Universitat de Girona

EFFECTS OF OPERATIONAL CONDITIONS ON THE PERFORMANCE OF A PARTIAL NITRITATION SBR TREATING HIGH NITROGEN LOADS

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PhD Thesis

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Author's contribution: Data monitoring and reactor performance. Writing the paper.

Abbreviations list

amoA	ammonium oxidation enzyme
anammox	anaerobic ammonium oxidation
AOB	ammonium oxidizing bacteria
bCOD	biodegradable organic matter
BOD	biochemical oxygen demand
BOD_5	biochemical oxygen demand (5 days)
BODu	biochemical oxygen demand ultimate (30 days)
COD	chemical oxygen demand
DO	dissolved oxygen
EUB	Eubacteria
FA	free ammonia
FISH	Fluorescence in situ hybridization
FNA	free nitrous acid
IC	inorganic carbon
MFC	microbial fuel cell
MLVSS	mixed liquor volatile suspended solids
nirK	nitrite reduction enzyme (K)
nirS	nitrite reduction enzyme (S)
NLR	nitrogen loading rate
NOB	nitrite oxidizing bacteria
nor	nitrate reduction enzyme
nosZ	nitrous oxide reduction enzyme
nox	nitrite oxidation enzyme
PCR-DGGE	polymerase chain reaction-denaturing gradient gel electrophoresis
PN	partial nitritation
PN-SBR	partial nitritation sequencing batch reactor
qPCR	quantitative polymerase chain reaction
sALR	specific ammonium loading rate
sNLR	specific nitrogen loading rate
sNPR	specific nitrite production rate
sNRR	specific nitrogen removal rate
sOLR	specific organic loading rate
sORR	specific organic removal rate
TKN	total kjeldahl nitrogen
TN	total nitrogen
TSS	total suspended solids
VSS	volatile suspended solids
WWTP	wastewater treatment plant

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El Dr. Maël Ruscalleda i Baylier, la Dra. Marilós Balaguer i Condom i el Dr. Jesús Colprim i Galceran, de la Universitat de Girona,

DECLAREM:

Que el treball titulat *Effects of operational conditions on the performance of a partial nitritation SBR treating high nitrogen loads*, que presenta en Jordi Gabarró i Bartual per a la obtenció de títol de doctor, ha estat realitzat sota la nostra direcció i que compleix els requisits per poder optar a Menció internacional.

I, perquè així consti i tingui els efectes oportuns, signo aquest document.

Dr. Maël Ruscalleda   Dra. Marilós Balaguer   Dr. Jesús Colprim

Girona, 29 de Maig de 2014

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Resum

El tractament biològic d'aigües residuals industrials que contenen altes concentracions de nitrogen (>1000 mg N L^{-1}) i baixa concentració de matèria orgànica biodegradable (bCOD) com són els lixiviats d'abocador és a dia d'avui un repte. El tractament convencional mitjançant nitrificació-desnitrificació d'aquest tipus d'aigües residuals industrials implica costos molt elevats degut a l'aeració i la necessitat de l'adició de bCOD externa. El procés de nitritació parcial (PN) combinat amb la oxidació anaeròbia d'amoni (anammox) resulta una alternativa més sostenible pel tractament biològic d'aquest tipus d'aigües. Els sistemes PN-anammox són tractaments totalment autotròfics que permeten reduir en un 40% els requeriments d'aeració i no necessiten l'adició de BCOD externa al procés. PN és el procés previ necessari per alimentar el posterior reactor anammox. L'objectiu del reactor de PN és el de produir un efluent apte pel reactor anammox. En el reactor PN, 57% de NH_4^+ contingut a l'afluent s'ha d'oxidar a NO_2^- per la obtenció de la proporció molar estequiomètrica NO_2^-:NH_4^+ de 1.32. L'eliminació de la BCOD és també un punt clau ja que els bacteris heterotròfics poden competir amb els bacteris anammox pel NO_2^- disponible.

El tractament de lixiviats d'abocador mitjançant el procés PN-anammox ha sigut demostrat anteriorment. No obstant, el reactor PN operava a alta temperatura (35°C) i es va prestar poca atenció a l'eliminació de bCOD. A més, les emissions d'òxid nitrós (N_2O) procedents de sistemes de nitrificació s'han convertit en un tema de preocupació. L'N_2O és un important gas d'efecte hivernacle amb un potencial 300 vegades major que el CO_2. Les condicions descrites que fan incrementar les emissions de N_2O són altes concentracions de NO_2^- i NH_4^+, fluctuacions d'oxigen dissolt i pH, baixa proporció de bCOD/N i també alta activitat dels bacteris oxidadors d'amoni (AOB). Totes elles presents en els reactors de PN.

La investigació presentada en aquesta tesi suposa canvis operacionals en el reactor PN com són la duració de les fases aeròbies i la baixada de la temperatura operacional en un reactor discontinu seqüencial (SBR) de PN tractant lixiviat d'abocador madur per reduir els requeriments energètics. La posada en marxa i operació del PN-SBR es va dur a terme en condicions completament aeròbies a 25 i 35°C i també incloent alimentacions anòxiques per promoure la desnitrificació heterotròfica via NO_2^- a 35°C. Especial atenció va ser posada en la qualitat de l'efluent, la selecció dels microorganismes usant tècniques moleculars i, per últim, la producció de N_2O en condicions anòxiques i aeròbies.

L'efluent del PN-SBR era adequat en tots els casos estudiats en termes de proporció molar NO_2^-:NH_4^+ i contingut en bCOD tot i el canvi de la temperatura operacional (25 i 35°C) i la inclusió d'alimentacions en condicions anòxiques. La posada en marxa i operació del PN-SBR tractant lixiviats d'abocador amb càrrega extrema de nitrogen (6 g N L^{-1}) va ser exitosament demostrada tant a 25°C com a 35°C. Un model cinètic es va implementar per obtenir informació sobre la reducció de l'activitat màxima dels AOB provocada per la concentració d'amoníac lliure (FA) i àcid nitrós lliure (FNA) així com també la limitació d'activitat deguda al HCO_3^-. En condicions plenament aeròbies a 35°C, l'activitat màxima dels AOB va ser reduïda per la combinació d'inhibició de FA i FNA i també la limitació de HCO_3^-. En canvi, en la operació del PN-SBR a 25°C els AOB eren parcialment inhibits per FNA i limitació de HCO_3^-.

En relació amb la producció de N_2O del PN-SBR, es va demostrar que la producció i posterior emissió de N_2O va ser de 3.6% del nitrogen total de l'afluent. La producció de N_2O va ser analitzada durant la operació del PN-SBR alternant alimentacions anòxiques amb fases aeròbies. L'N_2O va ser majoritàriament produït en condicions anòxiques (60%) degut a la desnitrificació heterotròfica incompleta. La velocitat anòxica de producció de N_2O era d'uns 10 mg N-N_2O $gVSS^{-1}$ h^{-1} mentre que l'aeròbia era d'uns 2 mg N-N_2O $gVSS^{-1}$ h^{-1}. Aquests resultats contrasten amb els resultats obtinguts per altres autors. Les condicions severes en termes de concentració de NH_4^+ i de NO_2^- i salinitat, van ser les causes majors per aquesta producció de N_2O. No obstant, la producció de N_2O del PN-SBR estava en el rang d'altres sistemes de PN-anammox tractant aigües residuals industrials.

Finalment, la selecció microbiològica obtinguda en el PN-SBR va ser avaluada mitjançant diferents tècniques moleculars com són *fluorescence in situ hybridization* (FISH), reacció en cadena de polimerasa (PCR), electroforesis amb un gel amb gradient desnaturalitzant (DGGE), PCR quantitativa i piroseqüenciació. En tots els experiments un filotip d'AOB va ser enriquit i estava ben adaptat a les condicions severes del PN-SBR. La comunitat heterotròfica era poc diversa però estava molt ben adaptada a les condicions del reactor. *Bacteroidetes* eren el grup de microorganismes dominants i estaven especialitzats en la degradació de bCOD lentament biodegradable. Una minoria del organismes heterotròfics tenia la capacitat genètica de desnitrificar completament l'N_2O a N_2.

En definitiva, els resultats d'aquesta tesi demostren que es va aconseguir exitosament el funcionament robust del PN-SBR tot i els canvis de temperatura i la inclusió de fases anòxiques. L'aplicació dels canvis realitzats en reactors d'escala real hauria de ser analitzada en termes de requeriments del reactor anammox posterior i la llei ambiental vigent (en el cas de l'N_2O). Els resultats inclosos a la tesi també demostren l'aclimatació dels microorganismes seleccionats en condicions severes i variables.

Summary

The biological treatment of industrial wastewater containing high nitrogen concentrations (>1000 mg N L^{-1}) and low biodegradable organic matter (bCOD), such as landfill leachate, is challenging these days. Conventional nitrification-denitrification of such wastewater implies high operational costs associated with aeration requirements and external bCOD supply. Partial nitritation (PN) combined with anaerobic ammonium oxidation (anammox) has become a more sustainable alternative treatment of this kind of industrial wastewater. PN-anammox systems are completely autotrophic treatments which reduce aeration requirements by 40% and eliminate adding an external bCOD source. PN is the preceding step to a subsequent anammox reactor whose objective is to acquire a suitable effluent to subsequently feed the anammox reactor. In the PN reactor, 57% of the influent's NH_4^+ must be oxidized to NO_2^- and reach a NO_2^-:NH_4^+ molar ratio of 1.32. The removal of the bCOD is also a key point because heterotrophic bacteria and anammox may compete for the available NO_2^-.

Landfill leachate treatment by the PN-anammox process has already been demonstrated some years ago, however, the PN reactor operates at a high temperature (35^{o}C) and little attention is paid to bCOD removal. Moreover, nitrous oxide (N_2O) emissions from nitrification systems have become a great concern. N_2O is an important greenhouse gas having 300 times more global warming potential than CO_2. Conditions leading to high N_2O emissions are described as high NO_2^- and NH_4^+ concentration, dissolved oxygen and pH changes, low bCOD/N ratio, as well as high ammonium oxidizing bacteria (AOB) activity. All of these conditions are typical in PN systems.

The research presented in this thesis involves changes to operational parameters to reduce energy requirements. These changes include aeration phase lengths and operational temperature decreases in a PN sequencing batch reactor (PN-SBR) treating mature landfill leachate. The PN-SBR startup and operation was assessed at fully aerobic conditions at 25 ^{o}C and 35^{o}C, as well as the implementation of anoxic feedings to promote heterotrophic denitrification via NO_2^- at 35^{o}C. Special attention was placed on effluent quality, microbial selection using molecular techniques and N_2O production under anoxic and aerobic conditions.

In all cases, the PN-SBR effluent was considered suitable despite the operational temperature (25 ^oC and 35^oC) and the inclusion of anoxic conditions in terms of both NO_2^- :NH_4^+ molar ratio and bCOD content. The startup and operation of the PN-SBR treating extremely high nitrogen concentration leachate (up to 6 g N L^{-1}) was successfully demonstrated at 25^oC and 35^oC. A kinetic model was implemented to obtain an AOB maximum activity decrease by free ammonia (FA) and free nitrous acid (FNA) concentrations as well as the HCO_3^- limitation availability. During fully aerobic conditions at 35^oC, maximum AOB activity was reduced through a combination of FA and FNA together with the HCO_3^- limitation. Besides, when the operational temperature was set at 25^oC, AOB were partially inhibited by FNA as well as HCO_3^- limitation.

N_2O production and later emission from the PN-SBR was demonstrated to be 3.6% of the influent's total nitrogen. N_2O production, including anoxic feedings and aerobic phases, was analyzed during the PN-SBR's performance. N_2O was mainly produced under anoxic conditions (60%) due to incomplete heterotrophic denitrification. The anoxic N_2O production rate was about 7 mg N-N_2O $gVSS^{-1}$ h^{-1}, whereas aerobic N_2O production was 2 mg N-N_2O $gVSS^{-1}$ h^{-1}. These results differ from previously reported studies. Stringent conditions, in terms of NH_4^+ and NO_2^- concentrations and salinity, were the main causes of N_2O production. Nevertheless, the N_2O production from the PN-SBR was in the same magnitude of order as other PN-anammox systems treating industrial wastewater.

Finally, the microbial selection achieved in the PN-SBR was also assessed by several molecular techniques such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), quantitative PCR and pyrosequencing. In all the experiments, one AOB phylotype was enriched and was well adapted to stringent conditions present in the PN-SBR. When anoxic feedings were included, the heterotrophic community was low diverse but well adapted to the PN-SBR conditions. *Bacteroidetes* were the dominant organisms and specialized in the degradation of slowly biodegradable bCOD. A minor fraction of the heterotrophic organisms had the genetic capability to completely denitrify NO_2^- to N_2.

The robustness of the PN process was successfully demonstrated, despite operational temperature being changed or anoxic feedings being included. Applying the operational changes studied in full scale reactors should be analyzed in terms of the requirements for the subsequent anammox reactor and environmental laws (in the case of N_2O emissions). The present thesis results also demonstrate bacterial acclimation to stringent and variable conditions.

Resumen

El tratamiento biológico de aguas residuales industriales que contienen elevadas concentraciones de nitrógeno (>1000 mg N L^{-1}) y baja concentración de materia orgánica biodegradable (bCOD) como son los lixiviados de vertedero es hoy en día un reto. El tratamiento convencional mediante nitrificación-desnitrificación de este tipo de aguas industriales implica costes muy elevados debido a la aeración y la necesidad de añadir bCOD externa. El proceso de nitritación parcial (PN) combinado con la oxidación anaeróbica del amonio (anammox) resulta una alternativa más sostenible para el tratamiento biológico de este tipo de aguas. Los sistemas de PN-anammox son tratamientos totalmente autotróficos que permiten reducir en un 40% los requerimientos de aeración y no necesitan la adición de bCOD externa al proceso. PN es el proceso previo necesario para alimentar el posterior reactor anammox. El objetivo del reactor de PN es el de producir un efluente apto para el reactor anammox. En el reactor de PN, 57% del NH_4^+ contenido en el influente se ha de oxidar a NO_2^- para la obtención de la proporción molar estequiométrica NO_2^-:NH_4^+ de 1.32. La eliminación de la bCOD es también un punto clave ya que las bacterias heterotróficas pueden competir con las bacterias anammox por el NO_2^- disponible.

El tratamiento de lixiviados de vertedero mediante el proceso PN-anammox ha sido ya demostrado anteriormente. No obstante, el reactor PN operaba a alta temperatura (35°C) y poca atención fue prestada a la eliminación de bCOD. Además, las emisiones de óxido nitroso (N_2O) procedentes de sistemas de nitrificación se han convertido en un tema de alta preocupación. El N_2O es un importante gas de efecto invernadero con un potencial 300 veces mayor al del CO_2. Las condiciones descritas que incrementan las emisiones de N_2O son altas concentraciones de NO_2^- y NH_4^+, fluctuaciones de oxígeno disuelto y pH, baja proporción de bCOD/N así como también alta actividad de las bacterias oxidadoras de amonio (AOB). Todas ellas presentes en los reactores de PN.

La investigación presentada en esta tesis supone cambios operacionales en el reactor de PN como la duración de las fases aerobias y la bajada de la temperatura operacional en un reactor discontinuo secuencial (SBR) de PN tratando lixiviado de vertedero maduro para reducir los requerimientos energéticos. El arranque y operación del PN-SBR fue llevado a cabo en condiciones completamente aerobias a 25 y 35°C y también incluyendo alimentaciones anóxicas para promover la desnitrificación heterotrófica vía NO_2^- a 35°C. Especial atención se

focalizó en la calidad del efluente, la selección de los microorganismos usando técnicas moleculares y por último, la producción de N_2O en condiciones anóxicas y aerobias.

El efluente del PN-SBR era adecuado en todos los casos estudiados, a pesar de la temperatura operacional (25 y 35^oC) y la inclusión de alimentaciones en condiciones anóxicas, en términos de proporción molar NO_2^-:NH_4^+ y contenido en bCOD. El arranque y operación del PN-SBR tratando lixiviados de vertedero con extrema carga de nitrógeno (6 g N L^{-1}) fue exitosamente demostrado tanto a 25^oC como a 35^oC. Un modelo cinético fue implementado para obtener información sobre la reducción de la actividad máxima de los AOB provocada por la concentración de amoníaco libre (FA) y ácido nitroso libre (FNA) así como también la limitación de actividad debido a la concentración de HCO_3^-. En condiciones plenamente aerobias a 35^oC, la actividad máxima de los AOB fue reducida por la combinación de inhibición por FA y FNA y también la limitación de HCO_3^-. En cambio, en la operación del PN-SBR a 25^oC los AOB eran parcialmente inhibidos por FNA y limitación de HCO_3^-.

En relación con la producción de N_2O del PN-SBR, se demostró que la producción y posterior emisión de N_2O fue de 3.6% del nitrógeno total del influente. La producción de N_2O fue analizada durante la operación del PN-SBR alternando alimentaciones anóxicas con fases aerobias. El N_2O fue mayormente producido en condiciones anóxicas (60%) debido a la desnitrificación heterotrófica incompleta. La velocidad anóxica de producción de N_2O era de unos 10 mg N-N_2O $gVSS^{-1}$ h^{-1} mientras que la aerobia era de unos 2 mg N-N_2O $gVSS^{-1}$ h^{-1}. Estos resultados contrastan con los resultados obtenidos por otros autores. Las condiciones severas, en términos de concentración de NH_4^+, NO_2^- y salinidad, fueron las mayores causas por dicha producción de N_2O. No obstante, la producción de N_2O del PN-SBR estaba en el rango de otros sistemas de PN-anammox tratando aguas residuales industriales.

Finalmente, la selección microbiológica ocurrida en el PN-SBR fue evaluada mediante distintas técnicas moleculares como son *fluorescence in situ hybridization* (FISH), reacción en cadena de polimerasa (PCR), electroforesis con un gel con gradiente desnaturalizante (DGGE), PCR cuantitativa y pirosecuenciación. En todos los experimentos un filotipo de AOB fue enriquecido y estaba bien adaptado a las condiciones severas del PN-SBR. La comunidad heterotrófica tenía baja diversidad pero estaba muy bien adaptada a las condiciones del reactor. *Bacteroidetes* eran los microorganismos dominantes y estaban especializados en la degradación de bCOD lentamente biodegradable. Una minoría de los organismos heterotróficos tenía la capacidad genética de desnitrificar completamente el N_2O a N_2.

En definitiva, los resultados de esta tesis demuestran que se logró de manera exitosa el funcionamiento robusto del PN-SBR a pesar de los cambios de temperatura y la inclusión de fases anóxicas. La aplicación de los cambios realizados en reactores de escala real tendría que ser analizada en términos de los requerimientos del reactor anammox posterior y de la ley ambiental (en el caso de la producción de N_2O). Los resultados incluidos en esta tesis también demuestran la aclimatación de los microorganismos seleccionados a condiciones severas y variables.

1. PARTIAL NITRITATION-ANAMMOX PROCESS BACKGROUND

1.1. PN-anammox technology: state of the art

Anaerobic ammonium oxidation (anammox) metabolism was first detected by Mulder et al. (1995) in a denitrifying biofilm that was treating urban wastewater in the Netherlands. Later, anammox stoichiometry (Eq. 1.1) was reported in 1999 by Strous et al. (1999b). Anammox bacteria belong to the *Planctomycetales* order and are strictly anaerobic and autotrophic organisms (Strous et al. 1999a); except *Anammoxoglobus propionicus* which have been described as being able to oxidize propionate (Kartal et al. 2007).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.066CH_2O_{0.5}N_{0.15}$$

(Eq. 1.1)

As a result of the discovery of anammox metabolism, research was then pointed towards the enrichment and application of anammox process and focused on nitrogen removal from NH_4^+ rich wastewater streams (Chamchoi and Nitisoravut 2007, Egli et al. 2001, López et al. 2008, Van Der Star et al. 2008). Anammox requires a NO_2^-:NH_4^+ molar ratio of 1.32 (Eq.1.1) and as such, a previous partial nitritation (PN) of the influent's NH_4^+ to NO_2^- is required. PN is carried out by ammonium oxidizing bacteria (AOB) which are also autotrophic organisms whose metabolism is governed by the stoichiometry given in Equation 1.2 (Ganigué et al. 2007).

$$NH_4^+ + 2\ HCO_3^- + 1.5\ O_2 \rightarrow NO_2^- + 3\ H_2O + 2\ CO_2$$ **(Eq. 1.2)**

Thus, the combination of PN and anammox emerges as a fully autotrophic nitrogen removal system which, when compared to conventional nitrification-denitrification systems, reduces the aeration requirement by 40% and excludes the external biodegradable organic matter (bCOD) supply (Ahn 2006). The PN-anammox process is usually applied to wastewater high in NH_4^+ and low in bCOD, for instance such as anaerobic digester liquor, swine piggery or landfill leachate, and consequently, has a low bCOD/N ratio (Strous et al. 1997b). The combination of the two linked processes (PN-anammox) can be achieved in one or two different reactors (Schmidt et al. 2003).

The PN-anammox operation in one single reactor can be successfully accomplished despite the different metabolic pathways (anoxic and aerobic), because oxygen inhibition over anammox biomass has been demonstrated to be reversible (Strous et al. 1997a). In this process, the dissolved oxygen (DO) gradient is stabilized in granules or biofilms which promote AOB activity in the aerobic zone and anammox activity in the anoxic zone (Sliekers et al. 2002).

Applying the PN-anammox process in two separate reactors isolates each process at their optimum conditions where the NO_2^- produced in the aerobic reactor is later fed to the anoxic reactor (van Dongen et al. 2001).

Successful application of PN-anammox process in a single reactor has been widely demonstrated at lab and full scale with several different configurations. The oxygen-limited autotrophic nitrification-denitrification system (OLAND) was first developed in the late 1990s by Kuai and Verstraete (1998) containing both AOB and anammox in biofilm. OLAND was successfully operated at lab scale treating synthetic media (Pynaert et al. 2002, Windey et al. 2005) and black wastewater which had an NH_4^+ concentration in the influent of 1000 mg N-NH_4^+ L^{-1} (Vlaeminck et al. 2009).

Additionally, complete autotrophic nitrogen removal over nitrite (CANON) reactors are granular sludge systems which also contain both AOB and anammox in the same granule. CANON experiments demonstrated excellent stability when treating synthetic water (Third et al. 2001) and, later, this was applied to treat a full-scale digester returns at 250 mgN-NH_4^+ L^{-1} (Kampschreur et al. 2009). It should also be noted the DEMON® process which was successfully implemented in a full-scale rejection water deammonification (1800 mg N-NH_4^+ L^{-1}) system at the WWTP Strass, Austria (Wett 2006). DEMON® consists of a sequencing batch reactor (SBR) intermittently aerated, controlled by pH and continuously fed. DEMON® was also successfully applied treating digested sludge liquor (1000 mg N-NH_4^+ L^{-1}) in an urban WWTP in Glarnerland, Switzerland. Nowadays, the company Veolia has developed a product called ANITA-Mox® which consists of suspended biofilm carriers treating mostly digested sludge liquor (Christensson et al. 2013).

In the case of landfill leachate treatment, Seyfried et al. (2001) first reported nitritation-deammonification in a rotating biological contractor (RBC) plant designed for conventional nitrification-denitrification treating leachate at 150-300 mg N-NH_4^+ L^{-1}. Furthermore, Cema et al. (2007) demonstrated the feasibility of combining heterotrophic denitrification and anammox processes in an RBC nitritation treating landfill leachate (<1500 mg N-NH_4^+ L^{-1}).

PN-anammox has also been successfully operated in two separate reactors. The Single reactor for High activity Ammonia Removal Over Nitrite (SHARON) was initially designed as a short cut for nitrogen removal via NO_2^- (Hellinga et al. 1998). NO_2^- build-up is achieved by favoring AOB in detriment to nitrite oxidizing bacteria (NOB) whose activity is undesirable. The SHARON process ensures NO_2^- build-up through operating temperatures and sludge retention time (SRT). The operating temperature of the SHARON process is 30-40°C as AOB present higher growth than NOB at temperatures above 25°C (Figure 1.1; (Hellinga et al. 1998)). No sludge retention system is used during the SHARON process. Thus, hydraulic retention time (HRT) fixed at 1-1.5 days is equal to SRT which is the convenient SRT to avoid NOB growth and to favor AOB growth. The SHARON process has nitritation limitation when treating digester sludge liquor due to the HCO_3^-:NH_4^+ molar ratio of the influent which is usually about 1. Therefore, only 50% of the influent's NH_4^+ could be oxidized to NO_2^- (Eq. 1.2). However, this particular characteristic of the SHARON effluent could be used as influent for a subsequent anammox reactor. Along these lines, van Dongen et al. (2001) demonstrated the feasibility of combining the SHARON process with the anammox process to treat digested sludge liquor.

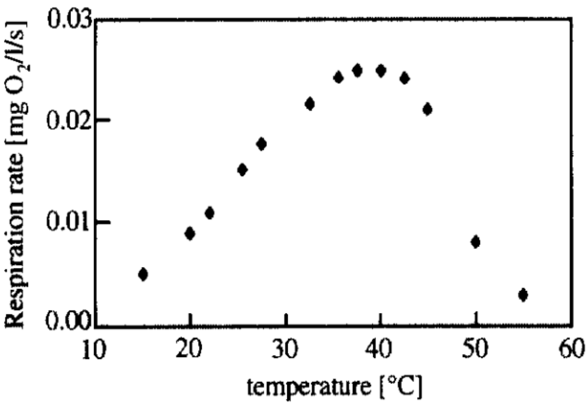

Figure 1.1. Temperature dependency of the maximum growth rate of AOB (Hellinga et al. 1998).

The SHARON-anammox process was also applied to treat pig manure with nitrogen concentrations of about 1000 mg N- NH_4^+ L^{-1} (Hwang et al. 2005), landfill leachate (Shalini and Joseph 2012, Vilar et al. 2010) and full-scale systems treating digester supernatant at 1500 mg N- NH_4^+ L^{-1} (Desloover et al. 2011, Kampschreur et al. 2008). However, the SHARON process can have operational problems when influent loads, such as landfill leachate, vary. From this perspective, Fux et al. (2003) reported that SBR configuration could also be used to obtain PN which has a higher biomass retention. Higher biomass concentration would ensure robustness when treating dynamic influents.

Other reactor configurations were applied in order to attain higher biomass retention. Yamamoto et al. (2008) reported PN satisfactorily treating digested pig manure in an upflow biofilm reactor with influent nitrogen concentrations of 2000-4000 mg N-NH_4^+ L^{-1}. Also, PN operated as an SBR treating landfill leachate at 2000-4000 mgN-NH_4^+ L^{-1} was carried out by several authors (Ganigué et al. 2007, Liang and Liu 2008, Liang and Liu 2007).

The treatment of landfill leachate deals with intrinsic leachate characteristics such as high NH_4^+ content, high COD with heterogeneous biodegradability of the organic components and high salinity (Renou et al. 2008). Also, variability of the leachate characteristics due to leachate pounds pumping can vary depending on needs of the operation of the landfill management. Anammox bacteria are more sensible to NH_4^+ loading shocks (Jin et al. 2012) and heterotrophic bacteria can compete for NO_2^- when bCOD is available (Ruscalleda 2011). AOB are autotrophic organisms more resistant to changes than anammox organisms. AOB can resist NH_4^+ loading shocks due to its high tolerance range to FA and FNA and also can coexist with heterotrophic bacteria competing only for oxygen which can be supplied into the reactor. Thus, landfill leachate is usually treated in two separate reactors because of its dynamic characteristics and the variability of its bCOD content. Along these lines, the Laboratory of Environmental and Chemical Engineering (LEQUIA) from the University of Girona has set up their investigations into the treatment of mature leachate via the Panammox® process.

1.2. Panammox® technology

Panammox® technology consists of a two steps PN-anammox system. PN and anammox are carried out in two sequential SBRs (Ganigué 2010, Ruscalleda 2011). This results in high biomass retentions and concentrations which in turn enables higher stability of the system. However, even though difficulties with the stability of the PN and anammox processes were detected, the processes have been optimized lasting recent years.

Anammox enrichment from several microbial sources such as activated sludge and sediments was investigated. Successful enrichment from two different urban nitrification-denitrification WWTPs was achieved (López et al. 2008). Anammox biomass was sequentially acclimated to leachate PN-SBR effluent by incrementing its content in the feed (Ruscalleda et al. 2008). Nevertheless, two challenges were detected during the anammox acclimation and the reactor's performance. Firstly, high salinity and leachate media could partially inhibit anammox activity (Scaglione et al. 2012) and secondly, competition between anammox and heterotrophic organisms for NO_2^- could lead to NH_4^+ accumulation, but also, and on the contrary, it has a positive affectation by the heterotrophic denitrification of the NO_3^- produced by the anammox activity (Ruscalleda et al. 2008, Ruscalleda et al. 2010). Thus, PN-SBR effluent had to ensure a good effluent in terms of NO_2^-:NH_4^+ molar ratio (1.32) and low bCOD.

The PN-SBR configuration to treat landfill leachate was first demonstrated by (Ganigué et al. 2007). The lab scale PN-SBR (20 L) was operated at 35^{o}C, at HRT of 1.5 days, and SRT of 5 days throughout the whole performance cycle. Influent was continuously fed and leachate was progressively added to the influent media during startup. The PN-SBR operation cycle ran for a total length of 8 hours: Those eight hours were divided into 360 minutes of aerobic feeding, 80 minutes of aerobic reaction, 15 minutes of settling and 25 minutes draw.

NOB growth suppression and activity inhibition was achieved by mean of free ammonia (FA) and free nitrous acid (FNA) (Ganigué et al. 2007) and accordingly, AOB were enriched in the PN-SBR sludge. However, FA and FNA also affected AOB activity but at higher concentrations than NOB (Anthonisen et al. 1976). Temperature and pH governs FA and FNA concentrations. Furthermore, pH is a key factor, with the optimum being pH 7.2, for AOB activity (Figure 1.2). Along these lines, Ganigué et al. (2008) reported that a step-feed strategy, compared to continuous feeding strategy, was a more efficient way of keeping the PN-SBR stable due to lower pH and bicarbonate concentration at the end of the reaction phase which caused higher limitations on AOB activity. A step-feed strategy was later applied initially during the startup and then during the stable operation of a pilot plant PN-SBR (250 L) treating raw leachate from the very first day (Ganigué et al. 2009).

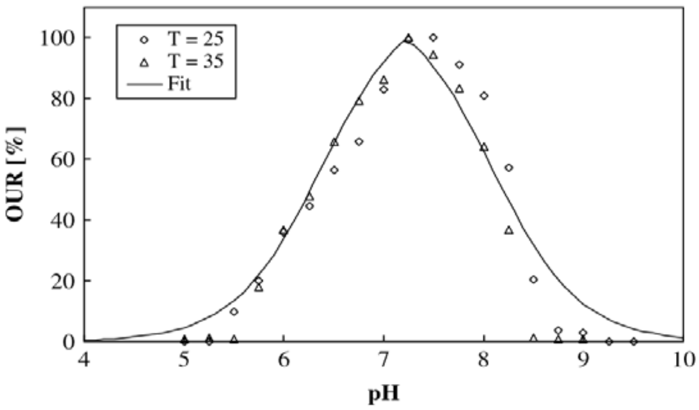

Figure 1.2. Influence of pH at 25°C and 35°C on the oxygen uptake rate (OUR) (Van Hulle et al. 2007).

NOB inhibition and successful NO_2^- build-up was achieved through high FA and FNA concentrations. HCO_3^- was proven to be a key parameter in controlling NH_4^+ oxidation to NO_2^- in thermophilic conditions (35^{0}C). Consequently, the influent's HCO_3^-:NH_4^+ molar ratio had to be adjusted to 1.14 in order to oxidize 57% of the influent's NH_4^+ to NO_2^-. The PN-SBR cycle configuration included anoxic feedings to promote heterotrophic denitrification (Ganigué et al. 2010), which led to low effluent bCOD concentration (<20 mg BOD_5 L^{-1}). However, little attention was paid to either the heterotrophic denitrification rates or the heterotrophic sludge community.

In summary, the PN process included in the Panammox® technology has been performed at 35°C and demonstrates high stability. Key parameters for successful PN performance were the FA and FNA concentrations, which inhibited NOB activity, and the influent's HCO_3^-:NH_4^+ molar ratio, which regulates the effluent NO_2^-:NH_4^+ molar ratio. High operating temperatures increase the energy requirements for heating. Thus, a decrease in the operational temperature of the process would imply lower treatment costs. However, temperature variation affects bacterial activity as well as chemical equilibriums such as NH_3/NH_4^+ and HNO_2/NO_2^- (Equations 1.3 and 1.4; (Anthonisen et al. 1976)) and the FA and FNA variation could significantly affect NOB inhibition as well as AOB activity.

$$FA\ (mgN \cdot L^{-1}) = \frac{TAN}{1+\left(\frac{10^{-pH}}{K_{e,NH_3}}\right)} \qquad \textbf{(Eq. 1.3)}$$

$where;\ K_{e,NH_3} = e^{\left(\frac{-6344}{273+T}\right)}$, TAN is the total ammonium as nitrogen (mg N L^{-1}), pH is the pH value and T is temperature in °C.

$$FNA\ (mgN \cdot L^{-1}) = \frac{TNO_2}{1+\left(\frac{K_{e,HNO_2}}{10^{-pH}}\right)} \qquad \textbf{(Eq. 1.4)}$$

$where;\ K_{e,HNO_2} = e^{\left(\frac{-2300}{273+T}\right)}$, TNO_2 is the total NO_2^- (mg N L^{-1})

Additionally, bCOD removal during anoxic feedings could involve N_2O formation due to uncompleted denitrification. N_2O formation is undesirable because of its high greenhouse effect.

1.3. Nitrous oxide (N_2O) production during PN

N_2O is a greenhouse gas with a global warming potential 300 times higher than CO_2 (IPCC 2001). The biological nitrification-denitrification process is responsible for the majority of anthropogenic N_2O emissions from wastewater infrastructures. To establish their contribution, several studies have monitored N_2O in conventional biological nutrient removal (BNR) and PN–anammox processes (Foley et al. 2010, Kampschreur et al. 2008). Experimental results between studies vary, largely due to the different operational conditions, and to date there is no consensus on their actual extent. However, fundamental research carried out in recent years has enabled the main conditions leading to N_2O accumulation and later emission, to be better understood. Figure 1.3 shows each and every possible chemical and biological pathway of nitrogen transformation known today (Desloover et al. 2012). Some reactions and pathways are still not clear and remain under investigation.

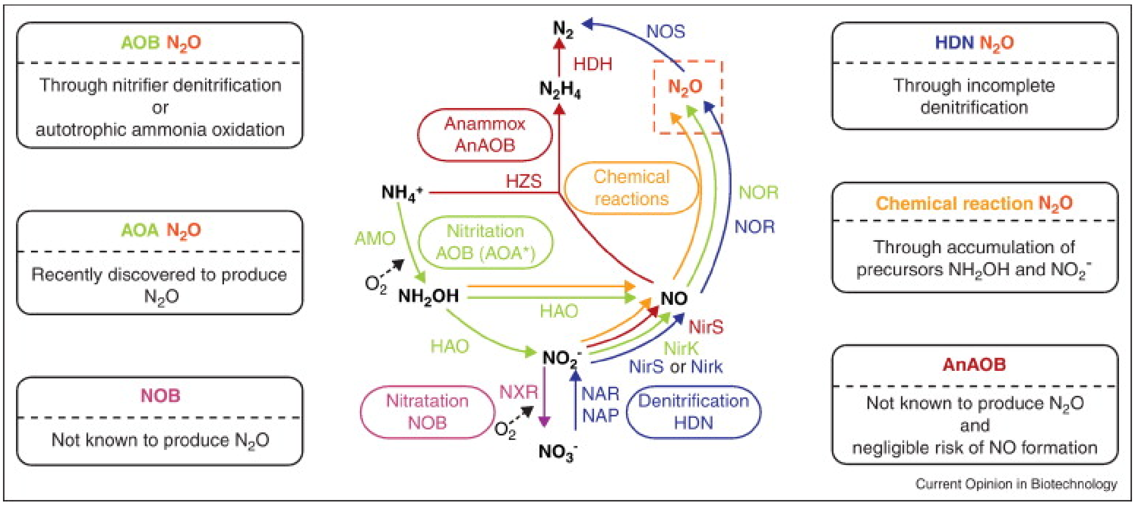

Figure 1.3. Conceptual overview of the N_2O production and consumption pathways during BNR, and the involved microbial communities and enzymes. The key microbial communities are aerobic ammonia-oxidizing bacteria (AOB), anoxic ammonia-oxidizing bacteria (AnAOB), ammonia-oxidizing archaea (AOA), heterotrophic denitrifying bacteria (HDN) and nitrite-oxidizing bacteria (NOB). The related enzymes are ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), hydrazine dehydrogenase (HDH), hydrazine synthase (HZS), periplasmatic nitrate reductase (NAP), membrane-bound nitrate reductase (NAR), Cu-containing nitrite reductase (NirK), cytochrome *cd1* nitrite reductase (NirS), nitric oxide reductase (NOR) and nitrous oxide reductase (NOS). *For archaeal nitritation, proposed intermediates are NH_2OH or HNO (Desloover et al. 2012).

On one hand, aerobic N_2O production is mainly caused by AOB activity via two metabolic pathways: hydroxylamine oxidation and autotrophic denitrification. The main parameters that promote N_2O production via AOB are high NO_2^- concentrations, high NH_4^+ loads in the influent and low DO concentrations (< 1 mg O_2 L^{-1}), among others (Kampschreur et al. 2008). On the other hand, anoxic N_2O production can be linked to two main pathways. During anoxic conditions both autotrophic and heterotrophic denitrification occurs. Heterotrophic denitrifiers can reduce NO_3^- to N_2 in four steps ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$; Fig. 1.3) using bCOD as the electron donor. A low bCOD/N ratio and electron competence between denitrification enzymes are defined as key parameters for N_2O production (Pan et al. 2013). Also, AOB can autotrophically denitrify NO_2^- to NO (Yu et al. 2010) which would then be available for further heterotrophic denitrification to N_2O and N_2.

Additionally, it is important to consider the plausible heterotrophic N_2O reduction inhibition by FNA. PN reactors operate under high NO_2^- concentrations which cause high FNA concentrations (up to 0.2 mg N-HNO_2 L^{-1}). Zhou et al. (2008) defined the 50% inhibition of N_2O reduction at 0.0007-0.001 mg N-HNO_2 L^{-1} and complete inhibition at 0.004 mg N-HNO_2 L^{-1} with denitrifying biomass operating at NO_2^- concentrations in the reactor lower than 10 mg N-NO_2^- L^{-1}. However, acclimated biomass in PN reactors could resist higher FNA concentrations like AOB community which has been observed to be enriched in phylotypes resistant to stringent conditions (Egli et al. 2003). N_2O reduction inhibition could cause large N_2O production and later emission during the performance of the PN-SBR treating high nitrogen loads.

2. OBJECTIVES

Stability in the treatment of high nitrogen loaded wastewater by PN in SBR configuration was successfully demonstrated by Ganigué (2010). However, by decreasing the operating temperature the operational costs of this process could be reduced, as could the anoxic oxidation of the bCOD by including anoxic feedings. Therefore, the main objective of this thesis was to study the effects of these two operational changes on the overall PN-SBR process performance in the context of bacterial activity stability and process robustness based on the effluent quality, as well as the production and later emission of the significant greenhouse gas N_2O.

To achieve this principal goal, several specific objectives were defined:

- To establish the effects of PN-SBR operating temperatures on FA and FNA inhibition and HCO_3^- limitation over AOB activity.

- To determine mechanisms for nitrogen conversion during changes of the operating temperature and intermittent aeration cycle configuration.

- To quantify aerobic and anoxic N_2O production of the PN-SBR operated under alternate aeration configuration and to identify the causes of N_2O accumulation.

- To characterize the microbial community ecology of the PN-SBR with intermittent aeration configuration treating mature leachate.

- To determine the suitability of including anoxic feedings regarding effluent quality, sodium bicarbonate and acid addition requirements and N_2O production.

3. MATERIALS AND METHODS

3.1. PN-SBR setup

The experiments were conducted in a pilot plant located at the Technological and Scientific Park of the University of Girona. The plant was situated in a container to facilitate its transport when necessary (Figure 3.1). The pilot plant for autotrophic nitrogen removal was placed inside the container (Figure 3.2). The principal elements were a pretreatment tank, a partial nitritation sequencing batch reactor (PN-SBR), anammox SBR and a control panel. The influent leachate came from the Orís and Corsa landfills (Catalonia, Spain) in 1000 L container tanks (Fig. 3.1.B) every 30-40 days.

Figure 3.1. External view of the pilot plant. **A.** Pilot plant container. **B.** Landfill leachate container tanks (1000L)

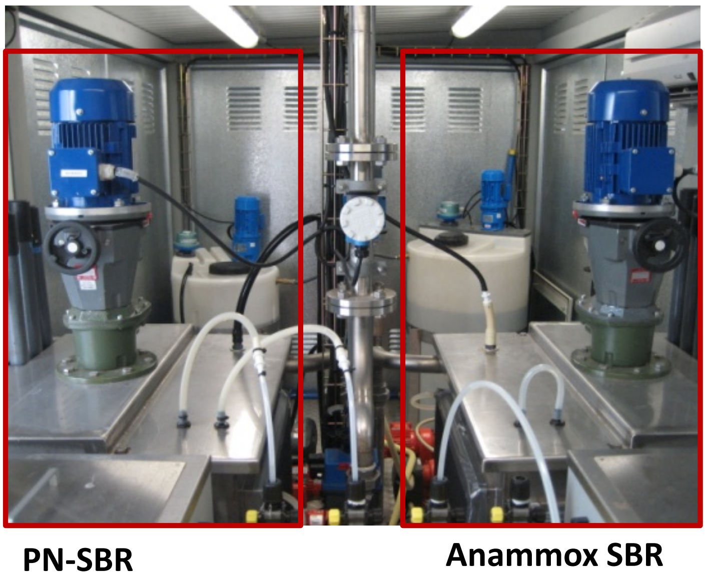

Figure 3.2. Inside view of the PN-anammox container.

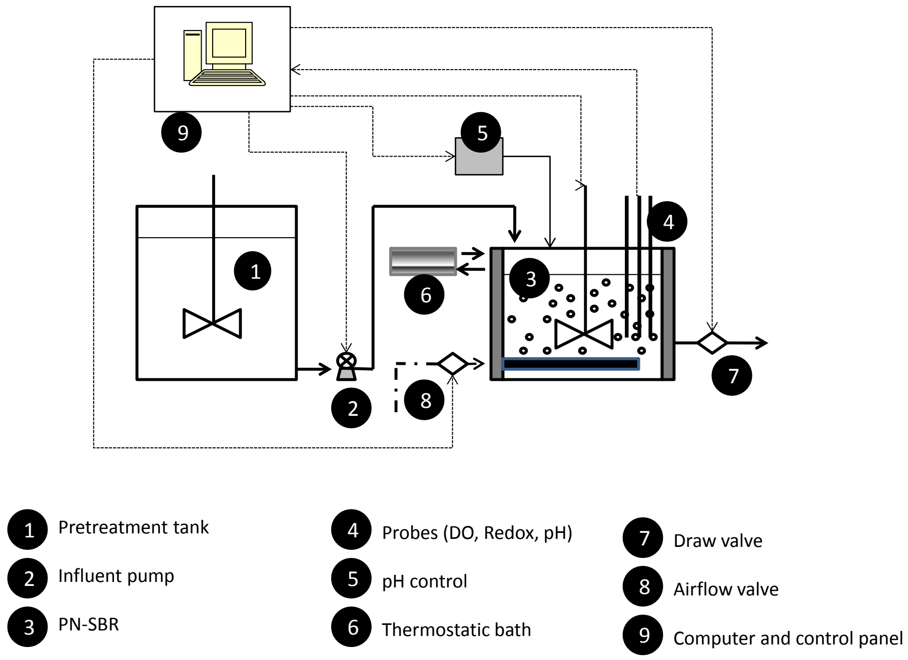

Figure 3.3. Schematic view of pilot plant

A scheme of the overall PN-SBR is presented in Figure 3.3. The leachate was first pumped into a 300 L pretreatment plastic tank equipped with a stirrer to ensure homogeneous influent conditions. When necessary, leachate was conditioned in this tank by solid sodium bicarbonate or hydrochloric acid addition (Fig 3.4.A). From this tank, the leachate was fed to the reactor with a peristaltic pump.

The PN-SBR was made of stainless steel and had a squared base, was 0.6 m long and 1.1 m high, and as a result had a maximum working volume of 250 L (Fig 3.4.B). The minimum volume was set at 111 L. An external water jacket ensured a constant temperature by recirculating water heated in a thermostatic bath and with the aid of a mechanical stirrer the mix liquor was mixed completely. Aeration was carried out by a blower connected to 3 air diffusers (Magnum, from OTT system GmbH & Co.) placed at the bottom of the reactor.

Figure 3.4. Inside view of the PN-SBR: **A)** Pretreatment tank. **B)** PN-SBR **C)** Control panel.

Data acquisition and PN-SBR control were managed via the control panel (Figure 3.4.C), which was composed of an interface card (PCI-1711 I PCLD-8710, Advantech, Taiwan) and computer controlled. The computer used an own created software developed by LabVIEW, which permitted the electronic on/off control of all the electrical devices and the control of the SBR cycle by another interphase card (PCI-885, Advantech). Control actions were focused on DO concentration (DO set point = 2 mg O_2 L^{-1}) during aeration phases and pH control to a maximum value of 8.0 by adding HCl (1M).

ORP, pH, dissolved oxygen concentration (DO) and temperature probe (CPF 82, CPF 81, OXYMAX-W COS-41) signals were sent to the control panel (Fig.3.4.C), allowing DO, pH and ORP to be monitored online. Furthermore, liquid N_2O concentration was measured online with an in-situ N_2O microsensor (Unisense, Aarhus, Denmark; Figure 3.5.A) during specific experiments. The microsensor was connected to an in-situ amplifier (PA 2000; Figure 3.5.B) via one single channel. This is a robust and compact system which ensures reliable data gathering. Data was stored every 5-30 seconds depending on the experiment. Signals from the microsensor were amplified and sent to a laptop. Unisense software (sensorTrace BASIC, Unisense) stored and depicted data during experiments. Calibration was linear and was performed by successively addition of saturated N_2O solution.

Figura 3.5. A) N_2O in-situ microsensor. **B)** Signal in-situ amplifier

3.2. Batch test set-up

To analyze the influence of specific factors on the PN-SBR sludge activity, batch tests were conducted in a 5 L fermenter (Fig. 3.6). The fermenter consisted of a 5 L glass vessel which was water jacketed to keep the temperature constant. In the reactor, the sludge was well mixed with a mechanical stirrer (250 rpm) and could be conditioned by sparging with nitrogen or air when needed. ORP, DO, T and pH probes allowed these parameters to be monitored and controlled via the fermenter and the connected computer. Maximum and minimum pH could be controlled by adding 1M acid (HCl) or base (NaOH) with peristaltic pumps.

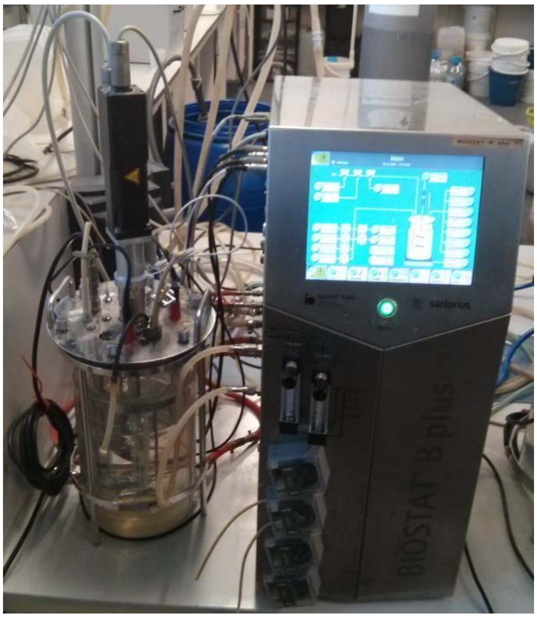

Figure 3.6. Fermenter set-up.

The fermenter screen permitted the control of variables through on-line measurement plots. The recipe to control batch conditions was set up with Sartorius® software on the PC connected to the fermenter. Data were stored and made available from the PC once the batch test was over. A schematic view of the set-up is shown in Figure 3.7.

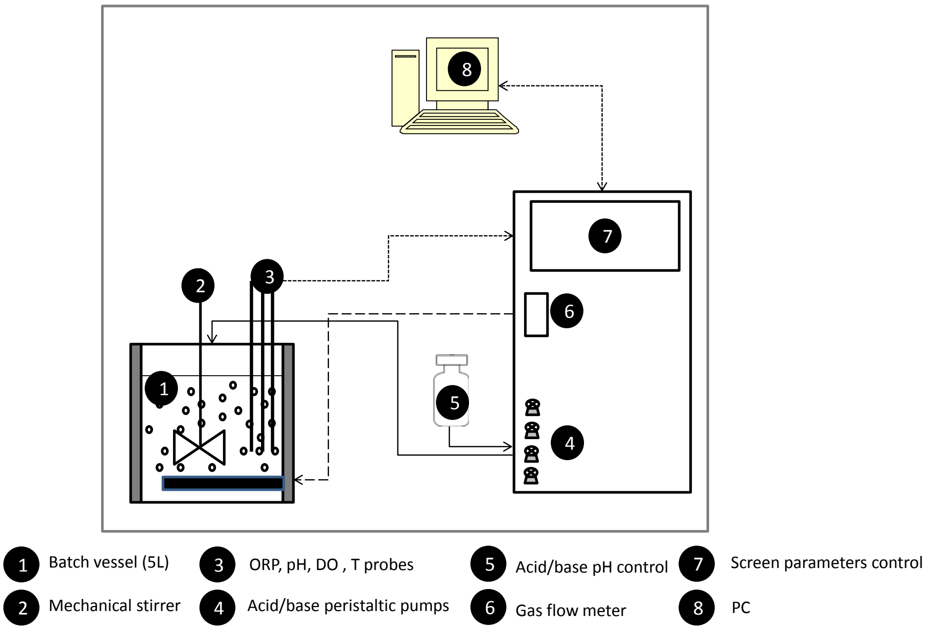

Figure 3.7. Schematic view of fermenter set-up. Solid lines refer to liquid, dashed to gas and dotted to data transfer.

4. CALCULATIONS

4.1. Free ammonia and free nitrous acid

The values of the free ammonia (FA; mgN L^{-1}) and the free nitrous acid (FNA; mgN L^{-1}) concentrations were calculated as a function of pH, temperature and total ammonium as nitrogen (TAN), for FA, or total nitrite (TNO_2), for FNA (Eq.4.1 and 4.2); Anthonisen et al. 1976).

$$FA = \frac{TAN}{1+\left(\frac{10^{-pH}}{K_{e,NH_3}}\right)} \qquad \textbf{(Eq. 4.1)}$$

$$where: K_{e,NH_3} = e^{\left(\frac{-6344}{273+T}\right)}$$

$$FNA = \frac{TNO_2}{1+\left(\frac{K_{e,HNO_2}}{10^{-pH}}\right)} \qquad \textbf{(Eq. 4.2)}$$

$$where: K_{e,HNO_2} = e^{\left(\frac{-2300}{273+T}\right)}$$

4.2. Observed and maximum nitrite production rate

The observed nitrite production rate (NPR_{obs}; mgN L^{-1} d^{-1}) was calculated as a function of daily influent flow (Q_d; ($L \cdot d^{-1}$)), nitrite [NO_2^-] (mgN·L^{-1}), nitrate [NO_3^-] (mgN·L^{-1}) and maximum volume of the reactor (V_{max}; (L)) according to Equation 4.3.

$$NPR_{obs} = \frac{Qd \cdot [NO_2^-] + Qd \cdot [NO_3^-]}{V_{max}} \qquad \textbf{(Eq. 4.3)}$$

The specific nitrite production rate (sNPR; mgN 10^{-10} $cells^{-1}$ d^{-1}) was calculated as a function of NPR_{obs} and the total AOB concentration [AOB_{tot}] (cells·L^{-1}) as given in Equation 4.4.

$$sNPR_{obs} = \frac{NPR_{obs} \cdot 10^{10}}{[AOB_{tot}]} \qquad \textbf{(Eq. 4.4)}$$

On the other hand, a maximum specific nitrite production rate ($sNPR_{max}$) (mg N·10^{-10}·cells^{-1}·d^{-1}) was calculated from Equation 4.4 following the kinetic expression and constants previously described in literature (Ganigué et al. 2007), which describes the $sNPR_{obs}$ to be dependent on the NPR_{max}, FA and FNA inhibition (modeled as Monod terms) as well as bicarbonate concentration (modeled as a sigmoidal term).

$$sNPR_{max} = \frac{sNPR_{obs}}{\frac{K_{I,NH_3}^{AOB}}{K_{I,NH_3}^{AOB}+NH_3}\cdot\frac{K_{I,HNO_2}^{AOB}}{K_{I,HNO_2}^{AOB}+HNO_2}\cdot\frac{e^{((HCO_3^- - k_{HCO_3^-})/a)}}{e^{((HCO_3^- - k_{HCO_3^-})/a)}+1}} \quad \textbf{(Eq. 4.5)}$$

Where: K_{I,NH_3}^{AOB} = FA inhibition concentration for AOB (605.48 mg N-NH_3 L^{-1}), K_{I,HNO_2}^{AOB} = FNA inhibition concentration for AOB (0.49 mg N-HNO_2 L^{-1}), a = kinetic constant (1.1), $k_{HCO_3^-}$ = bicarbonate half saturation constant (28.5 mg C L^{-1}). All these values were obtained from Ganigué (2010).

4.3. N_2O production and emission

N_2O generation (r_g; mg N gVSS^{-1} h^{-1}; Eq. 4.6), emission (r_e; mg N gVSS^{-1} h^{-1}; Eq. 4.7) and accumulation (r_a; mg N gVSS^{-1} h^{-1}; Eq. 4.8) rates were calculated using balances based on on-line N_2O liquid concentration measurements.

4.3.1. N_2O mass balance

$$r_g = r_e + r_a \quad \textbf{(Eq. 4.6)}$$

$$r_e = \frac{-K_L a \cdot \frac{V_{ref}}{V_r} \cdot \alpha \cdot \left(C_{N_2O\,liq} - C_{N_2O air}^{*}\right)}{VSS} \quad \textbf{(Eq. 4.7)}$$

Where: K_La = N_2O mass transfer coefficient (h^{-1}), V_{ref} = experimental reference volume for K_La (120 L), Vr = reactor volume α = correction coefficient (experimental determination is presented in SM), $C_{N2O\ liq}$ = N_2O liquid concentration (mg N L^{-1}), C^*_{N2Oair} = N_2O saturation concentration in the liquid in equilibrium with air (mg N L^{-1}; assumed as 0) and VSS = volatile suspended solids concentration (g VSS L^{-1}).

$$r_a = \frac{dC_{N_2O}}{dt \cdot VSS} \qquad \textbf{(Eq. 4.8)}$$

4.3.2. N_2O emission ($E_{i,xa}$)

$$E_{x,a} = \frac{\int_{t_0}^{t_n} r_e(t) \cdot dt}{1000} \cdot (VSS \cdot V_r) \qquad \textbf{(Eq. 4.9)}$$

Where: E_x = anoxic emission (g N), E_a = aerobic emission (g N), t_n = anoxic/aerobic time of sub-cycle (n).

4.3.3. N_2O production ($P_{i,xa}$)

$$P_{x,a} = \frac{\int_{t_0}^{t_n} r_g(t) \cdot dt}{1000} \cdot (VSS \cdot V_r) \qquad \textbf{(Eq. 4.10)}$$

Where: P_x = anoxic production, P_a= aerobic production.

4.3.4. Nitrite removal rate

$$r_{NO_2^-} = -\frac{d[NO_2^-]}{dt} \cdot V_r \qquad \textbf{(Eq. 4.11)}$$

Where: r_{NO2-} = nitrite removal rate (mg N h^{-1})

$$_s r_{NO_2^-} = \frac{r_{NO_2^-}}{VSS} \qquad \textbf{(Eq. 4.12)}$$

Where: $_s r_{NO2}^-$ = specific nitrite removal rate (mg N $gVSS^{-1}$ h^{-1}).

4.3.5. N_2O removal

$$_s r_{N_2O} = \ _s r_{NO_2^-} - r_g \qquad \textbf{(Eq. 4.13)}$$

Where: $_s r_{N2O}$ = specific N_2O removal rate (mg N $gVSS^{-1}$ h^{-1})

4.4. Specific nitrogen rates

PN-SBR specific activity was defined as the relative activity of the biomass, calculated as gVSS L^{-1}. Therefore, the specific NH_4^+ loading rate (sALR; g N $gVSS^{-1}$ d^{-1}) (Eq. 4.14), specific nitrite production rate (sNPR; g N $gVSS^{-1}$ d^{-1}) (Eq. 4.15) and specific nitrogen removal rate (sNRR; g N $gVSS^{-1}d^{-1}$) (Eq. 4.16) were calculated as function of daily influent flow (Q_d;Ld^{-1}), influent NH_4^+ concentration($[NH_4^+]_{inf}$; g N L^{-1}), effluent NO_x^- concentration ($[NO_x^-]_{eff}$; g N L^{-1}), influent and effluent TN ($[TN]_{inf}$, $[TN]_{eff}$; g N L^{-1}) maximum volume ofthe reactor (V_{max}; m^3) and the reactor VSS concentration($[VSS]_r$; gVSS m^{-3}).

$$sALR = \frac{Q_d \cdot [NH_4^+]_{inf}}{V_{max} \cdot [VSS]_r} \quad \textbf{(Eq. 4.14)}$$

$$sNPR = \frac{Q_d \cdot [NO_x^-]_{eff}}{V_{max} \cdot [VSS]_r} \quad \textbf{(Eq. 4.15)}$$

$$sNRR = \frac{Q_d \cdot ([TN]_{inf} - [TN]_{eff})}{V_{max} \cdot [VSS]_r} \quad \textbf{(Eq. 4.16)}$$

4.5. Specific organic rates

PN-SBR specific organic loading rate (OLR; gCOD $gVSS^{-1}$ d^{-1}) (Eq. 4.17) and the specific organic removal rate (ORR; gCOD $gVSS^{-1}$ d^{-1}) (Eq. 4.18) were calculated as a function of Q_d, V_{max}, $[VSS]_r$, influent COD concentration ($[COD]_{inf}$; gCOD L^{-1}) and effluent COD concentration ($[COD]_{eff}$; gCOD L^{-1}).

$$sOLR = \frac{Q_d \cdot [COD]_{inf}}{V_{max} \cdot [VSS]_r} \quad \textbf{(Eq. 4.17)}$$

$$sORR = \frac{Q_d \cdot ([COD]_{inf} - [COD]_{eff})}{V_{max} \cdot [VSS]_r} \quad \textbf{(Eq. 4.18)}$$

5. Effect of temperature on AOB activity of a partial nitritation SBR treating landfill leachate with extremely high nitrogen concentration

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Abstract

This study investigates the effects of temperature on ammonia oxidizing bacteria activity in a partial nitritation (PN) sequencing batch reactor. Stable PN was achieved in a 250 L SBR with a minimum operating volume of 111 L treating mature landfill leachate containing an ammonium concentration of around 6000 mg $N\text{-}NH_4+$ L^{-1} at both 25 and 35 °C. A suitable influent to feed an anammox reactor was achieved in both cases. A kinetic model was applied to study the influence of free ammonia (FA), the free nitrous acid (FNA) inhibition, and the inorganic carbon (IC) limitation. NH_4+ and NO_2- concentrations were similar at 25 and 35 °C experiments (about 2500 mg $N\text{-}NH_4+$ L^{-1} and 3500 mg $N\text{-}NO_2-$ L^{-1}), FA and FNA concentrations differed due to the strong temperature dependence. FNA was the main source of inhibition at 25 °C, while at 35 °C combined FA and FNA inhibition occurred. DGGE results demonstrated that PN-SBR sludge was enriched on the same AOB phylotypes in both experiments.

Keywords

Partial nitritation; Free ammonia (FA); Free nitrous acid (FNA); Landfill leachate; Temperature

6. Anoxic phases are the main N_2O contributor in partial nitritation reactors treating high nitrogen loads with alternate aeration

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Abstract

Partial nitritation (PN) reactors treating complex industrial wastewater can be operated by alternating anoxic-aerobic phases to promote heterotrophic denitrification via NO_2^-. However, denitrification under stringent conditions can lead to high N_2O production. In this study, the suitability of including anoxic phases in a PN-SBR treating real industrial wastewater was assessed in terms of process performance and N_2O production. The PN-SBR was operated successfully and, when the HCO_3^-:NH_4^+ molar ratio was adjusted, produced a suitable effluent for a subsequent anammox reactor. 10–20% of the total influent nitrogen was removed. N_2O production accounted for 3.6% of the NLR and took place mainly during the anoxic phases (60%). Specific denitrification batch tests demonstrated that, despite the availability of biodegradable COD, NO_2^- denitrification advanced at a faster rate than N_2O denitrification, causing high N_2O accumulation. Thus, the inclusion of anoxic phases should be avoided in PN reactors treating industrial wastewaters with high nitrogen loads.

Keywords

Anammox; N_2O; Industrial wastewater; Leachate; Anoxic feeding

Anoxic phases are the main N_2O contributor in partial nitritation reactors treating high nitrogen loads with alternate aeration

(Supplementary information)

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1. MATERIALS AND METHODS

1.1. PN-SBR cycle configuration

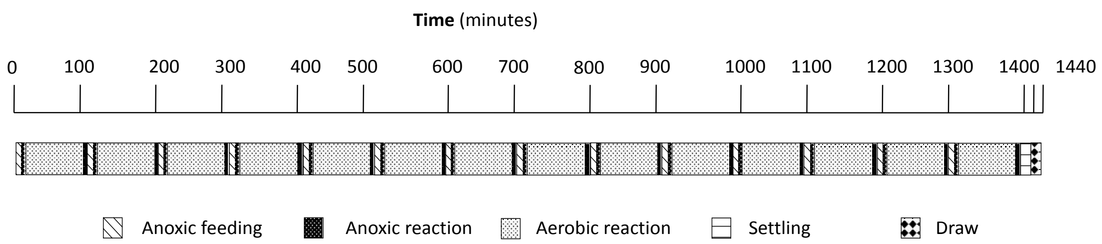

Figure S1. PN-SBR cycle configuration over 24 hours.

1.2. Landfill leachate characterization

Table S1. Raw landfill leachate mean and standard deviation (δ) characterization (n=30).

Compound	Units	mean	δ
Ammonium (NH_4^+)	mg N L^{-1}	2184	306
Nitrite (NO_2^-)	mg N L^{-1}	0	
Nitrate (NO_3^-)	mg N L^{-1}	<2	
Total nitrogen (TN)	mg N L^{-1}	2530	359
Alkalinity (Alk)	mg C-HCO_3^- L^{-1}	2384	335
HCO_3^-:NH_4^+	mol HCO_3^- mol^{-1} NH_4^+	1.40	0.08
Chemical oxygen demand (COD)	mg O_2 L^{-1}	6913	1578
5-day biochemical oxygen demand (BOD_5)	mg O_2 L^{-1}	732	411
Ultimate biochemical oxygen demand (BOD_u)	mg O_2 L^{-1}	1469	189
Conductivity (EC)	mS cm^{-1}	27.8	3.8
pH	-	8.25	0.22

1.3. K_La determination

One important term for N_2O emission and, consequently, its production is the determination of the K_La (Equation S1 (Tribe et al. 1995)).

$$\boldsymbol{k_{La}(t - t_0) = -ln\left(\frac{C_{N_2O}\,(t)}{C_{0_{N_2O}}}\right)} \qquad \text{(Equation S1)}$$

Where: K_La is the mass transfer coefficient, C_{N2O} (t) is the liquid N_2O concentration at time t and C_{0N2O} is the initial liquid N_2O concentration.

Special experiments to determine the PN-SBR K_La and the α term were carried out. Firstly, K_La from the PN-SBR was performed with tap water and a sodium chloride addition to obtain conductivity close to the mix liquor value (29 mS cm^{-1}). The total volume was 120 L (V_{ref}) during the experiments. The K_La results obtained are shown in Table S1.

Table S2. K_La determined from the PN-SBR (n=2)

Conditions	**K_La**	
	h^{-1}	
	mean	δ
mixing	0.82	0.01
aeration	39.36	1.92

Once K_La was determined, establishing the α term was needed because of the difference between the mix liquor and the salted tap water. Thus, several experiments were made in the 5L fermenter. Table S2 shows the experimental results from the experiments carried out with salted tap water as the PN-SBR K_La experiments. The second experiment was performed with salted water and paraformaldehyde (PFA 2%) to inhibit any possible bacterial activity. Finally, the mix liquor and PFA (2%) were also investigated.

Table S3. K_La determination in the 5L fermenter for α term calculation

Experimental water	**KLA**	
	h^{-1}	
	mean	δ
Salted tap water	59.82	0.59
Salted tap water + PFA	63.11	0.28
Mix liquor + PFA	25.44	0.08

From these experiments (Table S2) the α term, which was calculated to be 0.467, was able to be established. Additionally, experiments with differing VSS concentrations were also carried out to determine the VSS effects on KLA. However, K_La remained stable independently of the VSS concentration (data not shown).

1.4. DNA extraction, pyrosequencing and sequence processing

DNA from the PN-SBR reactor at day 94 was extracted and quantified according to Gabarró et al.(Gabarró et al. 2012) The bacterial community was analyzed by tag-encoded FLX-Titanium amplicon pyrosequencing using optimized protocols at the Research and Testing Laboratory (Lubbock, TX, USA)(Dowd et al. 2008). The DNA extract was used as a template in the PCR reactions using primers 341F/907R ((Muyzer and Ramsing 1995) and primers *nosZ*-F/nosZ1622R (Throbäck et al. 2004) complemented with 454-adapters and sample-specific barcodes in the forward primer sequences targeting the V3-5 regions of the bacterial 16S rRNA gene and the nitrous oxide reductase gene (*nosZ*), respectively. Demultiplexing of 16S rRNA and *nosZ* gene sequences according to sample barcodes, and denoising and chimera detection, respectively, were conducted in Mothur (Schloss 2009) using the Functional Gene Pipeline at the Ribosomal Database Project (RDP) (http://fungene.cme.msu.edu/FunGenePipeline/). All sequences were quality-filtered and sequences with quality scores <25 and <27 for 16S rRNA and *nosZ*, respectively; with lengths <250 bp or >900 bp and <270 bp or >500 bp for 16S rRNA and *nosZ* gene, respectively; or with uncorrectable barcodes, ambiguous bases or homopolymers >6 nt, were excluded. The FrameBot pipeline tool was applied to *nosZ* gene sequences to translate nucleotide sequences to protein sequences to detect and correct any frameshifts in the reads. Only *nosZ* nucleotide gene sequences with corrected frameshifts were used for further analysis. Sequence processing, taxonomic identification and classification of the16S rRNA and *nosZ* gene sequences was performed in Mothur using the Mothur files' trainset9_032012.rdp.fasta and trainset9_032012.rdp.tax, and the RDP *nosZ* gene database (http://fungene.cme.msu.edu/), respectively.

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7. Nitrous oxide reduction genetic potential from the microbial community of an intermittently aerated partial nitritation SBR treating mature landfill leachate

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Abstract

This study investigates the microbial community dynamics in an intermittently aerated partial nitritation (PN) SBR treating landfill leachate, with emphasis to the *nos*Z encoding gene. PN was successfully achieved and high effluent stability and suitability for a later anammox reactor was ensured. Anoxic feedings allowed denitrifying activity in the reactor. The influent composition influenced the mixed liquor suspended solids concentration leading to variations of specific operational rates. The bacterial community was low diverse due to the stringent conditions in the reactor, and was mostly enriched by members of *Betaproteobacteria* and *Bacteroidetes* as determined by 16S rRNA sequencing from excised DGGE melting types. The qPCR analysis for nitrogen cycle-related enzymes (*amo*A, *nir*S, *nir*K and *nos*Z) demonstrated high *amo*A enrichment but being *nir*S the most relatively abundant gene. *nos*Z was also enriched from the seed sludge. Linear correlation was found mostly between nirS and the organic specific rates. Finally, *Bacteroidetes* sequenced in this study by 16S rRNA DGGE were not sequenced for *nos*Z DGGE, indicating that not all denitrifiers deal with complete denitrification. However, *nos*Z encoding gene bacteria was found during the whole experiment indicating the genetic potential to reduce N_2O.

Keywords

Microbial community; Denitrification; *nos*Z; N_2O; Partial nitritation

Nitrous oxide reduction genetic potential from the microbial community of an intermittently aerated partial nitritation SBR treating mature landfill leachate

(supplementary materials)

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1. Materials and methods

1.1. PN-SBR setup

The usual PN-SBR performance during an operational subcycle is presented in Figure S1. pH remains invariable (6.8) during the preanoxic phase while dissolved oxygen concentration (DO) decreases being 0 mg O_2 L^{-1} at the beginning of the feeding (Fig. S1.A). During feeding, pH increases due to the alkalinity given by the influent reaching values about 7.2. During the anoxic reaction pH and DO remained stable. Later, at the aerobic reaction phase the pH increased during the first 20 minutes due to CO_2 stripping (up to 7.6) being 6.8 at the end of the phase due to both bicarbonate consumption and protons production by AOB.

On the other hand, Figure S1.B shows the temporal evolution of free ammonia (FA) and free nitrous acid (FNA) during the same subcycle operation. FA and FNA were calculated as Anthonisen et al. (1976) (total nitrite concentration and total ammonium concentration were used from the effluent data). Mainly, pH totally dominates FA and FNA concentration changes because temperature, nitrite and ammonium were kept constant. Thus, FA reached during the first minutes of the aeration phase values up to 40 mg $N\text{-}NH_3\ L^{-1}$ being about 6 mg $N\text{-}NH_3\ L^{-1}$ at the end of the aerobic phase. FNA had the opposite profile behavior. Maximum values were achieved at low pH and low at high pH.

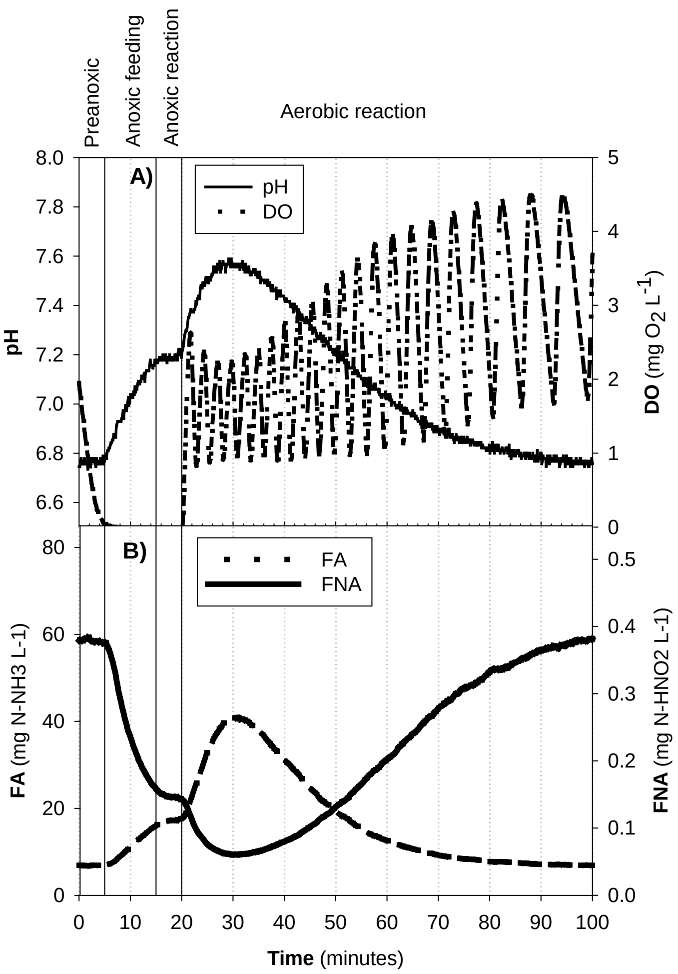

Figure S1. Operational subcycle temporal evolution of **A**) pH and dissolved oxygen (DO) **B)** free ammonia (FA) and free nitrous acid (FNA).

1.2. Fluorescence *in situ* hybridization (FISH)

A set of specific oligonucleotide probes are shown in Table S1. The hybridizations were performed in 35% formamide at 46 ºC for 1.5 h. Image acquisition was done with a Nikon CS1 90i Eclipse confocal laser-scanning microscope. Images were obtained using EZ-C1 software. The area containing cells targeted by the Cy3-labelled and Fluos-labelled specific probes

(NOBmix and AOBmix respectively) was quantified as a percentage of the area of Cy5-labelledEubacterial probe (EUBmix) within each image using a pixel counting program. The final quantification result was expressed as a mean percentage obtained from 30 imagesanalyzed along with a standard error. The standard error of the mean was calculated as the standard deviationof the area percentages divided by the square root of the number of images analyzed (Carvalho et al. 2007).

Table S1. Target groups, probes and astringent conditions used to determine general microbial community composition using FISH technique.

Group	Probe	Target organism	Sequence (5' – 3')	Dye	% Formamide	Reference
EUBmix	EUB338	Eubacteria	GCT GCC TCC CGT AGG AGT	Cy5	40	(Amann et al. 1990, Daims et al. 1999)
	EUB338II	*Planctomyces*phylum	GCA GCC ACC CGT AGG TGT	Cy5	40	
	EUB338III	*Verrucomicrobia*	GCT GCC ACC CGT AGG TGT	Cy5	40	
AOBmix	NSO190	Ammonia-Oxidizing*Betaproteobacteria*	CGA TCC CCT GCT TTT CTC C	Fluos	35	(Mobarry et al. 1996)
	NSO1225		CGC CAT TGT ATT ACG TGT GA	Fluos	35	
NOBmix	NIT3	*Nitrobacter*	CCT GTG CTC CAT GCT CCG	Cy3	35	(Daims et al. 1999)
	Ntspa662	*Nitrospira*like organisms	GGA ATT CCG CGC TCC TCT	Cy3	35	
	compNIT3	*Nitrobacter* competitors	CCT GTG CTC CAG GCT CCG	-	35	
	compNtspa663	*Nitrospira* competitors	GGA ATT CCG CTC TCC TCT	-	35	

Table S2. qPCR primers and thermal cycling conditions used for target genes quantification

Gene	Primer pair	Sequence (5' – 3')	Thermal Conditions	Reference
16S rRNA *Bacteria*	*341F*	CCTACGGGAGGCAGCAG	95°C, 15 min, 1cycle 95°C for 15 s, 55°C for 30 s, 72°C for 32 s, 35 cycles 95°C for 15 s, 60°C to 95°C, 1cycle	(Hallin et al. 2009)
	534R	ATTACCGCGGCTGCTGGCA		
amoA (AOB)	*amoA-1F*	GGGGTTTCTACTGGTGGT	95°C, 15 min, 1cycle 94°C for 30 s, 55°C for 45 s, 72°C for 32 s, 35 cycles 95°C for 15 s, 60°C to 95°C, 1cycle	
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
nirK	*nirK876*	ATYGGCGGVCAYGGCGA	95°C, 15 min, 1cycle 95°C for 15 s, 63 to 58°C for 30 s (-1°C by cycle), 72°C for 30 s, 6 cycles 95°C for 15 s, 60°C for 30 s, 72°C for 32 s, 40 cycles 95°C for 15 s, 60°C to 95°C, 1cycle	
	nirK1040	GCCTCGATCAGRTTRTGGTT		
nirS	*nirSCd3aFm*	AACGYSAAGGARACSGG	95°C, 15 min, 1cycle 95°C for 15 s, 65 to 60°C for 30 s (-1°C by cycle), 72°C for 30 s, 6 cycles 95°C for 15 s, 60°C for 30 s, 72°C for 32 s, 40 cycles 95°C for 15 s, 60°C to 95°C, 1cycle	
	nirSR3cdm	GASTTCGGRTGSGTCTTSAYGAA		
nosZ	*nosZ2F*	CGCRACGGCAASAAGGTSMSSGT	95°C, 15 min, 1cycle 95°C for 15 s, 65 to 60°C for 30 s (-1°C by cycle), 72°C for 30 s, 6 cycles 95°C for 15 s, 60°C for 30 s, 72°C for 32 s, 40 cycles 95°C for 15 s, 60°C to 95°C, 1cycle	
	nosZ2R	CAKRTGCAKSGCRTGGCAGAA		

Table S3. Target genes, PCR primers and thermal cycling conditions used in order to determine microbial community composition in PN-SBR

Gene	Primer pair	Sequence (5' – 3')	Thermal Conditions	Reference
16S rRNA *Bacteria*	*Eub357f-GC*	CCTACGGGAGGCAGCAG	94°C, 1 min, 1cycle 94°C for 1 min, 61°C for 45 s, 72°C for 1 min, 10 cycles	(Throback et al. 2004)
	Eub907r	CCGTCAATTCMTTTGACTTT	94°C for 30 s, 46°C for 45 s, 72°C for 1 min, 20 cycles 72°C, 10 min, 1cycle	
nosZ	*nosZf*	CGYTGTTCMTCGACAGCCAG	94°C, 2 min, 1cycle 94°C for 30 s, 60°C for 1 min, 72°C for 1 min, 35 cycles	
	nosZ1622R-GC	CGCRASGGCAASAAGGTSCG	72°C, 10 min, 1cycle	

2. Calculations

Total nitrogen (TN) was calculated as the sum of the TKN, NO_2^- and NO_3^-. Free ammonia (FA) and free nitrous acid (FNA) concentrations were calculated according to (Anthonisen et al. 1976) as function of pH, temperature, NH_4^+ and NO_2^- concentrations.

2.1. Specific nitrogen rates

PN-SBR specific activity was defined as the relative activity of the biomass, calculated as gVSS L^{-1}. Therefore, the specificNH_4^+ loading rate (sALR; g N $gVSS^{-1}$ d^{-1}) (Eq. 1), specific nitrite production rate (sNPR; g N $gVSS^{-1}$ d^{-1}) (Eq. 2) and specific nitrogen removal rate (sNRR; g N $gVSS^{-1}d^{-1}$) (Eq. 3) were calculated as function of daily influent flow (Q_d;Ld^{-1}), influent NH_4^+ concentration($[NH_4^+]_{inf}$; g N L^{-1}), effluentNO_x^- concentration ($[NO_x^-]_{eff}$; g N L^{-1}), influent and effluent TN ($[TN]_{inf}$, $[TN]_{eff}$; g N L^{-1}) maximum volume ofthe reactor (V_{max}; m^3) and the reactor VSS concentration($[VSS]_r$; gVSS m^{-3}).

$$sALR = \frac{Q_d \cdot [NH_4^+]_{inf}}{V_{max} \cdot [VSS]_r} \qquad \text{(Eq. 1)}$$

$$sNPR = \frac{Q_d \cdot [NO_x^-]_{eff}}{V_{max} \cdot [VSS]_r} \qquad \text{(Eq. 2)}$$

$$sNRR = \frac{Q_d \cdot \left([TN]_{inf} - [TN]_{eff}\right)}{V_{max} \cdot [VSS]_r} \qquad \text{(Eq. 3)}$$

2.2. Specific organic rates

PN-SBR specific organic loading rate (OLR; gCOD $gVSS^{-1}$ d^{-1}) (Eq.4) and the specific organic removal rate (ORR; gCOD $gVSS^{-1}$ d^{-1}) (Eq.5) were calculated as a function of Q_d, V_{max}, $[VSS]_r$, influent COD concentration ($[COD]_{inf}$; gCOD L^{-1}) and effluent COD concentration ($[COD]_{eff}$; gCOD L^{-1}).

$$sOLR = \frac{Q_d \cdot [COD]_{inf}}{V_{max} \cdot [VSS]_r} \quad \text{(Eq. 4)}$$

$$sORR = \frac{Q_d \cdot \left([COD]_{inf} - [COD]_{eff}\right)}{V_{max} \cdot [VSS]_r} \quad \text{(Eq. 5)}$$

3. Results

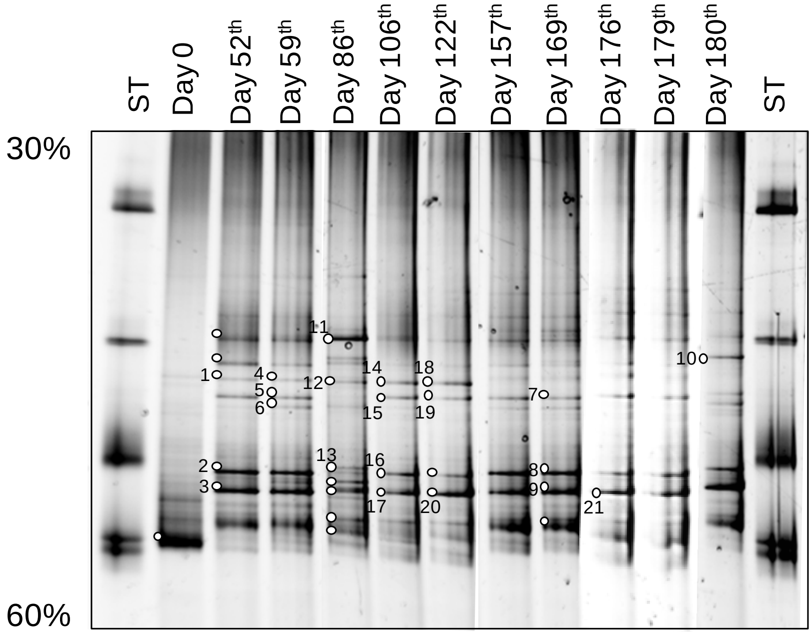

Figure S2. Negative image of a SybrGold-stained gel of 16S rRNA gene fragments (~560 bp) separated by DGGE. Mixed liquour samples were analyzed at different days during stable operation of PN-SBR. Symbols in the left margin of the lanes indicate the bands that were excised and sequenced, numbers indicate bands that yielded a validated good consensus sequence. The percentages on the left margin give the estimated concentrations of denaturant. ST, DGGE band position standard.

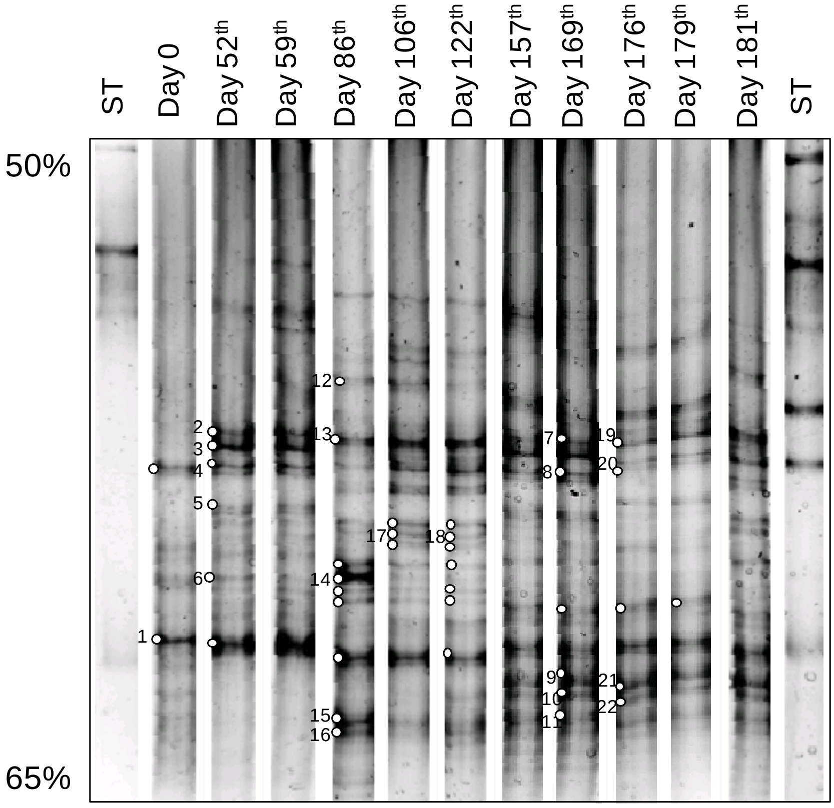

Figure S3. Negative image of a SybrGold-stained gel of nosZ gene fragments (~453 bp) separated by DGGE. Mixed liquor samples were analyzed at different days during stable operation of PN-SBR. Symbols in the left margin of the lanes indicate the bands that were excised and sequenced, numbers indicate bands that yielded a validated good consensus sequence. The percentages on the left margin give the estimated concentrations of denaturant. ST, DGGE band position standard.

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8. RESULTS AND DISCUSSION

8.1. PN-SBR performance

The PN-SBR has the clear objective of attaining suitable effluent with which to feed a subsequent anammox reactor (Ganigué et al. 2007). The PN-SBR effluent must achieve a NO_2^- :NH_4^+ molar ratio close to the stoichiometric value of 1.32 (Strous et al. 1997) along with a low biodegradable COD (bCOD) concentration to reduce any possible subsequent competition for NO_2^- between the heterotrophic and anammox bacteria (Ruscalleda et al. 2008). PN systems are usually operated at 30-35^{o}C because at these values AOB have higher growth rates than NOB (Hellinga et al. 1998).

Ganigué et al. (2009) demonstrated the stability of the PN-SBR process treating mature landfill leachate at 35^{o}C. The desired nitrogen conversion was driven by two different, but key, aspects. First, the NOB inhibition was obtained by the non-ionized forms of NH_4^+ and NO_2^-, free ammonia (FA) and free nitrous acid (FNA), respectively, which are affected by temperature and pH (Eq. 4.1 and 4.2). Second, the stoichiometric influent's HCO_3^-:NH_4^+ molar ratio was adjusted to 1.14 to obtain the desired effluent NO_2^-:NH_4^+ molar ratio of 1.32 (Ganigué et al. 2009). Although stable PN operation was achieved in these conditions, various modifications to the operating cycle could be applied to reduce energy requirements. Two different operational changes were assessed: i) temperature decrease and ii) the inclusion of anoxic feedings to promote heterotrophic denitrification. However, starting up and operating at lower temperatures as well as including anoxic feedings could in fact destabilize the performance of the process thus, the viability of the PN process in these conditions was analyzed.

8.1.1. Nitrogen conversions

Temperature affects bacterial activity as well as the chemical equilibriums of nitrogen species. Temperature has the opposite effect on FA and FNA equilibriums in their ionized forms present in the PN-SBR mixed liquor, and from which a higher FA but lower FNA emerges when temperature increases (Eq. 4.1 and 4.2). Both FA and FNA negatively affect AOB and NOB activity (Anthonisen et al. 1976). In this sense, an operational temperature change could affect the stability and robustness of the PN-SBR. The PN-SBR performance was evaluated under two different operating temperatures (35^{o}C and 25^{o}C) and was fed from day one of operation with mature leachate containing a mean NH_4^+ concentration of up to 6000 mg N-NH_4^+ L^{-1} (Gabarró et al. 2012).

A kinetic model was applied to investigate the influence of FA and FNA as inhibitors, as well as limitations from HCO_3^- availability. Table 5.1 summarizes maximum percentage reduction in AOB activity due to the inhibition and limitation. The AOB community was highly inhibited by FNA at 25°C, while a mix of FA and FNA inhibited AOB at 35°C. However, the influent's HCO_3^- concentration effect was similar for both experiments thus; limitations caused by HCO_3^- availability were also similar in both cases (about 45%).

Table 8.1. Contribution to AOB activity depletion at 25°C and 35°C from FA, FNA inhibition and in IC limitation kinetic terms (Gabarró et al. 2012).

Temp	FA inhibition			FNA inhibition			IC limitation			$sNPR_{max}$
°C	$1-\left(\frac{K_{I,NH_3}^{AOB}}{K_{I,NH_3}^{AOB}+NH_3}\right)$ (%)			$1-\left(\frac{K_{I,HNO_2}^{AOB}}{K_{I,HNO_2}^{AOB}+HNO_2}\right)$ (%)			$1-\left(\frac{e^{((HCO_3^- - k_{HCO_3^-})/a)}}{e^{((HCO_3^- - k_{HCO_3^-})/a)}+1}\right)$ (%)			mg N cells^{-1} d^{-1}
	mean	±	δ	mean	±	δ	mean	±	δ	mean
25	4.9	±	0.9	49.9	±	0.6	42.2	±	1.5	32.3
35	22.1	±	2.3	21.6	±	1.7	49.4	±	0.7	46.7

The highest specific nitrite production rates observed ($sNPR_{obs}$) during stable operation were 9.1 x 10^{-10} and 14.3 x 10^{-10} mg N cells^{-1} d^{-1} at 25 °C and 35°C, respectively. These values were compared to the theoretical maximum sNPR ($sNPR_{max}$) by applying the kinetic model, i.e., 32.3 10^{-10} and 46.7 x 10^{-10} mg N cells^{-1} d^{-1} at 25 °C and 35°C, respectively. Thus, the ratio $sNPR_{obs}$:$sNPR_{max}$ remained between 0.28 and 0.31 in the stable state. It could be demonstrated that AOB activity was decreased similarly in both runs, despite differences in AOB inhibition by FA and FNA. Therefore, as to the NO_2^-:NH_4^+ molar ratio of the effluent, it was demonstrated that mature leachate with extremely high influent nitrogen concentration could be successfully treated at 25°C (Table 5.2).

On the other hand, and taking into account changes in operating temperature and cycle configuration, NO_2^- build up was achieved in all of the PN-SBR conditions within about 10 days. The combination of FA and FNA were the main inhibition factors for NOB activity (Anthonisen et al. 1976) and NO_3^- concentration was below 10 mg N-NO_3^- L^{-1} for all tested configurations during the stable state. The operating changes applied to the PN-SBR (temperature and cycle configuration) did not diminish the quality of the PN-SBR effluent (Table 5.2).

During the fully aerobic runs, the adjustment of the influent leachate HCO_3^-:NH_4^+ molar ratio resulted in a good quality effluent in NO_2^-:NH_4^+ molar ratio terms. Besides, when anoxic feeding was applied, the effluent NO_2^-:NH_4^+ molar ratio was lower than expected because NO_2^- removal was mainly through heterotrophic activity (Table 5.2). Thus, higher HCO_3^-

influent concentration would be needed to reach the desired value of 1.32. However, anammox limitation by NO_2^- availability is better as the NH_4^+ accumulation has lower negative effects over anammox biomass than NO_2^- accumulation due to high NO_2^- inhibition over anammox biomass (Lotti et al. 2012, Scaglione et al. 2012). In this sense, an effluent NO_2^-:NH_4^+ molar of 1 could be considered as suitable for the subsequent anammox reactor.

Table 8.2. PN-SBR influent and effluent quality at differing temperatures and configuration cycles

PN-SBR cycle configuration	Temp (°C)	HCO_3^-:NH_4^+ inf (mol HCO_3^- mol^{-1} NH_4^+)	NO_2^-:NH_4^+ eff (mol NO_2^- $mol^{-1}$$NH_4^+$)	References
Aerobic	25	1.16 ± 0.06	1.33 ± 0.08	(Gabarró et al. 2012)
	35	1.12 ± 0.06	1.23 ± 0.07	
Aerobic/anoxic	35	1.17 ± 0.15	1.01 ± 0.14	(Gabarró et al. 2014)

Although a suitable PN-SBR effluent with a correct NO_2^-:NH_4^+ molar ratio was achieved, the bCOD removal prior to the anammox process (to diminish the possible NO_2^- competence between anammox and heterotrophic bacteria in the subsequent anammox reactor) was also a key point during the PN-SBR operation (Ruscalleda et al. 2008). COD contained in the leachate is heterogeneous in terms of biodegradability, with a larger proportion of non-biodegradable recalcitrant compounds, another part of slowly degradable bCOD (BOD_u) and low easily degradable bCOD (BOD_5) content (Anfruns et al. 2013). In summary, all the removed COD could be considered as bCOD. A fully aerobic cycle was applied during the experiments at 25°C and 35°C (Gabarró et al. 2012) and bCOD removal was achieved by heterotrophic aerobic oxidation using energy in aeration.

bCOD could be used as the electron donor during heterotrophic NO_2^- denitrification. bCOD oxidation in anoxic conditions decreases aeration requirements and also decreases the nitrogen load for the subsequent anammox reactor. The anammox NO_2^- inhibition risk decreases by lowering the nitrogen concentration of the anammox influent. In this light, anoxic feedings were included in the PN-SBR cycle to promote heterotrophic denitrification via NO_2^-. Anoxic feedings at 35°C resulted in 10-20% influent nitrogen removal (2300-2500 mg N-TN_{inf} L^{-1}). Nevertheless, heterotrophic denitrification via NO_2^- in the PN-SBR should be at low rates since NO_2^- can also be an inhibitor as well as leachate contented low bCOD concentration. Furthermore, NO_2^- removal can lead to partial denitrification and end up with undesired intermediates such as NO or N_2O, instead of N_2.

N_2O is a greenhouse gas with a potential 300 times higher than that of CO_2 (IPCC 2001) and can be emitted from nitrification-denitrification processes. Thus, N_2O production was studied during the PN-SBR performance at 35°C and including anoxic feedings.

8.1.2. N_2O production

N_2O can be produced under anoxic and aerobic conditions. Anoxic N_2O production might be induced by a low bCOD/N influent ratio, high NO_2^- concentration, pH variation and competition between denitrifying enzymes. Whereas, aerobic N_2O production by AOB is promoted by high NO_2^- concentration, low DO, dynamic influent NH_4^+ concentration and variable pH (de Graaff et al. 2010, Desloover et al. 2011, Joss et al. 2009, Kampschreur et al. 2008). Some of these conditions were present in the PN-SBR treating mature leachate (Gabarró et al. 2012, Gabarró et al. 2014).

The N_2O production in the PN-SBR with alternate aeration was calculated to be 3.6% of the total influent nitrogen (Gabarró et al. 2014), generated in both aerobic and anoxic conditions. Figure 5.1 outlines possible N_2O production routes under anoxic and aerobic conditions in the PN-SBR. Anoxic and aerobic phases contributed to total N_2O production by 60% and 40%, respectively. This finding was surprising as most of previous studies reported higher N_2O production during nitrification (Ahn et al. 2010, Kampschreur et al. 2008). Consequently, anoxic NO_2^- denitrification batch tests were carried out using the PN-SBR sludge to analyze heterotrophic denitrification rates (Figure 5, Chapter 5 (Gabarró et al. 2014)).

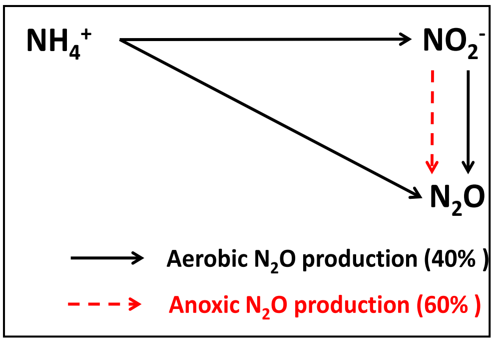

Figure 8.1. Metabolic scheme of N_2O production in the PN-SBR

Denitrification batch tests consisted of 60 minutes of bCOD limiting conditions, followed by an acetate spike used as easily degradable bCOD. Acetate addition was in excess (20%) to denitrify the initial NO_2^- concentration (100 mg N-NO_2^- L^{-1}). Consequently, two different conditions were achieved, one under bCOD limiting conditions and the other with no bCOD limitation. The NO_2^-denitrification rate (r_{NO2-}; 5.7-7.6 mg N gVSS^{-1} h^{-1}) was always higher than the N_2O denitrification rate (r_{N2O}; 0-4 mg N gVSS^{-1} h^{-1}) when NO_2^- was available despite the excess or limitation of bCOD. Additionally, it was observed that r_{N2O} depicted an acceleration phase under acetate excess or limitation, reaching its maximum when NO_2^- was completely removed. It is also noticeable N_2O denitrification stopped when acetate was spiked into the system.

Higher r_{NO2-} than r_{N2O} was also observed in the PN-SBR through high N_2O accumulation. Moreover, the mean anoxic N_2O production rate was calculated as 6.9 mgN-N_2O gVSS^{-1} h^{-1}. This value is in the range of the r_{NO2-} from the batch tests and so indicating that denitrification was mainly producing N_2O instead of N_2. This situation occurred despite enough bCOD being available in the system. Thus, the bCOD/N ratio was not the main cause for the N_2O accumulation. Then, it can be hypothesized that NO_2^- denitrification is carried out by even more microorganisms than N_2O denitrification or that denitrifying enzymes compete for electrons.

Another factor that could affect the heterotrophic denitrification under anoxic conditions is the possible inhibition by FNA of the N_2O reduction. Zhou et al. (2008) described the FNA inhibition concentrations with denitrifying sludge from a reactor fed with a nitrogen influent concentration of 250 mg N L^{-1}. Their results showed a 50% inhibition by 0.0007-0.001 mg N-HNO_2 L^{-1} and complete inhibition at 0.004 mg N-HNO_2 L^{-1}. The PN-SBR sludge was acclimated and selected under more stringent conditions of NO_2^-, NH_4^+ and salinity. The denitrification batch tests indicated that N_2O reduction rate increased by time despite FNA concentration and reached its maximum when NO_2^- was completely removed. Also, when acetate was spiked, N_2O reduction rate decreased to 0 mgN-N_2O gVSS^{-1} h^{-1}. This behaviour occurred despite FNA concentration which also decreased by time while NO_2^- was removed. Thus, results confirmed that FNA did not have the same affectation as Zhou et al. (2008) described for their denitrifying sludge. Further studies should be pointed to describe the FNA inhibition affecting the heterotrophic denitrification in the PN-SBR sludge.

Despite high N_2O production under anoxic conditions, the N_2O production from the PN-SBR (3.6%) was at the same magnitude of order as the municipal wastewater treatment plants in Australia (3.5%; (Foley et al. 2010)) or PN-anammox (operated in two steps) treating potato digestate (5.1-6.6%; (Desloover et al. 2011)) and digester liquor (2.3%; (Kampschreur et al. 2008)). Lab-scale PN reactor experiences have also been reported. In all the cases studied (Table 5.3), N_2O production was lower when treating synthetic and anaerobically treated black wastewater (de Graaff et al. 2010, Kong et al. 2013). However, NLR and influent NH_4^+ differed from the PN-SBR treating leachate presented in this thesis. Also, it is important to remark on the absence of bCOD in their influents.

PN-anammox can also be operated in one single reactor. Joss et al. (2009) reported a 0.4-0.6% N_2O emission of the NLR treating digestate liquor. In addition, Kampschreur et al. (2009) determined 1.23% of the NLR as N_2O emission. The PN-anammox configuration in one single reactor produces lower N_2O emissions than the two step PN-anammox since NO_2^- concentration is lower (Joss et al. 2009). However, complex industrial wastewaters such as landfill leachate treated by one single step PN-anammox have not yet been implemented and the experience with complex wastewaters is still under investigation. The impact of dynamic influents and bCOD content can destabilize the system when operating in a single reactor. In this sense, Panammox® technology has been demonstrated as a robust and stable technology for complex and variable influents.

The PN-SBR is only a part of the Panammox® process. It has been observed that the anammox process did not produce N_2O during denitrification (Desloover et al. 2011) and slight N_2O production from anammox reactors has been attributed to AOB denitrification (Kampschreur et al. 2008). Along these lines, the PN process would then be the main N_2O source matching in a subsequent anammox reactor such as the PN-SBR experiments discussed in this thesis. Thus, N_2O production from the PN-SBR could be taken as an approximate value for the whole Panammox® system. Nevertheless, further research should be pointed towards investigating N_2O production from the anammox reactor treating mature leachate.

The principal N_2O production in a PN-SBR operated under anoxic/aerobic conditions was obtained during anoxic conditions (60%). It is also known that aerobic N_2O production is exponentially related to AOB activity (Law et al. 2012) and at the same time, AOB activity is partially reduced by HCO_3^- limitation, FA and FNA concentrations. However, FA and FNA concentrations did not directly affect N_2O production by AOB (Law et al. 2011). In this light, further investigation should be pointed towards applying changes in the PN-SBR cycle to reduce N_2O production. The suppression of anoxic phases should be studied in N_2O production terms as well as HCO_3^- limitation over AOB.

Table 8.3. Summary of N_2O production from different wastewater treatment plants

Type of plant	Type of treated wastewater	NLR	Influent TN concentration	N_2O production	Highlights	References
		$Kg\ N\ m^{-3}\ d^{-1}$	$mg\ N\ L^{-1}$	%[a]		
12 BNR plants (USA)	Municipal	-	25-100	0.003 - 2.6	Aerobic treatment contributed significantly more to N_2O emission. N_2O production diminished by minimizing NH_4^+ and NO_2^- build up in the presence of O_2.	(Ahn et al. 2010)
7 BNR plants (Australia)	Municipal	-	47-114	3.5 ± 2.7	Positive correlation between NO_2^- accumulation and N_2O production at both aerobic and anoxic zones	(Foley et al. 2010)
Lab scale PN SBR	Synthetic	1	350	0.8 ± 0.4	N_2O emission rate was high in the initial phase of the aeration period, where hydroxylamine oxidation pathway accounted for 65% of the total N_2O production.	(Rathnayake et al. 2014)
Lab scale PN intermittently aerated SBBR	Synthetic	1.25	500	1.5 ± 0.2	The conditions in the intermittently aerated partial nitrification-SBBR are inherently more likely to induce some N_2O production.	(Kong et al. 2013)
Lab scale PN continuous reactor	Black water	34°C → 0.34 25°C → 0.08	1000-1500	0.6 – 2.6	Reduction of the aeration could lead to a reduction in N_2O emission. NO_2^- accumulation stimulated N_2O production.	(de Graaff et al. 2010)
Pilot scale intermittently aerated PN SBR	Mature landfill leachate	1.5	2300-3200	3.6 ± 0.4	NH_3 and HNO_2 inhibition and HCO_3^- substrate limitation caused low AOB activity and thus, moderate N_2O production.	(Gabarró et al. 2014)
Full-scale 2 stage PN-anammox	Industrial – potato processing factory – digestate	0.18-0.22	264	5.1-6.6	100% of the N_2O production from nitritation reactor. Anammox reactor emitted 0%. The critical parameters for the production of N_2O during nitritation include high nitrite and ammonium values, DO set point around 1.0mgO_2 L^{-1}	(Desloover et al. 2011)
Full-scale 2 stage PN-anammox	Digestate liquor	0.6	1500	2.3	The main process conditions influencing N_2O emission are DO, NO_2^- concentration and aeration rate. Denitrification by AOB was considered as the main source of N_2O emission from the nitritation and anammox reactor.	(Kampschreur et al. 2008)
Full scale single stage nitritation-anammox SBR	Digestate liquor	0.45	700	0.4-0.6	Lower nitrite concentration than other processes (<10 mgN-NO_2^- L^{-1}) caused lower N_2O emission.	(Joss et al. 2009)
Full scale single stage nitritation-anammox reactor	Digester liquor	1.2	250	1.23	In the absence of oxygen limitation, a linear relation between the nitrite concentration and the NO and N_2O concentrations in the off gas was found. Oxygen limitation (at levels between 0-2mgO_2/L) leads to a decrease in emission of NO and N_2O Over-aeration could lead to a dramatic increase of NO and N_2O emissions.	(Kampschreur et al. 2009)

[a] N_2O production/NLR

8.2. Microbial community

The inoculum of the PN-SBR was taken from a conventional nitrification/denitrification urban wastewater treatment plant (WWTP) located in Sils-Vidreres (Catalonia, Spain). For all the experiments presented, microbial analysis of the inoculum demonstrated diversity in both the nitrifying and the heterotrophic community. During the PN-SBR start-up period, enrichment of AOB and heterotrophic bacteria specialized in degrading complex bCOD was demonstrated (Gabarró et al. 2012, Gabarró et al. 2014, Gabarró et al. 2013). None of the stable state PN-SBR dominant species were dominant in the inoculum.

Several molecular techniques were used to identify and quantify microorganisms involved in the PN-SBR performance. Table 5.4 summarizes all of these molecular techniques used during the experimental period. In the long term (alternating anoxic and aerobic phases and at 35^{o}C), one only phylotype (*Nitrosomonas sp*) was found to be dominant (99%) in the *Nitrosomonadales* group using pyrosequencing (Gabarró et al. 2014) and also using PCR-DGGE (Gabarró et al. 2013). During the temperature effect experiments (at 25^{o}C and 35^{o}C), *Nitrosomonas europaea* was also the dominant AOB specie using PCR-DGGE. However, *Nitrosomonas eutropha* was also detected.

One possible explanation could be the primers that were used. On one hand, AOB 16S rRNA primers, which are specific for AOB detection, were used for the temperature experiments. On the other hand, general 16S rRNA primers, which are used to identify general bacteria that represent more than 7% of the total bacterial community, were used during the alternation of anoxic and aerobic phases experiment. Thus, it can be assumed that *N. eutropha* dominance was lower than 7% in the general eubacteria group detection. To the contrary, *N. europaea* was a significant specie in the PN-SBR. Also, it is interesting to highlight that it was found that the longer the experiment lasted, the lower the intensity of the second melting type (*N. eutropha*) (Gabarró et al. 2012) . In this sense, it is also plausible that over longer periods *N. eutropha* would eventually be washed out of the system.

Overall, *Nitrosomonas europaea* found in the PN-SBR and the only dominant phylotype was well adapted to the stringent conditions of FA, FNA and salinity in all cases. It is remarkable that this specie of *Nitrosomonas* was able to tolerate such a wide range of stringent conditions (FA, FNA and salinity) and dynamic influents (Gabarró et al. 2013). As a consequence, the PN-SBR performance was demonstrated to be robust, feasible and stable, thus ensuring a good quality effluent.

Table 8.4. Summary of molecular techniques used in the experiments presented in this thesis.

PN-SBR cycle configuration	Temperature (°C)	Molecular techniques used	References
Aerobic	25	PCR using AOB 16S rRNA primers AOB qPCR	(Gabarró et al. 2012)
	35		
Aerobic/anoxic	35	FISH PCR using general 16S rRNA primer PCR using nosZ primer AMO, nirK,nirS and nosZ qPCR	(Gabarró et al. 2013)
		Pyrosequing general 16S rRNA Pyrosequencing nosZ	(Gabarró et al. 2014)

Regarding heterotrophic community, the PN-SBR sludge was enriched to stringent conditions with resistant species which were not dominant in the inoculum. The leachate treatment at 35°C and combining anoxic and aerobic phases was assessed by PCR and DGGE fingerprinting of 16S rRNA and *nosZ* encoding genes to analyze eubacteria and denitrifiers phylogeny. Phylogenetic tree for 16S rRNA demonstrated that PN-SBR sludge was enriched by *Bacteroidetes* and *Betaproteobacteria*. Heterotrophic *Betaproteobacteria* were related to denitrification pathways as their closest cultured bacteria were *Comamonas nitrativorans* and *Castellaniella denitrificans*, whereas the *Bacteroidetes* identified were closely related to the cultured bacteria relatived to *Haliscomenobacter*, *Sphingobacter*, *Parapedobacter* and *Empedobacter*. Most of these were described as degrading complex organic molecules such as soluble microbial products (Rittmann et al. 2002).

Surprisingly, the *nosZ* gene marker revealed that only *Alphaproteobacteria* and *Betaproteobacteria,* both related to uncultured bacteria found in landfill leachate treatment and wastewater treatments, could genetically complete denitrification to N_2 (Gabarró et al. 2013). This observation was also confirmed by pyrosequencing. *Bacteroidetes* accounted for up to 49% of the 16Sr RNA gene sequences, while *Betaproteobacteria* and *Alphaproteobacteria* made up 25%. In the *nosZ* gene sequence pyrosequencing, 67% and 27% of the gene sequences were attributed as being *Betaproteobacteria* and *Alphaproteobacteria,* respectively. However, qPCR demonstrated the existence of microorganisms encoding the *nirK, nirS* and *nosZ* genes which, at the same time, demonstrated the capacity of the PN-SBR to denitrify both NO_2^- and N_2O species.

Based on the experimental results, the main nitrogen bio-transformation pathways in the PN-SBR operated under intermittent aeration are presented in Figure 5.2. AOB is the only one responsible for producing aerobic N_2O and it exponentially correlates with AOB activity (Law et al. 2012). Nevertheless, experimental results showed that aerobic production was about 1.5% of the NLR. This is because PN-SBR treating mature leachate operated at very low NH_4^+ oxidation rates (20 mgN-NH_4^+ $gVSS^{-1}$ h^{-1}; (Gabarró et al. 2014)) because of FA and FNA activity inhibition, as well as the HCO_3^- substrate limitation (Gabarró et al. 2012).

As for the anoxic pathways, AOB can denitrify NO_2^- during anoxic conditions but produces NO as the end-product (Yu et al. 2010) which could then be further reduced to N_2O by heterotrophic bacteria. On the other hand, in heterotrophic denitrification NO_2^- is sequentially reduced to NO, N_2O and finally N_2 using the bCOD from the anoxic feeding phases as the electron donor.

Experimental results showed significant N_2O generation during anoxic conditions because of a low dominance of heterotrophic organisms with the genetic capability to denitrify N_2O. Furthermore, the genetic potential of the PN-SBR community to denitrify N_2O was low because *Bacteroidetes* sequenced from mixed liquor samples did not have the *nosZ* gene (Gabarró et al. 2013). In this context, a small part of the microbial community (*Alphaproteobacteria* and *Betaproteobacteria*) would be able to completely denitrify N_2O to N_2 (Gabarró et al. 2013). Based on these findings, we propose an ecological equilibrium approach (Figure 5.3) where *Bacteroidetes*, which are specialized in the oxidation of slowly degradable bCOD (Gabarró et al 2013), could only denitrify NO_2^- to N_2O, while heterotrophic *Alphaproteobacteria* and *Betaproteobacteria* would be the mainly responsible for NO_2^-, NO and N_2O denitrification using bCOD from the influent leachate as the electron donor.

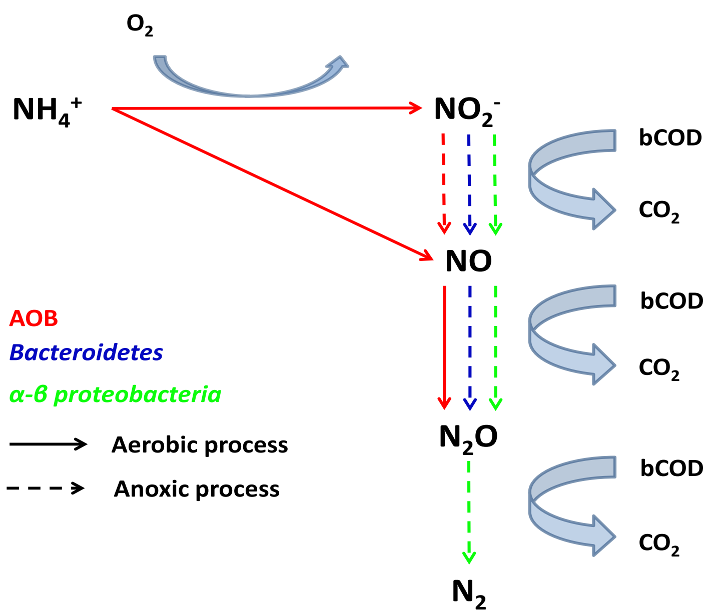

Figure 8.2. The biological pathways approach for nitrogen transformations in the PN-SBR. Different colors are used to separate these biological pathways. The ammonia oxidizing bacteria (AOB) pathway is in red, *Bacteroidetes* in blue, and *alpha* and *betaproteobacteria* in green. Aerobic processes are marked by solid lines and anoxic processes by dashed lines.

8.3. Implications of this thesis

From the discussion above, it has been seen that the PN-SBR operation was stable at high nitrogen concentrations in the influent, at temperatures of 25°C and 35°C and including anoxic feedings to promote heterotrophic denitrification.

The optimal temperature choice for the PN-SBR has to be related to the subsequent anammox reactor's working temperature. Lately, scientific efforts have been focused towards decreasing the operational temperatures during the anammox process. Dosta et al. (2008) reported on the short and long term effects of reducing operating temperature in an anammox SBR. After acclimation, the anammox SBR could be stably operated at 18°C. Furthermore, Daverey et al (2013) were successfully able to operate a simultaneous partial nitrification, anammox and denitrification (SNAD) reactor at 20°C. Nevertheless, high nitrogen load streams such as mature leachate require high anammox activity. Consequently, the treatment of landfill leachate by the anammox process has been usually done at temperatures of 30-35°C (Ruscalleda et al. 2008, Ruscalleda et al. 2010, Xu et al. 2010).

Working with a temperature of 25ºC in the Panammox® configuration would only depend on the anammox SBR performance, therefore the anammox SBR limits the running temperature of the overall process.

Anoxic feedings were also applied during the PN-SBR cycle configuration and extensively discussed presenting this thesis. However, the engineering implications of the results would mean that several considerations should be taken into account. The application of anoxic phases during the PN-SBR has two significant consequences: N_2O production and effluent NO_2^-:NH_4^+ ratio fluctuation.

In reference to PN-SBR N_2O production, anoxic feedings would be not recommended when treating mature landfill leachate because 60% of the total N_2O production was derived under anoxic phases. Thus, a fully aerobic cycle would be more appropriate. However, N_2O production under these conditions should be analyzed to corroborate the hypothesis of resulting in lower N_2O production.

On the other hand, including anoxic feedings in the PN-SBR cycle configuration could be considered as a good option when dealing with an influent that has a HCO_3^-:NH_4^+ molar ratio higher than the stoichiometric value of 1.14. NO_2^- denitrification would decrease the effluent NO_2^-:NH_4^+ molar ratio and reduce the use of chemicals such as HCl. Moreover, the addition of HCl should be diminished or even suppressed to balance the denitrified nitrogen with the PN-SBR effluent needs.

9. CONCLUSIONS

The results of this thesis have resulted in a deeper knowledge of the impacts of changes made in the PN-SBR operation. The configuration of the PN-SBR has been demonstrated as a robust and effective technology to deal with complex industrial wastewaters such as mature leachate containing high nitrogen concentration, as well as heterogonous COD. Stable PN was able to be maintained, despite stringent conditions and the changes made in the operation, and also, the PN-SBR ensured a suitable effluent for a subsequent anammox reactor.

Stringent conditions in the PN-SBR conditioned microbial community, bacterial activity and thus, nitrogen conversions. The principal conclusions reached in this thesis can be highlighted as:

- The PN-SBR can be operated at 25^{o}C and maintain similar specific AOB activity as it would at 35^{o}C. In both cases, the activity is considerably lower than the maximum. By applying a kinetic model to the experimental data, FA and FNA inhibition contributions along with HCO_3^- limitation were quantified. At 25^{o}C, AOB activity depletion was mainly caused by FNA inhibition (49%) and HCO_3^- limitation (42%). Whereas at 35^{o}C, a mix of FA and FNA caused inhibition (43%), although HCO_3^- limitation contributed considerably to AOB activity depletion as it did at 25 ^{o}C.

- Despite lower AOB activity, stable and robust PN-SBR performance was achieved at both temperatures and the desired effluent quality in NO_2^-:NH_4^+ ratio and bCOD content terms were guaranteed.

- FA and FNA effects on AOB activity depletion caused a low ammonium oxidation rate and consequently affected aerobic N_2O production.

- Stringent conditions led to having only one AOB phylotype (*Nitrosomonas europaea*) which was able to deal with extremely high NH_4^+ and NO_2^- concentrations as well as the high salinity present in the PN-SBR mixed liquor.

- The heterotrophic community was low in diversity but highly specialized. *Bacteroidetes* found in the PN-SBR were specialized in degrading low biodegradable organic molecules (slowly degradable bCOD).

- The rate of N_2O reduction to N_2 was lower than the NO_2^- denitrification rate, consequently causing an accumulation of N_2O during the anoxic phases in the PN-SBR.

- The inclusion of anoxic feedings in the PN-SBR cycle configuration would depend on the preferences of the operator. In terms of N_2O, fully aerobic cycles should be applied. Whereas, when the HCO_3^-:NH_4^+ molar ratio of the influent was higher than 1.14, anoxic feedings would be able to lower the use of acid for HCO_3^- concentration reduction of the influent.

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Curriculum Vitae

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EDUCATION

Feb'10 — **Master in Water Science and Technology**: "Partial nitritation of ammonium present in landfill leachates as a previous step of Anammox reactor: Start-up and operation" with the Laboratory of Chemical and Environmental Engineering (LEQUIA), University of Girona. Girona (Spain). Co-supervisors: Dr. Maria Dolors Balaguer and Dr. Jesus Colprim.

Sept'08 — **Bachelor in Environmental Sciences** for the University of Girona, Girona (Spain).

June'03 — **Technical degree in Environmental chemistry** for I.E.S. Narcís Monturiol of Barcelona (Spain).

COURSES

1. **Skills acquisition to write an european research project (European funds)**, organized by University of Girona (Girona, Spain, Feb 11th-15th 2013).
2. **Workshop "Unisense microsensors"**, organized by Unisense (Aarhus, Denmark, May 29th-31st 2012).

PUBLIC SCIENCE DISSEMINATION PARTICIPATION

1. Special guest presentation during greenhouse gas task group online meeting entitled "*N_2O production from the treatment of streams with high nitrogen load by PN-anammox*", organized by GHG IWA task group (Girona, February 28th 2014)

2. High school students visits during **science week**, organized by University of Girona (Girona, November 2012, 2013).

3. **Researchers' night (European funds FP7)**, organized by European universities, in Girona, organized by University of Girona (Girona, September 2010, 2011, 2012).

4. **Saló de l'ensenyament ("Educational meeting")**, organized by Catalan Government (Barcelona, March-April 2009, 2010, 2011, 2012).

EXPERIENCE

Feb'14 - Apr'14

Predoctoral stay at ModelEau Department of University of Laval (Quebec Ville, Quebec, Canada). **Research focus:** Nitrous oxide production from a partial nitritation SBR modelization by West DHI software. **Supervisor:** Dr. Peter Vanrolleghem.

Sep'11 – June'14

Part-time assistant professor at the University of Girona. Practical lessons of biotechnologic products, processes and projects in the 3rd year of Biotechnology Bachelor.

Feb'10 – Apr '14

Environmental Science PhD Student at the Laboratory of Chemical and Environmental Engineering (LEQUIA) of the University of Girona (Spain) Supervisors: Dr. Maria Dolors Balaguer, Dr. Ruscalleda and Dr. Jesus Colprim. **Thesis title**: "Effects of operational conditions on the performance of a partial nitritation SBR treating high nitrogen loads". **Predoctoral Fellowship** awarded from the University of Girona (Spain).

Sep'10 - Dec'10

Predoctoral stay at the Earth and Environmental Engineering Department of Columbia University (New York, USA). Supervisor: Dr. Kartik Chandran. **Research focus:** Nitrous oxide production from nitrifying sludge. **Research grant** founded by University of Girona (Spain).

Sep'08 – Sep'10 — **Environmental Science Master Student** at the Laboratory of Chemical and Environmental Engineering (LEQUIA) of the University of Girona (Spain) Supervisors: Dr. Maria Dolors Balaguer and Dr. Jesus Colprim. **Predoctoral Fellowship** awarded from the University of Girona (Spain).

Feb'08 - Aug'08 — **Environmental Science Ph.D. Assistant** at the Laboratory of Chemical and Environmental Engineering (LEQUIA) of the University of Girona (Spain). PhD student assisted: Ramon Ganigué. Supervisors: Dr. Maria Dolors Balaguer and Dr. Jesus Colprim. **Collaborating Fellowship** awarded from the University of Girona (Spain).

RESEARCH INTERESTS

Biological wastewater treatment, advanced nitrogen treatments, greenhouse gases production from wastewater treatments, modelling, programming.

PUBLICATION LIST: JOURNALS

1. **Gabarró, J.**, González-Cárcamo, P., Ruscalleda, M., Ganigué, R., Gich, F., Balaguer, M.D. and Colprim, J. (2014) Anoxic phases are the main N_2O contributor in partial nitritation reactors treating high nitrogen loads with alternate aeration. *Bioresource Technology* 163: 92-99.

2. **Gabarró, J.**, Hernández-del Amo, E., Gich, F., Ruscalleda, M., Balaguer, M.D. and Colprim, J. (2013). "Nitrous oxide reduction genetic potential from the microbial community of an intermittently aerated partial nitritation SBR treating mature landfill leachate." *Water Research* 47(19), 7066-7077.

3. A. Anfruns, **J. Gabarró**, Gonzalez-Olmos, S. Puig, M.D. Balaguer, J. Colprim (2013). "Coupling anammox and advanced oxidation-based technologies for mature landfill leachate treatment". *Journal of hazardous materials* 258-259, 27-34.

4. **Gabarró, J.**, Batchellí L., M.D. Balaguer, S. Puig, J. Colprim (2013). “On-site grey water treatment for irrigation reuse at a sports center: A case study”. *Environmental technology* 34 (11), 1385-1392.

5. **Gabarró J.**, R. Ganigué, M. Ruscalleda, M.D. Balaguer, J. Colprim (2012). “Effect of temperature on AOB activity of a partial nitritation SBR treating landfill leachate with extremely high nitrogen concentration.” *Bioresource Technology* 126 (2012): 283-289.

6. Mora X., M. Ruscalleda, **J. Gabarró**, Vilà A., M.D. Balaguer, J. Colprim (2011). “ Panammox: tratamiento de lixiviados de vertedero de residuos sólidos urbanos con alto contenido amoniacal mediante nitritación parcial y anammox.” *Tecnología del Agua* 326: 38-44.

7. Ganigue, R., **J. Gabarro**, H. Lopez, M. Ruscalleda, M. D. Balaguer and J. Colprim (2010). "Combining partial nitritation and heterotrophic denitritation for the treatment of landfill leachate previous to an anammox reactor." *Water science and technology* 61(8): 1949-1955.

8. Ganigue, R., **J. Gabarro**, A. Sanchez-Melsio, M. Ruscalleda, H. Lopez, X. Vila, J. Colprim and M. D. Balaguer (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 (23): 5624-5632.

PUBLICATION LIST: CONFERENCES–ORAL PRESENTATIONS

1. **J. Gabarró**, P.González-Cárcamo, M. Ruscalleda, R. Ganigué, M.D. Balaguer, J. Colprim (2014). “Lessons learnt from N_2O production during extremely high nitrogen streams treatment”. ***Keynote**. 2nd IWA specialized conference "EcoTechnologies for Sewage Treatment Plants -EcoSTP2014-“*, (June 23rd-25th Verona, Italy), Org: IWA.

2. A. Anfruns, Ruscalleda, M., Vilà, A., González-Cárcamo, P., **Gabarró, J.,** Balaguer, M.D., Colprim, J.(2014). “N And COD Removal Of Leachate Enhancing The COD Biodegradability Combining Panammox® And PhotoFenton”. *2nd IWA specialized conference "EcoTechnologies for Sewage Treatment Plants -EcoSTP2014-“*, (June 23rd-25th Verona, Italy), Org: IWA.

3. A. Anfruns, Ruscalleda, M., Vilà, A., González-Cárcamo, P., **Gabarró, J.**, Balaguer, M.D., Colprim, J.(2014). “Improving biodegradable organic matter availability in a combined partial nitritation-anammox/photo-fenton process for optimal N and COD removal from mature landfill leachates”. *4th International conference on industrial and hazardous waste management*, (Sept 2nd-5th Chania, Crete, Greece), Org: International waste working group.

4. M. Ruscalleda; A. Anfruns; **J. Gabarró**; R. Gonzalez-Olmos; X. Mora; M.D. Balaguer; J. Colprim (2013). "Advanced treatment of mature landfill leachates for refractory COD and full autotrophic N removal: applicability and cost-saving analysis". *International conference on nitrification*, (Sept 2nd-5th Tokyo, Japan), Org: The nitrification network.

5. Dumit, M., **J. Gabarró**, S. Murthy, R. Riffat, K. Chandran. (2011). "The impact of post anoxic dissolved oxygen concentrations on nitrous oxide emissions in nitrification processes". *84th annual Water Environment Federation Technical Exhibition and Conference,* (October 15th-19th Los Angeles, USA), Org: WEF.

6. **Gabarró J.,** L. Batchellí, M.D. Balaguer, S. Puig, J. Colprim. (2011). "On-site grey water treatment for irrigation reuse at a sports center: A case study." *8th IWA International Conference on water reclamation & reuse*, (September 26th-29th Barcelona, Catalonia), Org: IWA.

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

PUBLICATION LIST: CONFERENCES–POSTER PRESENTATIONS

1. R. Gonzalez-Olmos; A. Anfruns; **J. Gabarró**; S. Puig; M.D. Balaguer; J. Colprim (2013). "Combination of advanced oxidation and anammox processes to treat landfill leachate with high nitrogen and organic matter concentration". *3rd European conference on environmental applications of advanced oxidation processes*, (Oct 27th-30th, Almería, Spain), Org: CIEMAT-Universidad de Almería.

2. **Gabarró, J.**, P. Gonzalez-Carcamo, M. Ruscalleda, R. Ganigué, M.D. Balaguer, J. Colprim (2013). "N_2O dynamics in a PN-SBR treating mature landfill leachate". *Nutrient Removal and Recovery 2013: Trends in Resource Recovery and Use*, (July 28th-31st, Vancouver, Canada), Org: IWA, WEF.

3. **Gabarró J.**, A. Anfruns, S. Puig, R. González-Olmos, X. Mora, M. Ruscalleda, M.D. Balaguer and J. Colprim. (2013). "Coupling advanced chemical and biological technologies for extremely high contaminated mature landfill leachate". *Leading edge technologies congress,* (June 2nd-6th 2013, Bordeaux, France), Org: IWA.

4. **Gabarró**, **J.**, R.Ganigué, M. Ruscalleda, M.D. Balaguer, J. Colprim. (2010). "Temperature effects on partial nitritation treating extremely high ammonium levels of landfill leachate". Enzymology

and ecology of the nitrogen cycle, (15th –17th September 2010, University of Birmingham, UK), Org: Biochemical Society.

5. Batchellí, L., **J. Gabarró,** M.D. Balaguer, M. Laburu, S. Pui**g,** J. Colprim. (2010). On-site greywater treatment for sport center irrigation. *International IWA Specialized conference on Sustainable Solutions for Small Water and wastewater Treatment systems (S2Small2010)*. (April 19th-22th 2010; Girona, Spain), Org: IWA.

RESEARCH PROJECTS and R+D CONTRACTS WITH ENTERPRISES

The main research projects, that I have participated, were:

1. **ITACA- Investigation of treatment, reuse and control technologies for future sustainability of wastewater treatment.**

Financing and duration: Spanish Ministry of Science and Innovation. 2011/2014.

Principal researcher: Dr. Jesús Colprim

Participants: CESPA Waste Management.

Description: PANAMMOX© treatment of landfill leachate. Semi-industrial pilot plant startup and operation.

2. **NIMOX- Partial nitritation and anaerobic oxidation through Anammox biomass of ammonium originated on anaerobic sludge digestion of a urban wastewater treatment plant.**

Financing and duration: Spanish Ministry of Science and Innovation. 2008/2010.

Principal researcher: Dr. Jesús Colprim

Participants: Water Catalan Agency, CESPA Waste Management.

Description: High nitrogen concentrations and low biodegradable organic matter waste streams have high operational costs for their treatment. Anammox is a good alternative to conventional nitrogen treatments. The objective of this project was to study, and to evaluate the viability of the implementation of PANAMMOX® configuration treating returned wastewater from anaerobic sludge digestion.

3. **LEAMMOX- Landfill leachate biological treatment through partial nitritation and anaerobic oxidation of ammonium by PN-SBR and Anammox process.**

   Financing and duration: Spanish Ministry of Science and Innovation. 2009/2011.

   Principal researcher: Dr. Jesús Colprim

   Participants: CESPA Waste Management.

   Description: Landfill leachate presents high ammonium concentrations and low biodegradable organic matter. Anammox process has been recently studied for landfill leachate treatment due to its lower operational costs. The objective of this project is the development of a partial nitritation and an Anammox process applied to landfill leachate treatment.

3. **On-site greywater treatment for sport center irrigation***

   ** Not developed: financing problems due to global economic warning*

   Financing and duration: Spanish Ministry of Science and Innovation. Programa Nacional de Investigación Aplicada, Subprograma de Investigación Aplicada Colaborativa. CIT-310000-2009-39.

   Principal researcher: Dr. Sebastià Puig

   Participants: Catalan Institute for Water Research (ICRA; Spain) and Bombas Electricas S.A (BOELSA; Spain).

   Description: Greywater is the water flow that comes from showers, hand basins, kitchen sinks and washing machines excluding black wastewater. The objective of this project was the on-site greywater treatment assessment from sport center and its later reuse for irrigation. The idea was to develop a product for a market which combines knowledge from water treatment and reuse using products from the enterprise BOELSA.

4. **Consolidated groups: Laboratory of Chemical and Environmental Engineering (LEQUIA).**

   Financing and duration: Catalan Government. AGAUR (Comission for Universities and Research). 2005SGR 00406 (2005-2008).

   Participants: LEQUIA (Spanish acronym for the Laboratory of Chemical and Environmental Engineering) of the University of Girona (UdG) (Spain)

   Description: Grants for consolidated research groups.

LANGUAGES

Catalan: native

Spanish: native

English: fluent

French: intermediate

German: beginning

Computer skills

Scientific software: West DHI, Matlab, Sigmaplot

Other: MS office