aquest sentit, es va desenvolupar i aplicar un mètode basat en balanços de matèria per determinar la contribució de la heterotròfica en l'eliminació de nitrogen del sistema.

Amb les experiències adquirides amb l'enriquiment i el reactor anammox a escala de laboratori, es va procedir a dur a terme la posada en marxa d'un reactor anammox a escala pilot (0.4 m^3). L'estratègia utilitzada per a la posada en marxa es va basar en la dissolució de la matriu de lixiviats i l'aplicació d’una relació molar nitrit:amoni a l’influent per sota de 1.32, amb l’atenció centrada en la concentració de nitrit a l’efluent. L'objectiu era permetre una correcta aclimatació de la biomassa als lixiviats d'abocador i garantir un funcionament estable del procés sense pujades de nitrits. El reactor es va iniciar a una càrrega volumètrica de nitrogen molt baixa a causa la poca concentració de sòlids inicial en el reactor. No obstant això, la capacitat d'eliminació de nitrogen va augmentar ràpidament al cap de menys de 160 dies es va poder tractar un influent amb 100% de lixiviat procedent del reactor de nitritació parcial sense problemes i de forma estable, assolint una càrrega de 0.4 kg $N \cdot m^{-3} \cdot d^{-1}$.

En aquesta tesi es presenta el procés anammox integrat en sistema combinat de nitritació parcial i anammox en dos reactors SBR (procés PANAMMOX®) com una alternativa viable per al tractament biològic de lixiviats madurs d'abocadors de sòlids urbans. El treball identifica els paràmetres clau per al funcionament a llarg termini d’un reactor anammox SBT alimentat amb lixiviats i s'investiga l'efecte de les característiques dels lixiviats d’abocador sobre el funcionament del procés.

CHAPTER 1. INTRODUCTION

1.1 Urban landfill leachates: Characteristics and problems

Human activities produce huge amounts of solid waste. Their potential environmental impact depends on a wide range of factors, such as the amount produced, the composition, toxicity, or degradability, among others. Landfilling was the first solution for waste management and, despite the increasing of the fraction of solid wastes treated by incineration or reuse; it remains the most employed method and usually the unique final solution for solid waste management (Renou *et al.* 2008). Since no better alternative to landfilling is feasible nowadays (even incineration produces ashes that need a final treatment), the landfill sites have been improved with highly engineered facilities in order to optimize the space available and especially to minimize the negative impact on the surrounding environment. Among the negative effects caused by migration of biogas, generation of annoying odors, noise and visual nuisances, the possible contamination of surface water flows and groundwater has been the main concern in landfill sites over the years (EPA, 2000). For this reason, the solid wastes have been confined and isolated by several layers of draining and impermeable materials in order to minimize the percolation of rain water through the waste and the diffusion of landfill leachate and landfill gas to the surrounding water bodies and air.

Thus, one of the main concerns in landfill sites management is the production of landfill leachates. This highly contaminated wastewater is the result of the degradation and stabilization of the solid waste and possible percolation of rain through the landfill. The composition of leachates is highly variable and depends on several factors and may change significantly depending on the characteristics of the solid wastes dumped, the degree of separation of the moisture fraction in the municipalities served by the landfill site, the seasonality and also on the degree of solid waste stabilization (related to the age of the site and the decomposition stage in the landfill) (Kurniawan *et al.* 2006a). Due to the high diversity of waste materials deposed in landfills, this kind of wastewater contains a wide range of contaminants. Kjeldsen *et al.* (2002) grouped the pollutants into four categories: i) Organic matter (OM): dissolved OM, refractory compounds and volatile fatty acids (VFA); ii) Inorganic macrocomponents: sodium, potassium, calcium, iron, magnesium, manganese, chloride, sulphate, bicarbonate and ammonium; iii) Heavy metals: cadmium, copper, or lead among others and iv) Xenobiotics: traces of organic compounds like phenols or pesticides.

EPA (2000) reported a characterization of the changes on leachates composition with the age of the landfill. They divided the leachate maturation process into five successive dynamic periods that depend on the presence of suitable conditions (aerobic, hydrolysis and fermentation, acetogenesis, methanogenic and aerobic period) (Figure 1.1).

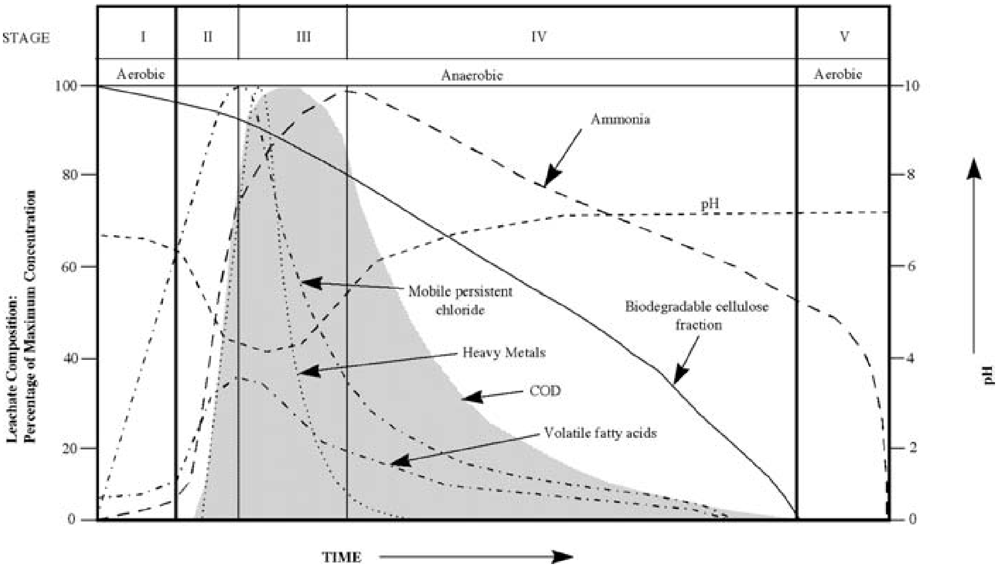


Figure 1.1. Changes on landfill characteristics over the time (EPA, 2000).

Generally, and following this pattern, young landfill sites produce leachates with a high presence of VFA. Thus, young landfill leachates are ammonium-rich wastewater with a high biodegradable fraction in the OM content. Ammonium is released by the OM decomposition and reaches the maximum concentration during the first stages of the waste degradation process. As the time goes by, the stabilization process results in the increase of the ammonium concentration and a reduction of the biodegradable fraction of COD. The leachates produced at the last stages are termed mature leachates. In this case, organics are basically humic and fulvic-like substances and the ammonium concentration remains high but may slightly decrease over the time (Kulikowska and Klimiuk 2008).

1.2 Conventional nitrogen removal treatments in leachates

1.2.1 Physicochemical treatments

The treatment of landfill leachates is a complex issue due to the wide range of pollutants to be removed and to the fact of requiring the application of advanced techniques. The elevated concentrations of ammonium in leachate up to 6000 mgNH_4^+-N·L^{-1} (Ganigué *et al.* 2009) and the potential risk for aquatic environments, place nitrogen as main priority concerning the treatment of landfill leachates (Robinson, 1995). Therefore, this study is focused on N removal from landfill leachates. Following, we discuss about the existing N removal techniques used for wastewater treatment. Physicochemical treatments are briefly described and attention is mainly put on biological methods.

Nowadays, physicochemical treatments are largely employed for landfill leachate treatment due to the strict conditions they present (extremely high ammonium, refractory COD and low available C/N ratio). There are several available treatments to remove N from this kind of wastewater, such as ultrafiltration processes (Ozturk *et al.* 2003) or adsorption in activated carbon (Abdul Aziz *et al.* 2004), but the most used methods are chemical precipitation (Li *et al.* 1999; Zhang *et al.* 2009b) and ammonia stripping (Calli *et al.* 2005).

1.2.1.1 Chemical precipitation

Chemical precipitation consists on the use of chemical reactions that convert the dissolved ions of a solution into insoluble solid phase that precipitates. This method can be used to remove the non-biodegradable organic compounds, ammonia and heavy metals from landfill leachate (Li *et al.* 1999). Regarding nitrogen removal, the method consists on the formation of struvite (magnesium ammonium phosphate) by adding magnesium chloride and sodium hydrogen phosphate as precipitants. The process does not require specific treatment facilities and can be performed *in situ* in the own leachate pool of the landfill site, meaning easy set-up. Moreover, struvite can be used as nitrogen source for fertilizers if the quality of the resulting sludge is good enough (without contaminants such as heavy metals). However, the reuse of struvite is commonly too expensive due to its low purity and is just a waste that needs a final treatment.

Moreover, the need of a high dosage of reactants and the sensitivity of the process efficiency to pH is the main drawbacks of this method (Kurniawan *et al.* 2006a).

1.2.1.2 Ammonia stripping

Ammonia (NH_3) can be stripped from landfill leachate in treatments that make use of the mass transfer from the liquid to the gas phase when leachate and air are forced to interact in a counter-current flow. Ammonia is the nitrogen specie removed from the waste stream and, therefore, the ammonia/ammonium equilibrium must be switched to ammonia by adjusting the pH of the leachate to alkaline values over 10. This method offers a high effectiveness and is the most-widely used treatment for ammonia removal from leachates (Kurniawan *et al.* 2006a). The efficiency of the process depends on the air transfer through the leachate. A full-scale plant for ammonia stripping can be seen in Figure 1.2.

Figure 1.2. View of an ammonia steam stripping plant for the treatment of landfill leachate in the United Kingdom.

According to EPA (2000) this process is a feasible alternative to deal with ammonium concentration above 100 mg NH_4^+-$N \cdot L^{-1}$. Advantages of this process are the simplicity of the technology and the possibility to assume wastewater fluctuations. However, the process does not remove nitrate/nitrite neither organics. In landfill sites without biogas recovery, it presents high energy consumption for heating. This treatment releases amounts of NH_3 gas to the environment that do not overpass the level of toxicity (1,700 $mg \cdot m^{-3}$) but produce detectable odor near the plant. To avoid this environmental impact, the NH_3 gas must be treated with HCl or H_2SO_4 and the cost of the process is increased.

In front of physicochemical methods, biological N-removal has been proved to be more efficient and offers significantly lower costs (mainly due to differences in investment cost and energy requirements) in domestic and industrial wastewater treatment (Tchobanoglous *et al*. 2004). However, when wastewater presents such extreme conditions as landfill leachates, conventional biological nitrogen removal processes become expensive and advanced technologies are required to develop more sustainable methods.

1.2.2 Conventional biological nitrogen removal processes

Activated sludge systems for nutrient removal have been explored and widely applied. During the last decades, most of the efforts have been focused on the development of new technologies to obtain better effluent quality, with special attention on N-removal and the reduction of treatment costs.

1.2.2.1 Background to the nitrogen cycle

Nitrogen is the fourth most abundant element in the biosphere and is essential for life. The N-cycle is covered by various catabolic processes (nitritation, nitratation, denitrification, dissimilatory nitrate reduction to ammonium and anaerobic ammonium oxidation; Equations 1-5), anabolic processes (ammonium uptake, assimilatory nitrate reduction and nitrogen fixation), and ammonification (a necessary result of the biological food chain) (Jetten *et al.* 2009). These processes are carried out by bacteria and have been engineered over the years in human built systems to perform biological nitrogen removal in WWTP. Ammonium in wastewater is released from ammonification (it comprises the abiotic hydrolysis of the particulate organic nitrogen to soluble organic nitrogen and its following conversion to ammonium by heterotrophic bacteria). The NH_4^+-N is the nitrogen source for the synthesis of new biomass and as the sole energy source for growth of ammonium oxidizing bacteria (AOB) (Henze *et al.* 1999). Nitritation to produce nitrite from ammonium (equation 1) is carried out by AOB. The next oxidation step to produce nitrate (nitratation, equation 2) results from nitrite oxidizing bacteria (NOB) activity. The complete oxidation of ammonium to nitrate by both AOB and NOB is globally termed nitrification. Recent studies have found that the occurrence of ammonium oxidation is not only performed by bacteria (both β- and γ-protobacteria). Ammonium oxidizing archaea (AOA) have been identified in many natural environments (such as the water column and sediments) (Taylor *et al.* 2010; Li *et al.* 2011) and wastewater treatments (Kasuga *et al.* 2010; Kayee *et al.* 2011; Yu *et al.* 2011) and seem to be important actors in the nitrogen cycle.

The effective nitrogen removal in WWTP is commonly achieved making use of heterotrophic denitrification. Denitrification is a sequential process with four steps, i.e. nitrate to nitrite, nitric oxide (NO), nitrous oxide (N_2O) and N_2 gas. Biological denitrification is carried out entirely by heterotrophic bacteria and requires a biodegradable organic C-source as electron donor (Schmidt *et al.* 2003). Since nitrite is an intermediate of both nitrification and denitrification processes, engineering solutions have been developed during the last years aiming to keep nitrate out of the system in order to save part of the aeration costs and external C-source dosage (van Loosdrecht and Salem 2006). It has been recently demonstrated that AOB contain the genes needed to perform denitrification processes under micro-aerophilic conditions. AOB denitrification is the main source of NO and N_2O emissions in WWTPs performing conventional nitrification-denitrification, but these nitrogen oxides can be also produced from incomplete denitrification (Yu *et al.* 2010; Flores-Alsina *et al.* 2011; Ni *et al.* 2011b). Both NO and N_2O production are linked to ammonium removal, but are unwanted due to the large greenhouse effect of these N-compounds, which is much higher than the one for CO_2 (Ni *et al.* 2011b).

The understanding of the N-cycle changed radically in the 90s when the anaerobic ammonium oxidation was discovered (Mulder *et al.* 1995). Anammox bacteria are chemolithoautotrophic microorganisms able to oxidize ammonium to N_2 gas in the absence of DO and with nitrite as the final electron acceptor (equation 5; Strous *et al.*, 1998).

Ammonia oxidation by AOB (nitritation)

$$NH_4^+ + 1.382\ O_2 + 1.982\ HCO_3^- \longrightarrow$$

$$0.018\ C_5H_7O_2N + 0.982\ NO_2^- + 2.927\ H_2O + 1.891\ CO_2 \qquad (1.1)$$

Nitrite oxidation by NOB (nitratation)

$$NO_2^- + 0.003\ NH_4^+ + 0.488\ O_2 + 0.010\ H_2CO_3 \longrightarrow$$

$$0.003\ C_5H_7O_2N + NO_3^- + 0.008\ H_2O \qquad (1.2)$$

Heterotrophic Denitrification

From nitrate

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \longrightarrow$$

$$0.056C_5H_7O_2N + 0.47N_2 + HCO_3^- + 1.68H_2O \qquad (1.3)$$

From nitrite

$$NO_2^- + 0.53CH_3OH + 0.67H_2CO_3 \longrightarrow$$

$$0.04C_5H_7O_2N + 0.48N_2 + HCO_3^- + 1.23H_2O \qquad (1.4)$$

Anaerobic ammonium oxidation by anammox bacteria

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \longrightarrow$$

$$1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (1.5)$$

In the following sections we discuss about the existing alternatives to deal with biological nitrogen removal from wastewater in general and their applicability on leachates treatment.

1.2.2.2 Combined nitrification-denitrification processes

Heterotrophic denitrification is the main metabolic route employed nowadays in environmental engineering for biological nitrogen removal in municipal WWTPs. Ordinary heterotrophic organisms grow on readily biodegradable substrate (dissolved organic matter) to reduce nitrate and nitrite to N_2 gas. Their growth rate depends on the biodegradability of the organic substrate (C-source), with typical maximum growth rate values around 2 d^{-1} (at 20 ºC) and anoxic yield coefficient of 0.63-0.67 g COD·g^{-1} COD (Henze 1987; Gujer *et al.* 1999). However, ammonium is commonly the main nitrogen form in wastewater, which implies the need for a previous nitrification step to supply nitrate or nitrite (the electron acceptors) for heterotrophic denitrification.

The applicability of nitrifiers and denitrifiers in engineering processes for N-removal from wastewater became widely recognized in the 1960s and nowadays, the combined treatment by aerobic autotrophic nitrification followed by anoxic heterotrophic denitrification remains the widest used method in WWTP (Ahn 2006). The classic bioreactor configuration to perform nitrification-denitrification consists in an anoxic tank followed by an aerobic tank and the secondary settler. The internal recirculation from the aerobic compartment to the anoxic tank serves to supply electron acceptors for denitrification (nitrate/nitrite) and the external recirculation from the secondary settler to the biological process inflow ensures the target biomass retention time and concentration (Figure 1.3). An important point is the aeration, which must be adjusted to provide enough DO for nitrification (4.57 g $O_2 \cdot g^{-1} NH_4^+$-N) but avoiding unnecessary energy consumption. The aeration requirements represent one of the main fractions of the treatment cost in WWTPs performing conventional nitrification/denitrification (Stowa, 1996).

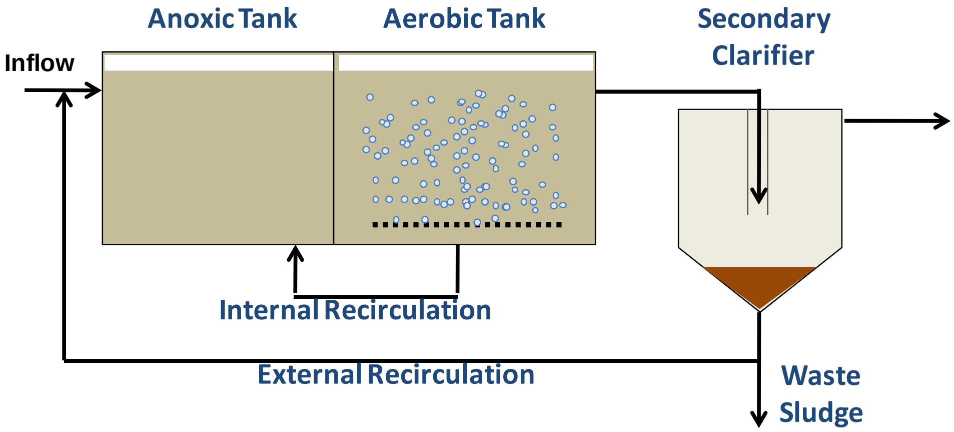


Figure 1.3. Scheme of the conventional activated sludge process

The denitrification potential, and therefore the N-removal efficiency, is mainly governed by the presence of biodegradable organic carbon. The minimum C/N ratio was calculated and set at 3.5-4.5 g $COD \cdot g^{-1}$ N (Henze *et al.* 1994) but in practice, values about 6-11 g $COD \cdot g^{-1}$ N are often necessary for complete denitrification(Sun *et al.* 2010). The minimum C/N ratio will vary depending on the quality of the C source. External carbon sources, such as methanol or ethanol (Puig *et al.* 2008), are commonly used to obtain an effluent nitrogen concentration under the discharge limits.

The effective C/N ratio (taking into account the biodegradable fraction of COD) in mature landfill leachate tends to be far from these optimal values and implies high expenses on OM dosage for denitrification. Methanol (CH_3OH) is the most used because of its relatively low cost (Ahn 2006), but better N-removal efficiencies are obtained by using a mixed C-source. To reduce the cost of the treatment, by-products and waste materials produced in large amounts and containing high biodegradable organic carbon content, such as piggery manure, have been explored as less-costly alternative C-sources for domestic wastewater treatment (Sun *et al.*, 2010). However, these waste products are complex mixtures and can introduce N-containing compounds, non-biodegradable C, toxics and other pollutants that could compromise the effluent quality.

Alternative configurations, such as simultaneous nitrification/denitrification (SND) (Gupta *et al.* 1994; Münch *et al.* 1996; Hao *et al.* 1997; Hocaoglu *et al.* 2011; Yang and Yang 2011)have been developed in activated sludge processes with effective reduction of the cost of the treatment thanks to the lower C-source consumption (22-40% less than separate tanks) lower and the reduction of the sludge yield by 30%. However, in the case of landfill leachates the use of SND is not enough to compensate the deficit of COD, and large amounts of chemicals are still needed to ensure complete denitrification. In addition, the range of salinity in landfill leachates makes the formation of flocular sludge or aggregates difficult and avoids the possibility of DO gradients formation.

1.2.2.3 Short-cut denitrification over nitrite

This alternative process is sustained on the basis that nitrite is an intermediate in both nitrification and denitrification and the production of nitrate is not necessary. Thus, the key factor in this process is to keep nitrate out of the treatment system and to promote the accumulation of nitrite (nitritation) for subsequent denitrification (Bae *et al.* 1997; Wang *et al.* 2004; Ruiz *et al.* 2006; Galí *et al.* 2007). Compared to complete nitrification/denitrification, the application of partial nitrification reduces the aeration demand by 25% and the biodegradable COD consumption by 40% (Magri *et al.* 2007). This process becomes highly cost-efficient for the treatment of wastewater with a low C/N ratio, even with the extremely typical low values of landfill leachates, since methanol addition is significantly reduced (Ahn 2006).

This alternative is a good solution to reduce costs of the treatments in existing landfill leachate treatment plants without the need of building new facilities.

NH_4^+ removal via NO_3^- (nitrification/denitrification)

$$NH_4^+ + 2\,O_2 + 4\text{ g COD} \longrightarrow 0.5\,N_2 + H_2O + H^+ + 1.5\text{ g biomass} \qquad (1.6)$$

Short-cut nitrification/denitrification (nitritation/denitritation)

$$NH_4^+ + 1.5\,O_2 + 2.4\text{ g COD} \longrightarrow 0.5\,N_2 + H_2O + H^+ + 0.9\text{ g biomass} \qquad (1.7)$$

The nitrite oxidation to nitrate is limited by using strategies based on the different physiological characteristics of AOB and NOB and their response temperature, DO and the concentration of free ammonia (FA) and free nitrous acid (FNA) (Ganigué *et al.* 2007). The specific growth rate of AOB at 35º C is approximately twice the growth rate of NOB and low SRT promotes enrichment with AOB. The SHARON (Single-reactor High activity Ammonia Removal Over Nitrite) process makes use of this effect in a chemostat operated at 35º C and a SRT of 1-1.5 days (Mulder and Van Kempen 1997; Hellinga *et al.* 1998; Mulder *et al.* 2001; Van Kempen *et al.* 2001). The affinity of AOB for DO is higher than NOB (half-saturation coefficients about 1.2-1.5 $mgO_2{\cdot}L^{-1}$ and 0.2-0.4 $mgO_2{\cdot}L^{-1}$ for NOB and AOB, respectively) and nitrite accumulation can be obtained in a reactor operated with low DO set-point. Finally, NOB are less tolerant to both FA and FNA concentrations compared to AOB (Anthonisen *et al.* 1976; Ganigué *et al.* 2007). Thus, in wastewater with high ammonium content, such as landfill leachates, NOB growth can also be limited by the presence of ammonium and nitrite in the reactor.

Short-cut denitrification reduces the demand of carbon source (the first denitrification step is not necessary) and aeration (ammonium is only oxidized to nitrite).

The use of heterotrophic denitrification for effective nitrogen removal implies always the need of available carbon source. In case of mature landfill leachates, almost all theorganic matter necessary for denitrification must be externally supplied and the cost is high, even using these alternatives. For this reason, alternative processes based on autotrophic nitrogen removal appears to be really cost-effective solutions to treat wastewater with extremely low C:N ratio. This thesis aimed the development of an alternative treatment making use of the combined partial nitritation-anammox process. This technology was implemented in a two step with a partial nitritation SBR followed by an anammox SBR (PANAMMOX® process). Following, the characteristics and advantages of the anammox process are discussed.

1.3 Fully autotrophic nitrogen removal

The discovery of the anaerobic ammonium oxidation has lead to the possibility of removing nitrogen from wastewater without the need of biodegradable organic matter. Anammox bacteria and their metabolism were unknown until the 90s. However (Broda 1977) demonstrated by thermodynamic calculations the possibility of ammonium oxidation under anoxic conditions. Mulder *et al* (1995) detected important ammonium losses in the anoxic tanks of the WWTP of Gist-Brocades (The Netherlands) and hypothesized about the anammox metabolism. The anammox process was rapidly engineered (Jetten *et al.* 1997; Strous *et al.* 1997b; Strous *et al.* 1998; Jetten *et al.* 2001) and the use of processes based on anammox has been extended to several applications to treat high ammonium content wastewater.

1.3.1 The anammox process

The accepted stoichiometry of the anammox process was experimentally proposed by Strous *et al.* (1998). Anammox bacteria oxidize ammonium to N_2 gas using nitrite instead of oxygen as the final electron acceptor (equation 1.5). In this reaction, 89% of the incoming nitrogen (NH_4^+ plus NO_2^-) is converted to N_2 gas, while the rest (11%) is converted to produce the required electron equivalents for the synthesis of new cell material (Strous *et al.* 1998).

To deal with anammox, a source of nitrite is required to satisfy the need of 1.32 mole $NO_2^-\cdot$mole^{-1} NH_4^+ (equation 1.5). Therefore, the process has to be coupled to a partial nitritation (PN) process, in order to aerobically oxidize 60% of the incoming ammonium nitrogen to nitrite. Despite of this, ammonium is only partially converted to nitrite and 63% less of oxygen demand is required compared to conventional nitrification/denitrification.

The main advantage with regard to conventional N-removal processes is the 100% saving of external C-source for denitrification (Jetten *et al.* 1998; Van Dongen *et al.* 2001). A very low production of sludge (Strous *et* al., 1998) and very low CO_2, N_2O and NO emissions with respect to the influent nitrogen load (Kampschreur *et al.* 2008; Pellicer-Nàcher *et al.* 2010) are other important advantages of the process.

Anammox bacteria present a high affinity to both ammonium and nitrite, with values for the substrate saturation constants between 0.2-0.75 g $N{\cdot}m^{-3}$ (Dapena-Mora *et al.* 2004b; Lackner *et al.* 2008; Ni *et al.* 2010a). The key characteristic of the anammox bacteria is their low growth rate around 0.065 d^{-1} (Strous *et al.* 1998), implying high doubling times, usually set above 8-11 days. This is the critical point for the start up of anammox reactors, since the availability of enriched inoculums is limited. However, the low biomass yield of anammox bacteria, around 0.159-0.165 g $COD{\cdot}g^{-1}$ NH_4^+-N results in low sludge production, which is advantageous at long term operation.

Anammox bacteria show high specific activities about 26-55 nmol $N_2{\cdot}mg^{-1}$ protein, leading to high nitrogen removal capacity in anammox reactors (Egli *et al.* 2001; Jetten *et al.* 2009). The activity of anammox bacteria is affected by pH and temperature. The range of pH for proper anammox activity was set at 6.7-8.3 by (Jetten *et al.* 1998; Strous *et al.* 1999), being the optimum with maximum activity at pH 8 (for *Candidatus Brocadia anammoxidans*). However, it seems that it is possible to adapt anammox bacteria to higher pH around 9.3 (Ahn *et al.* 2004). The optimal temperature for the anammox reaction was initially set between 37-40 ºC (Strous *et al.* 1999) but several works have reported good removal efficiencies at room temperature (Egli *et al.* 2001; Vázquez-Padín *et al.* 2011; Yang *et al.* 2011b; Yang *et al.* 2011a).

1.3.1.1 Effect of substrates and DO on anammox bacteria

Anammox bacteria are sensitive to several environmental conditions that can cause perturbations on Specific Anammox Activity (SAA; kg $N_2{\cdot}kg^{-1}$ $VSS{\cdot}d^{-1}$). Anammox activity is reversibly inhibited by DO levels from 0.25 to 2% saturation (Strous *et al.* 1997a; Egli *et al.* 2001). The initial activity is completely recovered as well as anoxic conditions are recovered. According to Strous *et al.* (1999) high ammonium and nitrate concentrations up to 1 g $N{\cdot}L^{-1}$ do not cause any negative impact on anammox bacteria.

With the concentrations commonly reached in anammox reactors, the possibility of ammonium inhibition on anammox biomass is usually not considered. On the contrary, severe nitrite inhibition on anammox activity has been reported in several studies and the nitrite concentration in anammox processes is of special concern. Strous *et al.* (1999) observed a decrease of the nitrogen removal efficiency with nitrite concentrations above 100 mg NO_2^--$N{\cdot}L^{-1}$. However, the thresholds of inhibition concentrations change among the different sources. Inhibition concentrations up to 180 mg NO_2^--$N{\cdot}L^{-1}$ are reported by Van de Graaf *et al.* (1996), Egli *et al.* (2001) and Schmidt *et al*. (2003), while Dapena-Mora *et al.* (2007) established the 50% inhibition concentration (IC_{50}) at 350 mg NO_2^--$N{\cdot}L^{-1}$.

Other authors found nitrite inhibition at lower levels, around 60-70 mg NO_2^--$N{\cdot}L^{-1}$ (Van Dongen *et al.* 2001; Arrojo *et al.* 2008). Fux *et al.* (2004) reported severe inhibition at lower nitrite levels (30-50 mg NO_2^--$N{\cdot}L^{-1}$) after six days of exposition. Nitrite inhibition was considered irreversible, but this is still not clear in the literature and seems that SAA can be at least partially recovered.

1.3.1.2 Effect of organic matter

Biodegradable OM does not affect directly the Anammox microorganisms but induces the growth of heterotrophic bacteria in the reactor. Only methanol has been found toxic to all known anammox bacteria (Dapena-Mora *et al.* 2007). Strous *et al.* (1997b) early demonstrated the possible coexistence of anammox and heterotrophic denitrification processes to treat wastewater with some organic matter content. Mass balances were used by Ahn *et al.* (2004) to determine the amount of nitrogen removed by denitrification in a UASB reactor treating piggery waste with a C:N ratio of 11 g $COD{\cdot}g^{-1}$ TN at a NLR of 0.66 kg $N{\cdot}m^{-3}{\cdot}d^{-1}$.

However, the low growth rate of anammox microorganisms gives competitive advantage to heterotrophic bacteria if enough carbon source is supplied and anammox bacteria could be limited for space and substrate (Chamchoi *et al.* 2008; Lackner *et al.* 2008). Several works have reported inactivation of anammox bacteria when acetate or other organic carbon is supplied, with critical C/N ratios set around 2 (Chamchoi *et al.* 2008; Molinuevo *et al.* 2009, among others). Ahn and Choi (2006) explored the effect of heterotrophic bacteria coexisting with anammox during the start-up of the anammox process and found a reduction of the nitrogen removal efficiency due to the heterotrophic denitrification activity.

Despite of this, the presence of a controlled denitrifying activity in anammox reactors can lead to a better effluent quality due to the lower nitrate and COD concentration (Ruscalleda *et al.* 2008; Wang *et al.* 2010).

1.3.1.3 Application of the anammox process to wastewater treatment

The anammox process has been tested and successfully applied mainly in lab-scale processes to treat a wide variety of ammonium concentrated streams with low C:N ratios, such as sludge digester effluents (Van Dongen *et al.* 2001; Caffaz *et al.* 2006; Vlaeminck *et al.* 2009), piggery waste (Ahn *et al.* 2004; Hwang *et al.* 2006; Molinuevo *et al.* 2009) and fish canning wastewater (Dapena-Mora *et al.* 2006), among others. The application of the anammox process to landfill leachate has been only explored in few works (Siegrist *et al.* 1998b; Egli *et al.* 2001; Zhang and Zhou 2006; Liang *et al.* 2009; Liu *et al.* 2010; Xu *et al.* 2010; Zhou and Yao 2010; Wang *et al.* 2011a).

The first full-scale anammox reactor was implemented in the Sluisjesdijk WWTP (Rotterdam, The Netherlands) to treat the sludge digestion returns. This anammox reactor has a total volume of 70 m^3 and a treatment capacity of 750 kg $N{\cdot}d^{-1}$ (van der Star *et al.* 2007). The high specific NH_4^+ consumption rate of anammox (45.5 $nmol{\cdot}mg^{-1}$ $protein{\cdot}min^{-1}$; Strous *et al.*, 1998) allows high TN removal rates in anammox reactors. The reported N-removal rates in anammox reactors treating real wastewater usually range between 1-3 $kgN{\cdot}m^{-3}{\cdot}d^{-1}$ in SBR, but extremely high values up to 25 $kgN{\cdot}m^{-3}{\cdot}d^{-1}$ were obtained in lab-scale anammox reactors fed with mineral medium (Yang *et al.* 2010; Tang *et al.* 2011).

The benefits of the process in terms of saving costs for chemicals and aeration have been largely demonstrated. However, there are still very few full-scale plants making use of anaerobic ammonium oxidation. The main handicap to implement the process is the initial cost on engineering facilities, since special configuration is required for the reactors, and the difficulty to obtain enough biomass to seed new reactors. Both problems are mainly a consequence of the slow growth rate of anammox bacteria (Strous *et al.*, 1998; Van der Star *et al.*, 2007). This fact results in long start-up periods and difficult anammox enrichments. The first full-scale anammox reactor in Rotterdam required more than two years of operation until anammox activity was detected and stable conversion of ammonium did not occur until day 1235 of the process (Van der Star *et al*., 2007).

Nowadays research is carried out to explore the viability of different seeding sludge, such as conventional activated sludge or anaerobic digestion sludge among others (Zheng *et al.* 2004; Trigo *et al.* 2006; Kieling *et al.* 2007; Liu *et al.* 2008; López *et al.* 2008; Tang *et al.* 2009; Wang *et al.* 2009a; Bagchi *et al.* 2010; Jeanningros *et al.* 2010; Ni *et al.* 2011e; Wang *et al.* 2011b) in order to make the start-up of new anammox reactors easier.

1.3.2 Combined partial nitritation-anammox processes

As above mentioned, the anaerobic ammonium oxidation needs a source of nitrite (equation 1.5) and is obtained by the partial nitritation (PN) of the incoming ammonium. Two main alternatives are used to perform the combined partial nitritation-anammox process. In single-tank processes, the PN is performed in the same tank as anammox, while in two step processes PN and anammox are performed in separate reactors.

1.3.2.1 PN-anammox processes in a single tank

By the coexistence of AOB and anammox bacteria populations, PN can be performed in the same tank as anammox reaction. All the alternatives combining PN and anammox in a single reactor are based on the integration of anammox organisms in structured biofilms or granules where DO gradients can be obtained, allowing activity of both AOB and anammox bacteria in aerobic and anoxic layers, respectively (Gong *et al.* 2008; Bao *et al.* 2009; Xiao *et al.* 2009; Vlaeminck *et al.* 2010).

This is obtained by operating the system at low DO set-point levels. Several technologies make use of this possibility: The Completely Autotrophic Nitrogen Removal Over Nitrite (CANON) (Kuai and Verstraete 1998; Third *et al.* 2001; Sliekers *et al.* 2003; Zhang *et al.* 2009a), the Oxygen Limited Autotrophic

Nitrification-Denitrification (OLAND) (Kuai and Verstraete 1998; Verstraete and Philips 1998; De Clippeleir *et al.* 2011) and the pH-controlled deammonification system (DEMON) (Wett 2007) are different alternatives to combine PN and anammox. The underlying principle for all these processes is the cooperation of aerobic nitrifiers and anammox bacteria under oxygen limited conditions (Schmidt *et al.* 2003; Vlaeminck *et al.* 2010).

One-step processes are based on the so called CANON concept. Among these alternatives CANON, OLAND and DEMON configurations use co-diffusion across the biofilm (i.e. both oxygen and ammonium are supplied from the bulk liquid into the biofilm). However, the coexistence of anammox bacteria and AOB can also be obtained in membrane-aerated bioreactors (MABR) that use counter diffusion biofilms, with ammonium diffused from the bulk liquid and DO from the membrane directly to the deeper layer of biofilm (Pellicer-Nàcher *et al.* 2010) In both co-diffusion and counter diffusion biofilm systems, nitrite is the limiting substrate for anammox growth.

The first full-scale application that used this concept was the DEMON technology implemented in a SBR treating wastewater at the WWTP Strass (Austria) with a nitrogen load up to 0.68 kg $N \cdot m^{-3} \cdot d^{-1}$ (Wett 2007). The results showed 40% saving on oxygen consumption and the energy demand was reduced from 2.9 $kWh \cdot kg^{-1}$ N to 1.16 $kWh \cdot kg^{-1}$ N (ammonium nitrogen). Referring to the treatment of landfill leachates, one-step systems have been successfully applied to deal with ammonium concentrations up to 1500 mg $NH_4^+\text{-}N \cdot L^{-1}$ (Helmer *et al.* 2001; Cema *et al.* 2007; Liu *et al.* 2010).

1.3.2.2 Two-step PN-anammox processes

In this case, the PN process takes place in a separate tank, before the Anammox reactor. Optimal operational conditions for each process are applied in the respective reactor. DO set-points around 1.5-2 mg $O_2 \cdot L^{-1}$ can be applied in the PN reactor and high ammonium and nitrite concentrations allow NOB inhibition, while the anammox reactor is kept under anoxic conditions to enrich each reactor with the specific bacterial population required for the biological process. In these sequential processes, around 60% of the influent amminum is converted to nitrite in the PN reactor. The nitritation efficiency in the PN reactor can be controlled by different strategies depending on the wastewater characteristics. The effective nitrogen removal is performed in the anammox reactor.

The first application of the combined PN-Anammox processes in a two-step system was the SHARON-ANAMMOX® process (Van Dongen *et al.* 2001) to treat sludge digestion returns in the WWTP Rotterdam-Dokhaven. The SHARON configuration has been widely used to carry out PN in two-step Anammox processes. However, the chemostat configuration has a low capacity to deal with variations of the N-load, due to stability problems caused by ammonium shocks. Moreover, there is no biomass retention and it is hard to control the SRT when the sludge settleability is low (Ganigué *et al.* 2008).

The SBR technology is a more flexible system compared with a chemostat and the effects of perturbations can be more attenuated due to higher biomass retention (Strous *et al.* 1998) and the possibility of including changes in the cycle configuration. Ganigué *et al.* (2007) tested the feasibility to deal with PN of landfill leachates in both SHARON and SBR configurations and obtained more stability and better ammonium conversion efficiencies with the SBR configuration. These authors also demonstrated a sustained nitrite build up in a PN-SBR after long term operation treating urban landfill leachate with extremely high NH_4^+ content (up to 6000 $mgN\text{-}NH_4^+\cdot L^{-1}$).

This PN-SBR was the first step of the PANAMMOX® process and produced a suitable effluent to feed an Anammox reactor (Ganigué *et al.* 2009). The SBR configuration was better to treat leachates with high conductivity (up to 64 $mS\cdot cm^{-1}$), because the loss of biomass was partially reduced thanks to the settling phase.

1.3.2.3 Application to landfill leachate treatment

Several works report the application of the anammox process to the treatment of landfill leachate. Table 1.1 summarizes the studies about leachate treatment in combined partial nitritation-anammox either in one and two step processes.

Table 1.1. Main studies reporting anammox processes for the treatment of landfill leachate.

Source	**Reactor type**	**Volume** m^3	**Ammonium** $gNH_4^+\text{-}N\cdot m^{-3}$	**Nitrite** $gNO_2^-\text{-}N\cdot m^{-3}$	**NLR** $kgN\cdot m^{-3}\cdot d^{-1}$	**EC** $mS\cdot cm^{-1}$
Hippen et al 1997	BC	-	1650	5.12	1.6$^{(1)}$	-
Siegrist et al 1998	RBC	33	0.1-0.4	-	1.5-4 $^{(1)}$	11.2-12.3
Schmid et al 2003	RBC	40	349	-	5.8$^{(1)}$	-
Cema et al 2007	RBC	0.014	1500$^{(2)}$	-	0.93	-
Liang & Liu 2008	FBBR	0.036	885	1011	0.11	-
Xu et al 2010	SBR	0.003	1442	-	0.96	-
Wang et al 2010	SBR	384	634	-	0.50	-

BC: Biological Contactor; RBC: Rotating Biological Contactor; FBBR: Fixed-bed Biofilm Reactor; SBR: Sequencing Batch Reactor. (1) NLR expressed as g $N\cdot m^{-2}\cdot d^{-1}$. (2) Total nitrogen (ammonium plus nitrite).

As it can be seen in Table 1.1, RBC is the main reactor configuration used in landfill facilities for autotrophic nitrogen removal. Most of these studies (Hippen *et al.* 1997; Siegrist *et al.* 1998a; Egli *et al.* 2001; Schmid *et al.* 2003) were carried out in reactors designed for nitrification-denitrification purposes. However, important nitrogen losses were detected, in spite of the absence of available organic matter for denitrification and further analysis revealed the presence of anammox bacteria. Thus, more research is needed for the start-up of new anammox reactors in view to a rapid and feasible application of the autotrophic nitrogen removal in landfill sites.

CHAPTER 2. OBJECTIVES

2.1 Problem definition

Biological nitrogen removal from urban landfill leachates by the conventional nitrification-denitrification process is expensive due to the need for external carbon source dosage, the high energy demand for aeration and the sludge production. Alternative processes based on the autotrophic nitrogen removal carried out by anammox are cost effective alternatives for the treatment of high ammonium content wastewater with low C:N ratio. A combined partial nitritation-anammox process is a feasible alternative to treat leachates. In previous studies, stable partial nitritation was achieved, but the treatment of landfill leachates in the anammox process needs to be tested and studied in more depth before future implementations of the process in landfill sites.

2.2 Objectives

The main goal of this PhD thesis is to study the feasibility of applying the anammox process to the treatment of mature urban landfill leachates previously conditioned in a partial nitritation process. This work is a part of the development of the PANAMMOX® process as an alternative to conventional nitrification-denitrification to perform biological nitrogen removal from leachates. The objectives of the work started at lab-scale level with an anammox enrichment focused on the study of the long term dynamics and key parameters for the control and operation of anammox SBR. Moreover, the characteristics of the anammox biomass were also investigated. Then, the treatment of landfill leachate by anammox was firstly studied at lab-scale for the acquisition of the basic knowledge for the scale-up of the process. Finally, the start-up of a pilot-scale anammox reactor was carried out in order to definitively test the feasibility of treating leachates in an anammox SBR.

The specific objectives to attain the main goal are following listed:

Work with the anammox enrichment was focused on:

- Process assessment by the knowledge obtained from the in-cycle and long term dynamics.
- Key parameters for the process stability relating to the influent nitrogen compounds concentration, effluent quality and biomass characteristics.
- Study of the granular biomass characteristics.

- Response to nitrite: evaluation of the effect of nitrite levels in continuous operation and specific anammox activity tests. Strategies to deal with nitrite accumulations.
- Exploration of the interaction between heterotrophic denitrifying and anammox bacteria in a system without organic matter supply.

Then, the process was applied to the treatment of urban landfill leachate, with the following objectives:

- Study of the effect of the leachate matrix on anammox granules.
- Treatment of an influent with leachate coming from a partial nitritation reactor and high nitrogen content to obtain stable and high nitrogen removal efficiency.
- Testing the process with different leachates to evaluate the adaptation of the granular biomass to the leachate matrix and the effect of sudden changes in the leachate composition on the process performance.
- Study of the effect of the organic matter content of the leachate on the nitrogen removal efficiency.
- Quantification of the contribution of heterotrophic denitrification on the nitrogen removal of the process.

Once the lab-scale objectives were accomplished, the process was finally scaled up to pilot-scale in order to:

- Definition of a proper start-up strategy for anammox SBR treating landfill leachates, according to lab-scale experience.
- Start-up of the pilot-scale anammox reactor with granular sludge from the anammox enrichment.
- Treatment of an influent with 100% leachate coming from partial nitritation.
- Stable operation of the pilot-scale reactor to reach high nitrogen removal efficiencies.
- Use of organic matter for a better effluent quality.

CHAPTER 3. MATERIALS AND METHODS

This chapter includes the general materials and methods used during all the work in the different chapters. Additional *Materials and methods* sections are included in chapters 4-6 to explain the specific experimental set-ups and techniques used in each chapter.

3.1 Chemical analysis

The analytical methods used in this study for the determination of the different parameters of wastewater are listed in Table 3.1. Most are in accordance with Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Only the biochemical oxygen demand analysis was based on a Euro Norm. To analyze the soluble species, samples were filtered beforehand with a pore size of 0.2 µm (NO_2^--N, NO_3^--N, NH_4^+-N, soluble COD).

Table 3.1. Analytical methods.

Analysis		Compound	Reference
Nitrogen	NH_4^+-N	Ammonium	APHA-4500-NH_3.B-C5220B
	NO_2^--N	Nitrite	APHA-4110B
	NO_3^--N	Nitrate	APHA-4110B
Organic matter	COD	Chemical Oxygen Demand	APHA-5220B
	BOD_n	Biochemical Oxygen Demand	Euro Norm EN 1899-1/1998
	TOC	Total Organic Carbon	APHA-S310
Alkalinity	IC	Inorganic Carbon	APHA-S310
Suspended solids	TSS	Total Suspended Solids	APHA-2540D
	VSS	Volatile Suspended Solids	APHA-2540E

3.2 FISH analysis

Fluorescence *in situ* hybridization (FISH) was used to identify the microorganisms present in the sludge samples and to calculate their relative abundance in respect to the total bacterial community. FISH analysis was performed following the procedure specified by Amann (1995). Samples were fixed in a 4%

paraformaldehyde (PFA) solution for 2-4 hours to stop the biological activity. Then, biomass samples were washed in a phosphate buffer solution (PBS) and stored in a PBS/ethanol (1:1) at -18º C for a maximum of 6 months.

For hybridization, samples were fixed on gelatin coated slides and sequentially dehydrated in 50%, 80% and 95% ethanol solutions during 3 minutes. Hybridization was carried out under stringent conditions and incubation at 46º C for 90 minutes. Then, specimens were immediately washed with washing buffer at 48º C. The probes used in this study are summarized in table 3.2. More probe details can be found in the ProbeBase (Loy *et al.* 2003). The probes were labeled with fluorescein isothiocyanate, CY3 and CY5 dyes.

Table 3.2. Probes used in hybridization for identification of bacteria.

Name	Specificity	Probe	Formamide (%)	Reference
EUBMIX	Bacteria domain	EUB338	0-70	Amman et al (1990)
		EUB338-II	0-50	
		EUB338-III	0-50	Daims et al (1999)
Anammox	*C. Brocadia* and *C. Kuenenia*	Amx-820	30-40	Schmid et al (2000)
	C. Kuenenia	Kst-157-a-A-18	25	Schmid et al (2001)

The probed sludge was examined using a Leica® confocal laser scanning microscope (CLSM). Qualitative information of Anammox and total bacteria was extracted using mathematical software for image analysis developed in Matlab®, which used the separated RGB (red, green, blue) channels and the identification of positive pixels in each image. Then, the area targeted with a specific probe was calculated and compared with the total area targeted with the general probe. Results were considered in a qualitative point of view since the number of images acquired per sample (less than 30) was not enough for quantification.

3.3 Specific Anammox Activity (SAA) tests

In this thesis the specific anammox activity (SAA) was measured using an adaptation of the manometric method reported by Dapena-Mora *et al.* (2007).

Manometric methods for the assessment of bioprocesses rates in gas-tight reactors are based on the simple principle that the metabolic rate of a poorly soluble gas component is proportional to the variation in pressure provided by a specific bioprocess. Therefore, this method can be applied to measure the specific N_2 production rate by the anaerobic ammonium oxidation (Dapena-Mora *et al.* 2007; Scaglione *et al.* 2009; Bettazzi *et al.* 2010). The N_2 gas production can be obtained by applying the ideal gas law to the data pressure collected during batch tests.

3.3.1 Batch tests procedure

The batch tests were carried out in glass bottles with a total volume of 120 mL sealed with a rubber septum. The vials were placed in a thermostatic shaker (Bibby Scientific) to ensure mixing conditions and temperature. The pressure was periodically measured in the head-space of the vials using a differential pressure transducer with a measuring range within 0-345 mbar and a resolution of 1.7 mbar (Centerpoint Electronics). The accuracy of the method was confirmed before the experimental campaigns by performing well-known pressure increments inside the bottles. The measured values presented average errors below 5% and the SAA measured in replicates showed an average variability lower than 8%, indicating good repeatability of the method.

The bottles were seeded with granular sludge from the anammox enrichment or the lab-scale anammox SBR and collected during the reaction phase. The sludge was re-suspended and washed three times with mineral medium prepared according to López *et al.* (2008) and supplemented with sodium bicarbonate (30 mM). The salinity in the mineral medium was adjusted with NaCl in order to fix the same values in the control and the test bottles to exclude possible effects of salinity in the SAA tests. The final VSS concentration in tests was set around 1 $gVSS\cdot L^{-1}$. DO was removed by flushing the bottles with a mixture of Ar (95%) and CO_2 (5%) and the initial pH was fixed between 7.6-7.7. Then, the bottles were immediately sealed.

Before starting the experiment, the bottles were kept well-mixed at 35º C and 150 rpm during 30 minutes, in order to reach the batch test conditions. After this incubation period, concentrated solutions of NH_4Cl and $NaNO_2$ were added to set the initial ammonium and nitrite concentrations and determined the beginning of the experiment (t=0h). From this point on pressure measurements were performed at 30 min intervals during 3-4 hours in order to determine the N_2 production rate. At the end of the test, ammonium nitrite and nitrate nitrogen concentration were measured and the pH was checked to be within the optimal range for the anammox activity. Finally VSS concentration was measured. The maximum N_2 production rate (kg N_2-N·m^{-3}·d^{-1}) during the tests was obtained from the maximum slope of the cumulative N_2 production curve and was adjusted with the VSS content to obtain the final SAA value (kg N_2-N·$kgVSS^{-1}$·d^{-1}), based on the calculations reported by Dapena-Mora *et al* (2007).

3.4 Stereomicroscope and scanning electron microscope observations

Images of the granular biomass were obtained with a digital camera (Nikon coolpix-4500) coupled to a stereomicroscope (ZEISS SteREO Discovery V12) using emitted and/or transmitted light (Schoot KL2500). Detailed images of the anammox granules were obtained with a ZEISS DSM 960 A (1993) scanning electron microscope (SEM) with a magnification capacity and a voltage ranging from 4 to 300000 and 0.49 to 30 kV, respectively. The biomass samples were fixed with glutaraldehyde 2.5% in phosphate buffer during 2-4 hours and then washed twice with phosphate buffer. Then, fixed samples were washed with RO water (<3 ppb TOC). A set of ethanol solutions from 40 to 100% ethanol content (v/v) was used to dehydrate the samples. Finally, samples were desiccated by means of critical point dehydration and shaded with gold before observation.

CHAPTER 4. LAB-SCALE EXPERIENCES ON ANAMMOX WITH MINERAL MEDIUM

4.1 Motivation

The feasibility of lab-scale anammox SBR treating synthetic wastewater was demonstrated in previous studies. The SBR used in this work was started up and enriched with granular anammox biomass using a mixture of common activated sludge coming from the anoxic tank of a domestic WWTP performing nitrification/denitrification for biological N-removal (López *et al.* 2008). These experiences constitute the background of this work, but more studies are needed to move the application from synthetic to real wastewater.

Several authors have reported inhibition of anammox bacteria due to the presence of different exogenous compounds. From an engineering point of view, the effect of substrates on anammox activity is of great interest. The effect of nitrite has been largely explored in the literature but there is a disparity of criteria about the threshold of the 50% and 100% inhibition concentrations for anammox bacteria. Initially Strous *et al.* (1999) reported inhibition of anammox activity with a nitrite concentrations above 100 $mgNO_2\text{-}N\cdot L^{-1}$. Egli *et al.* (2001) observed a complete loss of activity when the biomass was exposed to 185 $mgNO_2^-\text{-}N\cdot L^{-1}$. However Dapena-Mora *et al.* (2007) reported a 50% activity loss with high concentrations of nitrite (350 $mgNO_2\text{-}N\cdot L^{-1}$). Fux *et al.* (2004) introduced the exposure length vector and found severe inhibition after 6 days of exposure to only 30 $mgNO_2\text{-}N\cdot L^{-1}$. The variability among the different studies of nitrite concentrations causing 50% of inhibition (IC_{50}) indicates that other factors such as biomass characteristics or environmental conditions affect the occurrence of nitrite inhibition in anammox reactors, which could have a case-specific component (Fux *et al.* 2004; Jung *et al.* 2007; Bettazzi *et al.* 2010; Tang *et al.* 2010a).

Therefore, the effect of nitrite accumulations needs to be elucidated, in our case, with a reactor enriched with granular anammox biomass related to *Candidatus Brocadia Anammoxidans*. In addition, nitrite inhibition in anammox bacteria has been commonly considered irreversible. However, recent studies have indicated the possibility of activity recovery, but this with long recovery periods (Fernández *et al.* 2010). In order to move to landfill leachate treatment and to scale up the process, the possible irreversible inhibition and the strategies to deal with nitrite accumulations in the reactor must be studied.

Several works in lab-scale reactors reports the start-up and enrichment of the reactor, but it is necessary to study the stability of the long-term process in an SBR to detect long term dynamics and to define the tools to monitor and control of the process performance.

4.2 Objectives

In this chapter, long-term operation of a lab-scale anammox SBR treating synthetic wastewater (anammox enrichment) with high ammonium content is evaluated. The main function of this reactor with a mineral medium was to provide a source of enriched granular anammox biomass to seed new anammox reactors and/or to be used in activity assays.

The objective of this chapter is the assessment of SBR operation with simple tools that allow easy control of the process and the identification of key operational factors to evaluate possible procedures for a stable operation. Interactions between the autotrophic anammox biomass and heterotrophic denitrifying bacteria were also explored.

Since nitrite is one of the main concerns in the operation of anammox reactors, the effect of nitrite on the anammox biomass from this lab-scale reactor is investigated to know how the anammox activity changes when exposed to different nitrite levels and the contact time with high nitrite concentrations.

4.3 Materials and Methods

4.3.1 Experimental set-up

4.3.1.1 20L SBR pilot plant

The study was conducted in a lab-scale SBR pilot plant located in the Science and Technology Park of the University of Girona. The experimental set-up consisted of a 20L reactor and a control system, as shown in the Figure 4.1 scheme.

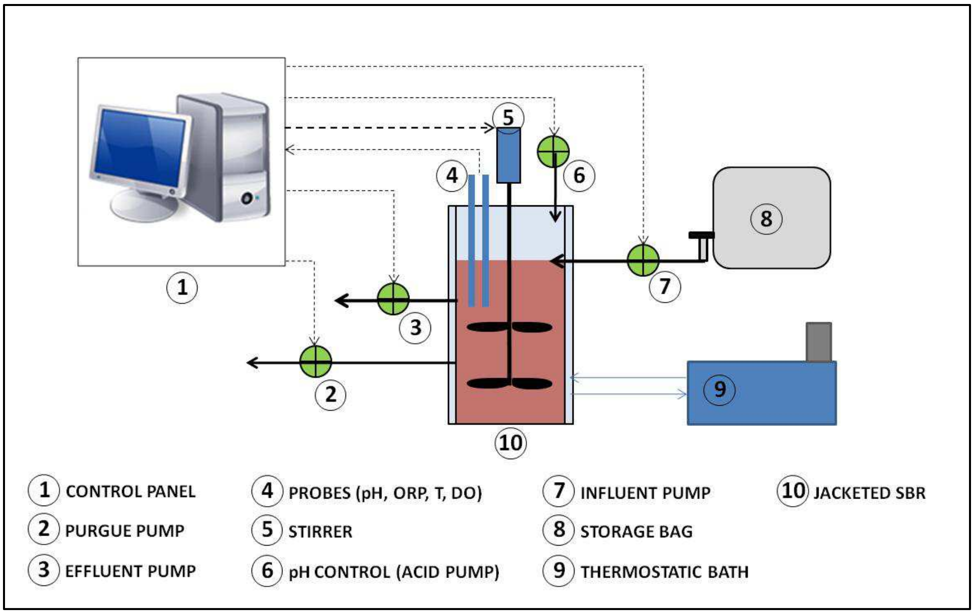


Figure 4.1. Schematic of the lab-scale set-up.

The reactor was water-jacketed for temperature control and was built from A-316 stainless steel with a cylindrical shape (height of 0.55m and internal diameter of 0.21m). A mechanical stirrer (Stuart Scientifics SS10) assured the complete mixture inside the reactor during the feeding and reaction phases. The influent was stored in a self-collapsible plastic bag to prevent air diffusion and was pumped to the upper part of the reactor with a peristaltic pump (Watson Marlow 505S). The minimum water volume (V_{min}) was set at 13.9 L, and the reactor was drawn by a peristaltic pump (Watson Marlow 505S) until the V_{min} was reached. A total of 2.0 ± 0.1L·cycle^{-1} of influent were fed from the top of the reactor.

The pilot plant was equipped with a monitoring and control system that received the online data provided by pH, oxidation reduction potential (ORP), dissolved oxygen (DO) and temperature probes (CPF81, CPF82 and OXYMAX-W COS-41; Endress-Hauser). The online data were acquired by means of an interface card (PCL-812 PG; National Instruments) and software developed in our own lab using Lab Windows® (Puig *et al.* 2005). This interface card also transmitted the program commands through a relay output board to switch the electrical devices on or off according to the operational cycle defined.

4.3.1.2 Operating conditions

The reactor was maintained under anoxic conditions and at 35 ± 1ºC. The mixed liquor was completely blended with a mechanical stirrer working at 250rpm during the feeding and reaction phases. An 8-hour cycle (Figure 4.2) was implemented and continuously repeated. The cycle consisted of a first phase of 360 minutes of continuous feeding, followed by an 85-minute reaction phase. The pH was maintained in a proper range by dosing 1M HCl spikes at the beginning of the cycle and halfway through the feeding phase. At the end of the reaction phase, a one-minute purge was performed to maintain the sludge retention time (SRT) in the reactor. The cycle ended with a 20-minute settling phase and 10 minutes of drawing. The resulting minimum settling velocity (MSV) was set at 0.045 m·h^{-1}.

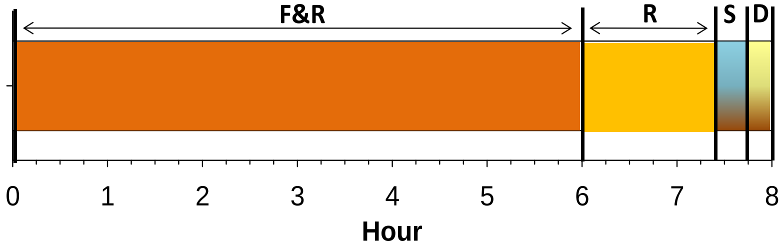


Figure 4.2. Schematic of the 8-hour cycle. F&R: Feeding and reaction; R: Reaction; S: Settling and D: Drawing.

Influent and effluent samples were periodically collected (2-3 per week) to analyze NH_4^+-N, NO_2^--N and NO_3^--N, alkalinity, TOC, and IC. In addition, the TSS and VSS in the reactor, the purge and the effluent were measured once a week.

4.3.1.3 Influent media

This anammox enrichment was always fed with synthetic media prepared according to Lopez *et al.* (2008) and consisting of a mixture of a mineral medium and concentrated solutions of $NaNO_2$ and NH_4Cl. The composition of the mineral medium is shown in Table 4.1, which depicts the concentration of nutrients. A 1.25 mL micro-element solution (prepared according to Van De Graaf *et al.* 1996) was added per litre of mineral medium.

Table 4.1. Nutrient contents in the mineral medium.

Nutrient	Source	Source concentration mg/L	Nutrient concentration mg/L
Ca^{2+}	$CaCl_2 \cdot 2H_2O$	3000	81.79
Mg^{2+}	$MgSO_4 \cdot 7H_2O$	2000	19.72
Fe^{2+}	$FeSO_4$	125	4.60
$P\text{-}PO_4^{3-}$	KH_2PO_4	63	1.42
$C\text{-}HCO_3^-$	$NaHCO_3$	1050	150.20

4.3.2 Batch experiments

4.3.2.1 Batch tests for modeling

Experiments with biomass from anammox enrichment were performed to model the presence of heterotrophic bacteria in the system. To perform the experiments, 2L of mixed liquor was collected from the SBR during the end of the reaction phase. The biomass was washed three times with a fresh mineral medium in order to remove the rest of substrates. The experiments were performed in a 2L water-jacketed reactor with a magnetic stirrer. Anoxic conditions were ensured by gasifying a mixture of Ar and 5% CO_2 before starting the experiment. The pH and DO were controlled during the experiment to guarantee the optimal ranges for anammox biomass.

Before starting the experiments, the temperature was set at 35ºC and the reactor was then maintained under homogeneous conditions for 10 minutes. The batch started with the spike of substrate consisting of the addition of concentrated solutions of NH_4Cl, $NaNO_2$ and $NaNO_3$. Samples for total and soluble COD, VSS and biomass to be fixed for further FISH analysis were collected at the beginning of the experiment. Samples were then periodically collected to follow the evolution of the nitrogen species. The amount of mixed liquor extracted for sampling was compensated by the expansion vessel in order to avoid DO penetration.

4.3.2.2 Specific anammox activity tests with nitrite

In this chapter, the effect of nitrite on anammox activity with mineral medium is described. Inhibition tests were used to evaluate the effect of a certain nitrite level on the specific anammox activity (SAA, Kg N_2-N·Kg^{-1} VSS·d^{-1}). These tests were performed with anammox biomass according to the procedure described in section 3.3 of the Materials and Methods section and consisted of a set of two to three controls and the replicates with the chemical compound studied (i.e., nitrite). Controls consisted of 120 ml bottles with mineral medium and an initial spike of 50 mg NH_4^+-N·L^{-1} and 50 mg NO_2^--N·L^{-1}, and were used to determine the reference activity. The rest of the bottles contained the nitrite concentration to be tested. The inhibition was calculated as the difference between the reference activity measured in the controls and the activity measured in the bottles with inhibitor (nitrite).

The effect of high nitrite concentrations was tested in two ways: 1) the effect of the nitrite concentrations themselves and 2) the effect of exposure over time to high nitrite concentrations. Moreover, the activity recovery after washing the biomass affected by nitrite inhibition was also explored to determine the reversible or irreversible character of the nitrite inhibition on anammox bacteria.

The effect of the nitrite concentration was tested in assays with bottles containing 50 mgNH_4^+-N·L^{-1} and 100, 200, 300, 400 and 500 mg NO_2^--N·L^{-1} with the respective replicates. The SAA was measured just after spiking the nitrogen compounds and after 24h of incubation at the respective nitrite levels. The effect of washing for activity recovery was tested with the biomass exposed for 48h to the different nitrite concentrations; the anammox biomass was then washed three times with mineral medium and a SAA test with an initial spike of 50 mgNH_4^+-N·L^{-1} and 50 mg NO_2^--N·L^{-1} was performed.

4.3.2.3 Batch tests for soluble microbial products production

A 0.8L up-flow biofilm anammox reactor located at the Technical University of Denmark (Lyngby, Denmark) operating for more than three months was used to study the production of soluble microbial products (SMP) in anammox cultures. A fiber with a total surface area of 12.1 dm^2 was placed inside the reactor as support for biofilm growth. The temperature was maintained at 36 ± 1ºC by a water bath and the pH was kept within the optimum range for the biomass (between 7.5 and

8.3). The reactor was continuously filled from the bottom with mineral medium prepared with distilled water and the concentrated solutions of substrates and trace elements. The substrate solution (5ml per liter of medium) was prepared by dissolving 10.8g KH_2PO_4, 46.35g $MgCl_2$, and 72g $CaCl_2$ in 2L of distilled water. The trace elements solution I (2ml per liter of medium) contained 2.5g EDTA and 4.58g $FeSO_4{\cdot}7H_2O$ in 1L of distilled water. Finally, the trace elements solution II (2ml per liter of medium) were prepared with 15g EDTA, 0.20g $ZnCl_2$, 0.24g $CoCl_2{\cdot}6H_2O$, 0.99g $MnCl_2{\cdot}4H_2O$, 0.17 g$CuCl_2{\cdot}2H_2O$, 0.24 g$Na_2MoO_4{\cdot}2H_2O$, 0.19g $NiCl_2{\cdot}6H_2O$, and 0.22g $Na_2SeO_4{\cdot}10H_2O$ in 2L of distilled water. A concentrated solution of NH_4Cl and $NaNO_2$ in a 1:1 ratio of NH_4^+-N and NO_2^--N was used as the substrate source. The carbon source was supplied by adding solid potassium bicarbonate (0.5g $KHCO_3{\cdot}L^{-1}$ of medium).

To analyze the fluorescent compound production in the system, two batch experiments were carried out. The aim of the batch experiments was to explore in more depth the SMP production dynamics during the both the growing and starvation phases. During the batch experiments the reactor was equipped a recirculation pump to ensure the complete homogenization of the bulk liquid and the total reactor volume was increased to 1.45L. Before starting the batch tests, the bulk liquid was washed out with a fresh mineral medium at 36ºC, prepared as described above. After 30 minutes of acclimatization, the substrate was added by a concentrated solution (10ml) of ammonium and nitrite. An initial spike of substrate (ammonium and nitrite) was applied to set the initial conditions at around 100 mgN-$NH_4^+{\cdot}L^{-1}$ and 100 mgN-$NO_2^-{\cdot}L^{-1}$ for Batch 1 and around 200 mgN-$NH_4^+{\cdot}L^{-1}$ and 200 mgN-$NO_2^-{\cdot}L^{-1}$ for Batch 2. The batch test started after 15 minutes of homogenization, when the first sample was collected.

4.3.3 Excitation-emission matrix (EEM) fluorescence spectrometry

EEM fluorescence was tested and used to explore the effluent dissolved organic matter (EfOM) from anammox reactors. The fluorescence spectrum was measured using a Varian Cary Eclipse Fluorescence Spectrophotometer. The wavelength range for excitation spectra was 240-450 nm, while for emission it was 300-600 nm, with a 5-nm and 2-nm steps, respectively. Excitation and emission slit widths were set to 5 nm and the photomultiplier tube voltage to 1000v. The excitation and emission spectra measured from each sample were combined to create excitation-emission matrices. Sample inner filter effects were also corrected using absorption measurements performed on a Varian Cary 50 Bio UV-visible Spectrophotometer in a 1 cm quartz cuvette and UV-visible spectra recorded from 240 to 700 nm with 0.5 nm slit width. The inner filter effect correction was followed by Raman calibration according to (Lawaetz and Stedmon 2009). The calibrated and corrected fluorescence data were then modeled using the DOMFluor Toolbox in MatLab® 7.0 (MathWorks Inc., USA) according to the procedure recommended in (Stedmon 2008). The number of fluorescence components was found by a validation method including split half and residual analysis.

4.4 Results

4.4.1 Long-term anammox SBR operation

After the completion of the start-up and enrichment periods (López *et al.*, 2008), the anammox SBR was operated continuously to test the long-term stability of the process treating high N-strength wastewater for more than three years. The reactor was also used as source of anammox inoculums for new anammox reactors and batch test experiments.

4.4.1.1 Nitrogen removal performance

The lab-scale anammox SBR performance is depicted in Figure 4.3 where the nitrogen loading rate (NLR, Kg $N\cdot m^{-3}\cdot d^{-1}$), nitrogen discharge rate (NDR, Kg $N\cdot m^{-3}\cdot d^{-1}$), and nitrogen removal efficiency (%, according to influent ammonium plus nitrite) are presented.

During this period, the NLR ranged between 0.22 kg $N\cdot m^{-3}\cdot d^{-1}$ and 1.14 kg $N\cdot m^{-3}\cdot d^{-1}$ (days 1594 and 1495, respectively), with an average of 0.78 ± 0.19 kg $N\cdot m^{-3}\cdot d^{-1}$. The NDR was kept as low as possible and was close to zero most days. Maximum discharge values were achieved on days 1075, 1210, 1461, and 1496 with an NDR of 0.08 kg $N\cdot m^{-3}\cdot d^{-1}$, 0.09 kg $N\cdot m^{-3}\cdot d^{-1}$, 0.20 kg $N\cdot m^{-3}\cdot d^{-1}$, and 0.09 kg $N\cdot m^{-3}\cdot d^{-1}$, respectively. Consequently, good nitrogen removal efficiencies were generally obtained during the entire experimental period. In terms of ammonium and nitrite, the average efficiency was 98.3 ± 2.8% (the removal efficiency of nitrite was 98.5 ±

3.0%). Considering the total nitrogen, the nitrogen removal efficiency obtained in the SBR was 82.2 ± 2.8%, which is close to the theoretical stoichiometric value (89%; taking into account the nitrate production).

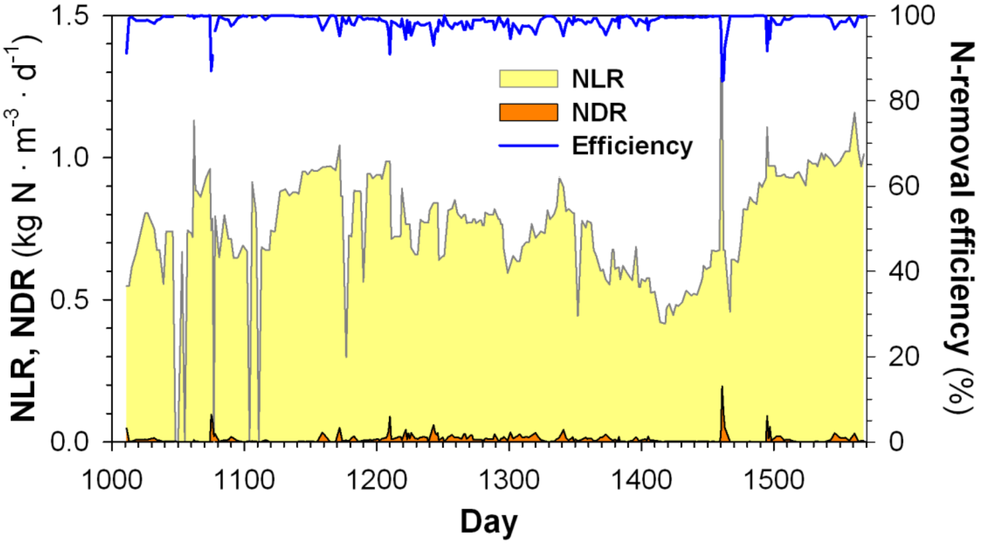


Figure 4.3.NLR, NDR and N-removal efficiency during the long-term operation. The three parameters are calculated as ammonium plus nitrite.

The influent total nitrogen and the effluent nitrogen species concentration are shown in Figure 4.4. The average total nitrogen in the influent wastewater treated during the study was about 1925.8 ± 476.4 mg N·L^{-1}. The influent was nitrate-free and contained average ammonium and nitrite concentrations of 841.7 ± 160.2 mg NH_4^+-N·L^{-1} and 1131.8 ± 233.2 mg NO_2^--N·L^{-1}, respectively. As shown in Figure 4.4-A the influent nitrite to ammonium molar ratio ranged between 0.85 and 1.75 mol NO_2^--N ·mol^{-1} NH_4^+-N, with the average value set at 1.34 ± 0.12. This ratio was maintained close to the stoichiometric value, but presented fluctuations over time, with an impact on the effluent quality.

The nitrogen content of the effluent as ammonium, nitrite and nitrate are depicted in Figure 4.4-B. Nitrate was the main nitrogen compound in the effluent according to the anammox reaction, ranging from 94.4 mg NO_3^--N·L^{-1} to 289.1 mg NO_3^--N·L^{-1}. Nitrite and ammonium concentrations in the effluent were normally below 50 mg N·L^{-1} and presented average values of 19.0 ± 41.1 mg NO_2^--N·L^{-1} and 17.3 ± 32.56 mg NH_4^+-N·L^{-1}, respectively. The high removal efficiencies obtained during the study and the low effluent nitrogen concentrations prove the good performance of the process.

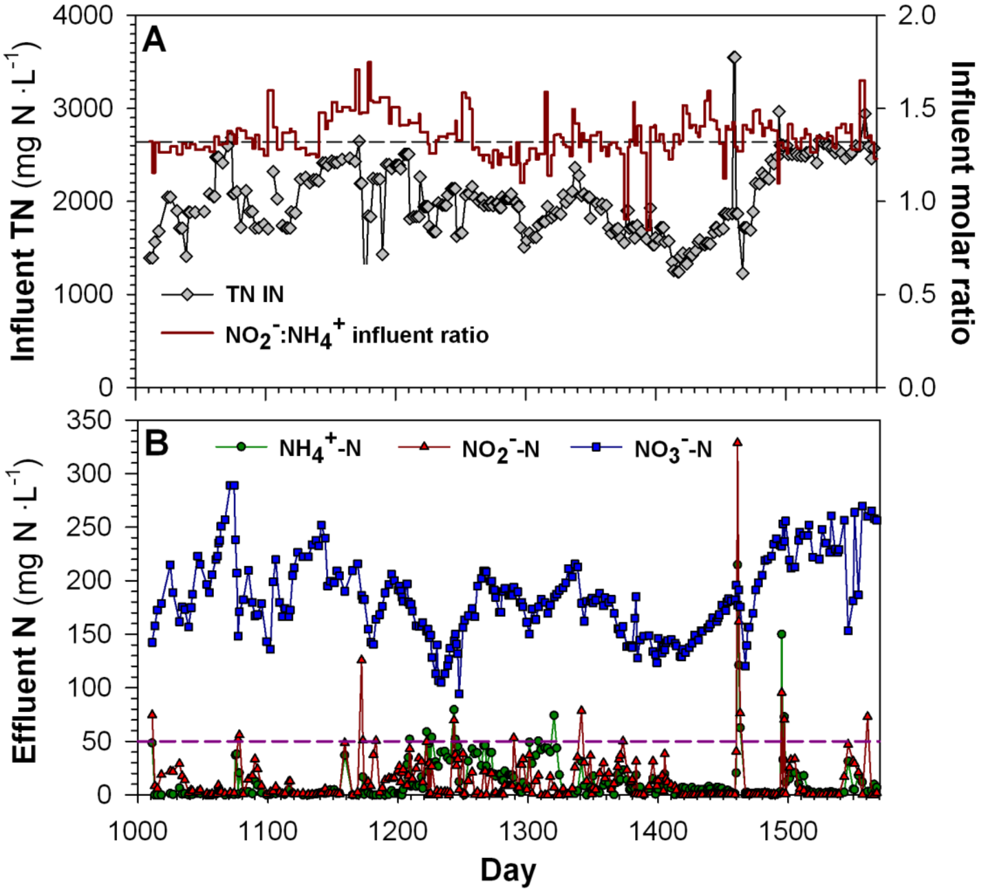


Figure 4.4. Evolution of the N content in the influent (A) and the effluent (B) of the anammox enrichment during the long-term operational period.

Figure 4.4-B shows some nitrite and ammonium accumulations as well as periods with an increase in nitrogen discharge. The main concern dealt with the nitrite accumulations. When the nitrite concentration in the reactor was more than 100 mg NO_2-N·L^{-1}, the reactor was fed with a mineral medium without substrate in order to rapidly wash away the nitrite accumulated inside (i.e., on Day 1172 and 1461).

On the contrary, accumulations between 50 and 100 mg NO_2^--N·L^{-1} (such as Days 1243 and 1495) were solved by increasing the duration of the reaction phase of the SBR cycle, allowing ammonium and nitrite consumption. In all cases, when the reactor was fed again, the NLR was adjusted according to the N removal capacity of the system. Table 4.2 summarizes the highest nitrite and ammonium effluent concentrations attained during the study, with the corresponding influent nitrite to ammonium ratio applied on those days.

Table 4.2. Maximum levels of nitrite and/or ammonium reached during the study inside the reactor.

Day	**Eff. Nitrite** $mgNO_2^-\text{-}N{\cdot}L^{-1}$	**Eff. Ammonium** $mgNH_4^+\text{-}N{\cdot}L^{-1}$	**Inf. Ratio** $NO_2^-: NH_4^+$
1011	74.5	48.4	1.32
1172	125.9	1.6	1.71
1243	69.7	79.6	1.36
1320	7.0	74.3	1.14
1341	78.6	31.2	1.42
1461	328.7	214.8	1.43
1495	95.5	150.0	1.10
1561	73.0	3.2	1.65

Generally, the increments of the effluent nitrite and ammonium contents were related to deviations of the influent ratio. Thus, in most cases an increase of influent nitrite to ammonium molar ratio over 1.32 resulted in nitrite accumulations. On the contrary, the effluent ammonium concentration was directly affected by influent ratio values below 1.32 which always resulted in the accumulation of ammonium in the reactor, such as on Day 1320.

Influent ratios above 1.32 were not always related to important nitrite presence in the effluent during the period between Days 1420 and 1490 influent ratios of about 1.39 ± 0.1 were applied (with maximum values of 1.53). In this case, the effluent nitrite concentration remained close to zero (0) in spite of an excess of nitrite according to anammox stoichiometry. In this context heterotrophic denitrification could be responsible for some nitrite removal, as will be forward tested in this chapter. The maximum effluent nitrogen concentrations were achieved on Day 1461, with nitrite and ammonium accumulations up to 328.7 mg NO_2^- -N·L^{-1} and 214.8 mg NH_4^+ -N·L^{-1} (Table 4.2). Such high effluent levels were achieved by a high influent concentration caused by undesired operational procedures.

4.4.1.2 Evolution of the mixed liquor suspended solids

Volatile suspended solid (VSS, mg/l) concentrations inside the reactor and in the effluent are presented in Figure 4.5, together with the corresponding VSS to TSS ratio. The desired VSS concentration was set around 1500 mg VSS·L^{-1}, and controlled by periodic purge events of the reactor. The biomass retention time was adjusted to 45 days. Nevertheless, because of the high attachment capacity of the anammox biomass to the reactor walls, the VSS concentration in Figure 4.5 is only an indicative value of the total amount of anammox biomass in the reactor.

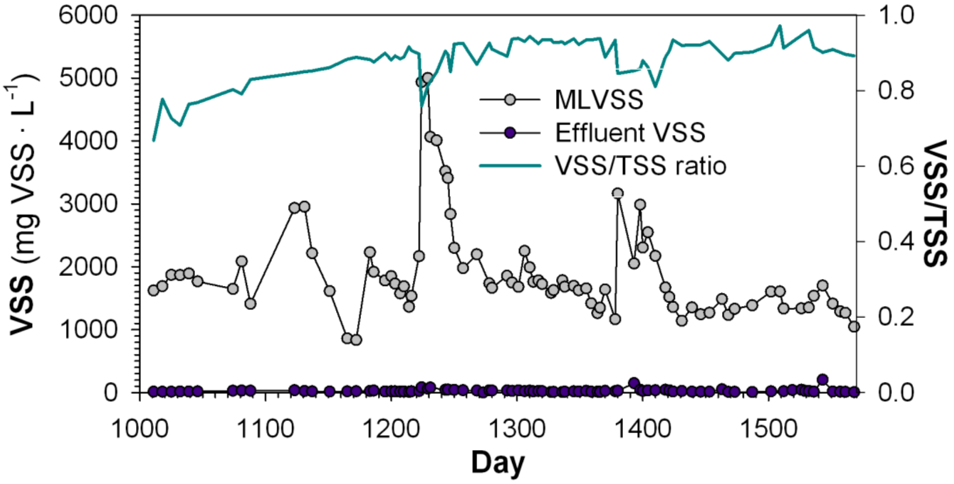


Figure 4.5. Evolution of the VSS content in the mixed liquor (grey dots) and the effluent (violet dots) of the reactor, with the corresponding VSS/TSS ratio (solid black line).

It can be observed in Figure 4.5 how the VSS content in the reactor suddenly changed on Day 1119, 1221 and 1380. During these days the reactor was stopped at the beginning of the 8-hour cycle and opened to clean the inlet parts. All the biomass attached to the reactor walls and elements such as probes and the stirrer were re-suspended in the mixed liquor. This way, important amounts of granular sludge were returned to the bulk liquid. These actions produced two effects in the reactor: the increase of the VSS concentration in the mixed liquor and a slight increase of the inert fraction in suspended solids, reflected in a reduction of the VSS/TSS. In order to recover a better quality of the sludge with a proper VSS/TSS ratio, the purge flow was increased until the MLVSS concentration remained constant. Day 1119 is an exception because the increase of the MLVSS was due to a spontaneous detachment of an important amount of biomass, but the walls of the reactor were not completely cleaned.

In SBRs, anammox granules tend to attach to the internal surfaces of the reactor in the zone comprised between the minimum (V_{min}) and maximum (V_{max}) of the cycle (Figure 4.6). In the SBR, this accumulation could form a layer of more than 1cm thick (Figure 4.6-A). The main biomass fixation zones are signaled in red in the scheme of the lab-scale reactor presented in Figure 4.6-C. This biomass attached to the walls of the reactor was less active because of the difficulty of the microorganisms to reach the substrate in the deeper layers (due to problems for the diffusion) and the fact that this zone was only covered by the bulk liquid during a part of the 8-hour cycle. Thus, this biomass could not contribute to N-removal all the time. Moreover, since the reactor was water jacketed, the drying of the biomass was very intense, promoting the presence of dead biomass close to the walls.

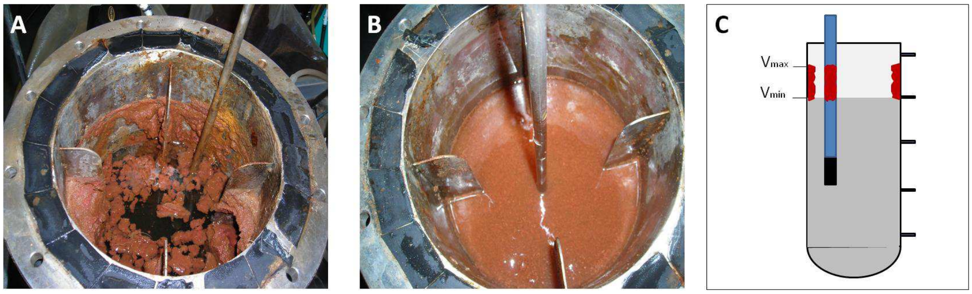


Figure 4.6. Views of the inside of the SBR before (A) and after (B) cleaning the walls. (C) Scheme of the reactor with the main biomass attachment zones (red areas).

The higher the difference between V_{min} and V_{max}, the higher the surface where the biomass will be attached. Thus, low volumetric exchange ratios (VER) in anammox SBR reduce the possibility of active biomass loss by this process. This is in accordance with the results reported by De Clippeleir *et al.* (2009), who obtained higher autotrophic N-removal when a lower VER was applied.

Several specific anammox activity (SAA) tests were performed during the long-term operation period with suspended solids from the SBR (Figure 4.7). The maximum SAA measured during the entire period was 0.85 ± 0.05 kg $N\text{-}N_2{\cdot}kg^{-1}$ $VSS{\cdot}d^{-1}$, achieved on Day 1154, when the reactor was removing 0.95 kg $N{\cdot}m^{-3}{\cdot}d^{-1}$. The average SAA measured during the entire operational period was set at 0.54±0.23 kg $N\text{-}N_2{\cdot}kg^{-1}VSS{\cdot}d^{-1}$. Low SAA was obtained in the experimental measurements carried out on Day 1252 and 1404, with 0.28 ± 0.03 kg $N.kg^{-1}VSS{\cdot}d^{-1}$ and 0.32 ± 0.05 kg $N.kg^{-1}VSS{\cdot}d^{-1}$, respectively.

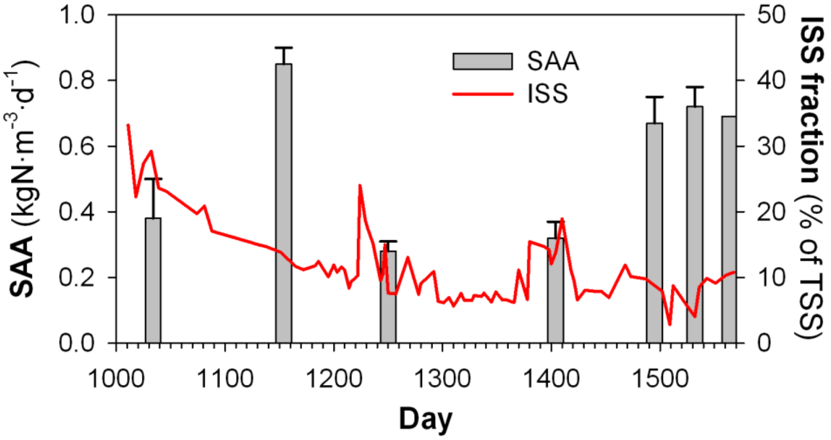


Figure 4.7. Results of SAA tests with SBR biomass (grey bar) and the evolution of the inert suspended solids (ISS) fraction of the total suspended solids (red line).

Figure 4.7 shows than when effluent nitrite levels are close to zero (i.e. Days 1154, 1490, 1532, and 1564), the SAA presents the highest values around 0.67kg $N\text{-}N_2\cdot kg^{-1}VSS\cdot d^{-1}$. Also, on operational days with sustained effluent nitrite concentrations between 20 and 50 mg $NO_2^{-}\text{-}N\cdot L^{-1}$ (i.e., 1019, 1250, and 1404), a lower SAA was detected with values between 0.28 and 0.38 kg $N\text{-}N_2\cdot kg^{-1}VSS\cdot d^{-1}$, which corresponds to an anammox activity reduction between 45 and 60%. As postulated by Fux et al. (2004), long exposure to relatively low nitrite accumulations resulted in a decrease in the experimental SAA.

The evolution of the inert fraction of suspended solids during the study is shown in Figure 4.7 (red line). It can be seen that the inert solid content was high during the beginning of the experimental period –with the ISS fraction up to 32.2% of the TSS and progressively decreased. During the rest of the study, the inert fraction represented around 8% of the TSS and increased only during reactor maintenance.

Table 4.3 shows the changes in the suspended solids composition after the cleanings performed on Day 1221 and 1383 in the anammox enrichment. It can be seen how an important amount of biomass can be attached to the walls of the reactor (more than 50%). The attached biomass presented an important presence of the inert fraction (more than 32% on Day 1221), much higher than the fraction of inert solids in mixed liquor suspended solids. This is indicative that the biomass attached to the zone above the minimum liquid volume had limited activity and was degraded due to the water jacket of the reactor.

Table 4.3. Changes in the suspended solids composition during the two days that the SBR was opened and cleaned.

Day	TSS_{before} mg·L^{-1}	VSS_{before} mg·L^{-1}	TSS_{after} mg·L^{-1}	VSS_{after} mg·L^{-1}	Attached % (1)	ISS_{before} mg·L^{-1}	ISS_{added} mg·L^{-1}	Inert MLSS % (2)	Inert Attached %(3)
1221	2416.2	2167.6	6496.0	4936.0	56.1	248.6	1311.4	10.29	32.14
1383	1249.1	1166.0	3742.9	3164.3	63.2	83.1	495.5	6.65	19.87

Before: value measured just before cleaning the walls. After: value after cleaning
(1) Percentage of biomass attached to the walls of the reactor the day of cleaning
(2) Percentage of inert solids in the mixed liquor TSS before cleaning
(3) Percentage of inert solids in the attached biomass.

In view of the scale-up of the process, these results suggest the necessity of applying measures to limit the amount of biomass attached to the internal parts of the reactor between the minimum and maximum volumes.

4.4.1.3 Characterization of the granular biomass

Sludge samples were collected periodically for further characterization of the granular biomass using the stereomicroscope and FISH analyses. Figure 4.8 shows two typical images obtained with the stereomicroscope from biomass samples from the SBR, in this case collected on Day 1371 (Figure 4.8-A; before opening) and Day 1412 (Figure 4.8-B; after opening).

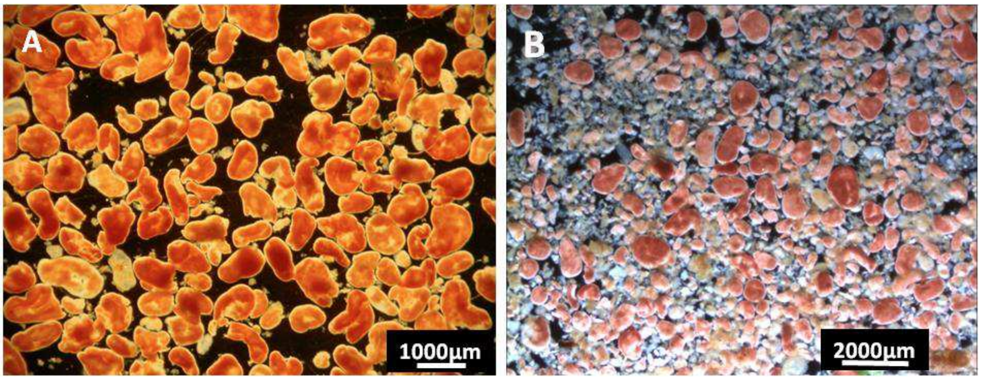


Figure 4.8. Images of the granules of SBR obtained by the stereomicroscope. A: sample collected before cleaning (12.5x); and B: after cleaning the reactor (25x).

The anammox granules can be clearly observed and present the typical red color of anammox. The granules were compact with a regular and elliptic shape. The percentiles 10 and 90 of the size distribution were set at 550µm and 980µm (the diameter of the equivalent sphere), respectively. These images confirm that the quality of the sludge was affected when the reactor was opened to re-suspend the biomass attached to the walls.

It can be seen how the Day 1371 sample contained only compact anammox granules. After cleaning the inlet walls of the reactor, the sample of Day 1412 also contained an important presence of debris suspended in the bulk liquid (Figure 4.8-B). This debris was attributed to the dead biomass re-suspended together with the active granules. This led in a decrease of the VSS/TSS ratio due to the presence of inert particulates. The debris was washed out of the reactor by increasing the purge flow during some days. The sludge volumetric index (SVI) of biomass was always around 90mL·gr^{-1}VSS, with the same values of SVI$_{30}$ and SVI$_{10}$, indicating the fast settling velocity of the sludge. The good settling properties were also reflected in the effluent VSS content, which was very low during the entire study (27.7 ± 27.1mg VSS·L^{-1}). This property is important in favoring the biomass retention in anammox SBR reactors.

Samples were punctually analyzed using the sequential electronic microscope (SEM) observation and FISH analysis. Figure 4.9 shows a typical image of a granule obtained in the SEM with a sample collected on Day 1163 (Figure 4.9-A) and an image of the FISH analysis (Figure 4.9-B).

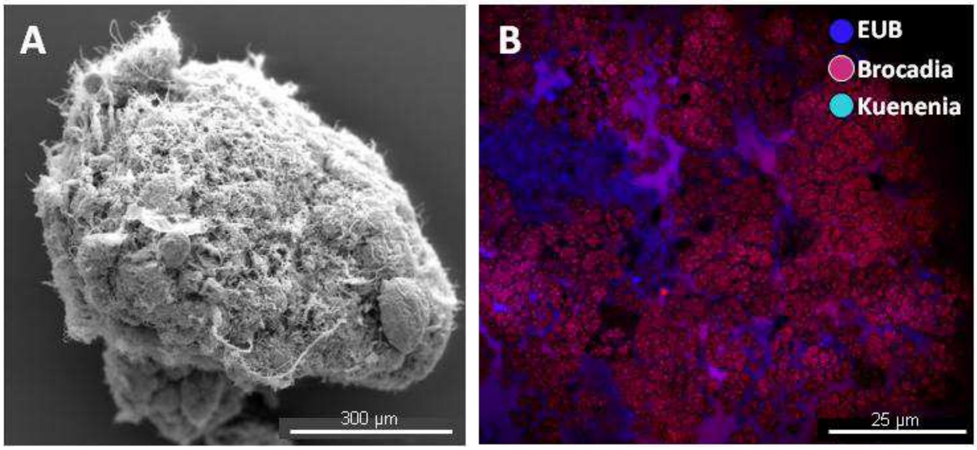


Figure 4.9. Results of the SEM observation (A) and FISH analysis (B) obtained from a sludge sample collected on day 1163 in SBR-1.

The analysis of granules in the SEM also allowed for exploring the relative composition of the granules (the relative abundance of metals with respect to the C content; mass to mass). The results revealed that the granules were rich in calcium. Calcium is sometimes used to promote granulation in biological processes in order to improve the sludge settling velocity, which is in accordance with our results (Van Der Star *et al.* 2008; De Graaff *et al.* 2011). In a minor proportion (three times lower than calcium), other compounds were present in the granules, mainly magnesium, phosphorus and sulfur.

The FISH analysis (Figure 4.9-B) proved that the system was enriched with anammox bacteria, with a relative abundance with respect to the total microbial community of about 89.1 ± 7.6%. Also, according to FISH analysis, the population of anammox bacteria was always related to *Candidatus Brocadia anammoxidans*, since no signal of *Candidatus Kuenenia stutgartiensis* was observed in any sample. So far, the microbial community in our anammox reactors always contains only one type of anammox bacteria.

4.4.1.4 Experimental process assessment of anammox SBR

A system enriched with anammox bacteria might present nitrite/nitrate to ammonium removal ratios close to the stoichiometric values (i.e., 1.32 moleNO_2^--$N_{removed}$·$mole^{-1}$$NH_4^+$-$N_{removed}$ and 0.26 moleNO_3^--$N_{produced}$·$mole^{-1}$$NH_4^+$-$N_{removed}$), since the nitrogen removal is attributed to anammox bacteria. The experimental ratios are shown in Figure 4.10.

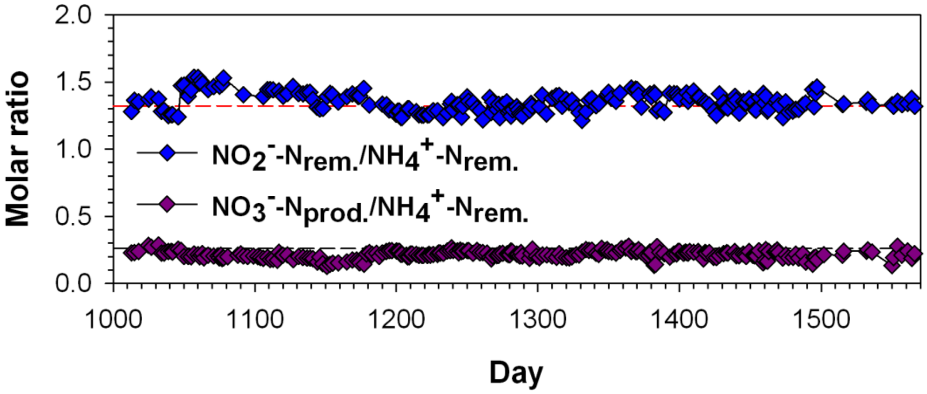


Figure 4.10. Experimental nitrite to ammonium and nitrate to ammonium ratios obtained during long-term operation. The dotted lines are indicating the stoichiometric values of the anaerobic ammonium oxidation.

The experimental ratios match the stoichiometric coefficients most days, but a significant deviation can be observed between Day 1050 and Day 1140, when experimental values present some deviation with higher nitrite consumption and a lower nitrate production than the specified for anammox. The average experimental values obtained in this study were 1.34 ± 0.07 NO_2^--N: NH_4^+-N and 0.22 ± 0.03 NO_3^--N: NH_4^+-N, respectively. These values are very close to the stoichiometric coefficients of the anammox reaction, but reveal some contribution of heterotrophic denitrification removing nitrite and nitrate.

Any source of organic carbon was supplied to the mineral medium (EDTA was used to prepare the mineral medium, but the influent total organic carbon -TOC- was always below the detection limit of the analysis, close to zero). However, the soluble microbial products (SMP) derived from the biomass decay metabolism of bacteria, as well as extracellular polymeric substances (EPS) and hydrolysis products, can sustain heterotrophic growth (Ni *et al.* 2011c; Ni *et al.* 2011d) without biodegradable organic matter in the influent wastewater.

Several studies report the presence of active heterotrophic denitrifiers in enrichments where bicarbonate is supplied as the only carbon source, such as autotrophic nitrifying systems (Matsumoto *et al.* 2010, among others). In these systems, heterotrophs grow on the organic carbon released from autotrophic bacteria. Referring to anammox reactors, Dapena-Mora *et al.* (2004b) included the heterotrophic denitrification processes in the modeling work of the start-up of anammox enrichment. Also in a modeling study, Lackner *et al*. (2008) identified heterotrophic activity in an anammox membrane-aerated biofilm reactor fed with mineral medium without organic matter supply.

4.4.1.5 Online profiles

Referring to the online parameter behavior, the evolution of pH, DO, ORP and T during three typical 8-hour cycles with the reactor working at optimal operational capacity are depicted in Figure 4.11. The initial conditions for each cycle are governed by the end conditions of the previous cycle. Since temperature was maintained by the water jacket of the reactor at 36 ± 1 ºC and no DO supply was applied, these parameters remained stable under normal operation. Thus, the pH and ORP were the main online control parameters.

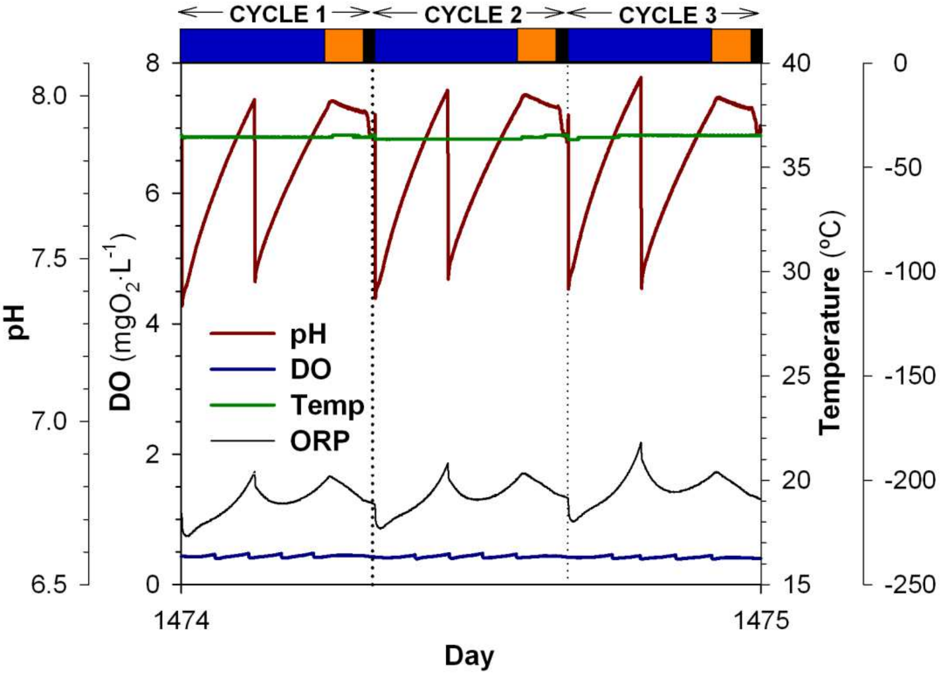


Figure 4.11. Profiles of the online parameters during a typical day of normal operation. The dotted lines indicate the end/beginning of the 8-hour cycles. At the top of the figure, the feeding (blue), reaction (orange) and settling and drawing (black) phases of the 8-hour cycle are indicated.

DO was only monitored to ensure anoxic conditions. The ORP was a control parameter of the system and varied in negative values around -200 mV, indicating the complete absence of DO and the presence of nitrate but very low levels of nitrite. Values down to -400 mV are a signal of practically no presence of either nitrite or nitrate (anaerobic conditions) with the risk of the development of sulfate reduction processes resulting in the production of toxic H_2S. The repetitive profiles during the 8-hour cycles is a consequence of the pH and the accumulation of some nitrite (below 10 $mgNO_2^-$-$N·L^{-1}$) during the feeding phase.

As can be seen in Figure 4.11, at the beginning of each cycle the pH was decreased by 1M HCl addition to the initial point (pH minimum). The continuous feeding phase started just after the acid pump stopped and the anammox reaction started at this point, when substrate was supplied. As postulated by the anaerobic ammonium oxidation stoichiometry (Equation 1.5), the oxidation of ammonium has a proton consumption associated with it, corresponding to 0.13 mole

$H^+_{removed}$·$mole^{-1}$ NH_4^+-$N_{removed}$. This leads to a pH upward trend in the system and requires of a proton source to maintain a proper pH level in the system.

The pH was maintained in the optimum range by acid addition (1M HCl) in two steps: the first addition at the beginning of the cycle and the second after 3 hours: in the middle of the feeding phase in order to avoid extreme pH changes. Thus, the feeding and reaction phase started at the minimum pH value. Meanwhile, the substrate was continuously fed, and the anammox reaction took place and consumed H^+. Figure 4.12 shows the pH profile obtained during one 8-hour cycle during a day with correct SBR operation (4.12-A), low substrate accumulation (4.12-B), and severe nitrite inhibition (4.12-C).

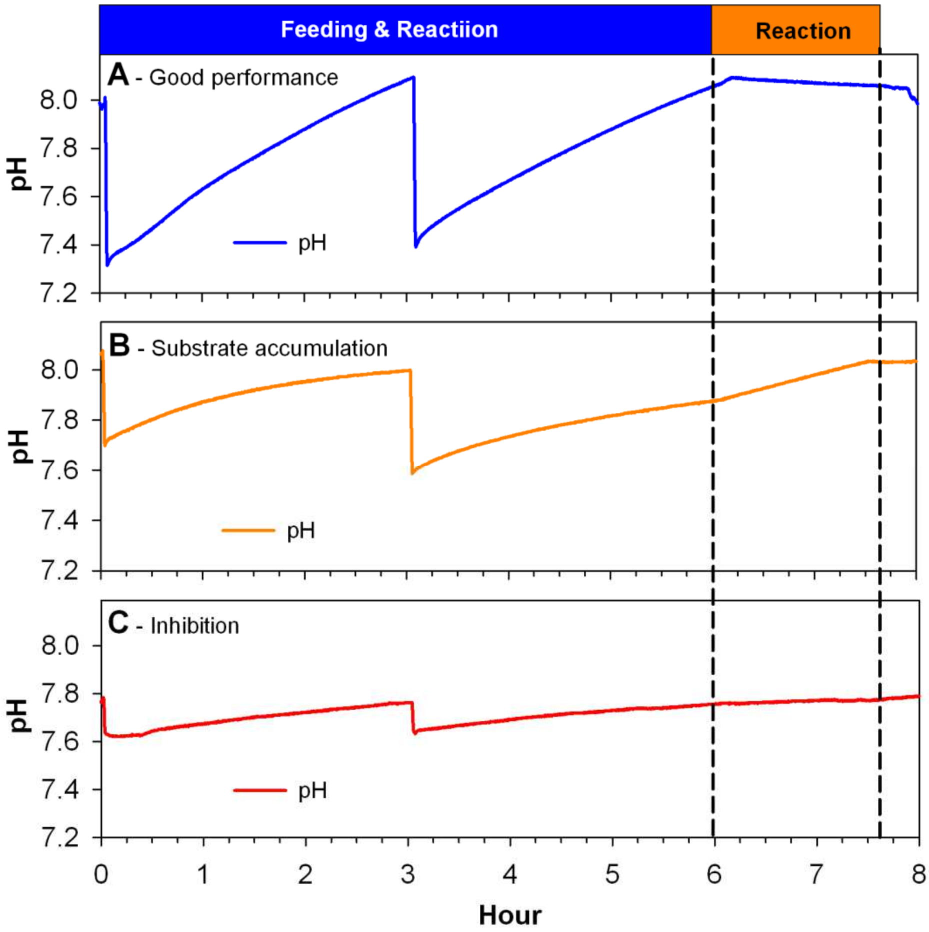


Figure 4.12. Profiles of pH during an 8-hour cycle of the anammox enrichment. A: correct operation (Day 1154); B: low substrate accumulation in the reactor (Day 1370); and C: low N-removal capacity (Day 1173). The dashed lines indicate the transition between cycle phases.

During Day 1154, the reactor presented a typical pH profile indicating good performance and producing an effluent with ammonium and nitrite concentrations close to zero. The pH profile on this day (Figure 4.12-A) indicates that substrate was removed practically at the same rate as it was loaded, since the pH upward trend stopped just after the end of the feeding phase. The effluent ammonium and nitrite concentrations measured on this day were both close to zero.

The pH profile of Day 1370 (Figure 4.12-B) shows that the NLR surpassed the treatment capacity of the reactor. In this case, it can be seen how the pH continued increasing until the end of the cycle, indicating that ammonium and nitrite were not completely removed during the reaction phase. On this day, the effluent contained a relatively low accumulation of ammonium and nitrite (12.6 $mgNH_4^+$-N·L^{-1} and 33.2 $mgNO_2^-$-N·L^{-1}, respectively), which did not constitute a high risk for the process.

Oppositionally, the pH profile obtained on Day 1173 (Figure 4.12-C) indicated a very low proton consumption rate, and it can be noted that the pH upward trend continued during the reaction phase, since substrate was not totally removed. These two examples prove that the behavior of the pH profile is a consequence of the anammox activity and constitute a good tool for monitoring the anammox SBR performance and easily detect problems in the reactor. The use of pH as indicator of the bacterial activity has been already reported in titration studies (Ficara and Canziani 2007).

4.4.2 The effect of nitrite concentration on specific anammox activity

The SBR was affected by several periods with the accumulation of substrate (either ammonium or nitrite), resulting in decreases of the nitrogen removal. The negative effects of high concentrations of nitrite on anammox bacteria have been widely reported in the literature, while it is known that ammonia does not affect SAA at concentrations below 20 mgFA·L^{-1} (Fernández *et al.* 2010), which represent ammonium concentrations up to 196 mg NH_4^+-N·L^{-1} at pH=8.0 or 577 mg NH_4^+-N·L^{-1} at pH=7.5.

The value of 50% inhibition concentration (IC_{50}) changes among the different studies reported in the literature and the effect of the exposure length has not been described well. Bettazzi *et al.* (2010) considered that the nitrite inhibition effect on anammox bacteria is case-specific and depends on several factors such as biomass characteristics or environmental conditions. Taking into account this framework, the behavior of the biomass from the lab-scale reactor was analyzed in two directions: first, the immediate effect of different nitrite concentrations was tested and second, the effect of the exposure at different nitrite levels during 24h or 48h was explored.

4.4.2.1 Response to nitrite exposure

Granular biomass from the lab-scale anammox SBR was collected and used in SAA assays. The biomass response to nitrite concentrations in the bulk liquid ranging from 100 to 500 mg NO_2^--N·L^{-1} was tested at short (assay conducted after the nitrite addition) and long exposure (24h of nitrite exposure). The results are presented in Figure 4.13 where the maximum SAA obtained for each nitrite concentration for both short and long exposures are contrasted with the control. As expected, the general trend in all the experiments was a progressive loss of activity by increasing the nitrite concentration. The activity loss for a similar nitrite level was higher after 24h of exposure than that observed after 3-4h.

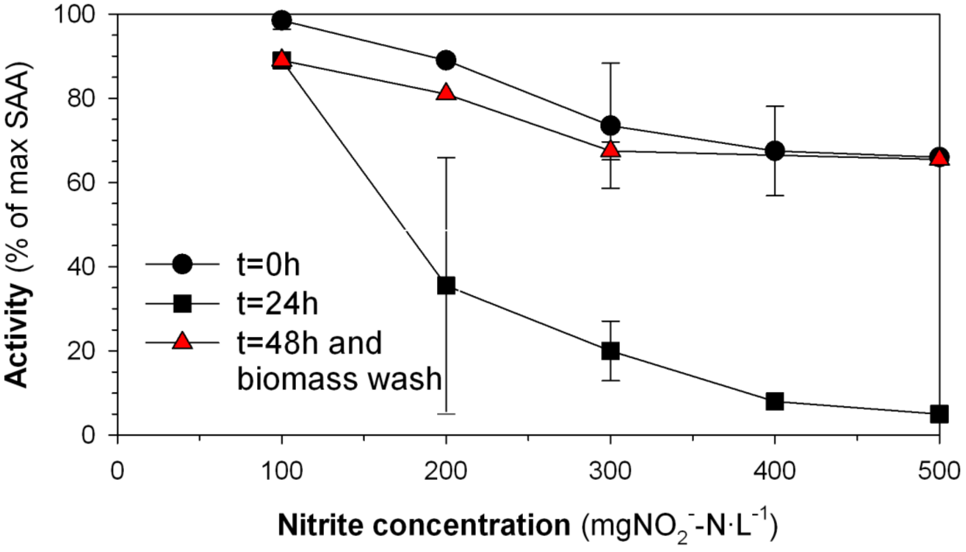


Figure 4.13. SAA measured at different nitrite levels for short and long exposure and after washing.

At 100 mgNO_2^--N·L^{-1}, no significant reduction is observed on SAA after a short exposure time (t=0 h, 10% SAA reduction after 24h). The effect of increasing the nitrite concentration is stronger after long exposure times. In this sense, in both short and long exposure cases the SAA decreases with higher nitrite concentration, but even at 500 mg NO_2^--N·L^{-1}, the SAA after a short time remains above 60% of the maximum SAA measured, while the biomass exposed during 24h at this nitrite level showed practically a complete loss of activity.

The vials measured after 24h exposure period were then maintained with the same level of nitrite one more day. After 48h of exposure to high nitrite concentrations, the biomass was washed with fresh mineral medium and a normal SSA test with control substrate concentrations (50 mgNH_4^+-N·L^{-1} and 50 mgNO_2-N·L^{-1}) was carried out immediately. The results are also shown in Figure 4.13 (t=48h and biomass wash) and demonstrate that the SAA can be recovered by washing the biomass, even though it is exposed during a long period of time to nitrite concentrations up to 500 mgNO_2^--N·L^{-1}. However, the activity was not totally recovered and only reached values close to the short-term exposure results.

The results confirm that nitrite inhibition at the conditions tested during these experiments was partially reversible. The effect of nitrite inhibition depends on the time that biomass is exposed to it.

4.4.2.2 Nitrite inhibition after 24h-exposure

Since several assays were performed at long exposure, the IC_{50} after nitrite load shock can be calculated. The calculated inhibition curve represents the percentage of inhibition {I(%)} caused on SAA by the exposure of nitrite concentrations up to 500 mgNO_2^--N·L^{-1} in this system and was estimated using the extended non-competitive inhibition model reported by Kroiss *et al.* (1992) (Equation 4.1).

$$I(\%) = 100 \cdot \left[1 - \frac{1}{1+(C_{NO2}/a)^b}\right] \qquad \text{(Eq. 4.1)}$$

where C_{NO2} is the nitrite concentration; and *a* and *b* are the fitting parameters and present calculated values of 173 and 3, respectively. Since parameter *a* is the IC_{50}, the estimated nitrite concentration that implies a decrease of the SAA by 50% after

24h-exposure was 173 mgNO_2^--N·L^{-1}. Figure 4.14 shows the loss of SAA (in percentage) at the nitrite concentrations tested experimentally and the fitted inhibition curve.

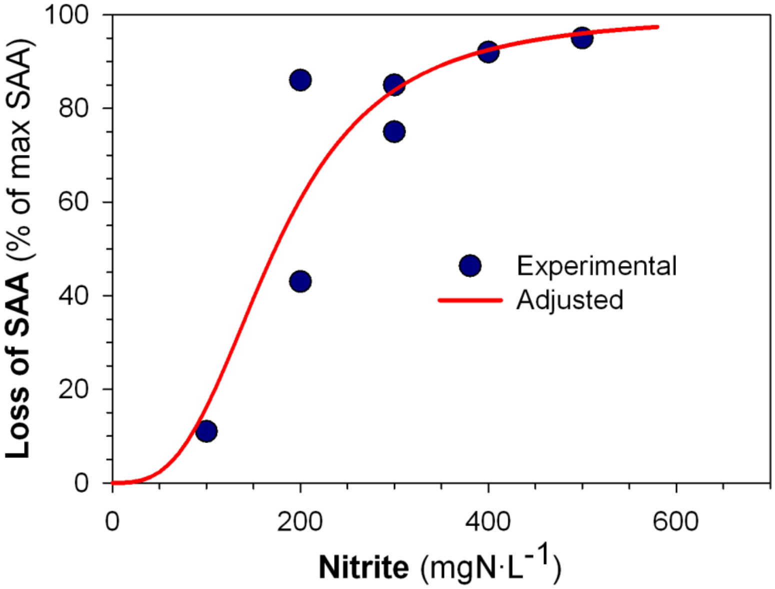


Figure 4.14. Estimation of the inhibition of SAA with respect to the nitrite concentration after 24h-exposure.

The IC_{50} found was higher than the value reported by Strous *et* al. (1998) (100 mgNO_2-N·L^{-1}) and below the value found by Dapena-Mora *et al.* (2007) after short exposure (350 mg NO_2-N·L^{-1}). However, these authors performed the experiments with anammox biomass identified as Kuenenia Stutgartiensis, while our studies were done using the *Brocadia* genera.

4.4.3 Heterotrophic bacteria growing in an anammox enrichment

In this section batch tests with biomass from the SBR and mineral medium are used to analyze the occurrence of heterotrophic denitrification in a system without organic carbon supply. The aim of the study was to correctly describe the dynamics of ammonium, nitrite and nitrate in order to explain both anammox and heterotrophic bacteria growth.

4.4.3.1 Model approach definition

The model is a modification of the activated sludge model No. 1 (ASM1; Henze *et al.*, 2000) that includes the anammox process and considers two-step denitrification (Sin *et al.* 2008). The model approach describes the relationships between five soluble species: ammonium nitrogen (S_{NH4}), nitrite nitrogen (S_{NO2}), nitrate nitrogen (S_{NO3}), dissolved nitrogen (S_{N2}) and readily biodegradable substrate (S_S); and four solid species: active anammox microorganisms (X_{AMX}), active heterotrophic bacteria (X_H), slowly biodegradable substrate (X_S) and inert biomass (X_I). All units are chemical oxygen demand (g COD·m^{-3}) except for S_{NH4}, S_{NO2}, S_{NO3} and S_{N2}, which are expressed as g N·m^{-3}. These relationships are shown in Figure 4.15.

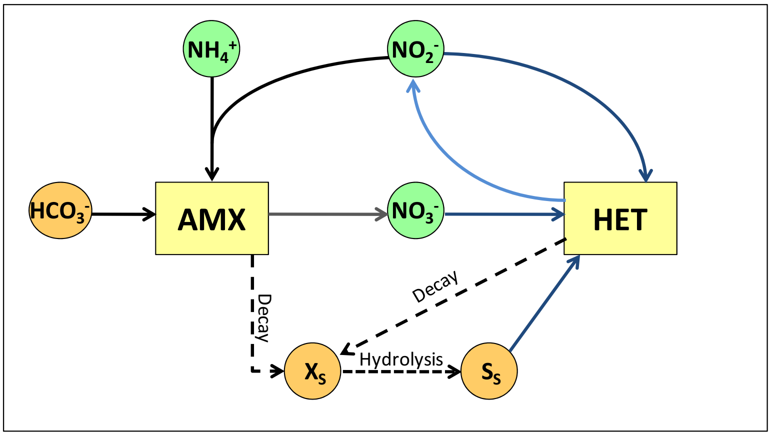


Figure 4.15. Scheme of the fate of the N-compounds in the biomass aggregates of anammox cultures.

The growth rates (Table 4.4) are described using Monod kinetics to express the dependency on substrate concentration. No nitrite inhibition term was included in the anammox growth rate, since the concentration during the batch tests was too low to cause anammox activity inhibition. On the contrary, the kinetic rate describing the growth of X_H on nitrate includes a nitrite inhibition Monod term to express the preference of X_H to growth on nitrate (the non-limiting electron acceptor, which is mainly available) rather than nitrite.

The microbial decay was described using the death-regeneration concept for both anammox and heterotrophic bacteria. This was preferred over the endogenous respiration concept of ASM3 because of the slow decay rate of anammox bacteria and to allow heterotrophic activity without biodegradable organic matter supply

(Dapena-Mora *et al.* 2004b; Ni *et al.* 2009a). Both nitrite and nitrate are possible electron acceptors for growth and energy production in X_H, using the biodegradable substrate produced during the hydrolysis of slowly biodegradable substrate.

Table 4.4. Kinetic rates used to describe the system in the model.

Process	**Kinetic rate expression**
Anammox bacteria	
1 Growth of X_{AMX}	$\mu_{AMX} \cdot \frac{S_{NH4}}{K_{NH4}^{AMX} + S_{NH4}} \cdot \frac{S_{NO2}}{K_{NO2}^{AMX} + S_{NO2}} \cdot X_{AMX}$
2 Decay of X_{AMX}	$b_{AMX} \cdot X_{AMX}$
Heterotrophic bacteria	
3 Anoxic growth on nitrite	$\mu_H \cdot \eta \cdot \frac{S_S}{K_S + S_S} \cdot \frac{S_{NO2}}{K_{NO2}^{H} + S_{NO2}} \cdot X_H$
4 Anoxic growth on nitrate	$\mu_H \cdot \eta \cdot \frac{S_S}{K_S + S_S} \cdot \frac{S_{NO3}}{K_{NO3}^{H} + S_{NO3}} \cdot \frac{K_I^H}{K_I^H + S_{NO2}} \cdot X_H$
5 Decay of X_H	$b_H \cdot X_H$
6 **Hydrolysis**	$K_H \cdot \frac{X_S/X_H}{K_X + X_S/X_H} \cdot X_H$

The values of the kinetic and stoichiometric parameters used in the model are listed in Table 4.5 (end of this section). The stoichiometry for anammox and heterotrophic bacteria and all the parameters used in the model are presented as a Peterson matrix in Table 4.6 (end of this section). For simulations Stella 7.0.3 Research software was used, which is a simple tool that allows for an easy definition of the model and flow scheme. The initial concentration of active anammox and heterotrophic bacteria was calculated using the particulate COD value measured at the beginning of the batch tests and the relative abundance of each population found by FISH analysis and CLSM observations from samples of biomass collected and fixed from the SBR the on the day of the batch.

4.4.3.2 Active heterotrophic bacteria in anammox culture

Different batch experiments were simulated to fit the model simulation results on the measured ammonium, nitrite and nitrate concentrations. Most of the kinetic parameters were obtained directly from the literature, and only Y_H, K_{NO2}^{AMX}, and K_I^H were specifically adjusted for this study. All batch experiments consisted of one or different spikes of nitrogen compounds without biodegradable substrate addition in all cases. Figure 4.16 shows the profiles of the model predictions and the experimental measurements in two batch experiments.

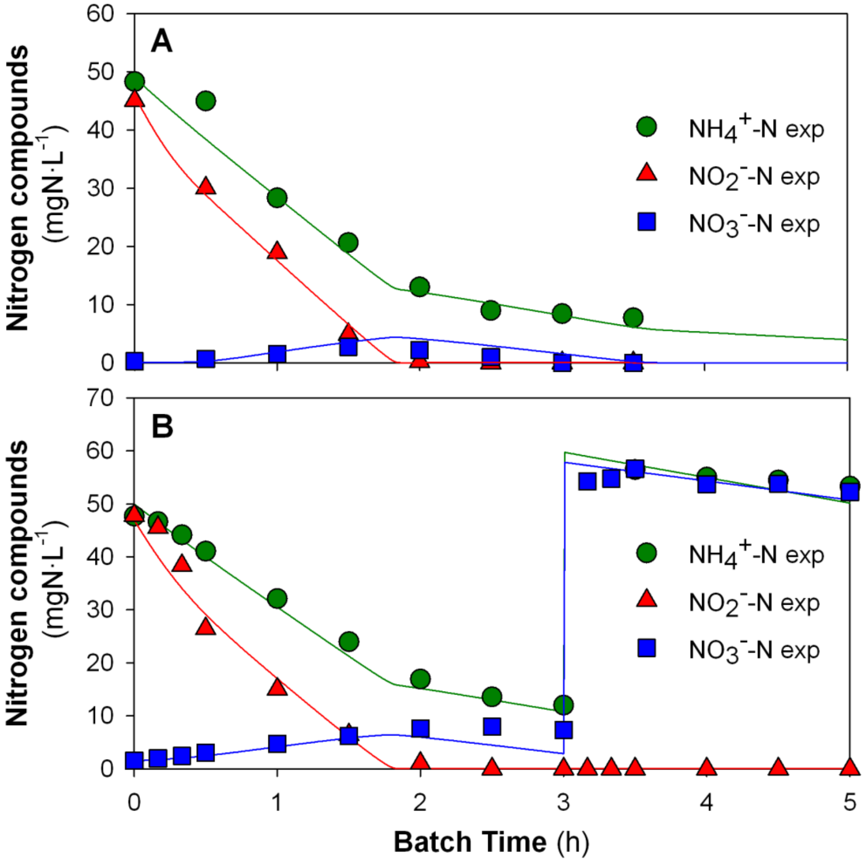


Figure 4.16. Experimental results of ammonium, nitrite and nitrate obtained during two batch tests (scatter) with the corresponding simulated data (lines). A: Batch 1 (pulse of ammonium and nitrite); B: Batch 2 (pulse of ammonium and nitrite and pulse of nitrate and ammonium at 3h).

The model correctly describes the growth of anammox bacteria using ammonium and nitrite, as well as the production of nitrate. All the experiments had a first period with ammonium and nitrite consumption and nitrate production. In all cases, a change in the nitrite profile slope can be observed, as a result of the low nitrate reduction by heterotrophic bacteria at nitrite concentrations above 30mg NO_2^--N·L^{-1}. Initially, nitrite is removed by both anammox and heterotrophic bacteria and the ratio between the removal rates of nitrite and ammonium is higher than 1.32. At lower nitrite concentrations, the velocity of the first denitrification step increases and nitrite is released, which can be used by anammox bacteria thanks to the higher affinity for nitrite than in heterotrophs.

At this point, the ratio between nitrite and ammonium consumption rates is lower than 1.32. Thus, anammox bacteria can also grow when no nitrite is detected in the bulk liquid due to heterotrophic activity, which releases nitrite from the growth on S_S using nitrate as an electron acceptor. For this reason the slope of ammonium profiles changes when no nitrite and no nitrate are detected, respectively. Without detectable nitrite, anammox bacteria could still grow, but that depends on the rate of the first denitrification step. When no more nitrate was detected, the ammonium content remained almost stable since nitrogen removal stopped. This was possible due to the higher affinity for nitrite in anammox bacteria (K_{NO2}^{AMX} set at 0.2 g N·m^{-3}) compared to heterotrophic microorganisms (K_{NO2}^{H} set at 0.5 g N·m^{-3}). This explains the remaining ammonium removal just after nitrite depletion during the batch tests. The results also showed that the nitrite levels reached during the batch tests can partially inhibit the growth of heterotrophic bacteria on nitrite. The results of the model evaluation are presented in Figure 4.17.

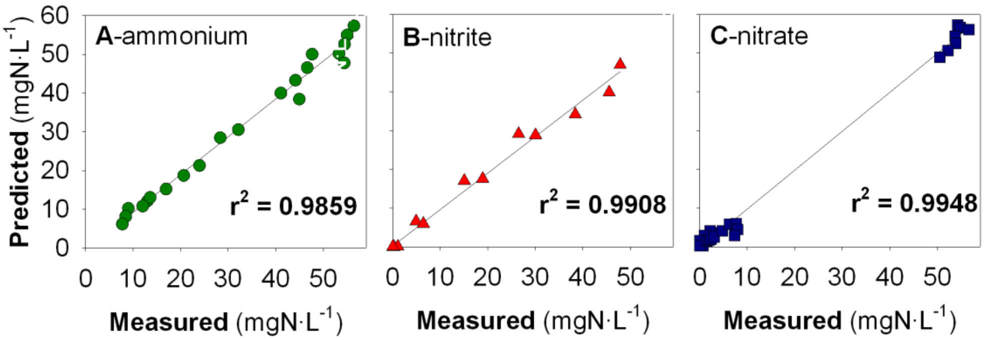


Figure 4.17. Verification of the model comparing the adjustment of simulated data with respect to the measured (experimental) values.

The model verification was performed comparing the experimental data obtained in both replicates of each batch test with the data obtained with simulations. It can be seen that in all cases the model was able to predict the nitrogen compounds evolution (Figure 4.17-A to C), with a good agreement between the predicted and measured results, suggesting the validity of the model used in this work. Despite including only the hydrolysis process as source of S_s in the system (without consideration for SMP production), the viability of active heterotrophic bacteria in this anammox enrichment was also proved with the modeling study.

Table 4.5. Kinetic and stoichiometric parameters of the model.

Parameter	Definition	Value	Unit	Source
Y_{AMX}	Yield for X_{AMX} growth	0.159	$gCOD \cdot g^{-1}N$	Dapena-Mora *et al* (2004)
Y_H	Anoxic yield for X_H growth	0.65	$gCOD \cdot g^{-1}COD$	This study
i_{NBM}	Nitrogen content in biomass	0.07	$gN \cdot g^{-1}COD$	Gujer *et al.* (1999)
f_I	Inert fraction in biomass	0.08	$gCOD \cdot g^{-1}COD$	Henze *et al.* (1987)
η	Anoxic reduction factor	0.6	--	Gujer *et al.* (1999)
μ_{AMX}	Maximum growth rate X_{AMX}	0.003	h^{-1}	Strous *et al.* (1998)
μ_H	Maximum growth rate X_H	0.5	h^{-1}	Henze *et al.* (1987)
b_{AMX}	Decay rate coefficient X_{AMX}	$1.6 \cdot 10^{-4}$	h^{-1}	Ni *et al.* (2009)
b_H	Decay rate coefficient X_H	0.05	h^{-1}	Henze *et al.* (1987)
K_{NO2}^{AMX}	NO_2 affinity constant X_{AMX}	0.2	$gN \cdot m^{-3}$	This study
K_{NH4}^{AMX}	NH_4 affinity constant X_{AMX}	0.3	$gN \cdot m^{-3}$	Dapena-Mora *et al* (2004)
K_{NO2}^{H}	NO_2 affinity constant X_H	0.5	$gN \cdot m^{-3}$	Henze *et al.* (1987)
K_{NO3}^{H}	NO_3 affinity constant X_H	0.5	$gN \cdot m^{-3}$	Henze *et al.* (1987)
K_I^H	NO_2 inhibition constant X_H	5	$gN \cdot m^{-3}$	This study
K_S	Affinity constant for S_S	20	$gCOD \cdot m^{-3}$	Henze *et al.* (1987)
K_H	Maximum hydrolysis rate	0.375	h^{-1}	Gujer *et al.* (1999)
K_X	Hydrolysis affinity constant	1	$gCOD \cdot g^{-1}COD$	Gujer *et al.* (1999)

Table 4.6. Stoichiometric matrix for the model.

Component	S_S	S_{NH4}	S_{NO3}	S_{N2}	S_S	X_S	X_{AMX}	X_H	X_I
Process	COD	N	N	N	N	COD	COD	COD	COD
1		$-\frac{1}{Y_{AMX}} - i_{NBM}$	$\frac{1}{1.14}$	$\frac{2}{Y_{AMX}}$			1		
2		$i_{NBM} - i_{NX} f_I$				$1 - f_I$	-1		f_I
3	$-\frac{1}{Y_H}$	$-i_{NBM}$	$-\frac{1 - Y_H}{1.71 Y_H}$						
4	$-\frac{1}{Y_H}$	$-i_{NBM}$		$\frac{1 - Y_H}{1.15 Y_H}$					
5		$i_{NBM} - i_{NX} f_I$			$1 - f_I$			-1	f_I
6	1								

4.4.4 Monitoring the production of soluble microbial products in an anammox enrichment with EEM fluorescence spectrometry

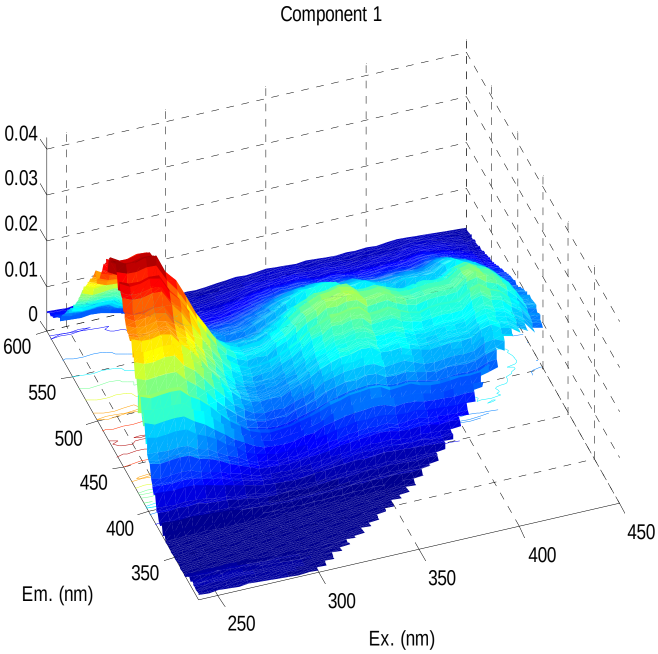
The nitrogen removal ratios obtained in the anammox enrichment and the model simulations of the previous section prove the presence of active heterotrophic bacteria in anammox cultures fed with mineral medium.

In this section, the production of SMP by active anammox biomass in anammox enrichments fed with a mineral medium without an organic carbon source was investigated. The aim of the work was the introduction of a new tool for the analysis of organic compounds in anammox reactors. This study was performed during a research stay at the Technical University of Denmark (DTU, Lyngby, Denmark).

SMP are present and often make up the majority of the effluent organic matter (EfOM) from biological treatment processes but are a mixture of unknown compounds that have been a challenge to measure and characterize (Ni *et al.* 2011a). In this section, the EfOM of an up-flow biofilm anammox reactor was characterized by an excitation emission matrix (EEM) fluorescence spectrometry combined with a parallel factor (PARAFAC) approach to determine the location of the fluorescence peaks.

So far, EEM fluorescence has been used to explore the production of soluble microbial products in conventional activated sludge processes, mainly in membrane bioreactors to investigate the causes and precursors of bulking and fouling phenomena (Chen *et al.* 2003; Ni *et al.* 2011a), but never applied to the anammox processes.

First, the effluent produced during the continuous operations of the anammox reactor was analyzed to identify the characteristics of the fluorescence components possibly making up the EfOM of the reactor. The results obtained after EMM fluorescence measurements and the application of PARAFAC analysis are presented in Figure 4.18, in which the corresponding excitation and emission wavelengths of the fluorescence peaks of the two components identified in the effluent of the anammox reactor can be seen. Thus, the results confirmed the presence of two effluent organic compounds that can be identified with EEM fluorescence spectrometry.



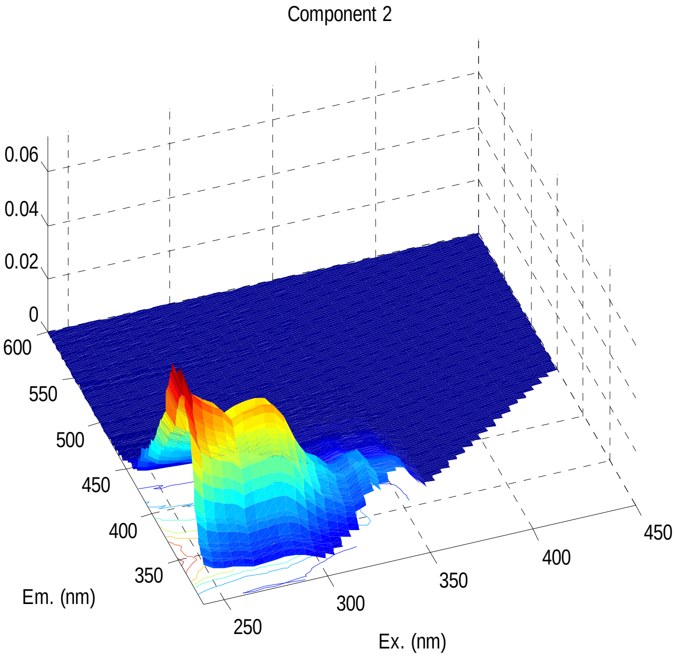


Figure 4.18. The two components of EfOM decomposed using the PARAFAC approach: component 1, located in the humic acid-like region (top); and Component 2, identified as a protein-like substance (bottom).

According to the regions of the EEM fluorescence spectra defined by Chen *et al.* (2003) (Figure 4.19), Component 1 is situated among the regions of humic acid-like and fulvic acid-like substances. The EEM peak of Component 2 is inside the regions comprising protein-like and soluble microbial product-like substances. The peak locations obtained in this work are close to those reported in other studies on EfOM from bioprocesses. Wang *et al.* (2009b) analyzed the dissolved OM in the effluent of an MBR and obtained three fluorescence compounds that were also identified as protein- and humic acid-like substances but displayed different peak wavelengths (Ex/Em peaks at 235-240/340-355 nm and 280-285/320-335nm for proteins and Ex/Em of 276-281/340-370 nm for humic acids). Similarly, protein-like components have been found in various types of wastewater treatment systems (Arunachalam *et al.* 2005; Sheng and Yu 2006; Li *et al.* 2008, among others).

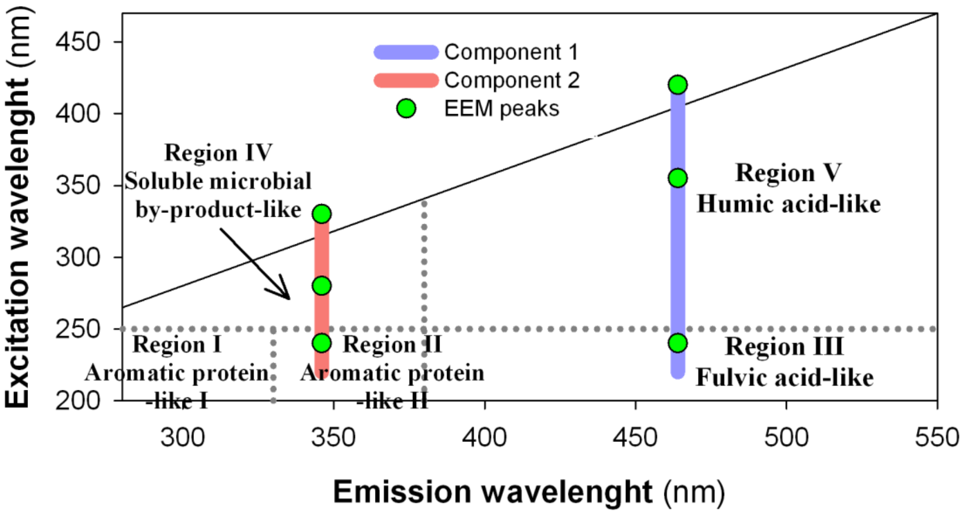


Figure 4.19. Location of the EEM peaks of Components 1 and 2 with respect to the five regions defined by Chen *et al.* (2003).

The reported substances with EEM fluorescence peaks located in the same regions as our components 1 and 2 are related to bacterial activity in activated sludge systems. However, the characteristics of both components 1 and 2 have not been reported so far in wastewater treatment and are therefore specific to the EfOM from the anammox culture. Moreover, in our work both the humic acid-like and the protein-like components exhibited triple excitation peaks, which have not been previously reported.

The production and dynamics of both Components 1 and 2 were investigated in two batch tests. During the experiments, a pulse of substrate (ammonium and nitrite) was added at the beginning, and the presence of the two components was monitored during the substrate up-take phase and a starvation period. Each batch was performed on different days when the reactor was treating 0.58kg $N\cdot m^{-3}\cdot d^{-1}$ (Batch 1) and 1.20kg $N\cdot m^{-3}\cdot d^{-1}$ (Batch 2). The initial concentration of ammonium and nitrite was set around 100mg $N\cdot L^{-1}$ in Batch 1 and increased up to 200mg$\cdot L^{-1}$ in Batch 2, since the removal capacity was higher. The exposure to such high nitrite concentrations was short and nitrite inhibition was not considered. The results obtained during the batch experiments are presented in Figure 4.20.

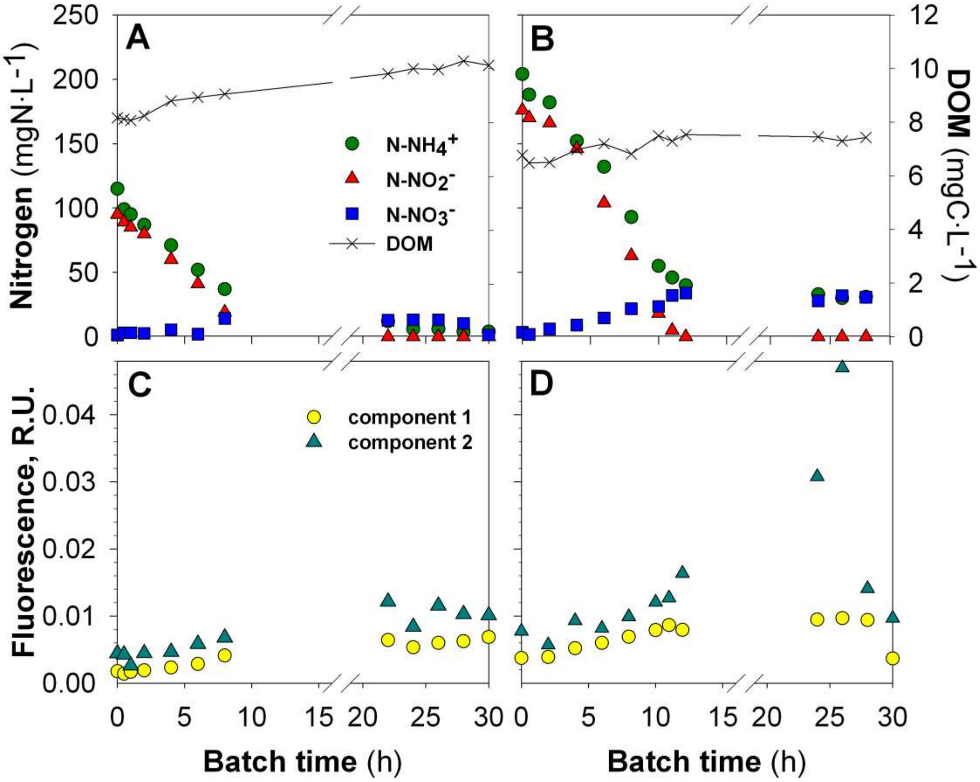


Figure 4.20. Batch test results for Batch 1 (Left) and Batch 2 (Right). Top: the profiles of ammonium, nitrite, nitrate and DOM (measured as non-purgeable volatile organic carbon). Bottom: fluorescence intensity for Components 1 and 2. A and C: initial spike at 100ppm of ammonium and nitrite; B and D: initial spike at 200ppm of ammonium and nitrite.

Figure 4.20 (top, A and B) presents the results of ammonium, nitrite and nitrate evolutions during the batch tests. In both cases, two periods can be observed. Initially, nitrite and ammonium were removed and nitrate, as well as organic carbon, was released in the solution. The nitrite to ammonium removal ratio considering only the first phase (while nitrite was available) was 1.02 NO_2^--N: 1.0 NH_4^+-N and 1.16 NO_2^--N: 1.0 NH_4^+-N for Batch 1 and Batch 2, respectively. The corresponding nitrate productions were 0.11 mole NO_3^--$N_{produced}$·$mole^{-1}$ NH_4^+-$N_{removed}$ and 0.22 mole NO_3^--$N_{produced}$·$mole^{-1}$ NH_4^+-$N_{removed}$. These values are indicative of some heterotrophic denitrifying activity during batch tests. During the second period, without nitrite, ammonium removal and nitrate production ceased since nitrite was no longer available (endogenous phase). A low degree of organic carbon production was observed during the batch, mainly during the substrate uptake period, with an increment of 2.0 mg C·L^{-1} and 1.05 mg C·L^{-1} for batch 1 and batch 2, respectively, which proves the availability of organic carbon source.

The fluorescence components found during the batch tests (Figure 4.20-D and C) were identical to those observed during continuous operation. The fluorescence intensity measured for each component is shown in Figure 4.20-C and 4.20-D. Components 1 and 2 were produced during active anammox growth, since the fluorescence intensity increased over time while nitrite was available. The fluorescence signal was higher for the two components in Batch 2 (Figure 4.20-D), when higher initial substrate concentrations were applied.

Component 2 was clearly correlated with anammox metabolism. The ratio between the production of this protein-like substance and the ammonium consumption during the nitrite uptake phase were $3.01 \cdot 10^{-5}$ R.U.·mg^{-1} NH_4^+-N and $3.12 \cdot 10^{-5}$ R.U.·mg^{-1} NH_4^+-N, and the rates of protein-like production to nitrate production were $1.82 \cdot 10^{-4}$ R.U.·mg^{-1} NO_3^--N and $1.63 \cdot 10^{-4}$ R.U.·mg^{-1} NO_3^--N for Batch 1 and Batch 2, respectively. This indicates that the production of this fluorescent component was proportional to the substrate up-take rate. In this sense our results are in agreement with those from Dong *et al.* (2010), who monitored a period of recovery of methanogenic activity and reported that the higher the activity in bioreactors, the higher the SMP content. The stability of this organic product during the starvation phase reveals its low biodegradability. The low biodegradability of SMP was reported by Ni *et al.* (2010b), who identified these substances as the main fraction of the EfOM in activated sludge processes.

Component 1 was also produced during the substrate uptake phase in both Batch 1 and 2. The concentration of this substance remained stable during the starvation phase in Batch 1, but presented an important variation during the same period in Batch 2. In this case, the concentration still increased at the beginning of the starvation phase and then decreased suddenly. This behavior could be an indication that Component 2 is produced by heterotrophic bacteria and could also be a substrate for the hydrolysis processes, which have low affinity for substrate and need a high concentration. However, more research is needed to identify the source and fate of component 2. Similar substances were reported and attributed to endogenous processes in previous works (Ni *et al.* 2009b).

The results of this work show that the EfOM composition in the anammox culture results from the microbial activity and is a first step toward opening the door for future applications of EEM fluorescence with respect to monitor the performance of anammox reactors. However, more research is needed to explore the presence of EEM components in real wastewater applications.

4.5 General discussion

4.5.1 Effect of nitrite concentration on anammox granules with Brocadia anammoxidans in mineral medium

In this chapter, the effect of a high nitrite concentration on anammox biomass is explored. The results obtained show that the activity loss measured in anammox sludge, due to a high nitrite concentration, depends on two factors: 1) the nitrite concentration and 2) the exposure time. In this sense, activity loss was relatively low even with nitrite levels up to 500 $mgNO_2^-$-N·L^{-1} (less than 40 %) if the biomass was exposed to such nitrite levels for a short time (t=0h). The effect of nitrite inhibition on anammox activity is rapidly augmented with the time and the specific anammox activity is significantly decreased after 24h of exposure. However, a reduction of the activity of about 30-40% in an anammox reactor treating an influent with high nitrite content can lead to a rapid accumulation of nitrite. This induces a retroactive effect of nitrite accumulation and activity loss that can result in a severe perturbation of the process. For this reason, in spite of the high IC_{50} values the nitrite concentration in the reactor must be maintained close to zero.

Taking these considerations into account, in an anammox SBR treating high nitrogen strength wastewater nitrite accumulations above 70-100 $mgNO_2^-$-N·L^{-1} should be avoided and rapidly corrected. In the experiments, it has been proven that anammox activity can be partially recovered if the nitrite concentration is reduced to normal values. In practical application, the reactor can be maintained just under the reaction phase to allow the consumption of the accumulated substrate without feeding. This way, the retroactive inhibition due to substrate accumulation is cut. In cases of extreme accumulations, this measure is not feasible, since the activity loss is higher and substrate consumption rate is not enough to prevent the negative effects of long-term exposure. At this time, a washing is necessary to remove the nitrite quickly. With regard to a future scale-up and the applications to real wastewater, the concentration of nitrite inside the reactor should be maintained as low as possible to avoid rapid accumulations of nitrite when treating landfill leachate, due to the high nitrite content in these streams after partial nitritation. Moreover, additional factors affecting the anammox activity should be studied.

The effluent quality is influenced by the influent nitrite to ammonium molar ratio. In this way, the reactor was very sensitive to influent ratios below 1.32, which resulted in a rapid accumulation of ammonium. However, on most days the influent ratio in this system was above 1.32, but the system showed some capacity to assimilate this nitrite excess. This behavior was a result of the presence of some heterotrophic bacteria in the system that contributed to nitrite removal, as confirmed by the nitrogen removal ratios. However, this capacity was limited (since the available biodegradable substrate was limiting), and high deviations above 1.32 or sudden increments of the influent ratio resulted in nitrite accumulation in the reactor.

4.5.2 Long-term operation of anammox SBR

Operating anammox reactors using an SBR configuration had some repercussions on the process performance. Including a reaction phase in the cycle configuration provides more capacity to assimilate slight variations of the loading rate without affecting the effluent quality. During the reaction phase the excess substrate can be totally consumed. In another way, the reaction phase combined with pH control by punctual acid addition used in this process allows pH profiles to be used as a good tool to monitor the process performance. The risk of substrate accumulation due to an extremely high loading rate can be predicted with the pH profiles, since a stable increment will be observed during the entire reaction phase.

The SBR allows better adjustment of the inflow rate according to the nitrogen removal capacity of the system and the influent nitrogen content than continuous flow reactors, because the mixing conditions are not dependent on the inflow.

During the long-term operation of the SBR, an important biomass attachment on the walls of the reactor was detected. This is common in all anammox reactor configurations, but in this case the biomass was mainly attached in the zone between the minimum and maximum water volumes. This enhances the degradation of the biomass and implies that an important fraction of the total biomass content in the reactor is only removing nitrogen during only part of the 8-hour cycle. These results showed that this biomass was less active than the suspended solids and imply the need to limit this dynamic in future reactors

Active heterotrophic denitrifying bacteria were detected in the anammox reactor during all the study in spite of the absence of a biodegradable carbon source in the mineral medium. The modeling work with batch test data showed that this heterotrophic activity could be sustained by the decay and hydrolysis processes. Thus, in an anammox enrichment heterotrophic bacteria can use the decay products to grow on nitrite or nitrate. Soluble microbial products are produced by the bacterial activity in the anammox reactors and results in some dissolved organic matter presence in the effluent. The analysis by EEM fluorescence revealed that low biodegradable organic compounds are released to the bulk liquid when ammonium is removed by anammox bacteria. These SMP presented excitation-emission peaks close to those reported for humic and fulvic-acids or proteins and did not constitute a sufficient biodegradable carbon source to sustain heterotrophic growth in such systems.

4.6 Conclusions

From the studies carried out in this chapter, we can conclude that:

- It has been possible to operate a lab-scale anammox SBR treating synthetic wastewater for more than three years. The results showed that high NLR between 0.5 and 1 kg $N \cdot m^{-3} \cdot d^{-1}$ can be sustained at long term. High N-removal efficiencies have been observed during the entire period with the measured SAA around 0.6 kg N·kg^{-1} VSS·d^{-1}.

- Anammox bacteria were always the dominant population in the system and the nitrite and nitrate to ammonium molar ratios were always very close to the stoichiometric coefficient values of the anaerobic ammonium oxidation. The characteristics of the biomass have been influenced by the stirring conditions and the strong attachment tendency of the anammox microorganism. Biomass was mainly attached to the internal surfaces between V_{max} and V_{min}.
- The presence of active heterotrophic bacteria was evident in the nitrogen removal ratios and FISH observations. The sustainment of heterotrophic activity by decay and hydrolysis products was proven using a modeling approach, which revealed that anammox can use nitrite released from heterotrophic growth on nitrate.
- Online profiles were used to assess the reactor's performance. The information about the performance of the process was mainly reported by the pH profiles. A stable pH value during the reaction phase was indicative of optimal operation, while pH uptrends until the end of cycle revealed a destabilization of the imbalance between NLR and NRR. ORP values give information about the possible risk of a complete consumption of NO_x^- and the possible transition to sulfate reduction processes under anaerobic conditions.
- Ammonium accumulations were mainly caused by a suboptimal influent nitrite to ammonium molar ratio, leading to ammonium excess. If only ammonium was accumulated above 100 mg NH_4^+-N·L^{-1} the process performance was not affected. Nitrite was also accumulated and two strategies were used depending on the nitrite concentration reached in the reactor. For concentrations below 50 mg NO_2^--N· L^{-1}, the reaction phase length was prolonged until the substrate was completely consumed (the pH uptrend stops). When extreme concentrations up to 350 mg NO_2^--N L^{-1} were reached in the reactor, the influent was changed and medium without nitrogen was fed.
- The effect of nitrite exposure was investigated in more depth by SAA assays. It was found that the effect on anammox biomass depends on the nitrite concentration and the duration of exposure. The IC_{50} after 24h in this reactor was set by calculations at 173 mg NO_2^--N L^{-1}. Nitrite inhibition was not totally irreversible and the activity was partially recovered (60% of

the initial SAA) after washing the biomass, even in the case of long exposure to 500 mg NO_2^--N L^{-1}.

- Anammox cultures in a mineral medium produce effluent organic matter without the presence of organic carbon in the influent. The production of soluble microbial products in anammox reactors was measured by EEM fluorescence, which revealed that anammox activity resulted in the presence of two organic compounds related to humid acid-like substances and soluble microbial products. More research is needed to investigate in more depth the characteristics of the effluent organic matter produced in anammox reactors, but the identification of soluble microbial products related to anammox metabolism could set the basis for the metabolic footprint as a tool for the assessment of the operation of anammox reactors.

CHAPTER 5. URBAN LANDFILL LEACHATE TREATMENT IN A LAB-SCALE ANAMMOX SBR

This chapter presents the results of the work carried out under the framework of the PANAMMOX project (Partial nitritation and anaerobic oxidation of ammonium in landfill leachates by PANI-SBR and ANAMMOX processes) that aimed for the development of an alternative technology for the treatment of high nitrogen loaded urban landfill leachates. This project resulted from the collaboration of the CESPA-GR and the LEQUIA group and was founded by the Spanish Government (CIT-310200-2007-90).

5.1 Motivation

With the knowledge and the experience acquired with the lab-scale granular anammox SBR, the next goal was the application of the anammox process in a treatment for autotrophic N removal from urban landfill leachates using the SBR technology. The literature includes many studies demonstrating the feasibility of applying the anammox process to treat high nitrogen content wastewater, exploring different start-up strategies (López *et al.* 2008; Wang *et al.* 2009a; Ni *et al.* 2010c; Ni *et al.* 2011e; Wang *et al.* 2011b), reactor configurations (Dapena-Mora *et al.* 2004a; Jin *et al.* 2008) and operational conditions (Strous *et al.* 1997a; Strous *et al.* 1999; Arrojo *et al.* 2008; van Hulle *et al.* 2011), as well as the effect of ammonium and nitrite concentration (Strous *et al.* 1998; Bettazzi *et al.* 2010; Fernández *et al.* 2010; Kimura *et al.* 2010; Tang *et al.* 2010b), COD (Güven *et al.* 2005; Chamchoi *et al.* 2008; Isaka *et al.* 2008; Lackner *et al.* 2008) and salts (Kartal *et al.* 2006; Dapena-Mora *et al.* 2007; Liu *et al.* 2008; Liu *et al.* 2009; Dapena-Mora *et al.* 2010). However, most of works were carried out in a mineral medium and real applications are still limited.

The start-up and operation of anammox reactors has been tested with a variety of high N-content streams: mainly sludge digestion returns (van Dongen et al., 2001; Wett, 2007, among others), but also pig manure (Ahn et al., 2004; Hwang et al., 2005; Molinuevo et al., 2009) and other industrial wastewater. The specific case of landfill leachate treatment by anammox has only been tested by a few authors (Siegrist *et al.* 1998b; Liang *et al.* 2009; Wang *et al.* 2010, among others). However these studies were carried out in biological reactors where anammox bacteria appeared spontaneously or reactors seeded with anammox bacteria acclimatized to landfill leachate. Thus, there is a lack of knowledge about the start-up of new anammox reactors without acclimatized biomass and the adaptation of this biomass to the leachate.

The composition of leachates is characterized by ammonium-rich content and high salinity, while the biodegradable organic matter (COD/N ratio) decreases over time due to the waste degradation and ammonification processes (EPA, 2000), but present short-term variability caused by seasonality, hydrology of the landfill site, and waste deposition (Kurniawan *et al.* 2006b). The effect of these variations on the performance of anammox reactors has not been studied before and will be also explored in this work. In addition, landfill leachates present some easily biodegradable organic matter, which can be removed in the partial nitritation process by aerobic conditions or even under anoxic conditions for denitrification purposes as proposed by Ganigué *et al.*·(2009), but the main fraction of the organic matter is generally slowly biodegradable or refractory. Liang *et al.* (2009) reported the degradation of humic substances by anammox treating landfill leachates and the mesophilic conditions commonly applied in anammox reactors (30-35ºC) favor hydrolysis of particulate and low biodegradable substances. Thus, the presence of heterotrophic bacteria coexisting with anammox bacteria in this kind of treatment is inevitable.

Typical growth rates of heterotrophic bacteria reported in the literature range between 1.5 and 2 d^{-1} and the heterotrophic anoxic yield over NO_x^- is generally set at 0.63-0.67 g $COD\cdot g^{-1}$ COD, both parameters considerably higher than in anammox bacteria (around 0.0066 d^{-1} and 0.159 g $COD\bullet g^{-1}$ N;Dapena-Mora *et al.* 2004b). The anammox population would practically disappear if enough biodegradable organic matter was supplied to the system (Chamchoi *et al.* 2008; Lackner *et al.* 2008). Negative effects have been reported when enriched anammox biomass was exposed to relatively high COD/N ratios, such as low N conversion (Hwang *et al.* 2005) or a progressive decrease of the ammonium conversion by increasing the COD/N ratio (Chamchoi *et al.* 2008). Lackner *et al.* (2008) estimated that heterotrophic bacteria could become dominant and anammox bacteria disappear at COD/N>2. The denitrification potential is mainly governed by the available biodegradable organic carbon source. Based on calculations, a minimum C/N ratio of about 3.5-4.5 g COD · g^{-1} N should be enough for complete denitrification (Henze *et al.* 1994).

This feedback implies the need to control the presence of heterotrophic bacteria in anammox processes where the availability of an organic carbon source is difficult to quantify due to the slowly biodegradable organic matter content in the influent. This is especially true in the case of landfill leachate treatment, since the seasonal variations and the internal dynamics of the landfill facilities can lead to sudden variations of the total organic matter content and the readily biodegradable fraction. In this study, the coexistence of anammox and heterotrophic bacteria is analyzed, and the tools for monitoring the denitrifying activity in the system are developed in order to detect possible variations in the respective abundance of both autotrophic and heterotrophic bacterial populations.

However, heterotrophs can also contribute to nitrogen removal and reduce the effluent load. This is of special interest in anammox reactors, where about 11% of the incoming nitrogen is converted to nitrate. Thus, the use of heterotrophic denitrification to remove the excess nitrate production of anammox reaction is also of interest in the treatment of landfill leachates.

5.2 Objectives

This chapter aims to describe start-up of the treatment of N-rich landfill leachates, previously conditioned by a partial nitritation process, in a lab-scale anammox SBR and evaluate the long-term operation and stability of the process.

Specifically, the study focuses on the analysis of the capacity of the anammox biomass coming from an anammox enrichment feed with mineral medium to remove nitrogen under the conditions of the landfill leachate matrix.

In addition, the long-term operation of an anammox SBR made it possible to treat leachates from two different landfill sites, both of them affected by seasonal variations in composition. The effects of these changes on the process performance are also studied.

In spite of the previous aerobic partial nitritation process, the influent leachate to the anammox reactor contains some biodegradable organic matter. The goal was to put the heterotrophic denitrification to good use to improve the effluent quality in terms of total nitrogen removal.

5.3 Materials and Methods

5.3.1 Experimental set-up

The experimental work was carried out in a lab-scale anammox SBR located in the Science and Technology Park of the University of Girona, consisting of a 2L cylindrical glass BIOSTAT B-PLUS (from Sartorius) (Figure 5.1). The reactor was water jacketed to control the temperature and was equipped with a mechanical stirrer for complete mixture. Air diffusion was avoided by maintaining the reactor with an atmospheric overpressure. The reactor was provided with pH, DO, ORP and temperature probes and monitored by a control unit for temperature, stirring velocity and pH control.

The influent leachate was stored in a 20-liter collapsible plastic bag to avoid air diffusion and was fed to the reactor by a peristaltic pump inserted in the control unit, as well as the effluent, base-addition and acid-addition pumps. The system was supervised by software that enabled online data acquisition and cycle control.

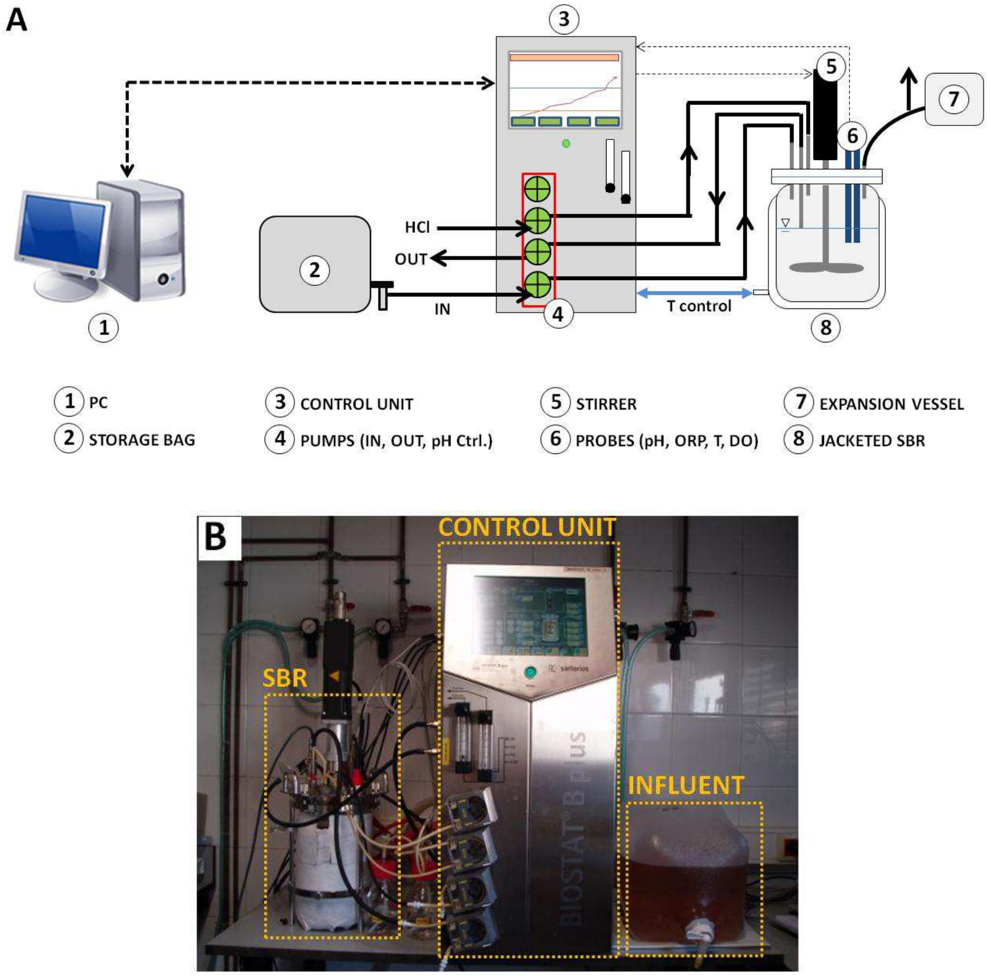


Figure 5.1. Scheme of the experimental set-up used in this study (A) and a picture of the 2L lab-scale anammox SBR (B).

5.3.2 Inocula

The system was seeded with granular anammox sludge coming from a 20-liter granular anammox SBR treating synthetic wastewater (Chapter 4). One liter of mixed liquor was taken from the SBR during the reaction phase, and the initial MLVSS content in the lab-scale SBR in the reactor was fixed at 547.3mg VSS·L^{-1}.

5.3.3 Operational conditions

The lab-scale anammox reactor was operated with the same 8-hour cycle configuration used in the anammox SBR described previously (Figure 5.2). Data for online monitoring of pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), and temperature were obtained from probes. The temperature was maintained at 36 ± 0.3 ºC, and the pH set-point was controlled at 7.5 ± 0.05 using a PID controller and adjusting the dosage of 1M HCl. The V_{min} was set at 1.5 L, and 0.350 L of leachate were fed in each cycle, giving a volumetric exchange ratio (VER) of 23.3%.

5.3.4 Urban landfill leachate

The leachate treated in this study came from a previous partial nitritation SBR (PN-SBR) process so as to provide the required ammonium and nitrite source. The effluent from the PN-SBR process was stored and then diluted and treated in the lab-scale anammox SBR. The leachate treated during the study came from two urban landfill sites: Corsa (Reus, Catalonia) and Orís (Orís, Catalonia).

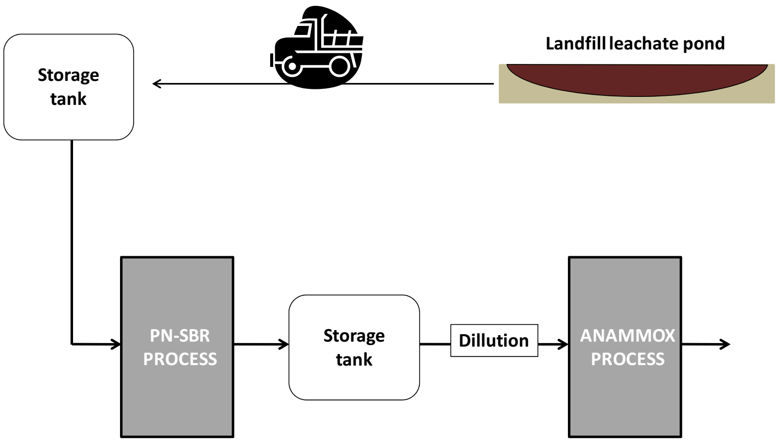


Figure 5.2 Scheme of the process to treat the urban landfill leachate in this study**.**

The experimental procedure is summarized in Figure 5.2. The leachate was collected in the leachate pond of the landfill site and transported to the lab in plastic containers of 1m^3 capacity. About 4-5 containers were received each time and stored in the lab facilities. This leachate was first conditioned in the PN-SBR process performed in a 250-liter SBR (Ganigué *et al.* 2010). The effluent of the PN-SBR process was stored again in plastic containers. The PN-SBR effluent was then diluted according to the needs of the anammox reactor and the influent nitrite to ammonium ratio was adjusted, if necessary. The influent of the anammox process was gasified with N_2 gas to remove the DO and was supplied from plastic bags to avoid air diffusion.

5.4 Results

In Chapter 4, the feasibility of autotrophic nitrogen removal from high nitrogen loaded wastewater in an SBR making use of the anammox process was tested and proven during long-term operation. Moreover, the main factors affecting the operation of the reactor and the biological and kinetic characteristics of the anammox biomass were explored to gain a better understanding of the process. However, the aim of this chapter was the treatment of urban landfill leachates by the anammox process. The characteristics of landfill leachates are very complex and varied compared to the mineral medium. The first requirement to achieve our goal was the operation of a stable partial-nitritation SBR (PN-SBR) to set the correct nitrite to ammonium molar ratio to the leachate before its treatment in the anammox reactor.

5.4.1 Characteristics of the partial-nitritation effluent leachates used in this study

The main characteristics of the three raw leachates used in this study are summarized in Table 5.1, where the content of nitrogen compounds and organic matter in the effluent of the partial nitritation process are presented. The parameter showing the main differences among the leachates used was the electrical conductivity (EC). The two leachates from the Corsa landfill site presented extreme conditions of EC, up to 64 $mS \cdot cm^{-1}$. In comparison, the conductivity of the leachate from Orís was lower. This difference is favored by the rainfall regimes of the two landfill sites, the one in Orís being wtter. Similarly, Corsa-1 had the highest total nitrogen concentration (up to 3268.8 mg $N \cdot L^{-1}$), followed by Corsa-2 (around 2500 mg N·L^{-1}). On the contrary, the COD content was similar in the three leachates, which presented BOD$_5$ values ranging from 12 to 56 mg O$_2$·L^{-1} and BOD$_{30}$>400 mg O$_2$·L^{-1}.

Table 5.1. Mean characteristics of the raw leachates used in this study after PN process.

		Leachate		
Parameter	**Units**	**CORSA-1**	**CORSA-2**	**ORÍS**
Time	days	0-113	114-476	477-END
NH_4^+-N	mgN·L^{-1}	2243.3±18.17	1150.9±34.3	849.9±52.0
NO_2^--N	mgN·L^{-1}	1006.6±44.8	1503.5±90.4	1350.1±56.5
NO_3^--N	mgN·L^{-1}	6.2±2.1	5.3±4.2	18.3±9.7
Sol.COD	mgO$_2$·L^{-1}	3279.4±278.1	2976.0±665.1	2898.9±799.1
Tot.COD	mgO$_2$·L^{-1}	3414.9±326.3	3082.5±683.4	3310.7±829.9
EC*	mS·cm^{-1}	62.5	64.8	26.1

*EC values measured the first day of application of the new leachate

The presence of heavy metals was also analyzed in the CORSA-2 and the ORÍS leachates, but no evidence of a significant presence was observed. The most abundant were metals such as Na, K, Fe, Ca, and Si in concentrations between 10^{-2} mg·L^{-1} and 10 mg·L^{-1}, after they were diluted to feed the anammox reactor.

5.4.2 The effect of the landfill leachate matrix on anammox granules adapted to mineral medium

The possibility of obtaining sludge enriched with anammox bacteria adapted to landfill leachate as inocula for new anammox reactors is very limited and therefore, the lab-scale anammox SBR could be seeded with the anammox sludge purged from the SBR described in Chapter 4. However, the effect of the landfill leachate matrix on the anammox biomass adapted to mineral medium was unknown, and previous studies were necessary to determine if biomass from the previous anammox SBR could be directly exposed to the landfill matrix.

Specific anammox activity (SAA) tests using granular biomass from the anammox SBR were carried out in series of controls with mineral medium and bottles with leachate (effluent from other biological reactors and without nitrogen). The aim was to explore any immediate effect on anammox biomass acclimated to the mineral medium when exposed to the leachate. The ammonium and nitrite concentrations at the beginning of the tests were both always set at 50 mgN·L^{-1} in all bottles and the environmental conditions (pH, T, IC) were the same in both control and leachate bottles. The salinity of the mineral medium in control bottles was always corrected with NaCl to set the same salinity as in the bottles with leachate. This way, the effect of salinity on the final SAA measurement was discarded. Different lots of leachate from the Corsa and Orís landfill sites were tested.

The results of the experiments are compiled in Table 5.2, where the loss of activity is calculated according to the nitrogen conversion rates obtained in the controls and leachate tests.

Table 5.2. Impact of different landfill leachates on specific anammox activity.

Landfill	**Matrix dilution**$^{(1)}$	**Initial NO_2^- and NH_4^+**	**Exposure**	**SAA Control**	**SAA Leachate**	**SAA loss**
		mgN·L^{-1}	h	gN-N_2·(gVSS·d)$^{-1}$	gN-N_2·(gVSS·d)$^{-1}$	%
Corsa	1:10	50	0	0.62 ± 0.14	0.54 ± 0.10	12.9
Corsa	1:4	50	48$^{(2)}$	0.77 ± 0.86	0.11 ± 0.01	85.7
Orís	1:5	50	0	0.66 ± 0.09	0.46 ± 0.23	30.3

(1) with tap water (v/v)
(2) Biomass incubated 48h with the leachate matrix before starting the test

The results presented in Table 5.2 show that the landfill leachate matrix has a clear impact on the SAA. The results prove that non-acclimated biomass loosed the SAA when it was exposed to the leachate matrix. The characteristics of the leachate as well as the dilution factor applied were significant factors in determining the SAA. The more the matrix was concentrated, the higher the biomass activity loss. Moreover, the impact was higher when the biomass was exposed longer to the leachate. Thus, granular biomass from a synthetic anammox SBR needs to be adapted to the new conditions in the leachate matrix.

5.4.3 Landfill leachate treatment in lab-scale anammox SBR: Start-up and operation

The main objective of this section is the application of the anammox process to the treatment of landfill leachate. The lab-scale anammox SBR was seeded with granular anammox sludge coming from the anammox enrichment described in the previous chapter.

5.4.3.1 Nitrogen removal

According to previous SAA assays, the effluent leachate of the partial nitritation process was diluted with tap water before it was fed into the anammox reactor. The hydraulic residence time (HRT) was fixed during the entire study at 1.76 days, and the nitrogen loading rate was adjusted by modification of the leachate dilution. The NLR was increased according to the treatment capacity of the system, and therefore, a higher NLR concluded with a higher matrix concentration in the reactor. This resulted in a progressive decrease of the dilution applied to the leachate coming from partial nitritation. The different leachates provided from the two landfill sites were changed without any transition period. The changes of leachate are marked with the vertical dashed lines in the figures of this section. Figure 5.3 presents the performance of the reactor in terms of N-removal. At the top of this figure, as well as in the following figures, the period with the three leachates are indicated and separated by vertical lines.

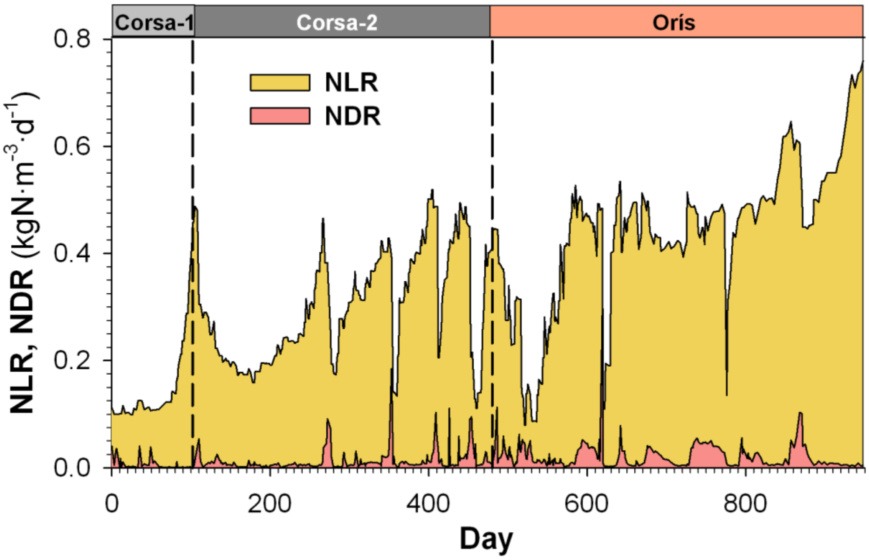


Figure 5.3. Evolution of the N-removal capacity of the lab-scale anammox SBR during the entire experimental period.

The NLR was initially set at 0.12kg $N \cdot m^{-3} \cdot d^{-1}$ and was adjusted during the study using the nitrogen removal rate (NRR) as a control parameter (with special consideration given to the effluent nitrite concentration due to its inhibition potential). The NLR during the entire study ranged between 0.082kg $N \cdot m^{-3} \cdot d^{-1}$ and 0.77kg $N \cdot m^{-3} \cdot d^{-1}$, with an average of 0.36 ± 0.14kg $N \cdot m^{-3} \cdot d^{-1}$. During the entire operational period, the NLR presented a general upward trend and was progressively increased until the maximum value of 0.78kg $N \cdot m \cdot^{-3} \cdot d^{-1}$ was achieved at the end of the study. The start-up of the reactor took 80 days. During this period, the NLR was maintaned at around 0.15kg $N \cdot m^{-3} \cdot d^{-1}$ in order to allow the biomass to adapt to the new environmental conditions. With the Corsa-1 leachate, a maximum value of 0.48kg $N \cdot m^{-3} \cdot d^{-1}$was achieved.

On the 100th day, the reactor was fed for one day with leachate from Corsa-2, but this leachate was definitively applied on Day 114. With the change of the leachate matrix, an adaptation period was required, and the NLR was reduced to 0.17kg $N \cdot m^{-3} \cdot d^{-1}$ (Day 178). Afterwards, the treatment capacity was recovered and the NLR was progressively increased, reaching a maximum value with Corsa-2 of 0.53kg $N \cdot m^{-3} \cdot d^{-1}$ on Day 404.

Similar behavior was obtained while treating the leachate from Orís. This leachate was applied on Day 477 and again a period of adaptation of the biomass to the new leachate matrix was required. The NLR decreased down to 0.08kg $N \cdot m^{-3} \cdot d^{-1}$ on day 520, but rapidly recovered and increased up to 0.78kg $N \cdot m^{-3} \cdot d^{-1}$. The stability of the process was high while treating the leachate from Orís, and the perturbances that occurred with Corsa-2 at NLR above 0.42kg $N \cdot m^{-3} \cdot d^{-1}$ were not present during this third period. All changes of leachate were applied without a transition period and resulted in a loss of activity, which made a huge decrease of the NLR necessary to reduce the effect of the leachate matrix by dilution.

Globally, a good process performance was obtained with the three tested leachates, and the treatment capacity was increased in all cases. The nitrogen removal was always close to the nitrogen loading rate with only some isolated falls caused by perturbances to the system. Consequently, the nitrogen discharge rate was very low with an average ammonium plus nitrite discharge rate of only 0.02 ± 0.02kg $N \cdot m^{-3} \cdot d^{-1}$. The total nitrogen discharge rate, including the effluent nitrate content, was also low (0.05 ± 0.03kg $N \cdot m^{-3} \cdot d^{-1}$), demonstrating the good performance of the process when treating urban landfill leachate.

Nevertheless, the system was affected by several accumulations of ammonium and nitrite that forced a reduction of the NLR in order to avoid inhibition of the anammox activity as a consequece of high nitrite concentrations. According to previous results, the way to proceed when ammonium and nitrite were rapidly accumulating was to switch the SBR to the reaction phase. This avoided higher accumulations, allowed the removal of the nitrogen accumulated, and made it possible to calculate the actual nitrogen removal capacity to adjust the NLR

As mentioned above, the influent leachate was diluted with tap water in order to adjust the influent TN content. Consequently, the dilution factor depended on the NLR applied and the TN content in leachates. The dilution applied to leachates during the experimental period is presented in Figure 5.4, which presents the percentage of leachate in the influent of the lab-scale anammox SBR (measured as v/v) and the total nitrogen content in the effluent of the partial nitritation process before dilution.

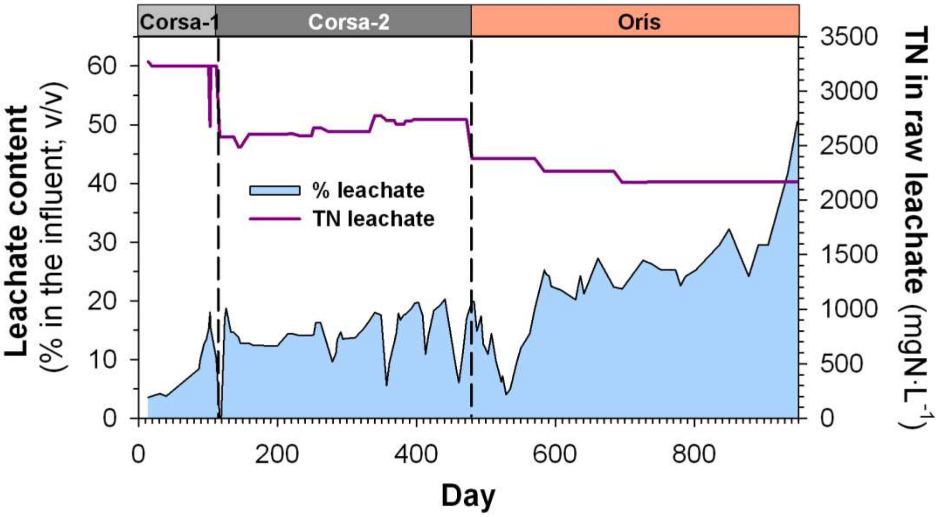


Figure 5.4. Percentage of leachate in the influent of the anammox process and TN content before the dilution in the leachate treated in the lab-scale anammox SBR.

During the start-up, the percentage of leachate in the influent of the lab-scale anammox SBR was very low, between 4 and 10%. This way, anammox biomass was not affected during the first days of operation by the strong leachate conditions and could remain active and growing. Then, as the NLR progressivelly increased, the dilution factor was reduced and the influent contained up to 50.2% of raw leachate at the end of the study. The leachate from Corsa had similar characteristics of the matrix in terms of organic matter content and conductivity, but the TN content in Corsa-2 was approximately 700mg N·L^{-1} lower than in Corsa-1 (Table 5.1), implying that for a similar nitrogen content in the influent of SBR-2, the leachate matrix was concentrated. In this sense, parameters such as the EC or the COD were increased by around 70% and 50% in Corsa-2 with respect to Corsa-1.

The leachate from Orís again presented a lower TN content than the previous (around 300mg N·L^{-1} less) and the leachate matrix was again concentrated. In this case, the conductivity of the leachate was lower than in Corsa but COD was still above 3000mg O_2·L^{-1} (Table 5.1). The maximum content of leachate achieved with both Corsa leachates was similar (18.1% and 20.2%, respectively), while the content of leachate during the third period (treating leachate from Orís) ranged between 4.8% and 50.2%. The concentration of ammonium and nitrite in the influent of the lab-scale anammox SBR is shown in Figure 5.5. Figure 5.5-A presents the evolution of the influent ammonium (green area) and nitrite (red area) concentrations.

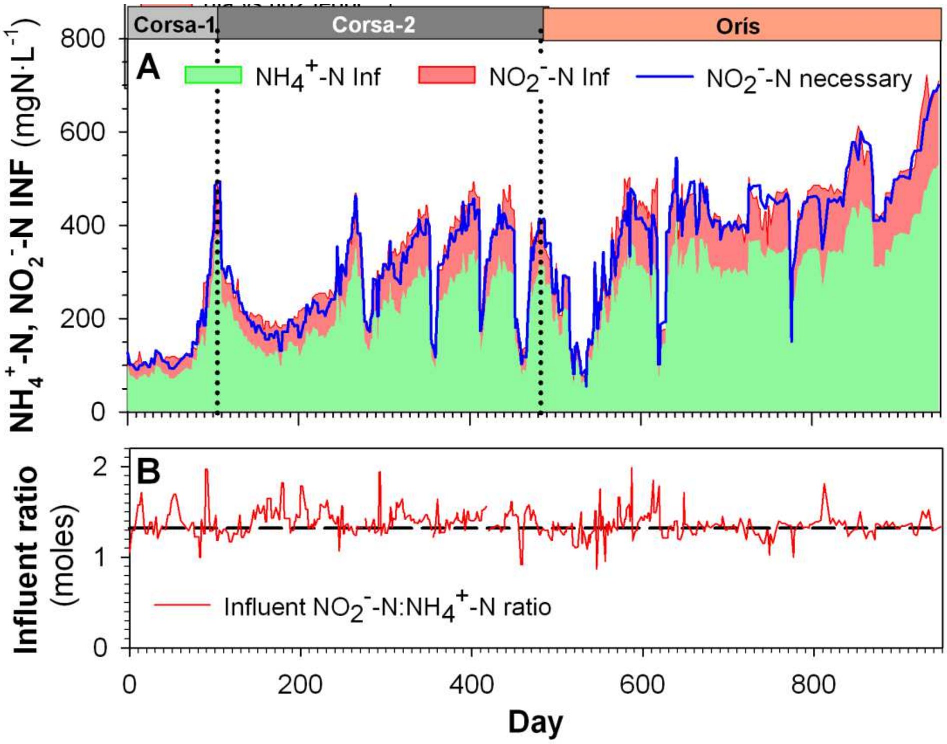


Figure 5.5. Nitrogen content in the influent. A: Experimental ammonium (green area) and nitrite (red area) contrasted with the theoretical nitrite demand (blue line) for the anammox process. B: Influent nitrite to ammonium molar ratio (the dashed line indicates the stoichiometric value of 1.32).

According to the anammox stoichiometry, 1.32 moles of NO_2^--N are required to oxidize 1 mole of NH_4^+-N. The blue line in Figure 5.5-A (NO_2^--N necessary) expresses the amount of nitrite necessary to oxidize the influent ammonium by anammox. The influent ammonium and nitrite concentrations during the operational period ranged between 41.4 - 530.6mg NH_4^+-N·L^{-1} and 67.8 - 722.3mg NO_2^--N·L^{-1}, respectively (Figure 5.5-A). Table 5.3 shows the average and maximum influent concentrations reached with each leachate (Corsa-2 and Orís) after the adaptation period. In can be seen that the average TN treated with Orís was higher than with Corsa-2 (746.1 ± 196.9mg N·L^{-1} and 566.3 ± 167.2mg N·L^{-1}, respectively), showing more stable operation of the process with the Orís leachate. The highest nitrogen contents reached with both leachates (up to 1240.0mg N·L^{-1}) are close to the nitrogen concentration that can be commonly found in mature landfill leachates, demonstrating the capacity of the process to treat high nitrogen-loaded landfill leachates(Cema *et al.* 2007; Ruscalleda *et al.* 2010; Xu *et al.* 2010; Yapsakli *et al.* 2011).

Table 5.3. Concentrations of ammonium and nitrite in the influent of the lab-scale anammox SBR, treated after the adaptation period to Corsa-2 and Orís leachates.

Leachate	Days	Average NH_4^+-N mgN·L^{-1}	Average NO_2^--N mgN·L^{-1}	Max NH_4^+-N mgN·L^{-1}	Max NO_2^--N mgN·L^{-1}	Max TN mgN·L^{-1}
Corsa-2	178-476	227.5 ± 71.0	319.7 ± 98.8	350.4	492.5	857.6
Orís	520-end	312.2 ± 86.2	421.4 ± 117.0	530.6	722.3	1261.5

Stable operation of the previous partial-nitritation process produced an effluent leachate with a good nitrite to ammonium molar ratio for the anammox process. In this sense, the average influent ratio in the lab-scale anammox SBR was set at 1.37 ± 0.15 mole NO_2^--N·$mole^{-1}$$NH_4^+$-N and ranged between 0.84 and 1.98. However, the experimental influent nitrite concentration was often slightly above the nitrite demand calculated to oxidize all the influent ammonia by anammox. The behavior of the influent nitrite to ammonium molar ratio is shown in Figure 5.5-B, where the optimal ratio of 1.32 is indicated by the dashed line. The main deviations can be observed during short periods, such as around Days 50-60, Day 230, Day 450, or Day 820.

Since the anammox biomass presents a lower tolerance to nitrite than to ammonium (Strous *et al.*, 1998; Dapena-Mora *et al.*, 2007); it is commonly preferred to operate anammox systems under nitrite limitations with the influent ratio set around 1 mole NO_2^--N·$mole^{-1}$ NH_4^+-N, leading to the presence of ammonium in the effluent of the anammox process. In this study, the influent ratio was aimed close to 1.32 to optimize the nitrogen removal efficiency of the process. The effluent concentrations of nitrite and ammonium are presented in Figures 5.6-A and 5.6-C, respectively, and contrasted with the nitrite balance (the difference between the influent nitrite concentration and the theoretical nitrite demand) (Figure 5.6-B).

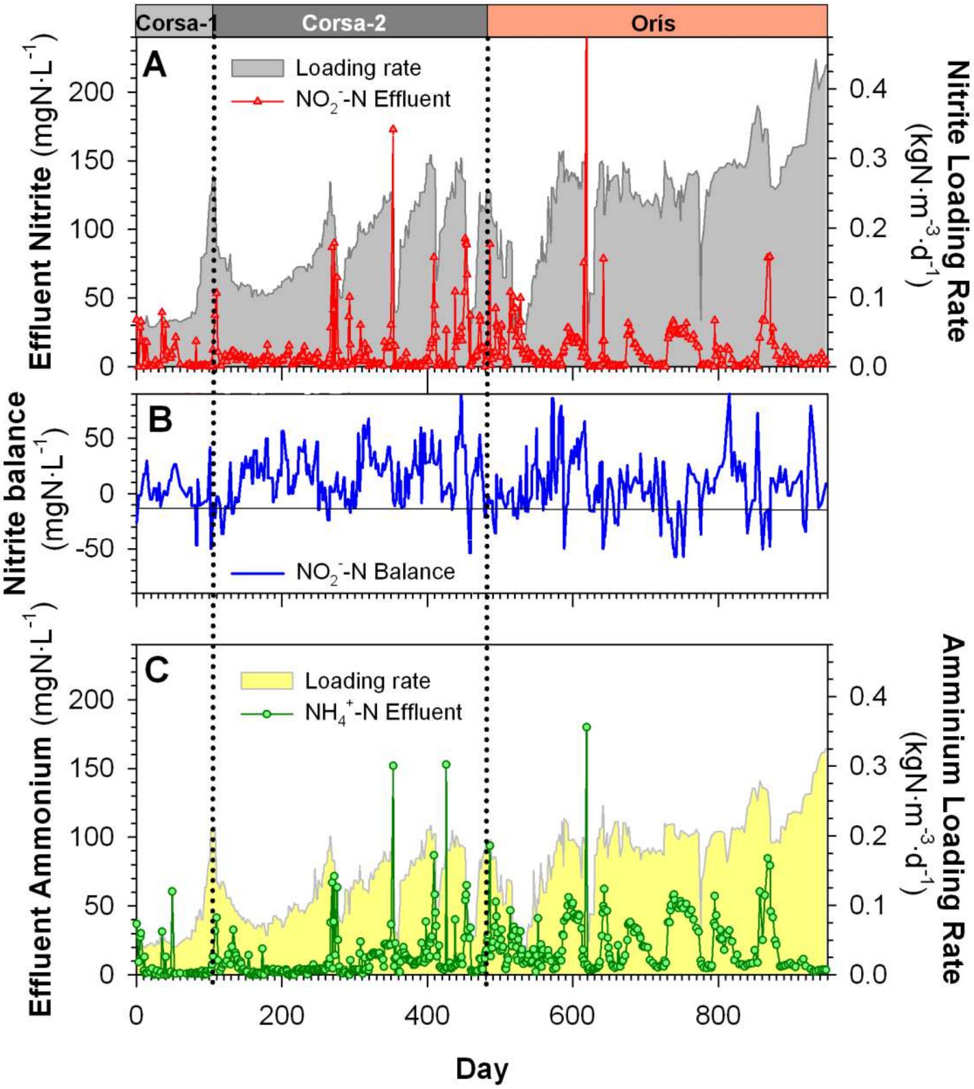


Figure 5.6. Effluent concentrations with respect to the influent conditions. A: Effluent nitrite concentration with the nitrite loading rate. B: Nitrite balance. C: Effluent ammonium concentration with the ammonium loading rate.

The system presented good removal efficiencies during most days, and the nitrogen removal capacity of the process increased progressively as the NLR was augmented, while the effluent concentrations of nitrite and ammonium were low.

The average effluent ammonium and nitrite concentrations during the entire experimental period were 16.5 ± 20.5mg NH_4^+-N·L^{-1} and 11.9 ± 21.6mg NO_2^--N·L^{-1}. The nitrogen (ammonium plus nitrite) removal efficiency of the treatment was on average set at 94.4 ± 7.8%, with specific removal efficiencies for ammonium and nitrite close to 95% and 97%, respectively.

Nitrite peaks indicate some destabilization periods that affected the performance of the process. Generally, nitrite peaks were accompanied by the proportional ammonium accumulation, which started in tandem with the nitrite accumulation. This fact indicates that in these cases the nitrite peaks were not caused by a deviated influent nitrite to ammonium molar ratio and were indicative of a biomass activity loss produced by the leachate matrix.

Thus, the increments of the effluent nitrite concentration were of special concern during the operation of the lab-scale anammox SBR. Dapena-Mora et al. (2007) established the 50% inhibition concentration (IC_{50}) for nitrite to 350 mg NO_2^--N·L^{-1} in batch tests performed with *Candidatus* Kuenenia stuttgartiensis. In Chapter 4, our explorations with bacteria belonging to *Candidatus* Brocadia anammoxidans resulted in an IC_{50} of 173 mg NO_2^--N·L^{-1} after 24h of exposure, and Fux *et al.* (2004) reported severe inhibition at only 30mg NO_2^--N·L^{-1} after six days of exposure. Thus, the persistent presence of relatively high effluent nitrite content could affect the activity of anammox bacteria. In addition, the results obtained with the previous assays (Section 5.4.2) showed that the leachate matrix can induce activity loss in the reactor. Considering all this feedback, and taking into account the high influent nitrite concentration normally applied, the effluent nitrite concentration was aimed below 10mg NO_2^--N·L^{-1}. This way, nitrite peaks can be detected and corrective measures can be applied before inhibitory conditions are reached. Severe nitrite inhibition occurred only on days 354 and 612.

In situations of substrate accumulation, such as during days 109, 271, 352, 413, 448, 490, and 858, the strategy used was to adjust the NLR according to the removal capacity measured in the process during the reaction phase. This action stopped nitrite accumulation and allowed the recovery of the anammox activity as soon as the nitrite concentration in the reactor dropped. The decrease of the NLR implied a reduction of the leachate matrix concentration in the influent of the process (Figure 5.4), which also benefited the biomass activity.

Only on day 292 was a clear increase of the effluent nitrite concentration measured without any peak in the effluent ammonium profile (Figure 5.6), indicating an excess of nitrite in the influent. On this day, the influent nitrite to ammonium molar ratio increased from 1.29 to 1.94, and the effluent ammonium concentration was close to zero. However, most of the fluctuations of the influent molar ratio above 1.32 occurring during the study were not directly reflected with peaks in the profile of the effluent nitrite concentration, indicating that the system had some capacity to assimilate this theoretical nitrite excess.

The influent and effluent nitrate concentrations are shown in Figure 5.7. Nitrate was supplied in the influent (about 20mg NO_3^--N·L^{-1}) until Day 470 as a security measure to avoid sulfate reduction activity. From this day on, the nitrate presence in the influent was due to some nitrification during the PN process or the leachate storage period. As expected, nitrate was produced in the reactor and the effluent nitrate concentration behavior followed the same pattern as the NLR. Nitrate was normally the main effluent nitrogen compound with an average concentration of 51.7 ± 17.8mg NO_3^--N·L^{-1}.

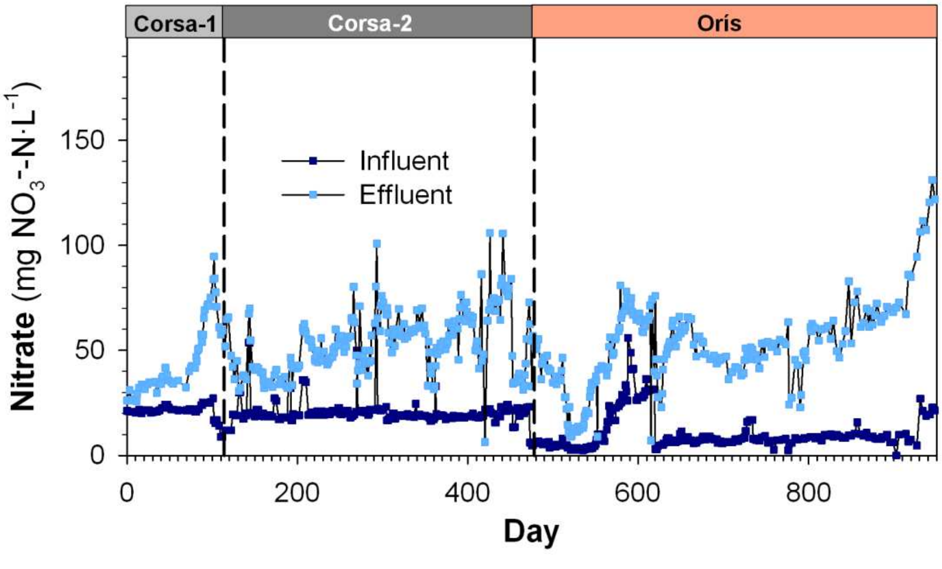


Figure 5.7. Evolution of nitrate concentration in the lab-scale anammox SBR.

As in the anammox enrichment (Chapter 4), the nitrogen removal ratios were calculated and followed during the study. Figure 5.8 shows the molar ratios obtained during the operation of the lab-scale anammox SBR. The average values for NO_2^--$N_{removed}$ / NH_4^+-$N_{removed}$ and NO_3^--$N_{produced}$ / NH_4^+-$N_{removed}$ ratios were 1.42 ± 0.18 moles and 0.15 ± 0.17 moles, respectively. There is a clear deviation of the experimental ratios with respect to the stoichiometric coefficient values with higher nitrite consumption and lower nitrate production than expected.

These ratios show that anammox was the main nitrogen removal route, but a significant contribution of heterotrophic bacteria was present during the whole study. The average nitrite to ammonium consumption ratio obtained during the study (1.42 ± 0.18) is higher than the average influent ratio (1.37 ± 0.15), indicating that in spite of the deviated influent ratio with a theoretical excess of nitrite in some periods, the process was globally operating under nitrite limitation.

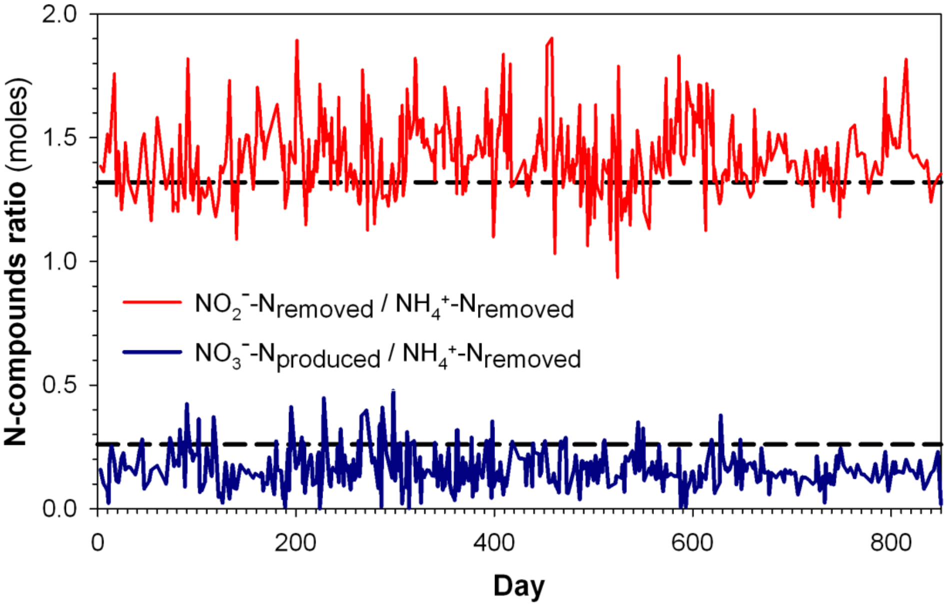


Figure 5.8. Experimental nitrite to ammonium (red solid line) and nitrate to ammonium (blue solid line) molar ratios obtained in SBR-2 with the respective stoichiometric values (dashed lines).

The effect of the landfill leachate on the biomass was tested again by an SAA test with biomass collected from lab-scale anammox SBR on Day 842 (Table 5.3). The test was performed with effluent of the lab-scale anammox SBR with a spike of 50mg $N \cdot L^{-1}$ of ammonium and nitrite and the respective controls in the mineral medium.

Table 5.4. Results of the later SAA test to evaluate the effect of leachate.

Leachate	Exposure	SAA Control	SAA Leachate	Activity loss
	h	g N-$N_2 \cdot g^{-1}$ VSS$\cdot d^{-1}$	g N-$N_2 \cdot g^{-1}$ VSS$\cdot d^{-1}$	%
Orís(1)	3-4	0.46 ± 0.08	0.20 ± 0.01	56

(1) Effluent of the lab-scale anammox SBR with 50 mg N$\cdot$L^{-1} of ammonium and nitrite.

The results showed a 56% higher SAA with the mineral medium than with leachate. This fact indicates that the maximum SAA in the anammox reactor treating landfill leachates was limited by the leachate matrix and reveals the presence of one or some components that cause partial inhibition of anammox bacteria activity, which grow slower than compared than in the mineral medium.

5.4.3.2 Organic matter removal

The three landfill leachates treated in this study contained organic matter (OM). Since the possible readily biodegradable OM available in the leachate was removed in the previous PN-SBR, the influent of the anammox process should contain only refractory OM.

The influent and effluent concentrations and the removal efficiency of soluble COD in the lab-scale anammox SBR are shown in Figure 5.9. The soluble COD in the influent leachate ranged from 100.3 mg $O_2 \cdot L^{-1}$ to 2500 mg $O_2 \cdot L^{-1}$ (average influent soluble COD set at 585.0 ± 319.6 mg $O_2 \cdot L^{-1}$). Some readily biodegradable OM (expressed by the BOD_5) was available in the influent, ranging between 10.2 mg $O_2 \cdot L^{-1}$ and 51.5 mg $O_2 \cdot L^{-1}$, and was attributed to the presence of VSS in the effluent of the PN-SBR and their hydrolysis during the storage period. The presence of VSS in the effluent of the previous PN process was also identified as a possible source of biodegradable OM to the anammox reactor in a two-step PN-anammox processes in previous studies (Van Dongen *et al.* 2001; Ganigué *et al.* 2007; van der Star *et al.* 2007). However, the BOD_{30} of the concentrated leachate (before dilution) showed values up to 3200 mg $O_2 \cdot L^{-1}$, indicating that part of the OM was slowly biodegradable and could be degraded in the reactor, favored by the high temperature.

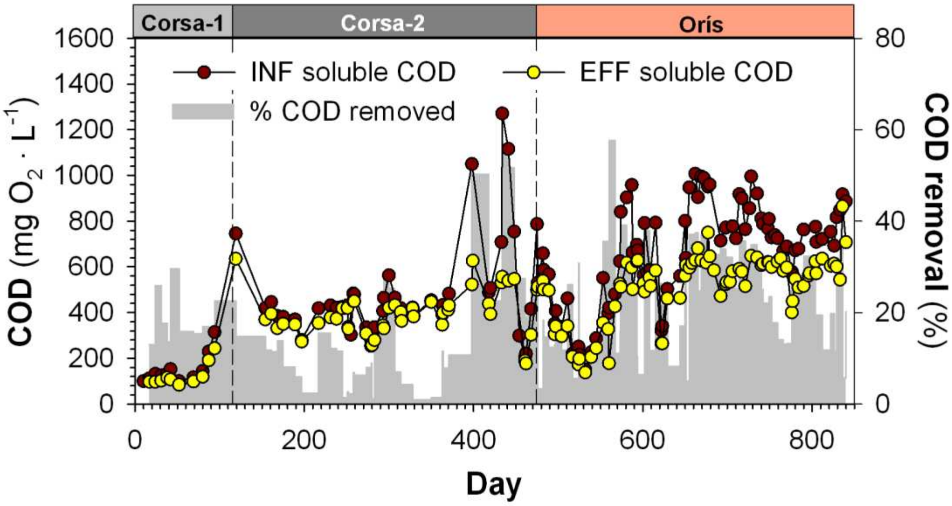


Figure 5.9. Soluble COD content of the influent landfill leachate and the effluent of the lab-scale anammox SBR, with the respective COD removal efficiency.

The OM content in the influent leachate depended on the characteristics of the leachate, which presented high variability over time and depending on the landfill site and the dilution applied to the leachate. Thus, sudden variations of the COD content are attributed to changes on the landfill leachate used and progressive increments are related to increments of the NLR with a decrease of the dilution factor. COD removal was detected during all the operational periods, indicating the presence of heterotrophic denitrifying activity in the anammox reactor. The average efficiency removal of soluble COD was set at 19.3 ± 12.7%, reaching maximum efficiencies up to 60% (Figure 5.9).

The presence of heterotrophic bacteria performing denitrification in the anammox reactor was also reflected in the nitrogen compounds removal molar ratios observed in Section 5.4.3.1. Experimental values differing with the stoichiometric coefficients are indicative of a consumption or production of nitrogen species by other microbial populations. In this study the biodegradable organic matter present in the landfill leachate was put to good use to improve the nitrogen removal capacity of the system. The presence of a limited denitrifying activity was beneficial for the system since nitrite excess was buffered and the effluent nitrate concentration was mitigated.

5.4.3.3 Biomass characteristics

As stated before, the lab-scale SBR was inoculated with granular sludge coming from the anammox enrichment at an initial MLVSS concentration of 547.3mg VSS·L^{-1}. Initially, the reactor was operated without purge (no SRT control) in order to ensure biomass retention and favor the enrichment of the system. In the beginning, the VSS content in the reactor decreased progressively due to the attachment of the biomass to the internal parts of the reactor (similarly observed in the anammox enrichment).

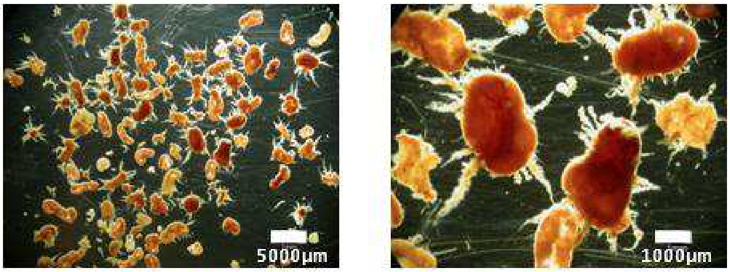
The minimum MLVSS concentration was reached on day 248 with 134.4mg MLVSS·L^{-1}. The reactor was then opened on day 269, and the biomass attached to the walls and internal elements of the reactor were re-suspended. After cleaning the walls and re-suspending the attached biomass, the MLVSS content was set to 1466.7mg VSS·L^{-1}. Thus, in spite of the low suspended sludge presence, the biomass content increased during this period.

From day 269 on, periodic cleanings were performed to maintain most of the sludge in suspension and were related to sudden increases of the MLVSS content in the system. After a cleaning the system was purged and the SRT fell below 50 days during some days in order to purge the inert particulate material and recover the sludge quality. Just after cleaning, the VSS/TSS ratio reached minimum values of 0.80, but was then maintained at 0.91 ± 0.04.

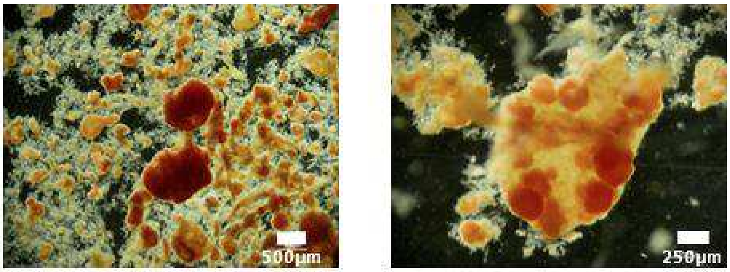
The maximum sludge concentrations above 4000mg VSS·L^{-1} were reached after the cleaning on Day 790. Between Days 860-890, the MLVSS decreased to around 2500mg VSS·L^{-1} due to the collection of biomass samples to seed the vials for SAA batch experiments. Different samples of granular sludge were collected from the lab-scale anammox SBR during the reaction phase in several days in order to examine the characteristics of the sludge.

Figure 5.10 shows some of the images obtained with the stereomicroscope. In all the pictures from the Corsa treatment period, it can be seen how the anammox granules (with a reddish-brown color) are surrounded by a matrix of heterotrophic bacteria and extracellular polymeric substances. Only at the end of the study, when the best stable operation was reached, did the sludge present compact granules with a regular shape, more similar to the results obtained in the anammox enrichment of Chapter 4.

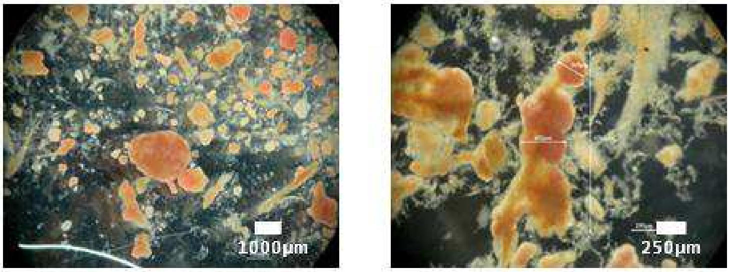
A: Day 46 (CORSA-1; 120 rpm)



B: Day 269 (CORSA-2; 250 rpm; Cleaning)



C: Day 284 (CORSA-2; 250 rpm)



D: Day 842 (ORÍS; 250 rpm)

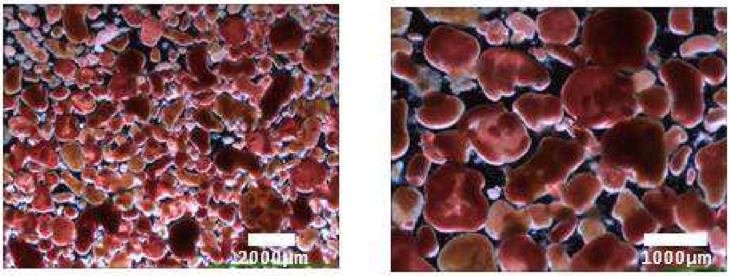


Figure 5.10. Images obtained by stereomicroscope of the granular sludge of the lab-scale SBR from different samples collected during the entire study. Left column: 12.5x; right column: 25x.

Figure 5.10-A corresponds to the initial period of the startup, when CORSA-1 leachate was treated and the stirrer velocity was 120rpm. After a cleaning of the reactor, the sludge was re-suspended. The re-suspended sludge contained important amounts of inert materials, as can be seen in the image of Figure 5.10-B taken from a sample collected two days after cleaning. As stated before, at this point the VSS/TSS ratio attained the lowest values (0.80 on Day 274), and the system was then purged. The particulate material was washed and two weeks later the quality of the sludge had improved (Figure 5.10-C). However, the anammox granules were still associated with heterotrophic matrix and filaments. After the period of acclimatization to the Orís leachate, a sample was collected again during stable operation of the reactor (Figure 5.10-D). Here, the presence of filamentous bacteria was much lower, and the sludge showed a more regular shape and size of the granules, with an average diameter of 1000 µm.

As in anammox enrichment, the biomass of this anammox SBR was also explored by molecular techniques to evaluate the changes throughout the experimental study. The microbial community was explored by FISH using the same procedure explained in Chapter 4, with a general probe targeting all bacteria and a specific probe for anammox microorganisms. Figure 5.11 shows the FISH images acquired from samples of biomass collected on Day 417.

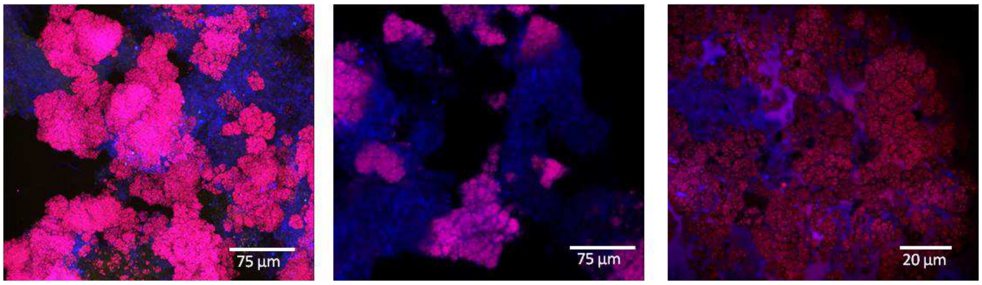


Figure 5.11. FISH images acquired by CLSM from biomass samples collected on Day 417. Blue: heterotrophic bacteria (general EUB-mix probe); Magenta: anammox bacteria (specific probe Amx-820).

As can be seen, the granular biomass was enriched with anammox bacteria but contained a significant fraction of heterotrophic bacteria. FISH images were used to quantify the relative abundance of anammox bacteria. The bacterial community was dominated by anammox bacteria with an average relative abundance of 78.3 ± 9.7%.

At the end of the operational period some granules were collected from the anammox SBR and were prepared to be observed in a SEM. The images obtained by SEM are shown in Figure 5.12.

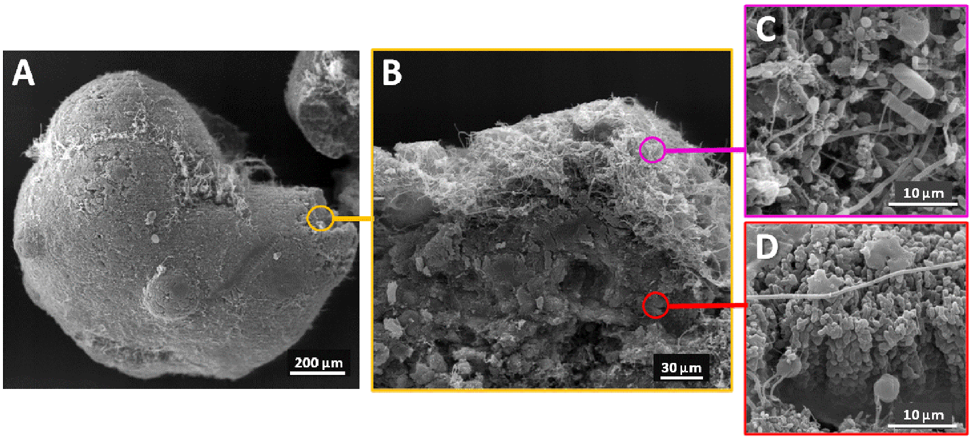


Figure 5.12. SEM images of the granular biomass of the lab-scale anammox SBR, collected at the end of the operational period. A: Image of a complete granule; B: zoom in A; C and D: zoom in B.

The results showed compact granules (Figure 5.12-A) with an important presence of filamentous bacteria, forming long structures. Figure 5.12-B zooms in on a broken part of the granule, which allows a section of the granule to be seen. In Figures 5.12-C and –D, augmented images from the surface and the internal zones of the granule are shown, respectively. A major presence of filamentous bacteria is observed in the external part, while compact structures are formed inside the granule.

5.4.4 Heterotrophic denitrifiers in granular anammox reactors

The N conversion ratios and the biomass analysis concluded that heterotrophic denitrifying populations were active in the lab-scale anammox SBR. Since the heterotrophic denitrification is supported by the presence of biodegradable OM, changes in the landfill leachate composition can alter the denitrification potential of the system. The possibility of compromising the anammox bacteria activity is real, especially in landfill sites where some zones are closed, but there are still zones receiving solid waste (and producing young leachates). Thus, the monitoring of the denitrifying capacity of the system is important to managing the anammox reactor in the treatment of landfill leachate.

5.4.4.1 Nitrogen mass balance: development of the method

In this section a new method based on soluble nitrogen species balances is proposed as a simple and useful tool for monitoring the contribution of heterotrophic denitrification on nitrogen removal in anammox reactors. The organic matter in the presence of nitrite and/or nitrate concludes with heterotrophic denitrifying biomass development. In an anammox reactor, this growth implies the coexistence of the autotrophic anammox and heterotrophic denitrifying cultures and, therefore, nitrogen removal is the result of the contribution of each process. To evaluate the contribution of each population to nitrogen removal, a methodology based on mass balances was developed. The stoichiometric parameters for the biological processes taken into account to calculate the mass balances are presented in Table 5.5.

Table 5.5. Stoichiometric matrix for biological N-removal processes included in the balance.

Component **Process**	**S_{NH4}** mg N·L^{-1}	**S_{NO2}** mg N·L^{-1}	**S_{NO3}** mg N·L^{-1}	**S_{N2}** mg N·L^{-1}	**S_S** mg COD·L^{-1}
Heterotrophic biomass					
Denitrification on NO_3^-	$-i_{N,BM}$	$-\frac{1-Y_H}{1.71 \cdot Y_H}$		$\frac{1-Y_H}{1.71 \cdot Y_H}$	$-\frac{1}{Y_H}$
Denitrification on NO_2^-	$-i_{N,BM}$	$\frac{1-Y_H}{1.15 \cdot Y_H}$	$-\frac{1-Y_H}{1.15 \cdot Y_H}$		$-\frac{1}{Y_H}$
Autotrophic biomass					
Anammox	-1	-1.32	0.26	2·1.02	

Parameters concerning the anammox process come from the stoichiometry reported by Strous *et al.* (1998). The denitrification mechanism has been considered as a parallel two-step denitrification according the concept reported by Sin *et al.* (2008). Thus, denitrification is a sequential reduction process of N-compounds. The coefficients for heterotrophic organisms are based on ASM 2 (Henze *et al.* 1999).

Focusing on nitrogen compounds, the mass balances of the system resulting from the anammox and heterotrophic denitrifier activities are defined in Equations 5.1, 5.2 and 5.3. As seen in Table 5.4, the ammonium decrement in the system (Eq. 5.1) is the consequence of ammonium removal (including the assimilation) by anammox bacteria and the ammonium assimilation in heterotrophic denitrification. The nitrate variation (Eq. 5.2) results from the difference between the nitrate production in the anammox metabolism and the nitrate reduction to nitrite by heterotrophic denitrifiers. Finally, the nitrite decrement is calculated from the nitrite generation by heterotrophic biomass due to nitrate reduction and nitrite removal by heterotrophic denitrification and anammox organisms (Eq. 5.3).

$$\Delta NH_4^+ = -NH_4^+{}_{ANAMMOX} - \frac{\mathrm{i}_{\mathrm{N,BM}}}{\frac{1-Y_H}{1.71 \cdot Y_H}} NO_2^-{}_H - \frac{\mathrm{i}_{\mathrm{N,BM}}}{\frac{1-Y_H}{1.15 \cdot Y_H}} NO_3^-{}_H \qquad \text{(Eq. 5.1)}$$

$$\Delta NO_3^- = 0.26 \cdot NH_4^+{}_{ANAMMOX} - NO_3^-{}_H \qquad \text{(Eq. 5.2)}$$

$$\Delta NO_2^- = NO_3^-{}_H - NO_2^-{}_H - 1.32 NH_4^+{}_{ANAMMOX} \qquad \text{(Eq. 5.3)}$$

The nitrogen ammonium variation (Eq. 5.1) is mainly affected by the anammox consumption parameter, because ammonium is the electron and energy source for anammox biomass. Heterotrophic denitrifiers only remove it as nitrogen assimilated for biomass production. Therefore, the contribution of the heterotrophic ammonium assimilation terms to the nitrogen ammonium balance is weaker compared to anammox ammonium removal. Moreover, the growth of heterotrophic biomass in an anammox reactor is limited by the low availability of biodegradable organic matter. Thus, ammonium is basically removed by the anammox route. This implies a low sensitivity of the mass balance to the heterotrophic biomass coefficients. For this reason, the accuracy of the Y_H value does not strongly determines the result of the nitrogen mass balances.

Nitrogen mass balances (Eq. 5.1, 5.2 and 5.3) contain three unknowns that correspond to 1) the nitrogen ammonium removal by anammox biomass ($NH_4^+{}_{ANAMMOX}$); 2) the nitrogen nitrate consumption by heterotrophic denitrifiers ($NO_3^-{}_H$); and 3) the nitrogen nitrite removal by heterotrophic biomass ($NO_2^-{}_H$).

These three parameters can be defined as the following expressions (Eqs. 5.4-5.5), after the equation system resolution:

$$NH_4^+{}_{ANAMMOX} = \frac{\dfrac{\dfrac{\mathrm{i}_{\mathrm{N,BM}}}{1-Y_H}}{1.71\cdot Y_H}\cdot\left(\Delta NO_3^- + \Delta NO_2^-\right) + \dfrac{\dfrac{\mathrm{i}_{\mathrm{N,BM}}}{1-Y_H}}{1.15\cdot Y_H}\cdot\Delta NO_3^- - \Delta NH_4^+}{1+0.26\dfrac{\dfrac{\mathrm{i}_{\mathrm{N,BM}}}{1-Y_H}}{1.15\cdot Y_H}+(0.26-1.32)\cdot\dfrac{\dfrac{\mathrm{i}_{\mathrm{N,BM}}}{1-Y_H}}{1.71\cdot Y_H}} \qquad \text{(Eq. 5.4)}$$

$$NO_3^-{}_H = 0.26\cdot NH_4^+{}_{ANAMMOX} - \Delta NO_3^- \qquad \text{(Eq. 5.5)}$$

$$NO_2^-{}_H = \left(0.26-1.32\right)\cdot NH_4^+{}_{ANAMMOX} - \Delta NO_3^- - \Delta NO_2^- \qquad \text{(Eq. 5.6)}$$

From Equations 5.4-5.6, the total nitrogen consumption by each kind of biomass and the total nitrogen removal in the reactor can be calculated. Concerning the anammox biomass, the total nitrogen removal is obtained through the stoichiometric coefficients of the process (Eq. 4.1). Therefore, 1.32 mol of N-NO_2^- are consumed and 0.26 mol of N-NO_3^- are generated for each mol of N-NH_4^+ removed by anammox (Eq. 5.7).

$$N_{ANAMMOX} = NH_4^+{}_{ANAMMOX}\cdot\left(1+1.32-0.26\right) \qquad \text{[Eq. 5.7]}$$

5.4.4.2 Contribution of denitrifiers on N-removal in granular lab-scale anammox SBR

The nitrogen mass balance method was used with the real data from the continuous operation of the lab-scale anammox SBR treating urban landfill leachate preconditioned in a PN-SBR and coming from different landfill sites, and revealed the total amount of nitrogen removed via anammox or heterotrophic denitrification in the anammox SBR.

The results obtained during the operation of the anammox reactor are presented in Figure 5.13, where the evolution of the fraction of the total N removed attributed to anammox or heterotrophic bacteria is shown.

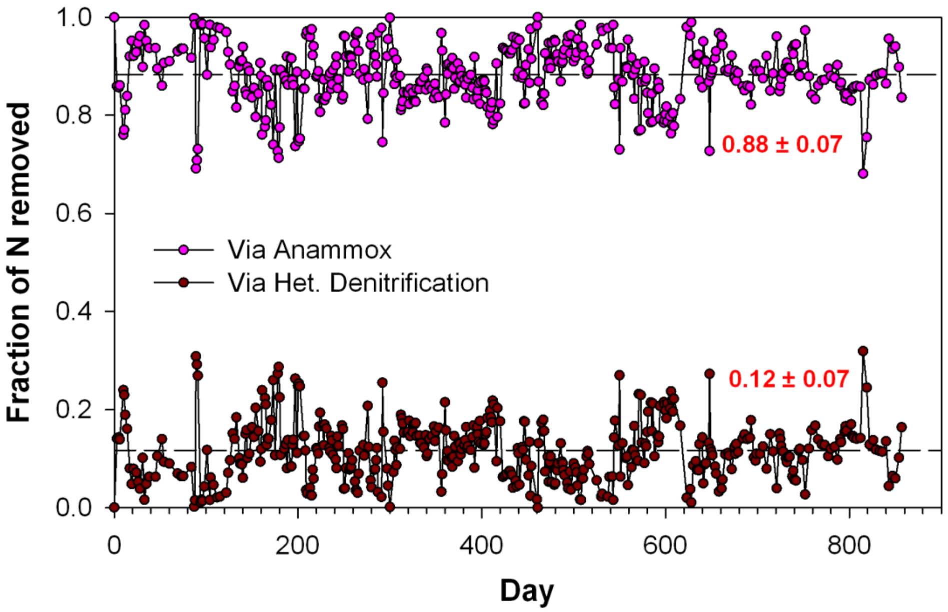


Figure 5.13. The fraction of total N removed autotrophically and heterotrophically during the operation of the lab-scale anammox SBR.

As can be seen, the anammox route was always the main N-removal pathway in this reactor, with an average coefficient of 0.88 ± 0.07. Heterotrophic denitrifiers were limited by the availability of biodegradable OM and depended on the dilution factor of the leachate and endogenous processes (hydrolysis and decay).

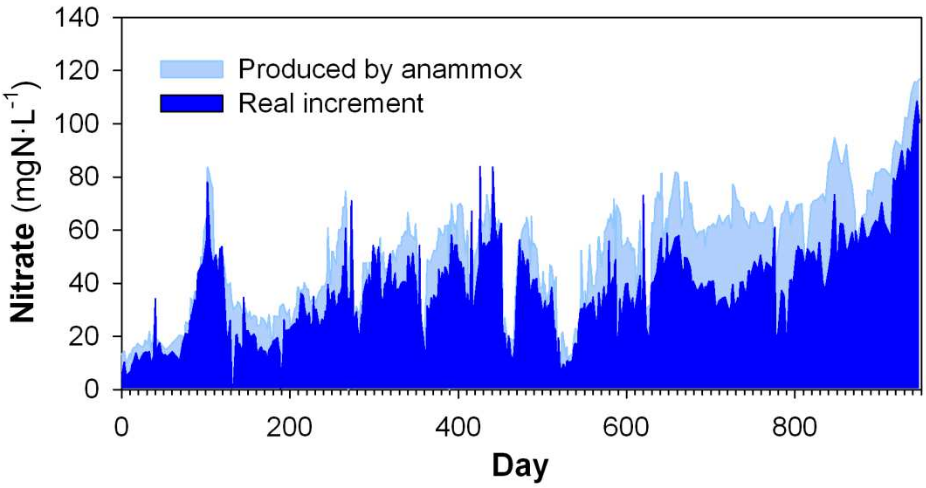


Figure 5.14. Production of nitrate by anammox reaction (blue) contrasted with the measured nitrite increment in the system.

Figure 5.14 shows the difference between the expected effluent nitrate concentrations taking into account the ammonium removed by anammox route and the actual nitrate increment in the system. It can be seen how in this reactor the contribution of heterotrophic denitrification resulted in a lower effluent nitrate concentration since part of the nitrate produced by anammox was removed. Thus, the coexistence of anammox and heterotrophic bacteria improved the nitrogen removal capacity of the treatment.

5.5 General Discussion

5.5.1 Biomass adaptation to landfill leachate

The results obtained during the operation of the lab-scale anammox SBR indicate that the effluent nitrite peaks were correlated with sudden changes of the leachate matrix composition. Thus, the biomass from a reactor fed with mineral medium cannot be directly applied to treat an influent with 100% leachate. The dilution of the leachate and a progressive increment of the leachate content in the anammox reactor is a good strategy to allow the acclimatization of the biomass to the leachate matrix. The adaptation period for the start-up of the reactor was less than 80 days, indicating a relatively rapid adaptation.

However, even in periods with the anammox sludge adapted to landfill leachate, drastic changes of the influent leachate required also a re-adaptation of the biomass to the new environmental conditions. In our case, the three landfill leachates presented different TN content, implying the need for different dilutions for similar NLR. However, the adaptation periods were progressively lower compared to the start-up period with Corsa-1. The change to Corsa-2 required 64 days before the NLR could be increased again and the change to Orís only 43 days, proving that the tolerance of the biomass to the landfill matrix is improved with time.

A stable process performance can be obtained in spite of variations of the influent leachate, but the maximum activity of the biomass adapted to landfill leachate increases in mineral medium without leachate. The characteristics of the leachate matrix reduce the maximum anammox activity. Thus, the enrichment of biological reactors with anammox biomass with landfill leachate is affected by the lower activity of anammox bacteria.

5.5.2 Organic matter in leachates

Negative effects on the nitrogen removal and the inactivation of the anammox route have been reported in several studies when the available organic carbon source increases (Chamchoi *et al.* 2008, among others). In this sense, the use of a two-step process to perform separately the PN step and the anammox step became a better option to treat urban landfill leachates or other wastewater with the possibility of isolated OM loading shocks.

The readily organic matter content in wastewater is easily measurable by testing the BOD_5. However, the readily biodegradable organic matter content in mature landfill leachates represents a small fraction of the total COD content. Thus, the organic compounds in mature leachates are basically refractory compounds and slowly biodegradable organic matter.

It has been proven in this chapter that a limited presence of denitrifying activity in the anammox reactor is advantageous in terms of nitrogen removal, since it contributes to nitrate and nitrite removal. Moreover, the COD is removed, also improving the effluent quality in terms of OM discharge. In this sense, the use of mass balances to monitor the contribution of heterotrophic denitrification to nitrogen removal is a useful tool to detect possible increments of the heterotrophic nitrogen removal in detriment of anammox. Under conditions of limited availability of organic carbon source for heterotrophs, anammox bacteria can use all the nitrite required without outside competition from denitrifiers.

The nitrogen mass balance method applied in this chapter can be used to prevent important perturbations in the performance process due to outside competition between both bacterial populations.

5.6 Conclusions

Thus, with the results obtained from the lab-scale experience with anammox SBR performance it can be concluded that:

- Mature landfill leachates can be treated in a two-step process with PN and anammox. It is not necessary to start up the anammox reactor with a huge amount (biomass and less than 0.6 g VSS·L^{-1} is enough). The process is stable and long-term operation can be maintained without the loss of anammox activity despite perturbations due to changes in leachate composition.
- The system can be operated in a nitrite to ammonium ratio close to 1.32, obtaining better effluent discharge compared with the typical 1:1 ratio used in these kinds of treatment.
- Thus, the treatment of landfill leachates by the anammox process is a feasible alternative to conventional biological treatment with complete heterotrophic denitrification because methanol addition is avoided and part of the slowly biodegradable organic matter is removed. This process also takes advantage of the physical treatments, since biological treatments have been proved to be cheaper.
- The nitrogen mass balance methodol developed in this chapter has been tested and applied to assess the contribution of each microbial population to nitrogen removal. The application of this tool to the results obtained in this lab-scale anammox reactor showed that 88% of the N-removal was attributed to anammox. This is a simple and effective method for

monitoring the presence of heterotrophic activity in anammox reactors integrated in two-step PN-anammox processes.

The results obtained and the experience acquired from the operation of this lab-scale reactor conform the basis of knowledge to further scale-up the process to a semi-industrial pilot plant.

CHAPTER 6. START-UP OF A PILOT-SCALE SBR WITH ANAMMOX BIOMASS TO TREAT LANDFILL LEACHATE

This chapter presents the results of the work carried out under the framework of the LEAMMOX project (biological treatment of LEAchates by partial nitritation and anaMMOX using PN and anammox SBRs) that aimed for the implementation of the PANAMMOX technology in a pilot-scale plant. This project resulted from the collaboration of the CESPA-GR and LEQUIA group and was founded by the Spanish Government (CIT-310000-2009-063).

6.1 Motivation

The feasibility of achieving efficient and stable nitrogen removal efficiency from urban landfill leachate in an anammox reactor was demonstrated at lab-scale using the SBR technology in Chapter 5. Raw landfill leachate was previously treated in a PN-SBR (Ganigué *et al.* 2009) to oxidize about half of the influent ammonium to nitrite. The PN-SBR process was tested and applied to treat mature landfill leachate maintaining nitrite build-up over long-term operation.

However, the leachate treated in the lab-scale anammox SBR (Chapter 5) was always diluted more than 50%, due to the operational strategy used in this reactor, based on a fixed HRT of 1.76 days. Drastic changes to the leachate characteristics resulted from rapid variations of the dilution factor or sudden changes of the leachate composition (i.e. salinity and COD). In this chapter the anammox process is implemented to a pilot-scale SBR and special efforts are made to maintain stable operation during the start-up. This chapter will also focus on the operational efforts to allow anammox biomass adaptation to the leachate matrix and to successfully treat the effluent of the PN-SBR.

The fast start-up of a pilot-scale anammox SBR to treat mature landfill leachate has not been reported so far in the literature. Future implementations of the anammox process at full-scale leachate treatment require the definition of a rapid and effective start-up strategy. Thus, from the previous work described in chapters 4 and 5, a start-up strategy for a pilot-scale anammox SBR will be developed.

Nowadays, some of full-scale leachate treatment plants initially designed to perform conventional nitrification-denitrification processes developed the presence of anammox biomass because of the dissolved oxygen gradients in the reactor (Siegrist *et al.* 2000; Cema *et al.* 2007; Wang *et al.* 2010). Even without applying optimal conditions, a very low amounts of anammox bacteria (about less than 1% of the total bacterial population) can be present in landfill leachate plants (Chiemchaisri *et al.* 2011; Yapsakli *et al.* 2011). These cases demonstrate the feasibility of using the anammox process to treat leachate even though the use of anammox biomass was not the initial goal of the treatment plant.

Referring to the start-up of anammox reactors, (Xu *et al.* 2010) started-up a lab-scale SBR using synthetic wastewater (mineral medium) and added a progressive higher fraction of leachate once the reactor was enriched with anammox biomass. However, the use of mineral medium to enrich a full-scale plant cannot be considered. Liang and Liu (2008) operated a two-step process with PN and anammox processes performed separately in up-flow fixed bed biofilm reactors followed by soil filtration. The leachate treated contained 1,400-2,800 mg NH_4^+-$N{\cdot}L^{-1}$ and was diluted with tap water after the PN process to reduce the TN in the anammox influent. In this study the nitrogen loading rate in the anammox reactor was below 0.17 Kg $N{\cdot}m^{-3}{\cdot}d^{-1}$.

There is a lack of knowledge about the method to set up new anammox reactors for treatment of mature landfill leachates. Factors such as the adaptation of the biomass to the high salinity of the leachate and the components of the leachate matrix have to be considered. Moreover, the studies carried out in lab-scale reactors use active anammox sludge to seed the reactors at an initial MLVSS concentration above 2 g $VSS{\cdot}L^{-1}$ (Xu *et al.* 2010; Ni *et al.* 2011e). However the availability of high amounts of anammox biomass to seed pilot- or full-scale reactors is still limited it is extremely difficult to obtain anammox sludge adapted to landfill leachate.

6.2 Objectives

The aim of this section is the start-up of a pilot-scale anammox reactor to be integrated in the PANAMMOX® process for the treatment of landfill leachate. Specifically, the study seeks to achieve a successful start-up by seeding the reactor with granular anammox biomass adapted to freshwater (coming from a lab-scale reactor treating synthetic wastewater).

The treatment of an influent containing 100% leachate previously conditioned in the PN-SBR process and the assessment of the process performance are also defined as goals of this chapter. In this section the experience acquired in the lab-scale processes is applied and evaluated to define the method and needs for full-scale implementation.

6.3 Materials and Methods

6.3.1 Experimental set-up

The experimental work was carried out in a pilot plant located in the facilities of LEQUIA in the Science and Technology Park of the University of Girona. Urban landfill leachate was provided from the landfill sites managed by CESPA-GR in 1000-liter cubicontainers. The landfill leachate used in this study was primarily conditioned in the pilot-scale PN-SBR in order to convert about 50% of the ammonium to nitrite. The effluent of the PN-SBR was stored again, conditioned and treated in the anammox reactor. The pilot plant (Figure 6.1-A) consisted of a 0.4m^3 stainless steel SBR completely sealed and equipped to operate under over pressure. The reactor was a column reactor with a cylindrical shape and a height to diameter ratio of about 5:1. Influent and effluent leachates were stored in cubicontainers at room temperature. The gas produced by the biological reactions in the reactor was compressed and stored in an expansion vessel further used to control the pressure during the drawing phase and to supply the gas sparger.

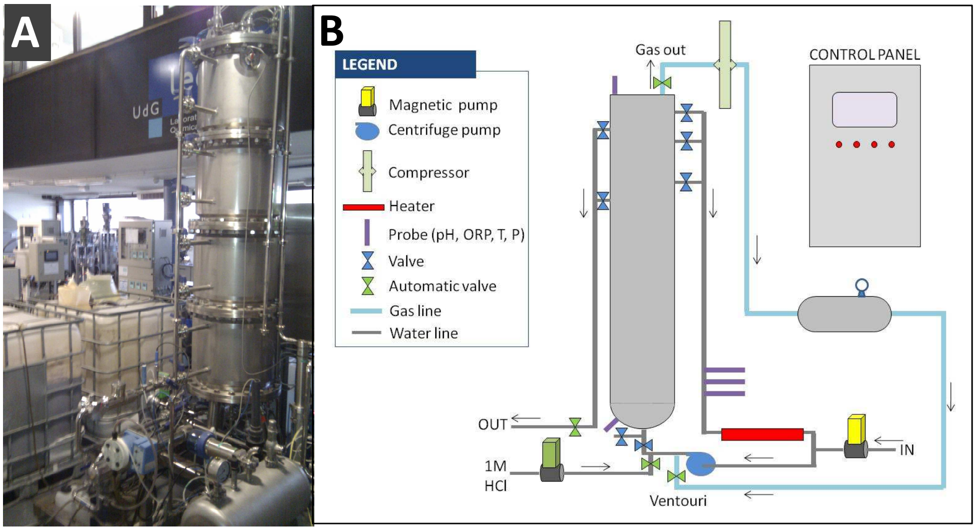


Figure 6.1. View of the pilot plant with the 0.4 m^3 anammox SBR (A) and schematic of the pilot-scale SBR (B).

A control panel with a PLC controlled all the automatic devices according to a cycle defined by the user. The online signals from the temperature, pH, ORP and pressure probes were collected and stored in the same PLC. The cycle was divided into different feeding and reaction phases, depending on the flow rate and NLR applied. All cycles were ended by the settling and drawing phases, with a length of

20 and 5 minutes, respectively. The number of cycles was increased according to the minimum and maximum volumes desired and the evolution of the treatment capacity. The MSV in this reactor ranged from 0.054 m·h^{-1} to 1.200 m·h^{-1}, when the reactor was operating with a VER of 1% and 22%, respectively.

Figure 6.1-B shows a schematic of the pilot-scale anammox SBR. The homogeneous conditions and complete mixing of the liquor were ensured by an internal recirculation. The recirculation flow was achieved by a centrifuge pump and passed through the different probes for pH, ORP and temperature control. Temperature was controlled by a heater installed in the recirculation and maintained at 35 ± 1 ºC; pH was controlled at 7.5 ± 0.1 by 1M HCl additions. The reactor was operated at 1 bar during the feeding and reaction phases, and the pressure was decreased to 0.5 bars during the settling phase to promote the gas release before the drawing phases. The operation under over pressure ensured complete anoxic conditions.

The reactor was operated with 24-hour cycles until Day 97 (Figure 6.2-A). The cycle consisted initially of a feeding phase at the beginning of the cycle followed by a continuous reaction phase until the end of the cycle, when the system was switched to settling and draw phases. To progressively increase the NLR, feeding phases were included during the reaction phase. This fact implied the enlargement of the difference between minimum and maximum volumes. When the maximum volume exchange ratio was reached, the cycle configuration was changed to 8-hour cycles, with the same structure as before (Figure 6.2-B). This way, additional feeding phases could be included and the NLR increased.

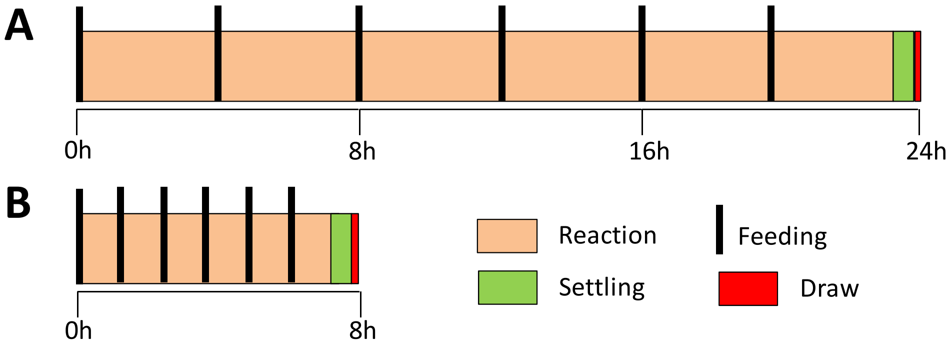


Figure 6.2. Scheme of the cycle configurations used in the SBR during the study. A: 24-hour cycle used until Day 97; and B: 8-hour cycle implemented at the end of the study.

6.3.2 Inoculum

The reactor was seeded with sludge from the anammox enrichment that was concentrated and stored at 4ºC from periodic purge events. The sludge was collected periodically from the purge of this reactor and was kept with the effluent of this reactor (mineral medium with high nitrate concentration). Some substrate (ammonium and nitrite) was added in spikes every one or two months to reactivate the anammox activity.

6.3.3 Experimental procedure

The raw landfill leachate coming from different landfill sites was pre-conditioned in the PN-SBR and stored in the effluent tank of this process. This leachate was then used to feed the anammox reactor. Thus, the duration of the storage period between the two steps was variable and depended on the production of the PN-SBR and the demand of the pilot plant anammox SBR. The NLR of the anammox reactor was increased according to the nitrogen removal capacity of the pilot plant anammox SBR, always using the effluent nitrite concentration as the key criteria to increase the flow rate according to Chapter 5. The effluent ammonium, nitrite, and nitrate concentrations in the pilot-scale SBR were measured 2-3 times per week in order to monitor the removal nitrogen performance of the process.

6.3.4 Urban landfill leachate

The leachate treated during the study was previously conditioned in the pilot-scale PN-SBR and came from two different urban landfill sites: Corsa and Xixona, Spain. Although the leachate came from two landfill sites, three different characteristic leachates were considered in this study: Corsa, recycled-Corsa and Xixona. The supply of influent leachate for the anammox process was subjected to the operation of the PN-SBR. Due to the independent research program of the PN-SBR, during a few days we recycled treated leachate from the pilot-scale anammox SBR (i.e., recycled-Corsa leachate). In this case, additions of NH_4Cl and $NaNO_2$ were used to supply the nitrogen content to the recycled-Corsa leachate to reach suitable influent values. This special operation was maintained for 20 days (from day 133 to day 153). After that, we moved to leachate from the Xixona landfill site. The characteristics of the three influents are presented in Table 6.1.

Table 6.1. Characterization of the influent leachates treated in SBR-3.

	Leachate	**CORSA**			**RECIRCULATION CORSA**			**XIXONA**		
	Days	0-133			133-153			153-end		
Parameter	**Units**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
NH_4^+-N	$mgN\cdot L^{-1}$	551.5 ± 49	487.4	618.6	510.8 ± 18	463.3	521.7	472.3 ± 39.9	424.3	537.6
NO_2^--N	$mgN\cdot L^{-1}$	568.1 ± 28	505.5	617.9	556.3 ± 34	522.3	597.0	530.4 ± 54.4	437.7	609.1
NO_3^--N	$mgN\cdot L^{-1}$	19.9 ± 19	8.5	76.1	100.1 ± 51	70.6	234.7	130.6 ± 70.3	25.0	197.8
Sol. COD	$mgO_2\cdot L^{-1}$	997.5 ± 357.3	595.9	1688.6	812.1 ± 210	665.3	1053.4	2923.1 ± 892.2	1859.8	3898.7
Tot. COD	$mgO_2\cdot L^{-1}$	1071.7 ± 357	707.9	1788.6	922.7 ± 183	797.3	1133.4	3051.1 ± 977.4	1969.8	4168.7
TOC	$mgC\cdot L^{-1}$	300.1 ± 52.0	241.4	360.5	215	-	-	1533.2 ± 278.0	1274	2003
IC	$mgC\cdot L^{-1}$	216.7 ± 25.2	188.2	244.7	178	-	-	270.1 ± 106.7	110.2	406.1
Alkalinity	$mgCaCO_3$	1111.5 ± 63.9	1022.0	1224.0	987.7 ± 110	860.0	1052.0	1382.6 ± 387.3	992	1789
Tot. BOD_5	$mgO_2\cdot L^{-1}$	51.7	-	-	5.3	-	-	132.2	-	-
Tot BOD_{30}	$mgO_2\cdot L^{-1}$	>800	-	-	>800	-	-	>400		
Leachate	% (v/v)	58	-	-	58	-	-	100	-	-
EC	$mS\cdot cm^{-1}$	19.1	17.3	19.5	22.9 ± 5	19.3	26.4	28.6 ± 5.3	24.8	32.3

6.4 Results

The initial MLVSS concentration was set at 106 mg VSS·L^{-1}.The pilot-scale anammox SBR was directly fed with leachate (preconditioned in a pilot-scale PN-SBR) coming first from Corsa and second from Xixona landfill sites.

The leachate was initially diluted with tap water to reduce the leachate matrix effects. According to the results obtained in Chapter 5 (lab-scale anammox SBR treating diluted leachate), it was decided to start-up the anammox pilot-scale SBR treating an influent with a leachate content of about 58% (close to the minimum dilution applied in the lab-scale SBR).

This dilution concluded with an influent conductivity of about 19.3 mS·cm^{-1}. This value was higher than the conductivity measured in the anammox enrichment (Chapter 4), which was between 7 and 10 mS·cm^{-1}. Nevertheless, according to Kartal *et al.* (2006) there is a possible adaptation of anammox bacteria from freshwater to high salinity by a step-wise increase of the conductivity from 10 to 20 mS·cm^{-1}. Thus, the initial conductivity applied in the pilot-scale SBR should be tolerated by the seeding anammox biomass.

6.4.1 SBR performance

The results of the start-up of the pilot-scale anammox SBR are presented in Figure 6.3. The NLR and NDR of the process are shown in Figure 6.3-A, together with the HRT evolution during the experimental period. Figure 6.3-B presents the influent nitrite to ammonium molar ratio. Finally, the concentration of the nitrogen species in the influent and the effluent of the process is depicted in Figures 6.3-C and D, respectively. The evolution of the effluent conductivity is also presented.

The dashed lines in the figures show the important changes during the pilot-scale anammox SBR operation. The short dashed line indicates the change of the cycle duration, from 24h to 8h. The changes of landfill leachate (Corsa; recycled-Corsa and Xixona) are indicated in all figures by vertical long dashed lines.

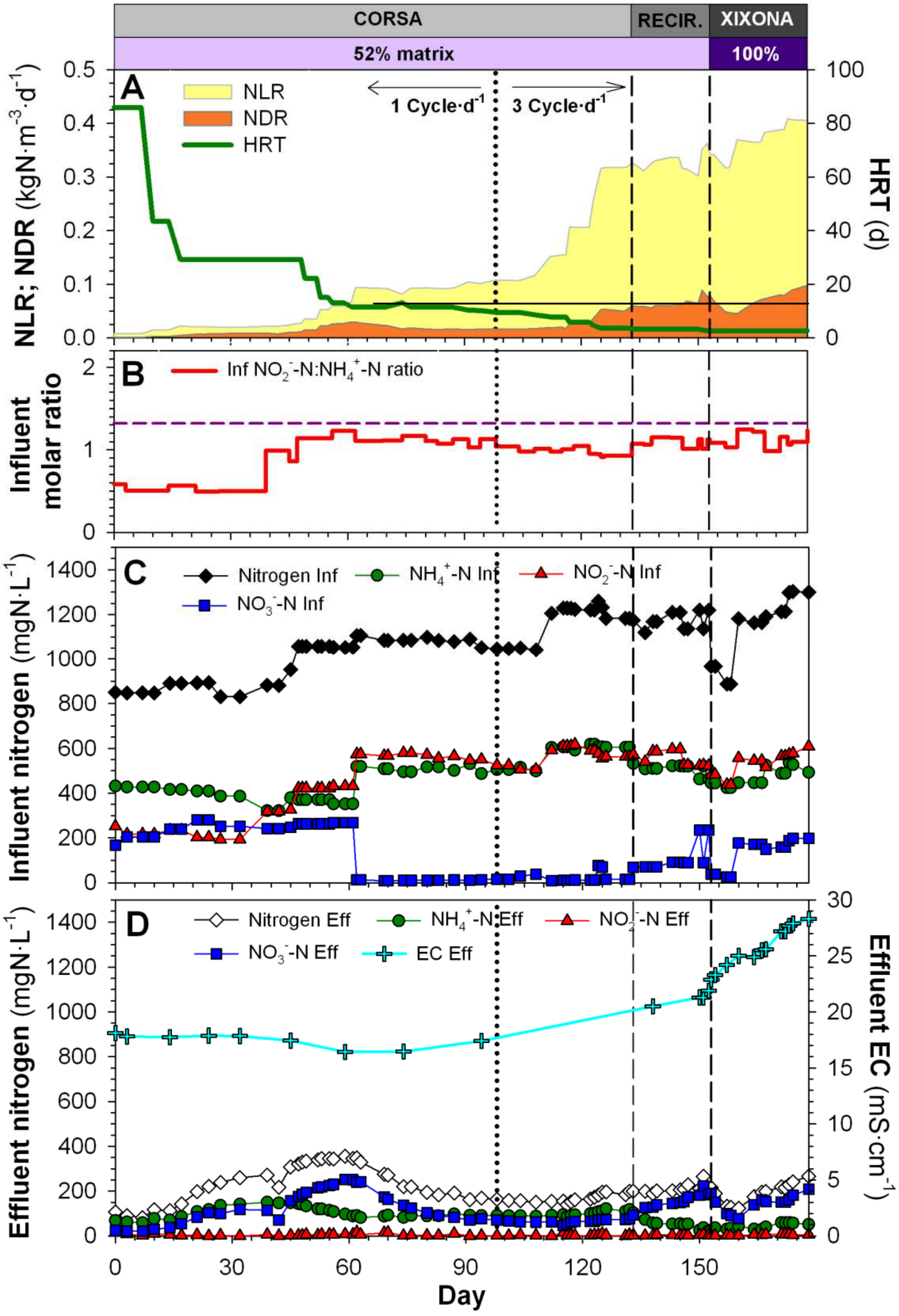


Figure 6.3. N-removal performance in anammox SBR. A: NLR, NDR and the HRT. B: Influent nitrite to ammonium molar ratio. C-D: Influent and effluent nitrogen concentrations, respectively.

The initial NLR was set at 0.007 kg $N\cdot m^{-3}\cdot d^{-1}$ due to the low initial anammox biomass used as inoculum (i.e., 106 mg $VSS\cdot L^{-1}$ in a maximum reactor volume of 400 liters) and was increased taking the effluent nitrite levels as control parameter, according to the experiences described in chapter 4 and 5. Thus, the minimum water volume of the reactor was initially set at 367.2 liters treating 4.32 $L\cdot cycle^{-1}$ (VER, 1%), and this volume was progressively increased by including several feeding phases equally distributed throughout the reaction phase. From the starting point to day 60, the leachate composition was 431 mg NH_4^+-$N\cdot L^{-1}$ and 251.6 mg NO_2^--$N\cdot L^{-1}$ with an important nitrate concentration of 167.6 mg NO_3^--$N\cdot L^{-1}$ (an initial TN of 850.5 mg $N\cdot L^{-1}$; Figure 6.3-A). The initial nitrite to ammonium ratio applied (0.6 mole NO_2^--N· $mole^{-1}$ NH_4^+-N) was selected as a prevention of nitrite accumulation while the nitrate concentration prevented the sulfate reduction activity during the start-up period. Since the system operated under nitrite limitation, ammonium had accumulated up to 149 mg NH_4^+-$N\cdot L^{-1}$ (Figure 6.3-D). In this way, on day 30, the nitrite to ammonium ratio was manually adjusted from 0.60 to 0.98. On day 47, because of the low effluent nitrite concentration observed, the influent ratio was increased to 1.1 mole NO_2^--$N\cdot mole^{-1}$ NH_4^+-N.

During only two months of operation (day 60), the NLR of the reactor was increased from 0.007 to 0.07 kg $N\cdot m^{-3}\cdot d^{-1}$ and the system presented clear anammox activity (i.e. high ammonium removal and pH up-trend during cycles, according to chapter 4). At this point, the influent nitrate concentration was reduced to 12.6 mg NO_3^--$N\cdot L^{-1}$ and the nitrite and ammonium levels increased to 519.3 mg NH_4^+-$N\cdot L^{-1}$ and 574.3 mg NO_2^--$N\cdot L^{-1}$, respectively, with a TN of 1106.2 mg $N\cdot L^{-1}$ (Figure 6.3-C). In order to increase the NLR, the minimum reactor volume was reduced from 367.2 to 345.2 liters by applying a VER of 14%. From day 98, the increased anammox activity concluded with a cycle change from 24 to 8 hours cycle as described above.

From day 133 to day 152, the effluent from the pilot-scale anammox SBR was recycled with external nitrogen additions (i.e., recycled Corsa leachate). On day 153, the reactor was fed again with effluent from the PN-SBR, but in this case with leachate from the Xixona landfill and without any dilution applied (i.e., influent with 100% leachate). From this point, the new leachate from Xixona was characterized by a higher conductivity (29.6 $mS\cdot cm^{-1}$, according to Table 6.1) than the 20 $mS\cdot cm^{-1}$ treated up to that day. Nevertheless, no negative effects on the reactor performance were detected, and stable operation with a progressive increase of the NLR was maintained until the end of the study, reaching a final NLR of 0.4 kg N·m^{-3}·d^{-1}.

During the entire experimental period, the effluent nitrite concentration was maintained at close to zero (< 4.3mg NO_2^--N·L^{-1}), while the ammonium excess was accumulated in the reactor. Consequently, the effluent ammonium concentration ranged between 21.9mg NH_4^+-N·L^{-1} and 150.7mg NH_4^+-N·L^{-1}, with an average of 84.3 ± 33.6mg NH_4^+-N·L^{-1}. The dynamics of the effluent nitrate concentrations were conditioned to the variations of the influent nitrate concentration.

The total nitrogen removal efficiency during the final period with 8-hour cycles was up to 89.4% due to the accumulation of ammonium and nitrate in the system. Nevertheless, the nitrite removal efficiency during this period was 96.3 ± 2.1%. The maximum values of 0.18 kg NH_4^+-N·m^{-3}·d^{-1} and 0.22 kg NO_2^--N·m^{-3}·d^{-1} for ammonium and nitrite, respectively, at the end of the study prove the successful compilation of the start-up of this pilot-scale anammox reactor.

6.4.2 Anammox Biomass

Homogeneous conditions of the mixed liquor inside the pilot-scale anammox reactor were achieved by both internal recirculation and periodic gas sparging. This also allowed the presence of a turbulent flow that kept the granules suspended.

In spite of the initial inoculation of anammox biomass with a resulting VSS of 106 mg·L^{-1}, the measured VSS during the whole operation period was maintained as low as 44.6 mVSS·L^{-1}. Such low values indicated the attachment growth of anammox biomass to the internal surfaces of the reactor and recycling tubes.

Figure 6.4 shows images of the biomass in the different forms that can be found in the reactor: forming aggregates (Figure 6.4-A) and fixed on the internal walls of the recycling tubes (Figure 6.4-B). Figure 6.4-A shows a filter with anammox aggregates after the filtration of a mixed liquor sample collected from the purge of the reactor under completely homogeneous conditions and maximum reactor volume.

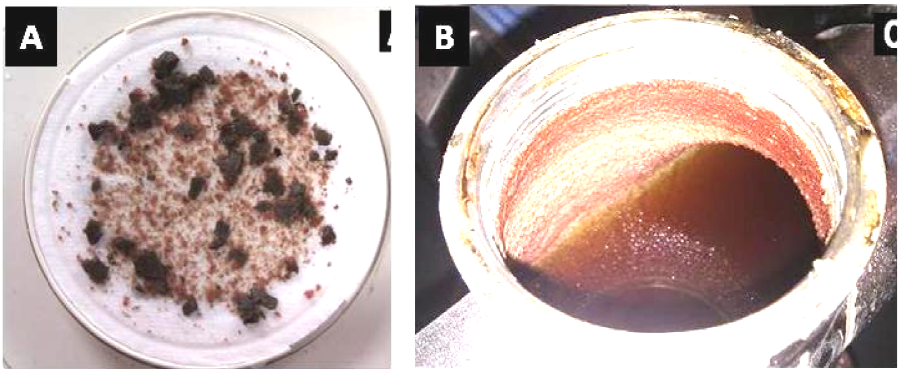


Figure 6.4. Pictures of the anammox biomass from the pilot-scale reactor. A: filter with aggregates and granules collected in the recirculation. B: Probe-hole showing the biomass attached in the internal parts of the tubes.

Figure 6.4-A shows different particle sizes. Large aggregates are probably biomass recently detached from the reactor walls and compact granules around 1mm diameter. Thus, because of the high amount of attached biomass, the mixed liquor VSS content cannot be used as a tool for monitoring the biomass growth in the early stages of operation.

6.4.3 Effect of the COD of leachate

As observed in the lab-scale anammox SBR, part of the COD content of the landfill leachate was removed in the pilot-scale anammox reactor. The readily biodegradable organic matter fraction of the raw leachate was removed in the PN-SBR. Thus, the influent leachate to the anammox reactor should be free of organic substrate for denitrification. However, the leachate contains an important amount of slowly biodegradable organic compounds that can be degraded and used in the anammox reactor due to the high temperature and HRT. Moreover, the effluent of the PN-SBR contained an important amount of VSS (400-800 mg VSS·L^{-1}) during some periods (mainly the first 60 days of operation) due to low settling properties of the biomass during the settling phase. The evolution of the organic matter content in the pilot-scale reactor is shown in Figure 6.5.

During the first 60 days, the leachate from Corsa presented a high amount of particulate COD (up to 1200 mg O_2·L^{-1}) resulting in a total COD of about 3100 mg

$O_2 \cdot L^{-1}$. This particulate organic matter was mainly from PN-SBR biomass and was considered to be slowly biodegradable. The influent particulate COD was practically eliminated on day 60 when the presence of VSS in the effluent of the PN-SBR was reduced. The soluble COD was considered mainly non-biodegradable, but presented an important fraction of slowly biodegradable organic matter. This can be clearly observed from Figure 6.6, where the effluent COD starts from 480 mg $O_2 \cdot L^{-1}$ and increases to 2000 mg $O_2 \cdot L^{-1}$ because of the initial dilution effects (i.e. the pilot scale anammox SBR was started up with a full volume of leachate matrix at 480 mg $O_2 \cdot L^{-1}$).

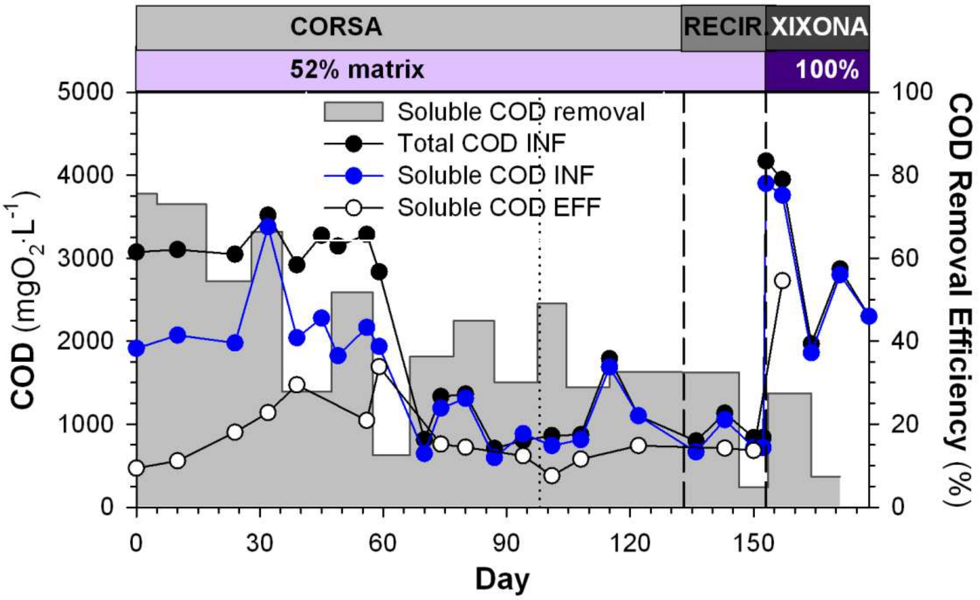


Figure 6.5 Organic matter presence in the pilot-scale anammox SBR and soluble COD removal efficiency during the study.

Moreover, the COD removal efficiency was dependent on the HRT. As can be observed in Figure 6.5, the soluble COD removal efficiency was higher at the beginning of the study when the HRT presented the higher values. The high HRT allowed the degradation of the slowly biodegradable OM. In this sense, the leachate from Corsa presented a BOD_5 of around 50 mg $O_2 \cdot L^{-1}$, but the BOD_{30} was always above 800 mg $O_2 \cdot L^{-1}$. On day 62 the particulate COD content of the leachate practically disappeared, with the soluble COD being almost equal to the total COD. From day 62 on, during the operation with Corsa leachate the soluble COD efficiency remained around 32%. Only at the end of the recycled-Corsa period (around day 145) was the COD removal very low (5%) due to the use of the effluent to feed the pilot-scale anammox reactor again.

The COD content presents a sudden increase of up to 4100 mg $O_2 \cdot L^{-1}$ on day 153 when the influent was changed to Xixona leachate. The BOD_5 of this leachate (132.2 mg $O_2 \cdot L^{-1}$) was higher than in leachate from Corsa, but the low HRT of 2.9 days achieved during this operational period limited the soluble COD removal efficiency to below 30%. The average total COD removal in the pilot-scale anammox SBR during the entire experimental period was 36.0 ± 21.0 mg $O_2 \cdot L^{-1}$.

Thus, the HRT and the temperature of the reactor (35ºC) favored the hydrolysis of the organic matter that released the carbon source necessary for a possible heterotrophic denitrification. Again, this reactor presented heterotrophic denitrifiers coexisting with anammox bacteria activity. The relative contribution of both autotrophic and heterotrophic pathways was calculated according to the method presented in chapter 5 and is shown in Figure 6.7.

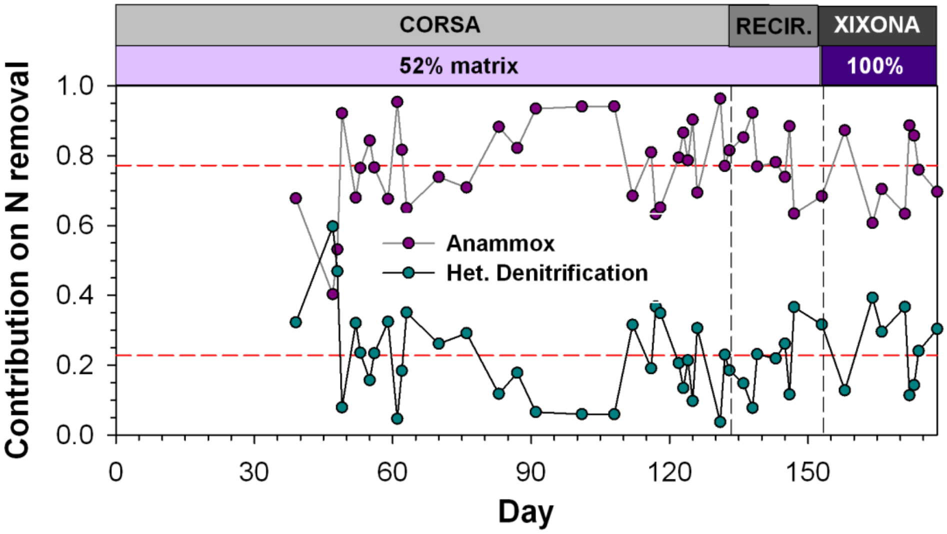


Figure 6.6. Fraction of nitrogen removed through anaerobic ammonium oxidation and heterotrophic denitrification pathways in the pilot-scale SBR. Dashed lines indicate the average values.

The results show the dominance of the anammox process, which was responsible for the 77.2 ± 12.0% of the nitrogen removal in the system. Thus, despite the changes of the influent content of organic matter, heterotrophic denitrifying activity was always limited in this reactor.

6.5 General discussion

Anammox bacteria have been detected in several full-scale plants with biological nitrogen removal to treat urban landfill leachate. In most cases, anammox bacteria are present in very low concentrations and can be detected only by molecular techniques (Yapsakli *et al.* 2011), since the optimal conditions for anammox growth are not applied. This spontaneous presence of anammox bacteria in biological treatments of landfill leachate indicates that the extreme conditions of the leachates can be tolerated by anammox microorganisms. However, the start-up of new anammox reactors to treat high nitrogen content leachates is still a challenge. The work of Van der Star *et al.* (2007) is only reference to the start-up of an anammox reactor integrated in a two-step partial nitritation-anammox process. There are a limited number of full-scale plants treating landfill leachate in a single aerated reactor in processes based on the CANON concept (Siegrist, *et al.*, 1998; Wang *et al.*, 2010, among others). In these cases, anammox bacteria was detected after long-term operation due to important nitrogen losses, but such processes were not started up to develop anammox. In this chapter we elucidate a strategy to start-up a pilot-scale anammox reactor for the treatment of urban landfill leachate, based on the control of the effluent nitrite concentration and the adaptation of the biomass to the leachate matrix.

In this chapter it has been proven that anammox biomass coming from an anammox enrichment fed with mineral medium can be adapted to the landfill matrix. The previous experience described in chapter 5 revealed that the anammox biomass was sensitive to drastic changes of the leachate, but its tolerance to the leachate matrix increased over time. In this sense, the initial conditions applied in the pilot-scale anammox SBR were fixed on these experiences. The dilution of the leachate during the first period of operation with low activity in the reactor ensured rapid adaptation of the biomass to the new conditions of the leachate and allowed the growth of bacteria. Thus, in spite of using a low amount of biomass to seed the reactor, the NLR could be rapidly increased from 0.007 to 0.4 Kg $N \cdot m^{-3} \cdot d^{-1}$.

The use of a low influent nitrite to ammonium ratio prevents the possibility of rapid nitrite accumulations, especially during the period with very low NLR. The progressive increase of the nitrite fraction reduces the nitrite limitation when the treatment capacity of the system increases and avoids substrate limitation for anammox growth. The literature reports other experiences about the start-up of anammox reactors for the treatment of landfill leachates, but only at lab-scale processes. Xu *et al.* (2010) started-up a single-step process for partial nitritation-anammox in a 3-litre lab-scale SBR. To start-up the process, they used mineral medium with up to 280 mg NH_4^+-N·L^{-1} during 85 days, in order to enrich the reactor with anammox biomass. Then, they moved to an influent with 20% of leachate until 100% of leachate was treated after 209 days of operation, with 1450 mg NH_4^+-N·L^{-1}. Similarly, Liu *et al.* (2010) started-up an 8.5-litre lab-scale anammox reactor integrated in a two-step partial nitritation-anammox process. They also used synthetic wastewater to enrich the reactor for 260 days until an NLR of 0.59 kg N·m^{-3}·d^{-1}. Then diluted leachate with up to 310 mg NH_4^+-N·L^{-1} and 420 mg NO_2^--N·L^{-1} was treated reaching a NLR of 0.91 N·m^{-3}·d^{-1} at the end of the study. The use of mineral medium is feasible for the start-up of lab/pilot-scale reactors, but is too expensive for full-scale application. Liang and Liu (2008) reported the start-up of a 36-liter anammox reactor treating the diluted leachate after a PN process. They increased the NLR from 0.06 to 0.11 kg N·m^{-3}·d^{-1} in 180 days, treating an influent with a TN content of up to 1896 mgN·L^{-1}. However, the nitrogen removal efficiency was limited to 64% and the ammonium removal was lost when the NLR was increased above 0.12 kg N·m^{-3}·d^{-1}.

Table 6.2. Comparison of the results of this work with other start-up studies**.**

			REFERENCE			
	Parameter	**Units**	**Liang and Liu, 2008**	**Xu et al., 2010**	**Liu et al., 2010**	**This Study**
PROCESS	**Steps**		2	1	2	2
	Reactor		FBBR	SBR	UASB	SBR
	Volume	Liters	36	3	8.5	400
MINERAL MEDIUM	**Application**	Days	0	85	260	0
	TN inf	mgN·L^{-1}	-	56-280	370	-
LEACHATE	**Application**	Days	180	124	69	178
	NH-N inf	mgN·L^{-1}	506-885	1320-1451	180-310	423.3-618.6
	NO-N inf	mgN·L^{-1}	441-1011	-	190-420	437.7-617.9
	NLR	kgN·m^{-3}·d^{-1}	0.06-0.11	0.96	0.59-0.91	0.007-0.4
	Leach. Content	%	100	20-100	50	58-100

All these works reflected the difficulty of a successful start-up of anammox reactors for the treatment of leachate. In our work, the reactor was directly fed with leachate and a stable operation was maintained until the end of the study, in spite of changing the influent conditions. An important increase of the NLR from 0.007 to 0.4 kg N·m^{-3}·d^{-1} was obtained, with nitrogen removal efficiencies close to

90%. The results prove the feasibility of a rapid start-up of anammox reactors for leachate treatment.

6.6 Conclusions

The final conclusions obtained in this chapter are listed below:

- A rapid start-up of a pilot-scale anammox SBR to treat landfill leachate was successfully carried out. The start-up strategy tested in this chapter was planned in view of a future scale-up of the process to the industrial scale. It has been proven that the dilution of the influent is a good option to adapt the biomass from freshwater to the leachate conditions
- The conductivity of the leachate is an important parameter to consider during the start-up. Nevertheless, increments of the leachate conductivity up to 10 $mS \cdot cm^{-1}$ can be tolerated by anammox biomass.
- An initial concentration of VSS of around 0.1 $gr\ VSS \cdot L^{-1}$ was viable to deal with the start-up of the pilot-scale reactor. Despite the initial loading rate set at only 0.007 $kg\ N \cdot m^{-3} \cdot d^{-1}$, the removal capacity of the system increased rapidly to 0.36 $kg\ N \cdot m^{-3} \cdot d^{-1}$ in less than 6 months.
- The biomass from the anammox enrichment was adapted to leachate matrix in less than 153 days, when an influent containing 100% of leachate (v/v) was treated in the pilot-scale SBR.
- The use of PN-SBR effluent with a nitrite to ammonium molar ratio below 1.32 allowed the prevention of nitrite accumulations in the system during the first days with the lowest nitrogen removal capacity
- The previous PN process influenced the presence of biodegradable OM in the influent of the anammox process. The results obtained reported an important fraction of slowly biodegradable organic matter in the leachate, which can be used by heterotrophic bacteria. In this sense, higher hydraulic retention times favored the removal of COD in the system.
- The presence of denitrifying activity in the system was high and accounted for about 23% of the nitrogen removal in the reactor. This fact proves the importance of monitoring the contribution of heterotrophs to avoid the out-competence of anammox bacteria if enough carbon sources are

available, since it is difficult to determine the fraction of the influent COD subjected to removal.

- This chapter demonstrates the feasibility of a treatment for landfill leachate based on a combined two-step partial nitritation-anammox process carried out in separate SBRs.

CHAPTER 7. GENERAL CONCLUSIONS

7.1 General Conclusions

The necessity of CESPA for an alternative process for the treatment of urban landfill leachates concluded in several research projects for the development of the PANAMMOX® process. This process is a two step combined partial nitritation-anammox treatment performed in two SBR. After the implementation of the partial nitritation in a pilot-scale PN-SBR obtaining a stable nitritation of the 57% of the incoming ammonium with step-feed strategy, the next step was the treatment of the effluent leachate in an anammox SBR.

This thesis deals with the treatment of mature landfill leachates by the anammox process, as the compilation of the second step of the global PANAMMOX® process. The results of the study demonstrate the feasibility of this technology for the treatment of mature landfill leachates. The work covered initial studies with the anammox enrichment, to study the characteristics of the anammox granules and the key operational parameters and evolved then from the lab-scale studies to test firstly the process, to a successful start-up of a pilot-scale anammox SBR treating leachate.

7.1.1 Start-up of anammox SBR to treat leachate

The conductivity of the leachate is an important paramenter to take into account for the start-up. Mature landfill leachates present extremely high conductivities and this parameter must be adjusted to start-up a reactor with biomass adapted to freshwater conditions, such as mineral medium. Is has been observed that increments up to 10 $mS \cdot cm^{-1}$ can be tolerated by the anammox biomass without negative impact and, therefore, it is possible to apply an initial conductivity around 20 $mS \cdot cm^{-1}$. The conductivity determines the initial dilution applied to the leachate to start-up the reactor. According to the experiences obtained with the lab-scale and the pilot-scale anammox SBR, the biomass from an anammox enrichment treating mineral medium requires around 60-80 days of adaptation to the landfill matrix before the SAA can increase. Similar adaptation periods were obtained with initial influent leachate contents set at 10% or 60%.

The application of a low influent nitrite to ammonium molar ratio (around 0.6) avoids nitrite accumulations in the reactor during the first stage. A progressive increase influent nitrite content can be applied when the anammox acitivity increase in order to avoid severe nitrite limitation. The operation of with influent nitrite to ammonium molar ratio close to 1 reduces the risk of fast nitrite accumulations in the system and the resulting SAA inhibition, but concludes in a lower effluent quality, with higher ammonium content.

It is possible of starting-up the anammox process with low initial VSS concentration (106 mg $VSS \cdot L^{-1}$ in this study) by applying a low NLR. This proves that low amounts of anammox biomass can be used to start-up new anammox reactors and there is no need to use inoculums adapted to landfill leachate.

7.1.2 Treatment of landfill leachate in anammox SBR

The feasibility of treating mature landfill leachate coming from a partial nitritation process was tested firstly in lab-scale experiments and further applied to pilot-scale. In both cases it was proven that high nitrogen removal efficiencies can be obtained with the application of the anammox process to treat this kind of wastewater.

The landfill leachates are characterized by a complex matrix with a highly variable composition. The anammox biomass not adapted to landfill leachate (i.e. coming from an anammox enrichment feed with synthetic wastewater) present a rapid and severe activity loss when is exposed to the leachate matrix. This effect is lower with diluted leachate and therefore, un-acclimated anammox biomass can be maintained active treateing diluted leachate. The results from the lab-scale experiments indicated the possibility of acclimatizing the anammox biomass to landfill lechate by applying a progressive reduction of the leachate dilution. The study at lab-scale also demonstrated the feasibility of treating leachate from different origins and, therefore, with different composition. The tolerance of the biomass to the leachate matrix increases with time, but sudden changes of the leachate characteristics results in a period of adaptation with a decrease of the SAA.

The pilot-scale experiments demonstrated that it is feasible to treat an influent containing leachate coming from the PN-SBR without previous dilution, proving that autotrophic nitrogen removal from mature landfill leachate is possible in a two step partial nitritation-anammox treatment in SBRs (PANAMMOX® process).

All the landfill leachates tested in this study were characterized by a high COD content with an important fraction of slowly biodegradable organic matter. The readily biodegradable substrate is removed in the previous PN-SBR. Both lab-scale and pilot-scale experiences, demonstrated that the presence of active heterotrophic bacteria coexisting in the anammox reactor is increased when treating landfill leachate. Despite of being mainly slowly biodegradable organic matter, the presence of organic carbon source in leachate allowed significant contributions of the heterotrophic route on nitrogen removal. This allowed better effluent quality, with lower nitrate and COD content.

The presence of a limited heterotrophic denitrifying bacteria population benefits the process but could compromise the anammox activity if more carbon source was available. The organic matter content in leachate depends on several factors and is variable. Therefore, the monitoring of the heterotrophic activity in the reactor can be used to predict possible anammox out competition.

High nitrogen removal efficiencies close to 95% can be obtained in the anammox reactor working with an influent nitrite to ammonium ratio close to 1.32. This ratio allows optimal nitrogen removal efficiencies but make the system more susceptible to nitrite accumulations.

The effluent leachate treated in the anammox reactor contains mainly nitrate and some ammonium, which concentration depends on the operational influent nitrite to ammonium molar ratio. Due to the common high conductivity values of landfill leachates, the total nitrogen and COD concentrations still above the discharge limits and the possible presence of toxics, this effluent cannot be directly versed to the receiving water bodies and needs to be forward treated. When possible, this effluent stream can be treated in an urban WWTP. Since the nitrogen content of the leachate has been removed, the contribution of leachate on the NLR of the plant is not significant and the nitrogen removal is not compromised. The contaminants of the leachate are removed if possible or, at least, diluted in the main stream of the WWTP. Reverse osmosis (RO) is a more effective solution, but also the most expensive due to the high energy consumption. However, the cost for the management of the RO waste product is reduced if nitrogen is previously removed. This solution can be used when there is no possibility to verse the leachate into a sewage system to a WWTP.

The CLONIC project (LIFE03 ENV/000140, 2003-2007) is part of the background of this thesis and aimed to study the application of partial nitritation-anammox combined with a thermal drying process to treat landfill leachates. The treatment of the anammox reactor effluent leachate by a thermal drying process offers several advantages compared with RO. The waste of the process is a solid waste (mainly salts) in contrast with the important amounts of concentrated streams produced in a RO. Moreover, the energy requirement to heat up the leachate can be solved by using the biogas produced in the landfill site. According to Vives *et al.* (2007), the cost per liter of leachate treated by this combined treatment is about 0.0133 $€·L^{-1}$, lower than the 0.0257 $€·L^{-1}$ of a conventional process with heterotrophic denitrification.

7.1.3 Key parameters for anammox SBR operation

One of the main concerns working in anammox is the nitrite concentration in the reactor, especially when treating an influent with high nitrite content in a SBR. The effect of nitrite accumulations is highly dependent on the time of exposition. In this sense, the impact of punctual nitrite concentration shocks is low with short exposure time (i.e. only a few hours), even at nitrite concentrations up to 500 mg NO_2^--N·L^{-1} (less than 40% of activity loss). However, after one day of exposure the SAA is reduced by 50% at nitrite concentration around 150-200 mg NO_2^--N·L^{-1}. Nitrite inhibition is partially reversible and the activity of biomass affected by severe nitrite inhibition can be partially recovered by washing the biomass to remove nitrite.

The experience obtained with the long term operation demonstrated that it is possible to deal with punctual nitrite accumulations up to 100 mg NO_2^--N·L^{-1} by giving extra time to the process with a longer reaction phase. Short term high nitrite accumulations are not critical if rapid measures are applied to solve the problem (i.e. washing the nitrite out). However, an effluent nitrite concentration sustained around only 30 mg NO_2^--N·L^{-1} during several days results in a reduction of the SAA.

The pH profiles are a simple and effective indicator of the process performance. In this sense, in reactors with a reaction phase at the end of the cycle a sustained pH up-trend until the settling phase is indicating a possible substrate accumulation due to a NRR lower than the NLR applied. On the contrary, stable pH during the entire reaction phase reveals that the NLR can be increased.

Treating landfill leachate, drastic changes in the leachate characteristics caused a negative impact on the process performance and implied the need of a re-adaptation of the anammox biomass to the new leachate matrix. In this sense, the presence of a previous tank (before the PN-SBR) to buffer the variation of the influent can be a good solution to prevent perturbations caused by sudden variations.

7.2 Future perspectives

More research efforts are necessary in this field to finally move the application of the anammox process to treat landfill leachate to full-scale. In this sense, a study among different landfill sites is necessary to determine the optimal landfilling centers for the application of the process according to the leachate characteristics. In the PN-SBR process leachates with extreme ammonium content and conductivity were successfully treated. The limitations of the anammox step must be explored in more depth in order to determine the range of leachates that can be treated and the seasonal variability of the leachate composition that can be assumed by the treatment.

The experiences acquired with the lab-scale and pilot-scale experiments will be applied to the design and start-up of a full-scale PANAMMOX® plant for the treatment of landfill leachate during a 3 years project in collaboration with CESPA.

The identification of the components in the leachate matrix that partially reduce the SAA could give an additional criterion to determine the viability of the process among different landfill sites.

In another way, other parameters relied to the process, such as the green house gases emissions, will be investigated in the future. Literature reports few studies about the nitrogen oxides emissions in partial nitritation-anammox process, most of them carried out in plants treating sludge digestion returns. Thus, the effect of the process operation and the landfill leachate characteristics on NO and N_2O emissions needs to be investigated.

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Wang, C. C., M. Kumar, C. J. Lan and J. G. Lin (2011a). "Landfill-leachate treatment by simultaneous partial nitrification, anammox and denitrification (SNAD) process." *Desalination and Water Treatment* **32**(1-3): 4-9.

Wang, S. Y., D. W. Gao, Y. Z. Peng, P. Wang and Q. Yang (2004). "Nitrification-denitrification via nitrite for nitrogen removal from high nitrogen soybean wastewater with on-line fuzzy control." *Water Science and Technology* **49**(5-6): 121-127.

Wang, T., H. Zhang, F. Yang, S. Liu, Z. Fu and H. Chen (2009a). "Start-up of the Anammox process from the conventional activated sludge in a membrane bioreactor." *Bioresource Technology* **100**(9): 2501-2506.

Wang, T., H. Zhang, D. Gao, F. Yang, S. Yang, T. Jiang and G. Zhang (2011b). "Enrichment of Anammox bacteria in seed sludges from different wastewater treating processes and start-up of Anammox process." *Desalination* **271**(1-3): 193-198.

Wang, Z., Z. Wu and S. Tang (2009b). "Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy." *Water Research* **43**(6): 1533-1540.

Wett, B. (2007). "Development and implementation of a robust deammonification process." *Water Science and Technology* **56**(7): 81-88.

Xiao, Y., G. M. Zeng, Z. H. Yang, Y. S. Liu, Y. H. Ma, L. Yang, R. J. Wang and Z. Y. Xu (2009). "Coexistence of nitrifiers, denitrifiers and Anammox bacteria in a sequencing batch biofilm reactor as revealed by PCR-DGGE." *Journal of Applied Microbiology* **106**(2): 496-505.

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Yang, J., L. Zhang, Y. Fukuzaki, D. Hira and K. Furukawa (2010). "High-rate nitrogen removal by the Anammox process with a sufficient inorganic carbon source." *Bioresource Technology* **101**(24): 9471-9478.

Yang, J., L. Zhang, D. Hira, Y. Fukuzaki and K. Furukawa (2011a). "High-rate nitrogen removal by the Anammox process at ambient temperature." *Bioresource Technology* **102**(2): 672-676.

Yang, J., L. Zhang, D. Hira, Y. Fukuzaki and K. Furukawa (2011b). "Anammox treatment of high-salinity wastewater at ambient temperature." *Bioresource Technology* **102**(3): 2367-2372.

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Yu, R., M. J. Kampschreur, M. C. M. Van Loosdrecht and K. Chandran (2010). "Mechanisms and specific directionality of autotrophic nitrous oxide and nitric oxide generation during transient anoxia." *Environmental Science and Technology* **44**(4): 1313-1319.

Yu, T., D. Li, R. Qi, S. T. Li, S. W. Xu and M. Yang (2011). "Structure and dynamics of nitrifier populations in a full-scale submerged membrane bioreactor during start-up." *Applied Microbiology and Biotechnology* **90**(1): 369-376.

Zhang, H. G. and S. Q. Zhou (2006). "Treating leachate mixture with anaerobic ammonium oxidation technology." *Journal of Central South University of Technology (English Edition)* **13**(6): 663-667.

Zhang, J., K. M. Fu, X. S. Cao, D. Li and X. Z. Meng (2009a). "Performance of CANON process in a sequencing batch biofilm reactor and influence of temperature." *Zhongguo Huanjing Kexue/China Environmental Science* **29**(8): 850-855.

Zhang, T., L. Ding and H. Ren (2009b). "Pretreatment of ammonium removal from landfill leachate by chemical precipitation." *Journal of Hazardous Materials* **166**(2-3): 911-915.

Zheng, P., F. M. Lin, B. L. Hu and J. S. Chen (2004). "Start-up of anaerobic ammonia oxidation bioreactor with nitrifying activated sludge." *Journal of Environmental Sciences* **16**(1): 13-16.

Zhou, S. and J. Yao (2010). "Rapid enrichment and acclimation of anaerobic ammonium oxidation by using activated sludge from a landfill leachate treatment plant." *Journal of Food, Agriculture and Environment* **8**(2): 1133-1137.

Curriculum vitae

Maël RUSCALLEDA BEYLIER

Surname(s):	RUSCALLEDA BEYLIER
Name:	MAËL
Date of birth:	28/06/1982
Address:	Laboratory of Chemical and Environmental Engineering (LEQUIA) Facultat de Ciències Campus Montilivi s/n 17071-Girona (Catalonia, Spain)
Land phone:	(+34) 972 183 249
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Training received

University Education

Current thesis in the Experimental Sciences and Sustainability PhD program for the University of Girona (2008-2011)

Project title: *Treatment of mature urban landfill leachates by anammox process*.

Supervisors: M. D. Balaguer and J. Colprim.

Master thesis in Water Science and Technology for the University of Girona (2006-2008)

Project title: *The anammox process combined with heterotrophic denitrification for the treatment of high nitrogen content landfill leachates*.

Supervisors: M. D. Balaguer and J. Colprim (January 2008).

Bachelor in Environmental Sciences for the University of Girona (2001-2006)

Project title: *Implementation of a system for the selective collection of municipal solid wastes in Girona (Spain)*.

Supervisors: A. Bonmatí and J. Comas (June, 2006).

Courses

The Second Course on Individual-based Modeling of Microbial Interactions & Processes Using iDynoMiCS

Lyngby (Denmark). 21th to 26th June, 2009. Organized by the Technical University of Denmark.

1st Summer School of the NOVEDAR-Consolider: "Model-based design, operation and control of urban WWTPs"

San Sebastian (Spain). 14th to 18th July, 2008. Organized by CEIT.

XIII course of confocal and spectral microscopy

Cerdanyola del Vallès (Spain). 25th to 28th February, 2008. Organized by the Universitat Autònoma de Barcelona (UAB).

Course of basic security in laboratories: good procedures, first aid, wastes and emergency plan

Girona (Spain). 5th February, 2008. Organized by the University of Girona.

Training Experience

Laboratory of Chemical and Environmental Engineering (LEQUIA), Institute of the Environment, University of Girona (2008-2011)

Predoctoral Scholarship of the Catalan Government, AGAUR, within the FI program (2010FI B2 00104).

CESPA-Gestión de Residuos SA (2007-2008)

Predoctoral Scholarship of the Catalan Government, AGAUR, within the FIE program (2006FIE 00035).

Laboratory of Chemical and Environmental Engineering (LEQUIA), Institute of the Environment, University of Girona (2006 -2007)

Collaborating Scholarship.

Scientific and technological activity

Participation in R&D&I projects founded by public bodies

Name of the project: **Tratamiento biológico de lixiviados mediante una nitrificación parcial y oxidación anaeróbica con el proceso PANI-SBR y ANAMOX**

Body where project took place: Universitat de Girona

Head(s) researcher(s): Jesus Colprim Galceran

Funding body or bodies: Ministerio de Industria, Turismo y comercio

Code according to the funding body: CIT-310000-2009-63

Start date: 2009, 2 years

Total amount: 129.716

Name of the project: **NIMOX - NItrificación parcial y oxidación anaeróbica mediante biomasa anamMOX del amonio procedente de los retornos de digestión de una EDAR urbana.**

Body where project took place: Universitat de Girona

Head(s) researcher(s): Jesus Colprim Galceran

Funding body or bodies: Ministerio de Educación y Ciencia (MEC)

Code according to the funding body: DEX-560620-2008-149

Start date: 2008, 3 years

Total amount: 173.820

Name of the project: **Nitritación parcial y oxidación anaeróbica del amonio de lixividados de vertederos mediante procesos PANI-SBR y Anammox**

Body where project took place: Universitat de Girona

Head(s) researcher(s): Jesus Colprim Galceran

Funding body or bodies: Ministerio de Educación y Ciencia (MEC)

Code according to the funding body: PET2006_0604

Start date: 2007, 3 years

Total amount: 115.366

Name of the project: **Nitritación parcil y oxidación anaeróbica del amonio de lixiviados de vertederos mediante processos PANI-SBR y Anammox**

Body where project took place: Universitat de Girona

Head(s) researcher(s): Jesus Colprim Galceran

Funding body or bodies: Ministerio de Educación y Ciencia (MEC)

Code according to the funding body: CIT-310200-2007-90

Start date: 2007, 2 years

Total amount: 114.250

Name of the project: **CLOsing the NItrogen Cycle from urban landfill leachate by biological nitrogen removal over nitrite and termal treatment (http://www.lifeleachate.com)**

Body where project took place: Universitat de Girona

Head(s) researcher(s): Jesus Colprim Galceran

Funding body or bodies: European Union (EU)

Code according to the funding body: LIFE03 ENV/E/000140

Start date: 2003, 5 years

Total amount: 230.701

Residencies in R&D&I centers

Department of Environment & Resources (DTU Environment), Technical University of Denmark (Lyngby, Denmark) (2009-2010).

Interactions between anammox and heterotrophic bacteria in biofilms and N_2O emissions in activated sludge processes. Supervision: Prof. B. F. Smets. September, 2009-April, 2010.

Assistance to conferences

1st Water Research Conference. Lisbon (Portugal), 11-14th April, 2010. Organized by IWA.

2nd IWA Specialized Conference on Nutrient Management in Wastewater Treatment Processes. Krakow (Poland), 6-9th September, 2009. Organized by IWA.

5th IWA Leading-Edge Conference and Exhibition on Water and Wastewater Technologies. Zurich (Switzerland), 1-4th June, 2008. Organized by IWA.

4th Sequencing Batch Reactor Technology Conference (SBR-4). Rome (Italy), 7-10th April, 2008. Organized by IWA.

CLONIC Final Workshop. Barcelona (Spain), 19-20th April, 2007. Organized by CESPA and LEQUIA.

ANAMMOX Workshop. State of the art. Barcelona (Spain), April 18th, 2007. Organized by LEQUIA.

Book chapters

Ruscalleda, M.; Ni, B.-J.; Sun, S.-P.; Pellicer-Nacher, C.; Wang, R.-C.; Balaguer, M.D.; Colprim, J.; Smets, B.F. (2011). Biological Nitrogen Removal from Domestic

Wastewater. 2nd Ed. Comprehensive Biotechnology. Elsevier B. V. (UK). ISBN: 978-0-444-53352-4

Lopez, H.; Ruscalleda, M.; Ganigué, R.; Puig, S.; Balaguer, M.D.; Colprim, J. (2008) Effects of low biodegradable organic matter on granular anaerobic ammonium-oxidizing bacteria.SIDISA 08 - International Symposium on Sanitary and Environmental Engineering.(Italy). ISBN 978-88-903557-0-7

López, H.; Puig, S.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2007). Enrichment and growth of granular anammox biomass to treat high nitrogen load wastewaters using SBR technology.CLONIC Final Workshop'07.1,pp. 82 - 90. (Spain). Legal deposit: B-12215-2007

Ganigué, R.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J.(2007). Start-up and operation of a Partial Nitritation-SBR (PN-SBR) treating urban landfill leachate to achieve a suitable influent for an anammox reactor.CLONIC Final Workshop'07.1,pp. 32 - 41. (Spain). Legal deposit: B-12215-2007

Journal papers

Ni, B.-J.; Ruscalleda, M.; Pellicer-Nàcher, C.; Smets, B. F. (2011). Modeling nitrous oxide production during biological nitrogen removal via nitrification and denitrification: a simple xtension to the general ASM descriptive models. *Environmental Science & Technology* **45**, pp. 7768 - 7776.

Mora, X.; Ruscalleda, M.; Gabarró, J.; Vilà, A.; Balaguer, M.D.; Colprim, J. (2011). Panammox process: Treatment of urban landfill leachate with high ammoniacal content by partial nitritation and Anammox process | [Panammox: Tratamiento de lixivados de vertedero de residuos sólidos urbanos con alto contenido amoniacal mediante nitritación parcial y Anammox]. *Tecnologia del Agua* **31**, pp. 38-45.

Ruscalleda, M.; Puig, S.; Mora, X.; Lopez, H.; Ganigué, R.; Balaguer, M.D.; Colprim, J. (2010). The effect of urban landfill leachate characteristics on the coexistence of anammox bacteria and heterotrophic denitrifiers. *Water Science and Technology*. **61**, pp. 1065 - 1071.

Ganigué, R.; Gabarró, J.; Lopez, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2010).Combining partial nitritation and heterotrophic denitritation for the treatment of landfill leachate previous to an anammox reactor. *Water Science and Technology*. **61**, pp. 1949 - 1955.

Ganigué, R.; Gabarró,J.; Sànchez-Melsió, A.; Ruscalleda, M.; Lopez, H.; Vila, X.; Colprim, J.; Balaguer, M.D. (2009). Long-term operation of a partial nitritation pilot plant treating leachate with extremely high ammonium concentration prior to an anammox process. *Bioresource Technology.***100**, pp. 5624 - 5632.

Ruscalleda, M.; Lopez, H.; Ganigué, R.; Puig, S.; Balaguer, M.D.; Colprim, J. (2008). Heterotrophic denitrification on granular anammox SBR treating urban landfill leachate. *Water Science and Technology*. **58**, pp. 1749 - 1755.

López, H.; Puig, S.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2008). Start-up and enrichment of a granular anammox SBR to treat high nitrogen load wastewaters. *Journal of Chemical Technology and Biotechnology*. **83**, pp. 233 - 241.

Ganigué, R.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J.Operational strategy for a partial nitritation-sequencing batch reactor treating urban landfill leachate to achieve a stable influent for an anammox reactor. *Journal of Chemical Technology and Biotechnology*. **83**, pp. 371.

Conference proceedings

Pellicer-Nacher, C.; Franck, S.; Ruscalleda, M.; Terada, A.; Smets, B. F. (2011). Who is Who? Assessing the microbial deiversity in wastewater treatment biofolms for completely autotrophic nitrogen removal. *IWA Biofilm conference 2011: Processes in Biofilms (IWA Biofilm2011)* (27th-30th October, Shangai, China). *Oral presentation.*

Gabarro, J.; Ganigue, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2011). Temperature effects on partial nitritation treating extremely high ammonium levels of landfill leachate. *Enzymology and ecology of the nitrogen cycle* (15th-17th September, Birmingham, UK). *Poster presentation.*

Vilà, A.; Ruscalleda, M.; Balaguer, M. D.; Colprim, J. (2011). Assessment of CFD and biological modelling with a multiphase Euler-Euler model for an anammox reactor. *8th IWA Symposium on Systems Analysis and Integrated Assessment (Watermatex 2011)* (20th-22th June, San Sebastian, Spain). *Oral presentation.*

Vilà, A.; Ruscalleda, M.; Balaguer, M. D.; Colprim, J. (2011). Modelling Anaerobic Ammonium Oxidation and Heterotrophic Denitrification applied to SBR Technology. *CAIP'2011 - 10º Congreso Interamericano de Computación Aplicada a La Industria de Procesos* (30th May-3th June, Girona, Spain). *Oral presentation.*

Vilà, A.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2011). Assesment of CFD and biological modelling with a multiphase Euler-Euler model for an Anammox reactor. *2nd National Young Water Professionals Conference*. (15th-17th June, Madrid, Spain). *Oral presentation*

Ruscalleda, M.; Seredynska-Sobecka, B.; Ni, B.-J.; Arvin, E.; Smets, B.F (2010). Characterizing the dissolved organic matter of anammox arocess by asing three-dimensional excitation-emission matrix fluorescence spectroscopy. *1st IWA Water Research Conference* (11th-14th April, Lisbon, Portugal). *Poster*.

Pellicer-Nacher, C.; Ruscalleda, M.; Terada, A.; Smets, B.F. (2010). Microbial community stratification in membrane-aerated biofilm reactors for completely autotrophic nitrogen removal. *1st IWA Water Research Conference* (11th-14th April, Lisbon, Portugal). *Poster*.

Ruscalleda, M.; Puig,S.; Mora, X.; López, H.; Ganigué, R.; Balaguer, M.D.; Colprim, J. (2009). The effect of urban landfill leachate characteristics on anammox bacteria and heterotrophic denitrifiers coexistence. *2nd IWA Specialised Conference.Nutrient Management in Wastewater Treatment Processes* (6th-9th September, Krakow, Poland). *Oral presentation.*

Ganigué, R.; Gabarró, J.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2009). Combining partial nitritation and heterotrophic denitritation for the treatment of landfill leachateprevious to an anammox reactor. *2nd IWA Specialised Conference.Nutrient Management in Wastewater Treatment Processes* (6th-9th September, Krakow, Poland). *Oral presentation.*

Ruscalleda, M.; López, H.; Ganigué, R.; Puig, S.; Balaguer, M.D.; Colprim, J. (2008). Heterotrophic denitrification on granular anammox SBR treating urban landfill leachate. *5th IWA Leading-Edge Conference and Exhibition on Water and Wastewater Technologies* (1st-4th June, Zurich, Switzerland). *Oral presentation*.

Ganigué, R.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2008). Partial nitritation-SBR treating landfill leachate, previous to anammox process. *4th Sequencing Batch Reactor Technology (SBR-4)* (7th-10th April, 2008, Rome, Italy). *Poster*.

López, H.; Puig, S.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2008). The pH control outcome as indicator of anammox SBR activity. *4th Sequencing Batch Reactor Technology (SBR-4)* (7th-10th April, 2008, Rome, Italy). *Poster*.

López, H.; Puig, S.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2007). Operational strategy of a partial nitritation-SBR (PN-SBR) treating urban landfill leachate to achieve a stable influent for an anammox reactor. *Nutrient*

Removal 2007: State of the Art (4th-7th March, Baltimore, United States of America). *Oral presentation*.

Ganigué, R.; López, H.; Ruscalleda, M.; Balaguer, M. D.; Colprim, J. (2007). Operational strategy of a partial nitritation-SBR (PN-SBR) treating urban landfill leachate to achieve a stable influent for an anammox reactor. *Nutrient Removal 2007: State of the Art* (4th-7th March, Baltimore, United States of America). *Oral presentation*.

López, H.; Puig, S.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2007). Enrichment and growth of granular anammox biomass to treat high nitrogen load wastewaters using SBR technology. *CLONIC Final Workshop '07. Closing the Nitrogen Cycle from Urban Landfill Leachate by Biological Nitrogen Removal over Nitrite and Thermal treatment. LIFE03 ENV/E/000140* (19th-20th April, Barcelona, Spain). *Oral presentation*.

Ganigué, R.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. (2007). Start-up and operation of a Partial Nitritation-SBR (PN-SBR) treating urban landfill leachate to achieve a suitale influent for an anammox reactor. *CLONIC Final Workshop '07. Closing the Nitrogen Cycle from Urban Landfill Leachate by Biological Nitrogen Removal over Nitrite and Thermal treatment. LIFE03 ENV/E/000140* (19th-20th April, Barcelona, Spain). *Oral presentation*.

Colprim, J.; López, H.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D. (2007). Condicionament dels retorns de processos de tractament de fangs de depuració a partir de procesos de Nitritació Parcial i Oxidació Anaeròbia de l"Amoni (Anammox). *III Jornades Tècniques de Gestió de Sistemes de Sanejament d"Aigües Residuals* (17th-18th October, Barcelona, Spain). *Oral presentation.*

In preparation

Ruscalleda, M.; Mora, X.; Balaguer, M.D.; Colprim, J. Start-up of a pilot-scale anammox SBR to treat landfill leachate. (*Journal, in preparation*).

Scaglione, D.; Ruscalleda, M.; Ficara, E.; Balaguer, M.D.; Colprim, J. Nitrite inhibition and recovery response of anammox granular biomass adapted to mineral medium and landfill leachate. (*Submitted to Journal of Hazardous Materials*)

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