

FUGITIVE GREEN-HOUSE GAS EMISSIONS
DURING BIOLOGICAL WASTEWATER
TREATMENT:
INVESTIGATING SOURCES AND MITIGATION
STRATEGIES IN LABORATORY AND FULL-SCALE
SYSTEMS

Adrián Rodríguez-Caballero

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Doctoral Thesis

**Fugitive green-house gas emissions during biological
wastewater treatment: Investigating sources and
mitigation strategies in laboratory and full-scale
systems**

Adrián Rodríguez Caballero

2015

Supervisor: Dr. Maite Pijuan Vilalta

Tutor: Dr. Ignasi Rodríguez-Roda Layret

Thesis submitted in fulfilment of the requirements for the degree of Doctor from the University of Girona
(PhD Programme: Water Science and Technology)

MAITE PIJUAN VILALTA, investigadora de l'Institut Català de Recerca de l'Aigua (ICRA) i **IGNASI RODRÍGUEZ-RODA LAYRET**, professor del Departament d'Enginyeria Química, Agrària i Tecnologia Agroalimentària de la Universitat de Girona i investigador de l'ICRA

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A mis padres,
por todo el apoyo, el cariño,
y porque las cosas importantes de la vida
no las aprendí en los libros.

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Muchos años después de defender la tesis doctoral, habría de recordar que el día en que llegué a Girona, *el Mundo era tan reciente que muchas cosas carecían de nombre, y para nombrarlas había que señalarlas con el dedo*. Y poco a poco, Maite Pijuan hacía realidad las ideas como cuando los artistas comienzan a darle golpes de pincel a un cuadro enorme. Y aquel garaje diáfano con olor a plástico nuevo se transformó en el laboratorio L06. Y lo llenamos de ilusión y de experimentos. Y así pudimos comenzar a ponerle nombre a las cosas. Decía Walter Bonatti que hay muchas montañas en la vida de los hombres, y desde luego aquella montaña que fue escribir una tesis doctoral, la pude escalar gracias a la ayuda inmensa y al esfuerzo de Maite. Después vimos llegar a mucha gente con más ilusión y más energía, como Anna, Olga, Eliza, Celia... Gente excepcional que convirtió el ICRA en un buen centro de investigación con mucho futuro. Y así, casi sin darnos cuenta, el Mundo ya no era tan reciente y mi tesis fue tomando forma. Habría de recordar éstas y otras muchas cosas más en aquel refugio inhóspito, en medio de las montañas más altas, mientras Martha me escuchaba.

Recordaría que toda aquella historia de la tesis doctoral empezó a escribirse mucho tiempo antes de llegar a Girona, junto al profesor Carl Pålson, que me abrió las puertas de su laboratorio de microbiología de par en par, para enseñarme que ciencia y arte no eran tan diferentes como pudiera parecer. Todo empezó a la orilla del lago Mälaren, junto a buenos colegas y amigos de la pequeña universidad de Mälardalen (Elena, Iana, Johan, Erik, Emma, Monica...). En aquella época, el frío de los inviernos era muy intenso, y sólo podía combatirse con el calor de la gran familia Kazimierzak-Leis y todos aquellos amigos de Västerås y de Stockholm, que siempre supieron darme los abrazos más sinceros. Muchos años después de defender la tesis, volvería a recordar la mañana de febrero en que me subí a un avión en Suecia y aparecí en Girona. Me recibieron el calor de mi vieja amiga Carol y la enorme sonrisa de Neus, que llenaba todo el despacho. No pude tener una mejor bienvenida que la que me dieron Quim, Meri y Anna en aquella casa enorme rodeada de bosque. Y en el ICRA estaban Serni, Pau, Gigi, Uri, Gemma, Mariona, Vicenç y muchos otros compañeros que hicieron que desde el primer día me sintiera como en casa. Y un día entró por la puerta un ingeniero loco, Ignasi, que quiso ser un gran amigo, y con el que compartí muchas horas de trabajo y, lo más importante, muchísimas risas.

Llegué a Girona con ganas de volver. De poder vivir con Martha los inviernos al sol y el lado mediterráneo de la vida. Mucho tiempo después de la mañana en que defendí la tesis, volví a recordar que en Girona y Catalunya tuve siempre la sensación de estar en mi tierra, porque yo nunca entendí las patrias y siempre tuve sitio para muchas banderas. *I em sentía molt català*, igual que antes fui castellano en Salamanca, sueco en Estocolmo y africano en Ciudad del Cabo. Y eso siempre sin dejar de ser cántabro en todas partes, porque había encontrado la fórmula mágica para quitarme de encima todas las etiquetas. Volví a recordar que la vida era una aventura constante desde las calas más recónditas de la Costa Brava hasta los picos más altos del Pirineo. No podría olvidar los días de escalada con tantos amigos en las paredes de caliza de Sadernes o en el bosque mágico de Savassona; el hielo en la cara norte del Gra de Fajol, con la *tramuntana* vigilándonos la espalda y congelándome las manos; las esquizadas desde el Bacivers, el Bastiments y tantos otros gigantes cubiertos de nieve recién traída por una intensa *llevantada*; tantas y tantas horas corriendo por los bosques de Les Gavarres, preparándonos para otras batallas en las montañas más altas. Recordaría todos esos paisajes compartidos con amigos como Maria, Pep y Anna, Ivan y Brigida, Rovira, Edu, Adri y otros "micos", Joel, Canimas, Riuró, Met y muchos otros corredores de montaña.

Años después de la defensa de la tesis, volvería a revivir algunos momentos muy antiguos, luchando en la guerra perdida de los recuerdos resbaladizos que se nos escapan, para poder contarle a Martha que nunca había visto a nadie agarrarse a la vida con tanta fuerza como lo hacía mi padre la mañana de septiembre en que ascendimos, muy despacito, el Col de Grand Ferret, junto a Juanjo y el resto de los bomberos. Y de cómo aquello fue la lección más importante que aprendí: Que el secreto de los héroes más valientes es nunca dar por perdida la sonrisa. Y también recordaría el amor inconmensurable de mi madre, que supo siempre demostrarme que estaría a mi lado

incondicionalmente, y eso fue muchas veces, el único flotador que encontré cuando arreciaban las peores tormentas; fue sin duda gracias al esfuerzo de mis padres, que pude recorrer el camino sinuoso que me llevó a defender una tesis doctoral.

Jamás podría olvidar el olor a serrín y mar Cantábrico de mi abuelo “Totoño”, o aquella tarde en que la ciudad lloraba lágrimas de salitre al verle remar por última vez en una trainera llena de flores. O las tardes a la sombra de los árboles frutales en la Ávila natal de mi abuela Amparo, que también era mi madre, y de todo lo que aprendí con ella y con Marysol, Adri y aquella bisabuela que recordaba el África de su juventud, pero que decidió olvidarse de su vejez. Y la energía infinita de la abuela Maricarmen mirando las olas golpear el paseo de La Maruca y nuestros incontables miércoles de arroz blanco, o el sabor secreto a coco y nata fresca de la tarta que nos preparaba en cada cumpleaños. O los veranos en que segábamos la hierba *a dalle* cerca de la cabaña del “tío Teo”, aquel olor a verde empapado de lluvia y a pelo de caballo, recogiendo las ciento una endrinas que se necesitaban para hacer el mejor pacharán.

Volvería a recordar que un mes antes de cumplir diez años, me llevaron a conocer a mi hermano Álex, el bicho de rizos negros que trajo las sonrisas a la familia y al que nunca supe agradecer lo suficiente haberme apartado de la soledad. Aparecerían en mi cabeza recuerdos que se mantienen muy nítidos, muy vivos, como las noches en que Maribel me ayudaba a transcribir una pequeña novela histórica que meses más tarde me llevaría a suspender matemáticas y a recorrer los lugares más insospechados de Latinoamérica y el Mundo, a correr con los *Rarámuris* en las montañas mexicanas, y a compartir caminos con buenos amigos y amigas de la Ruta Quetzal, los mejores compañeros de viaje. Y las tardes pescando en La Punta de La Mesa con el *tato* Carlos, viendo las olas romperse en mil pedazos contra las rocas, intentando que picaran las Lubinas, aunque al final siempre sacábamos Chaparrudos y Porredanos. Muchos años después, también me acordaría de las vueltas a casa desde tan lejos, desde Salamanca, Suecia o Sudáfrica. De volver a sentir, cada vez, el cariño de una gran familia, de todos mis tíos, tías, primos y primas que ya no podía contar con los dedos de las manos, pero sí con los latidos del corazón.

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Muchos años después de defender la tesis, habría de recordar todas estas cosas junto a Martha, tirados en aquel rincón de un refugio. Exhaustos. Con la cara quemada por el sol y el viento, las piernas cansadas, las manos curtidas, con olor a roca y hielo. Felices. Y volvería a recordar que, muchos años antes, aquel viaje al país de las auroras boreales le dio un vuelco a mi corazón, porque tuve la suerte de encontrar a aquella chica de sonrisa eterna y valentía infinita con la que compartir las aventuras y la vida, y que quiso acompañarme mientras escribía las cien páginas de soledad de mi tesis doctoral.

List of publications

The research work presented in this thesis (chapters 4 to 7) has been redrafted from a group of scientific publications listed below:

Rodriguez-Caballero, A., Pijuan, M. 2013. N₂O and NO emissions from a partial nitrification sequencing batch reactor: Exploring dynamics, sources and minimization mechanisms. *Water Research* **47**(9), 3131-3140.

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Rodriguez-Caballero, A., Ribera, A., Balcázar, J.L., Pijuan, M. 2013. Nitritation *versus* full nitrification of ammonium-rich wastewater: Comparison in terms of nitrous and nitric oxides emissions. *Bioresource Technology* **139**, 195-202.

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Rodriguez-Caballero, A., Aymerich, I., Poch, M., Pijuan, M. 2014. Evaluation of process conditions triggering emissions of green-house gases from a biological wastewater treatment system. *Science of the Total Environment* **493**, 384-391.

Author's Contribution: The monitoring campaign and data analyses with contributions from the other authors. Writing the paper, with contributions from the other authors.

Rodriguez-Caballero, A., Aymerich, I., Marques, R., Poch, M., Pijuan, M. 2015. Minimizing N₂O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor. *Water Research* **71**, 1-10.

Author's Contribution: The monitoring campaign and data analyses with contributions from the other authors. Writing the paper, with contributions from the other authors.

Additional relevant publications not included in this thesis:

Pijuan, M., Tora, J., **Rodriguez-Caballero, A.**, Cesar, E., Carrera, J., Perez, J. 2014. Effect of process parameters and operational mode on nitrous oxide emissions from a nitritation reactor treating reject wastewater. *Water Research* **49**, 23-33.

Castro, C., **Rodriguez-Caballero, A.**, Volcke, E., Pijuan, M. 2014. Effect of nitrite on the N₂O and NO production on the nitrification of low strength ammonium wastewater. Submitted to *Bioresource Technology*.

List of acronyms

<i>AOB</i>	<i>ammonia oxidizing bacteria</i>
<i>BOD</i>	<i>biological oxygen demand</i>
<i>C</i>	<i>carbon</i>
<i>CH₄</i>	<i>methane</i>
<i>CO₂</i>	<i>carbon dioxide</i>
<i>COD</i>	<i>chemical oxygen demand</i>
<i>DO</i>	<i>dissolved oxygen</i>
<i>FISH</i>	<i>fluorescence in situ hybridization</i>
<i>GHG</i>	<i>green-house gas</i>
<i>GWP</i>	<i>global warming potential</i>
<i>HRT</i>	<i>hydraulic retention time</i>
<i>MLSS</i>	<i>mixed liquor suspended solids</i>
<i>MLVSS</i>	<i>mixed liquor volatile suspended solids</i>
<i>N</i>	<i>nitrogen</i>
<i>N₂O</i>	<i>nitrous oxide</i>
<i>NH₂OH</i>	<i>hydroxylamine</i>
<i>NH₃</i>	<i>ammonia</i>
<i>NH₄⁺</i>	<i>ammonium</i>
<i>NO</i>	<i>nitric oxide</i>
<i>NO₂⁻</i>	<i>nitrite</i>
<i>NO₃⁻</i>	<i>nitrate</i>
<i>NOB</i>	<i>nitrite oxidizing bacteria</i>
<i>PLC</i>	<i>programmable logic controller</i>
<i>SBR</i>	<i>sequencing batch reactor</i>
<i>SRT</i>	<i>sludge retention time</i>
<i>TKN</i>	<i>total Kjeldahl nitrogen</i>
<i>TN</i>	<i>total nitrogen</i>
<i>TP</i>	<i>total phosphorous</i>
<i>WWTP</i>	<i>wastewater treatment plant</i>

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We have to realize that all of us – all the seven billions – are one family that live on the same planet

Gustaf Olsson

Summary

The exponential increase of the atmospheric concentration of green-house gases due to human activities is responsible for the acceleration of global warming and climate change. Recently, scientific studies have pointed at wastewater treatment systems as relevant sources of fugitive green-house gases (GHGs) such as nitrous oxide (N_2O) and methane (CH_4). Nitric oxide (NO) can also be emitted during wastewater treatment, and it is a potent ozone-depleting compound and a precursor of N_2O . Due to the high global warming potential of N_2O and CH_4 , emission of these gases (even at low levels) may be relevant and increase severely the overall carbon footprint of a wastewater treatment system.

The exact factors triggering N_2O and NO production in biological wastewater treatment systems are still under debate. However, it is known that N_2O and NO can be produced during the biological oxidation of ammonia (NH_3) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and during the reduction of nitrate (NO_3^-) or NO_2^- to nitrogen gas (N_2) by denitrifying bacteria. Differently, CH_4 is produced as a consequence of the degradation of organic matter under anaerobic conditions, and is given along several metabolic pathways in methanogenic archaea. Fugitive GHG emissions during wastewater treatment need to be understood and accurately accounted for in the global emissions budget, and mitigation strategies in wastewater treatment facilities should be designed and implemented.

This thesis collects and presents a group of research studies performed with the general goal of identifying potential minimization strategies for N_2O and CH_4 emissions from different wastewater treatment systems. The work carried out during the elaboration of this thesis can be divided into two main sections, namely lab- and full-scale studies. On one hand, laboratory experiments were carried out on sequencing batch reactors (SBRs) with enriched nitrifying bacterial populations. These experiments allowed the identification of some of the most important factors triggering production of N_2O and NO during partial and full nitrification of ammonium (NH_4^+)-rich wastewater, simulating processes that are commonly applied in the treatment of reject wastewater in real scenarios. On the other hand, two monitoring campaigns targeting N_2O and CH_4 were performed in municipal wastewater treatment plants (WWTPs). These campaigns allowed the identification of process conditions that lead to N_2O and CH_4 peak emission events.

In the first lab-scale study, both the concentration of NH_4^+ and NO_2^- in the bulk liquor of the partial nitrification SBR were shown to be key parameters related with N_2O and NO production and emissions. It was concluded that most of the N_2O originated during settling was due to biological reactions, and was emitted during the first minutes of each cycle, upon aeration. The complete oxidation of NH_4^+ (or most likely hydroxylamine) as a result of sufficient aeration time was suggested as a potential minimization strategy for N_2O emissions in partial nitrification systems. In the second set of experiments, partial and full nitrification of NH_4^+ -rich wastewater were compared for N_2O and NO emissions. Partial nitrification led to higher N_2O and NO emissions than full nitrification, likely due to the combined effect of lower NO_2^- and NH_4^+ concentrations in the bioreactor. Therefore, the application of partial nitrification in real-scale facilities need to be evaluated, taken into account the increase of gas emissions and its implications in the carbon footprint of the system.

The full-scale monitoring campaigns were carried out at the municipal WWTPs of Granollers and La Roca del Vallès, near Barcelona (Spain). The biological wastewater treatment at Granollers WWTP is performed in two parallel plug-flow activated sludge reactors. The activated sludge basins of this plant are coupled to a sludge treatment facility for biogas production and electricity generation. CH_4 emissions mainly occurred due to air stripping in the first part of the bioreactor, and were mostly related with the influent and reject wastewater flows entering the bioreactor from the anaerobic sludge digester. On the other hand, N_2O emissions were given along all the aerated parts of the bioreactor and were strongly dependant on the occurrence of process disturbances such as periods of no aeration or nitrification instability.

The biological wastewater treatment at La Roca del Vallès WWTP is performed in two SBRs. In contrast to Granollers WWTP, CH₄ emissions at La Roca del Vallès WWTP were insignificant, due to the absence of an anaerobic sludge digestion facility releasing reject wastewater. However, N₂O emissions were large, accounting for up to 60% of the total carbon footprint of the plant. At the SBR in La Roca del Vallès WWTP, cycles with long aerated phases showed the largest N₂O emissions, with the consequent increase in carbon footprint. An operational strategy was proven to severely mitigate N₂O emissions from La Roca del Vallès WWTP. It consisted of intermittent aeration (short oxic and anoxic phases of 20-30 min) being applied in the sequencing cycle configuration. This strategy led to the overall minimization of the carbon footprint of the WWTP, without compromising the process performance.

Resum

L'acceleració en l'escalfament global i el canvi climàtic són conseqüència de l'increment exponencial de la concentració de gasos d'efecte hivernacle en l'atmosfera degut, en part, a l'activitat humana. Estudis científics publicats recentment apunten a alguns sistemes de tractament d'aigües residuals com a fonts rellevants de gasos d'efecte hivernacle com l'òxid nitrós (N_2O) i el metà (CH_4). A més, l'òxid nítric (NO), un potent destructor de la capa d'ozó a més de ser un precursor del N_2O , també pot ser emès durant el tractament d'aigües residuals. Degut a l'alt potencial d'escalfament atmosfèric del N_2O i el CH_4 , l'emissió d'aquests gasos (inclús a baixes concentracions) pot ser rellevant, incrementant de forma significativa la petjada ecològica dels sistemes de tractament d'aigües residuals.

Els factors responsables de la producció del N_2O i NO en sistemes de tractament biològic d'aigües residuals estan subjectes a debat. No obstant, es coneix que tant el N_2O com el NO poden produir-se durant l'oxidació biològica de l'amoniac (NH_3) a nitrit (NO_2^-), duta a terme pels bacteris oxidants de l'amoni, i durant la reducció del nitrat (NO_3^-) o NO_2^- a nitrogen gas (N_2), duta a terme per bacteris desnitrificants. Per altra banda, el CH_4 es produeix com a conseqüència de la degradació de la matèria orgànica en condicions anaeròbies per part de microorganismes metanògens. Les emissions de gasos d'efecte hivernacle durant el tractament de l'aigua residual han de ser avaluades en profunditat i haurien d'incloure's en els càlculs globals d'emissions. A més, hi ha una necessitat creixent d'identificar i implementar estratègies de mitigació d'aquestes emissions en les estacions depuradores d'aigües residuals (EDARs).

Aquesta tesi doctoral compren una sèrie d'estudis científics realitzats amb l'objectiu general d'identificar estratègies de minimització de les emissions de N_2O i CH_4 en diferents sistemes de tractament d'aigües residuals. El treball descrit en aquesta tesi està dividit en dues seccions: i) estudis a escala laboratori i ii) estudis a escala real. Per una banda, es van realitzar una sèrie d'experiments en diversos reactors discontinus seqüencials a escala laboratori, enriquits amb bacteris nitrificants. Mitjançant aquests experiments es van poder identificar alguns dels factors més importants implicats en la producció del N_2O i el NO durant la nitrificació parcial d'aigua residual amb una altra concentració d'amoni, simulant un procés aplicat durant el tractament d'aigües de rebuig. Per altra banda, es van portar a terme dues campanyes de monitorització del N_2O i el CH_4 en dues EDARs urbanes. Aquestes monitoritzacions van facilitar la identificació de condicions d'operació que originen importants pics d'emissió d'aquests dos gasos.

En el primer estudi de laboratori es va demostrar que tant la concentració d'amoni com la de nitrit en un reactor de nitrificació parcial són paràmetres estretament lligats a la producció de N_2O i NO . Es va concloure que la major part del N_2O detectat, es produïa durant l'etapa de sedimentació a través de reaccions biològiques i era emès durant els primers minuts d'aeració al principi de cada cicle. L'oxidació completa de l'amoni (o més probablement de la hidroxilamina (NH_2OH)) com a resultat d'aplicar un temps d'aeració més llarg es pot proposar com una estratègia de minimització de les emissions de N_2O en sistemes de nitrificació parcial. En un segon grup d'experiments es van comparar les emissions de N_2O i NO durant la nitrificació parcial i completa d'una aigua residual amb un alt contingut d'amoni. En aquest segon estudi, l'aplicació de la nitrificació parcial va donar lloc a unes emissions majors de N_2O i NO comparant amb les emissions detectades durant la nitrificació completa, possiblement degut a l'efecte combinat de les concentracions d'amoni i nitrit molt majors en la nitrificació parcial. Per tant, l'aplicació d'aquest procés en sistemes reals hauria de ser avaluada tenint en compte les emissions de N_2O i NO associades i el seu impacte a la petjada de carboni de la planta.

Les campanyes de monitorització de gasos d'efecte hivernacle a escala real es van portar a terme a les EDARs de Granollers i La Roca del Vallès. El tractament biològic de l'EDAR de Granollers es porta a terme en dos reactors tipus flux pistó. A part de l'aigua residual que arriba a l'EDAR aquests reactors també tracten el corrent d'aigües de rebuig que s'origina durant el centrifugat de fangs

provinents del digestor anaerobi present a la planta. Els pics d'emissió de CH_4 detectats en aquesta EDAR eren conseqüència de la transferència a la fase gas del CH_4 dissolt present en aquest corrent d'aigües de rebuig que es produïa quan aquesta aigua arribava a la primera zona airejada del reactor flux pistó. Per altra banda, les emissions de N_2O es produïren al llarg de totes les zones aeròbies del reactor. Aquestes emissions estaven estretament relacionades amb episodis que provocaven desajustos en el procés, com per exemple fases sense aeració períodes d'inestabilitat en la nitrificació.

En l'EDAR de la Roca del Vallès, el tractament biològic de l'aigua residual es realitzava en dos reactors discontinus seqüencials (SBRs). Al contrari que l'EDAR de Granollers, les emissions de CH_4 en aquest cas varen ser insignificants, degut a l'absència d'aigües de rebuig provinents del digestor anaerobi. No obstant, les emissions de N_2O van ser elevades contribuint en un 60% a la petjada de carboni de l'EDAR. Es va observar que els cicles del reactor amb fases d'aeració llargues resultaven amb emissions de N_2O més altes. Per minimitzar aquestes emissions, es va aplicar una estratègia d'operació que consistia modificar els cicles del reactor aplicant seqüències d'aeració intermitent (fases aeròbies i anòxiques de 20-30 minuts). Aquesta modificació va resultar en una minimització important de les emissions de N_2O , mantenint el correcte funcionament del reactor.

Resumen

La aceleración del calentamiento global y el cambio climático son consecuencia del incremento exponencial de la concentración de gases de efecto invernadero en la atmósfera, debido en parte a actividades humanas. Estudios científicos recientes apuntan a algunos sistemas de tratamiento de agua residual como fuentes relevantes de gases de efecto invernadero tales como el óxido nitroso (N_2O) y el metano (CH_4). El óxido nítrico (NO) también puede ser emitido durante el tratamiento de agua residual, y es un potente agente causante de la disminución de la capa de ozono, además de ser un compuesto precursor del N_2O . Debido al alto potencial de calentamiento atmosférico del N_2O y el CH_4 , la emisión de estos gases (incluso a niveles bajos) puede ser relevante e incrementar de forma importante la huella ecológica de un sistema de tratamiento de agua residual.

Los factores causantes de la producción de N_2O y NO en sistemas de tratamiento biológicos de agua residual están aún sujetos a debate. Sin embargo, se sabe que tanto el N_2O como el NO pueden ser producidos durante la reacción de oxidación biológica del amoníaco (NH_3) a nitrito (NO_2^-), que es realizada por las bacterias oxidantes del amoníaco (AOB, en sus siglas en inglés), y durante la reducción del nitrato (NO_3^-) ó NO_2^- a nitrógeno atmosférico (N_2), llevada a cabo por bacterias desnitrificantes. De forma distinta, el CH_4 se produce como consecuencia de la degradación de la materia orgánica en condiciones anaeróbicas, y se da a lo largo de distintas rutas metabólicas en organismos metanogénicos del dominio *Archaea*. Las emisiones de gases de efecto invernadero durante los tratamientos de agua residual deben ser evaluadas en profundidad e incluidas en los cálculos globales de emisiones. Además, se han de diseñar e implementar estrategias de mitigación de estas emisiones en las estaciones depuradoras de agua residual.

Esta tesis doctoral reúne una serie de estudios científicos realizados con el objetivo general de identificar posibles estrategias de minimización de las emisiones de N_2O y CH_4 en distintos sistemas de tratamiento de agua residual. El trabajo descrito en esta tesis ha sido dividido en dos secciones: estudios a escala de laboratorio y estudios a escala real. Por un lado, una serie de experimentos fueron realizados en reactores discontinuos secuenciales (SBRs, en sus siglas en inglés) a escala laboratorio, con poblaciones enriquecidas de bacterias nitrificantes. A través de estos experimentos se pudieron identificar algunos de los factores más importantes implicados en la producción de N_2O y NO durante la nitrificación parcial de agua residual con alto contenido amónico, simulando un proceso comúnmente aplicado en el tratamiento de aguas de rechazo en plantas reales. Por otro lado, dos campañas de monitorización de N_2O y CH_4 fueron llevadas a cabo en estaciones municipales de depuración de agua residual (EDAR). Estas monitorizaciones facilitaron la identificación de algunas condiciones que desencadenan importantes picos de emisión de N_2O y CH_4 durante los distintos procesos implicados en los tratamientos biológicos del agua residual.

En el primer estudio de laboratorio se demostró que, tanto la concentración de ión amonio (NH_4^+) como la de NO_2^- en el licor de mezcla de un reactor, son parámetros de alta importancia relacionados con la producción y emisión de N_2O y NO durante la nitrificación parcial de agua residual. Se concluyó que la mayoría del N_2O se originaba durante la etapa de sedimentación a través de reacciones biológicas, y se emitía a lo largo de los primeros minutos de aireación en cada ciclo. La oxidación completa del NH_4^+ (o más probablemente de la hidroxilamina (NH_2OH)) como resultado de la aplicación de un tiempo de aireación suficientemente extenso puede ser sugerida como una estrategia potencial de minimización de las emisiones de N_2O en sistemas de nitrificación parcial. En un segundo grupo de experimentos se compararon las emisiones de N_2O y NO provocadas durante la nitrificación parcial y completa de agua residual con alto contenido en NH_4^+ . En este segundo estudio, la aplicación de nitrificación parcial desencadenó emisiones de N_2O y NO más altas que la nitrificación completa, posiblemente debido al efecto combinado de unas concentraciones más bajas de NO_2^- y NH_4^+ en el reactor biológico. Por lo tanto, la aplicación de nitrificación parcial en sistemas reales debería ser debidamente evaluada, teniendo en cuenta el incremento de la emisión de gases de efecto invernadero y su impacto en la huella ecológica de los propios sistemas.

Las campañas de monitorización de emisiones de gases de efecto invernadero se llevaron a cabo en las EDARs de Granollers y La Roca del Vallès, cerca de Barcelona (España). El tratamiento biológico de agua residual en la EDAR de Granollers se lleva a cabo en dos reactores paralelos de lodos activados, con estructura de flujo-pistón. Los reactores de lodos activados en esta planta están conectados con un digestor anaerobio donde los lodos son tratados para su aprovechamiento como fuente de biogás de cara a la generación de electricidad. Las emisiones de CH_4 se desencadenaron generalmente como consecuencia del arrastre provocado por la aireación en la primera sección del reactor, y fueron relacionadas con los flujos de agua de rechazo que se liberaban en el propio reactor, procedentes del digestor anaerobio. Por otro lado, las emisiones de N_2O se produjeron a lo largo de todas las zonas del reactor biológico sujetas a aireación. Estas emisiones estaban fuertemente relacionadas con eventos que provocaban desajustes en el proceso, como fases sin aireación o periodos de inestabilidad de la nitrificación.

En la EDAR de La Roca del Vallès, el tratamiento biológico del agua residual se realiza en dos reactores discontinuos secuenciales. Al contrario que en la EDAR de Granollers, las emisiones de CH_4 en este caso fueron insignificantes, debido a la ausencia de aguas de rechazo procedentes de un digestor anaerobio. Sin embargo, las emisiones de N_2O fueron elevadas, y se calculó que contribuían en un 60% a la huella ecológica de la planta. En el reactor biológico de La Roca del Vallès, los ciclos con fases de aireación largas se correspondían con los periodos de mayor emisión de N_2O , con un consecuente incremento de la huella de carbono. Durante la monitorización de la EDAR de La Roca del Vallès, una estrategia que minimizaba de forma evidente las emisiones de N_2O pudo ser comprobada. Esta estrategia consistió en aplicar secuencias de aireación intermitente (fases óxicas y anóxicas de 20-30 min) durante los ciclos, dando lugar a una minimización general de las emisiones de la EDAR, sin alterar el correcto funcionamiento del tratamiento biológico.

SECTION I

LITERATURE REVIEW, AIMS AND RESEARCH APPROACH

Chapter 1

General introduction

1.1 Importance of non-CO₂ fugitive green-house gas emissions during wastewater treatment

Increased green-house gas (GHG) emissions from anthropogenic sources are modifying earth's climate by accelerating global warming, with a variety of consequences such as ice melting in the poles and glaciers, raising sea level, extreme regional weather conditions and a wide range of health-related problems affecting the human population among others. Carbon dioxide (CO₂) (mostly emitted as a consequence of fossil fuels utilization) is the main contributor to climate change. However, emissions of other GHGs such as methane (CH₄), nitrous oxide (N₂O) and ozone depleting substances (i.e. hydrofluorocarbons (HFCs), chlorofluorocarbons (CFCs) or nitric oxide (NO)) are also known to severely affect earth's climate. Figure 1.1 shows the contribution of the most important GHGs to the global radiative forcing, which is a measure of the influence of these gases on the energy balance in the earth-atmosphere system.

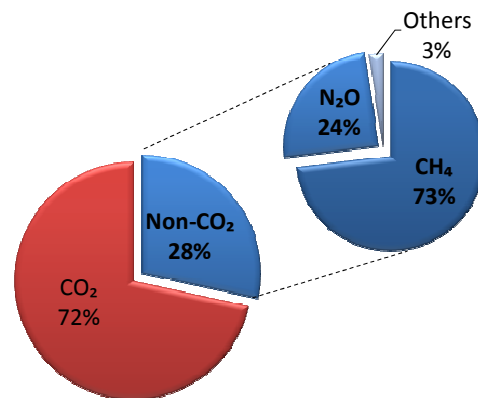


Figure 1.1 Contribution of anthropogenic GHGs to global radiative forcing (W/m²) (Data source: USEPA (2012))

According to USEPA (2012), anthropogenic CH₄ and N₂O emissions account for ~28% of the global radiative forcing, while CO₂ contributes in over 71%. The atmospheric concentration of CH₄ and N₂O has increased to levels unprecedented in the past. From the pre-industrial era, global concentrations of CH₄ and N₂O have increased by 150 and 20%, respectively. In addition, CH₄ and N₂O have a global warming potential (GWP) which is 28 and 265 times larger than the one attributed to CO₂ on a 100-year scope (IPCC, 2013), putting into perspective the relevance of these gases. This means, for example, that the warming effect of 1 ton of N₂O equals the one exerted by 265 tons of CO₂. Therefore, emission mitigation strategies targeting non-CO₂ GHGs could be more effective in counteracting climate change than the ones targeting CO₂ emissions (Montzka et al., 2011). It is therefore essential to unravel the sources of non-CO₂ GHGs and to implement emission mitigation measures.

According to data collected by the IPCC (2014), waste and wastewater contributes to almost 3% of direct GHG emissions, as shown in figure 1.2, with the energy supply, agriculture and forestry, and the industry sectors leading the global emissions ranking.

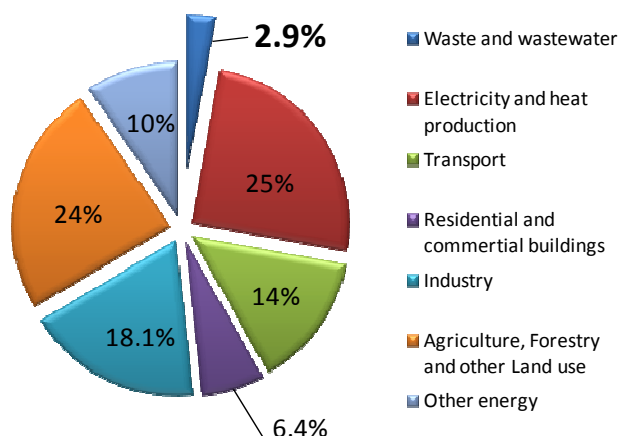


Figure 1.2 Global direct green-house gas emissions by source, in 2010 (data source: IPCC (2014)).

The waste sector is therefore a relevant source of GHGs in general and the third largest contributor of non-CO₂ GHG emissions after the energy and the agriculture sectors, according to data provided by the USEPA (2012) (Fig. 1.3).

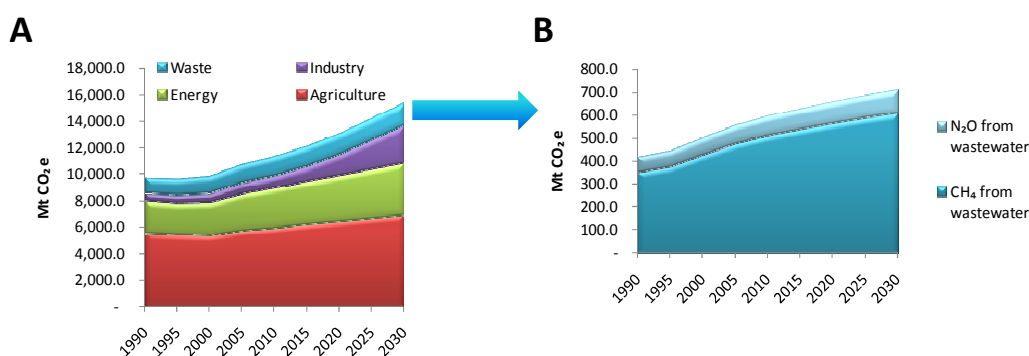


Figure 1.3 (A) Total global non-CO₂ emissions by sector and (B) CH₄ and N₂O emissions from wastewater (within the waste sector) from 1990 to 2005, and from 2005 to 2030 as estimated by calculations based on real country data, emission factors (IPCC, 2006) and projections. Emissions are expressed in CO₂ equivalents (CO₂ e), which is a unit that describes the GWP of any GHG, utilizing the functional equivalence of the GWP of CO₂. (Data source: USEPA, 2012).

Large quantities of CO₂ are also emitted from the waste sector, but carbon that is present in waste is generally considered to be biogenic (withdrawn from the atmosphere and utilized by food crops). Thus, emissions of CO₂ from waste represent no net flux to the system (IPCC, 2013). The two largest sources of non-CO₂ GHGs within the waste sector are land-filling of solid waste and wastewater, with around 93% emissions contribution. Within the waste sector, up to 35% of the non-CO₂ GHGs emissions can be attributed to wastewater treatment and handling, and global emissions of these gases are expected to increase due to the raising need of wastewater treatment by a continuously growing global population. In 2010, CH₄ and N₂O emitted from wastewater treatment systems contributed by 4 and 2% to the global account of each gas, respectively, according to data published by the USEPA (2012). By the year 2030, global CH₄ and N₂O emissions from wastewater are expected to be approximately 600 and 100 Mt CO₂ e, respectively (USEPA, 2012) (Fig. 1.3), if minimization strategies are not implemented worldwide. Emission calculations in the report by the USEPA (2012) are based on the standard emission factor applied by the IPCC (2006), previously reported by Czepiel et al. (1995) (3.2 g N₂O/person/year or 0.035 % of the N load) due to the lack of

real full-scale data. This emission factor is then multiplied by real country data on protein consumption, population and wastewater generation when available. Calculating N_2O emissions with a fixed and standardized factor presents obvious limitations that need to be taken into account, as full-scale emissions could be higher or lower, depending on the different process conditions and characteristics.

Besides the obvious environmental concern related with the fugitive emissions of non- CO_2 GHGs from wastewater treatment systems, there are also economical issues that nowadays are gaining relevance. Environmental taxes aiming at discouraging companies from emitting GHGs are applied worldwide, and non- CO_2 GHGs from wastewater treatment systems are starting to be included in these taxes, in some cases. Some of the countries pioneering the environmental taxing of CH_4 and N_2O from wastewater treatment utilize standard emission factors estimated by the IPCC in the 90s, which are now obsolete or non-accurate enough. Real emission data needs to be collected on a case-specific basis for environmental policies and taxes to be applied on a more accurate manner. This would also support wastewater treatment plants (WWTPs) managers in the design and implementation of mitigation strategies by identifying the exact source of fugitive GHG gas in each system. According to a report recently published by the USEPA (2013), there is a large potential for cost-effective mitigation measures targeting non- CO_2 GHGs emissions from wastewater treatment systems, although the uncertainty related with the different abatement methods and cost-benefit analyses is still a limitation for the sector. The implementation of wastewater treatment is essential for the protection of the environment and the sustainability of the human society, but it should be carried out taking into consideration GHG emissions and its global consequences.

1.2 N_2O sources and production pathways during wastewater treatment

N_2O can be produced through **nitrification** and/or **denitrification** (Fig. 1.4), which are two biochemical processes commonly applied with the purpose of removing nitrogen (N) compounds from wastewater. **Denitrification** is the biochemical reduction of ionic nitrogen oxides such as NO_3^- and NO_2^- to nitric oxide (NO) and N_2O with dinitrogen (N_2) as end product. N_2O is therefore an intermediate molecule in the denitrification reaction. In wastewater treatment, denitrification is attributed to a very diverse group of heterotrophic bacteria that couple the oxidation of organic or inorganic matter with the reduction of N compounds under anoxic conditions.

During denitrification, several factors have been shown to influence N_2O production. DO concentrations as low as 0.1 to 0.3 mg/L have been proven to promote N_2O production due to the inhibition of denitrifying enzymes such as the N_2O reductase (Schulthess et al., 1994; Otte et al., 1996; Tallec et al., 2008). Some authors also reported an increase in N_2O production under low COD/N ratios (lack of organic matter) during denitrification (Schalk-Otte et al. 2000; Chung and Chung, 2000). Under such conditions, the denitrifying enzymes compete for electrons resulting in denitrification imbalances. The effect of different carbon sources has been the focus of other set of studies with various results (Hanaki et al. 1992; Christensson et al. 1994; Hallin and Pell, 1998) but it still remains unclear the degree of dependency between N_2O production and the type of substrate being utilized by each denitrifying population. High NO_2^- and free nitrous acid (HNO_2) concentrations were reported to lead to N_2O accumulation due to the inhibition of the N_2O reductase enzyme in denitrifying cultures (Itokawa et al., 2001; Zhou et al., 2008). However, Schulthess et al. (1995) suggested that it is NO the compound that inhibits N_2O reduction rather than NO_2^- or HNO_2 , putting into perspective the relevance of NO as an important precursor of N_2O emissions in wastewater treatment systems.

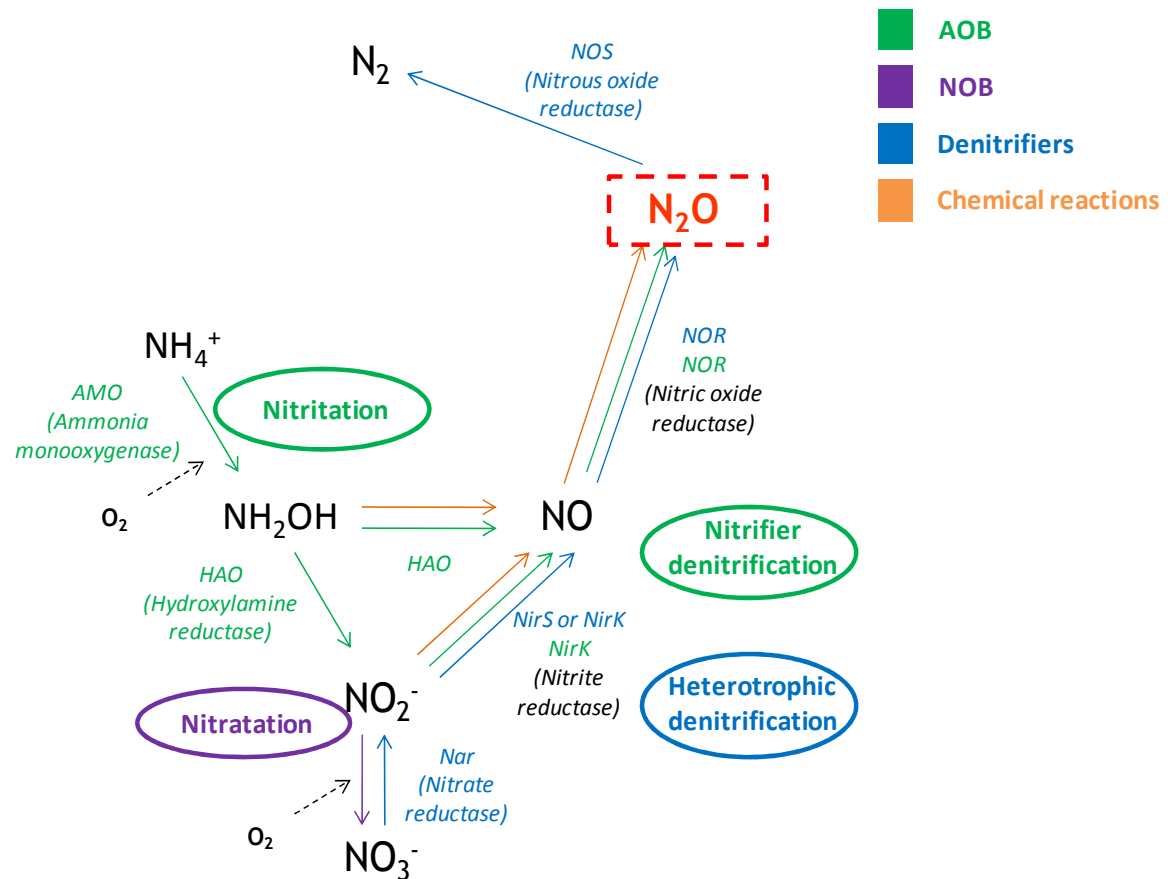


Figure 1.4 Graphical representation of N₂O production and consumption pathways during biological nitrogen removal, and the microbial communities and enzymes involved (modified from Desloover et al., 2012)

Observations made in lab-scale experiments and full-scale studies have consistently shown a high degree of discrepancy, especially when targeting bacterial populations as diverse as the denitrifying ones. In general however, it seems that N₂O production by denitrifiers is limited at the real-scale level, as reported by Ahn et al. (2010a), and nitrification is nowadays considered responsible for the majority of N₂O emissions in municipal WWTPs.

Nitrification consists of two coupled reactions: first, ammonium (NH₄⁺) is oxidized to hydroxylamine (NH₂OH) and NO₂⁻ through the process of nitritation by ammonia oxidizing bacteria (AOB). Then, NO₂⁻ is oxidized to nitrate (NO₃⁻) by nitrite oxidizing bacteria (NOB) through a reaction known as nitratation, although it is widely accepted that NOB don't contribute to N₂O production (Fig. 1.4). To date, several pathways have been suggested to be responsible for the production of N₂O during nitrification. One of the possibilities involve NH₂OH, an intermediate product of the oxidation of NH₃ to NO₂⁻ (Arp and Stein, 2003; Schmidt et al., 2004a) as a potential source of N₂O. After NH₃ oxidation by the ammonia monooxygenase (AMO) enzyme, NH₂OH is formed. Then, the hydroxylamine oxidoreductase (HAO) enzyme converts NH₂OH to the nitrosyl radical (NOH) which is further transformed into NO₂⁻ (Igarashi et al., 1997). It has been suggested that NO can be generated during the enzymatic conversion of NOH to NO₂⁻, while the unstable breakdown of NOH can lead to N₂O production (Poughon et al., 2001), although this hypothesis needs to be confirmed. Furthermore, besides the chemical oxidation of NOH, NO produced during NH₂OH oxidation can be biologically reduced to N₂O (Stein, 2011). These two sources of N₂O production need further research to be completely understood, but their relative importance in full-scale systems has been suggested to be minor (Wunderlin et al., 2012). It has been proposed however, that nitrification systems with high

NH_4^+ loading or elevated N conversion rates could support N_2O production through NH_2OH oxidation (Law et al., 2012b;) either from the chemical breakdown of NOH or from the reduction of NO being formed.

Alternatively, several authors have suggested denitrification by AOB (nitrifier denitrification) as the predominant source of N_2O in nitrifying systems (Goreau et al., 1980; Kampschreur et al., 2008a and 2008b; Kim et al., 2010; Wunderlin et al., 2012). Through this pathway, N_2O is produced by the activity of nitrifier-encoded nitrite reductase (NirK) and nitric oxide reductase (Nor) enzymes, responsible for the reduction of NO_2^- to NO and N_2O (Bock et al. 1995; Wrage et al. 2001; Kampschreur et al., 2008b; Kim et al., 2010), as represented in Figure 1.4. The genes encoding for the N_2O -reductase haven't been found in the genome of any of the species of AOB described to date, so N_2O is thought to be the end product of nitrifier denitrification. The key parameter regulating N_2O production through nitrifier denitrification is the oxygenation level (Tallec et al., 2006). In particular, anoxic or sub-oxic conditions (low DO concentrations) have been suggested to trigger the denitrification pathway in AOB (Goreau et al., 1980; Zheng et al., 1994; Chuang et al., 2007). Additionally, transient changes in the DO concentration have also been proven to increase N_2O production (Kester et al., 1997; Kampschreur et al., 2008b; Peng et al., 2014). In contrast, other authors postulated that it was the recovery from anoxic conditions rather than the imposition of anoxia the trigger for N_2O production (Ahn et al., 2010a; Yu et al., 2010). Many aspects of the specific N_2O production mechanisms under transient anoxic conditions and different DO levels are still to be unraveled. The effect of different factors on N_2O production during nitrification has traditionally been subjected to a certain degree of controversy, because many of these parameters interact with each other. When studying one isolated factor, attention needs to be paid as other parameters may also change, probably affecting N_2O production.

Other factors such as NH_4^+ and NO_2^- concentrations have also been identified to play a key role in N_2O production through nitrifier denitrification. Yu et al. (2010) showed that, upon recovery from anoxia, N_2O production increased and was correlated with NH_4^+ being accumulated during the anoxic period in a nitrifying culture. In the same study, it was suggested that the shift from low to high activity levels promoted N_2O production mechanisms. The effect of elevated NO_2^- concentrations on N_2O production has been on focus in several studies. Many authors have shown that N_2O production rates in nitrifiers are indeed correlated with NO_2^- accumulation (Sümer et al., 1995; Tallec et al., 2006; Kampschreur et al., 2008a and 2009; Foley et al., 2010; Desloover et al., 2011) both at the lab and full-scale levels. Novel methodology applied by Wunderlin et al. (2013) showed N_2O being mainly produced through NO_2^- reduction in mixed bacterial populations. In a different work however, Law et al. (2013) reported reduced N_2O production rates with increasing NO_2^- concentrations in an enriched AOB culture. Exceedingly high NO_2^- could be a source of inhibition in the nitrifier denitrification pathway for N_2O production in AOB. From this perspective, the potential adaptation of different bacterial communities to high NO_2^- concentrations needs further attention from the scientific community. The high NO_2^- concentrations found in processes that apply or enhance partial nitrification or nitritation for the treatment of NH_4^+ -rich wastewater, and its relation with N_2O production and emissions have been object of research in this thesis, as described below.

1.3 N_2O and NO emissions from nitritation systems

Wastewater treatment facilities performing anaerobic sludge digestion for biogas production are in need to implement strategies for the treatment of reject wastewater (effluent from the anaerobic digesters). The more stringent regulatory demands in terms of nutrient discharge from wastewater treatment facilities, as well as the need to reduce operational costs and energy consumption have resulted in nitritation or partial nitrification (oxidation of NH_4^+ to NO_2^-) of reject wastewater being a common side-stream process. Reject wastewater is usually characterized by high NH_4^+

concentrations ($\sim 1\text{g NH}_4^+\text{-N/L}$) as well as low COD content. When coupled with conventional denitrification or anammox, nitritation is considered as an attractive (low-cost) option for the treatment of NH_4^+ -rich wastewater, but has been shown to release higher amounts of N_2O than full nitrification systems (Ahn et al., 2011), probably as a side effect of the accumulation of NO_2^- . Law et al. (2011) detected an emission factor of 1% N_2O in a lab-scale sequencing batch reactor (SBR) treating synthetic reject wastewater. In their SBR the N_2O accumulated during the idle phases contributed to 94% of the total N_2O emitted during the first 15 minutes of the aerated phase. Kampschreur et al. (2008a) measured 3.4% of the ammonium converted emitted as N_2O in a full-scale continuous partial nitrification reactor treating reject wastewater. Similarly, Ahn et al. (2011) reported that 1.9% of the nitrogen load was emitted as N_2O (with an NH_4^+ conversion of around 80%), these emissions being measured under transition from full to partial nitrification mode. When partial nitrification was stabilized in their system, the N_2O emissions decreased to 0.57% of the nitrogen load. In another study with a partial nitritation system, Desloover et al. (2011) found that N_2O emissions were 5.1-6.6% of the nitrogen load, with 45-47% of the incoming nitrogen oxidized to NO_2^- and 13-15% oxidized to nitrate (NO_3^-).

In general, the implementation of nitritation with NO_2^- accumulation might increase not only N_2O but also NO emissions, the last one being a potent ozone-depleting substance and a precursor of N_2O , as mentioned before. In contrast with N_2O , NO has received little attention and its emission has been only measured in few studies with different results being reported from laboratory (Law et al., 2011; Ahn et al. 2011) and full-scale installations (Kampschreur et al., 2008a; Desloover et al., 2011). For example, Kampschreur et al. (2008a) found that 0.2% of the nitrogen load was emitted as NO (full-scale), while Ahn et al. (2011) described a partial nitrification lab-scale bioreactor in which the emissions of NO decreased to 0.07% of the nitrogen load after a stabilization period. In another study from a full-scale deammonification plant, 0.1% of the N output was reported to be emitted as NO (Weissenbacher et al. 2010). NO is not only a threat to the environment but also plays an important role in ammonia (NH_3) oxidation, with some regulatory effects being reported (Schmidt et al., 2004b). Thus, further analyses of NO emissions from wastewater treatment should be carried out.

In an extensive experiment, Ahn et al. (2011) compared N_2O and NO emissions from a lab-scale bioreactor operated sequentially in full-nitrification and partial-nitrification modes and found an increase in these emissions when operating in partial nitrification conditions. However, in their study, the transition from full to partial nitrification was achieved by reducing the DO concentration and the sludge residence time (SRT) which alone could have already caused an effect on N_2O production. They also reported a change on the predominant AOB population when operating under full and partial nitrification modes respectively, which could also have an effect on the overall emissions detected. The problems and uncertainties related with the application of partial nitrification for the treatment of NH_4^+ -rich wastewater was taken as a research niche during the elaboration of this thesis. Two studies were performed with the aim of unraveling the sources and dynamics of N_2O and NO production and emissions when treating high NH_4^+ concentration wastewater in lab-scale bioreactors, performing both nitritation and full nitrification (**chapters 4 and 5**). Both studies targeted the combined dynamics of both NO and N_2O released from partial nitrification processes, and the interactions between them, as this issue has not been extensively approached by the scientific community to date.

1.4 N_2O and CH_4 emissions from full-scale WWTPs

In wastewater treatment systems, not only N_2O but also CH_4 have been identified as the main non- CO_2 GHGs being emitted. Figure 1.5 shows the major potential sources of CH_4 and N_2O in a conventional municipal WWTP, namely the sewer networks and sludge treatment facilities for CH_4 and the biological treatment basins (activated sludge) and settlers in the case of N_2O .

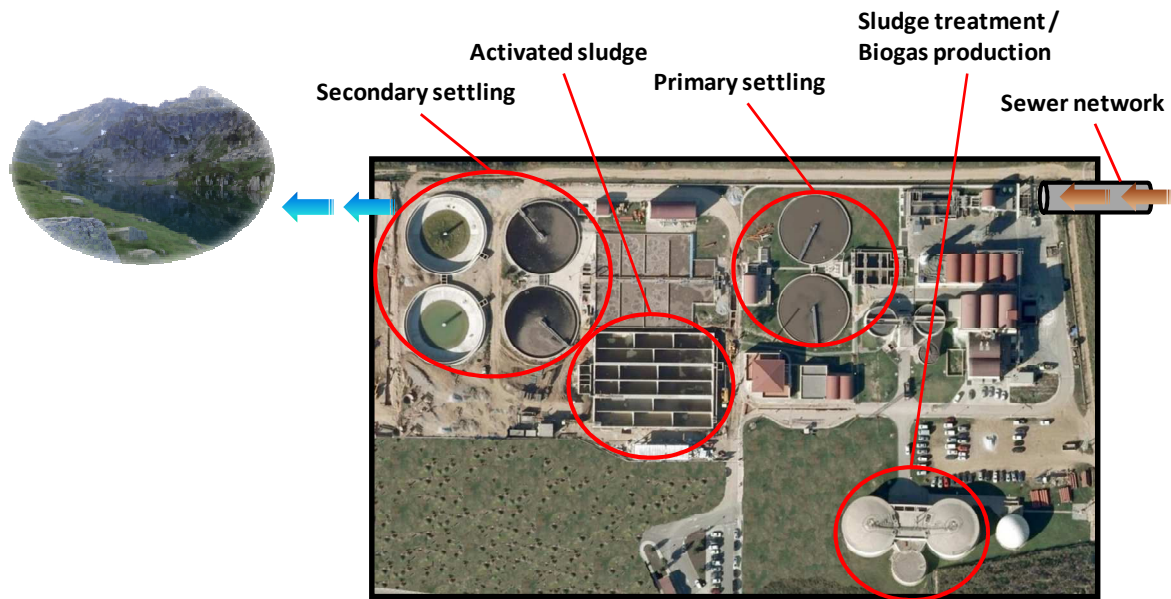


Figure 1.5 Potential sources of fugitive GHG emissions in a conventional WWTP.

In the last years, data on N_2O emissions from full-scale WWTPs has been collected in different countries. However, the reported results are highly variable, and consensus is yet to be achieved on the exact causes of N_2O emissions. In general, the methodology utilized for quantifying the emissions can be in itself a source of data variability. Firstly, the configuration of the bioreactors needs to be taken into consideration when choosing the monitoring methodology. Very recently, Ye et al. (2014) proposed a novel method to quantify N_2O emissions in bioreactors with surface aerators. It was proven that the commonly utilized gas hood method would lead to highly inaccurate results when applied to this type of reactors. Another source of inaccuracy is the sampling strategy, with studies based on grab samples leading to an over- or underestimation of the emissions depending on the time and location of the measurements (Kampschreur et al., 2009). The typical dynamic patterns and large fluctuations described by N_2O emissions along the different stages of the bioreactors and over time show the importance of on-line monitoring for accurate N_2O monitoring (Kampschreur et al., 2008a). Long-term (over one year) on-line sampling has been shown to be the only strategy that allows not only identifying seasonal tendencies, but also an accurate calculation of the average N_2O emissions from a particular WWTP (Daelman et al. 2013a). However, the majority of full-scale monitoring campaigns being performed to date have been carried out on a short-term basis (up to 1-2 months), including the research work presented in this thesis (**Chapters 6 and 7**). Short-term on-line monitoring campaigns are efficient in unraveling diurnal patterns. For research purposes, diurnal patterns may be very helpful to identify the mechanisms behind the N_2O emission, since many operational parameters of a WWTP also show diurnal variability, as pointed out by Daelman et al. (2013a). In order to correlate these parameters with emissions, high frequency data of both the emission and the parameters are needed, and that is sufficiently achieved through short-term on-line monitoring.

The latest published results in terms of N_2O emissions have been obtained through intensive continuous measurements, facilitating the evaluation of temporal patterns and dynamics, as well as favoring the comparison between studies. Ahn et al. (2010a) presented N_2O emissions data from 12 different WWTPs located in different areas of North America, with results ranging from 0.01 to 1.8% of the influent total Kjeldahl nitrogen (TKN). The variability of the reported emissions was indeed high, although all the campaigns were performed under the same monitoring protocol. In the same

study however, a trend was found, with processes that favored transient or permanent build up of NH_4^+ and/or NO_2^- positively correlating with higher emission levels. Moreover, N_2O emissions were generally found to be strongly correlated with the influent TKN loading (Ahn et al. (2010b)). More recently, Aboobakar et al. (2013) reported a clear diurnal pattern associated with the inflow of wastewater and with the NH_4^+ loading. In their study, 0.036% of the total nitrogen (TN) load was found to be released as N_2O from two full-scale activated sludge plug-flow reactors, contributing with a 13% increase to the carbon footprint of the WWTP. Aboobakar et al. (2013) found a negative, direct correlation between DO and N_2O emissions, highlighting the importance of considering these emissions when designing energy optimization strategies aiming at lowering the aeration levels. Long-term (16-month) research performed by Daelman et al. (2013b) in a fully-covered municipal WWTP delivered values as high as 2.3% of the incoming N being released as N_2O , representing three quarters of the carbon footprint of the WWTP under study. A seasonal trend was found in this case, relating water temperature and N_2O emissions, but the reason behind this trend remained unclear. In general, the latest reports have contributed to put into perspective the high relevance of N_2O emissions and their impact on the carbon footprint exerted by wastewater treatment facilities, as well as the necessity to investigate the potential for implementing minimization measures.

Besides N_2O emissions, during collection and treatment of wastewater anaerobic conditions may occur, resulting in CH_4 production. As shown in figure 1.6, organic matter (represented as chemical oxygen demand, COD) can be converted into different compounds such as acetate, propionate or hydrogen. These substances are then utilized by methanogenic archaea with production of CH_4 . In wastewater treatment systems, anaerobic processes such as sludge digestion for biogas production and electricity generation can be considered as an important source of CH_4 . Sewer systems have also been proven to be a source of CH_4 from which it can be transferred and released (Guisasola et al., 2008; Sudarjanto et al., 2014). Agitation and aeration during wastewater treatment facilitates CH_4 stripping to the atmosphere.

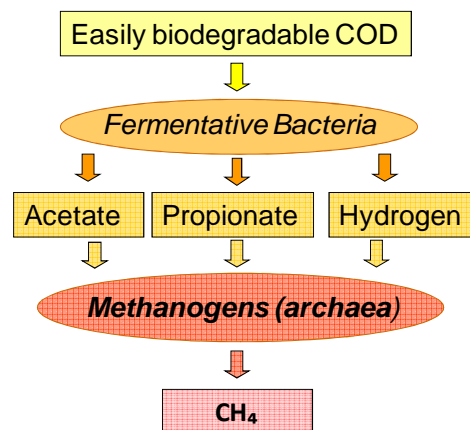


Figure 1.6 Graphical representation of CH_4 production, including compounds and groups of microorganisms involved.

Before the publication of this thesis there was only one published study reporting on-line CH_4 emissions from a domestic WWTP. Daelman et al. (2012) reported that 1.13% of the influent chemical oxygen demand (COD) of the WWTP of Kralingseveer (Netherlands) was emitted as CH_4 . About three quarters of these emissions were originated during primary and secondary sludge digestion. In that specific case, the CH_4 -related footprint of the sludge digester was larger than the CO_2 emissions that were avoided by using biogas for energy generation (Daelman et al., 2012), putting into context the impact that uncontrolled CH_4 emissions can have on the overall carbon footprint of wastewater treatment processes. Previous research by other authors (Czepiel et al.,

1993; Wang et al., 2011) also focused on measuring CH₄ emissions from WWTPs (with no anaerobic sludge digestion). These studies reported values between 0.08 and 0.16% of the influent organic load (biological oxygen demand (BOD)) or COD being emitted as CH₄. In both studies however, the analyses were based on grab samples not fully representative of the dynamics of the WWTPs and thus, the relevance of these results is more limited for comparative purposes. Recently, the potential aerobic oxidation of CH₄ in activated sludge has been explored through mathematical modeling and simulation (Daelman et al., 2014). This opened the possibility of achieving the desired effluent quality in terms of COD and N, while reducing the concentration of CH₄ which could be transformed to CO₂ by methanotrophic bacteria, under particular process conditions. However, these results need previous calibration and validation in full-scale studies in order to be applied.

The high variability of green-house gas emissions reported in full-scale studies has spread the general idea of these emissions being strongly bounded to specific configurations and operating conditions applied (Law et al., 2012a). Furthermore, the differences in configuration, operation and performance of each WWTP difficult the comparison between systems and minimization strategies may need to be approached on individual basis. To identify the most important operating conditions affecting the emission of N₂O and CH₄ is the key to develop mitigation strategies that allow reducing fugitive gas emissions in WWTPs. In this thesis, two different municipal WWTPs were on focus in two independent studies. The main difference between both facilities was the configuration of the biological treatment system, one of them consisting of a conventional plug-flow activated sludge reactor coupled to a sludge digestion facility (**chapter 6**), and the other consisting of an SBR (**chapter 7**). This last study is highly relevant at an international level, since no other comprehensive study reporting on-line N₂O emissions from full-scale SBRs has previously been performed, despite their application for the treatment of wastewater worldwide. Both studies represent the first monitoring campaigns carried out at a national level in Spain.

Chapter 2

Objectives and structure of the thesis

The main objective of this thesis was to *investigate N_2O production and emission dynamics* during wastewater treatment in order to *define* feasible *mitigation strategies*.

Together with the goal mentioned above, the following *sub-objectives* were also defined:

1. To assess the different factors affecting N_2O and NO production and emission dynamics during **nitritation** and **full-nitrification** of reject wastewater in lab-scale systems, and to explore operational strategies that mitigate these emissions.
2. To evaluate process operation configurations that play a relevant role on N_2O and CH_4 emissions in **full-scale municipal wastewater treatment plants (WWTP)** and to suggest operational strategies that could lead to fugitive GHG emissions mitigation.

In accordance with these objectives, the research in which this thesis is based has two well defined approaches. On one hand, **lab-scale studies (chapters 4 and 5)** were performed in SBRs where target bacterial populations were grown. On the other hand, N_2O and CH_4 emission dynamics were investigated in two **full-scale wastewater treatment plants (chapters 6 and 7)**. Figure 2.1 shows a graphical representation of the main content of the thesis, stressing the interrelationships of the main studies in which the thesis is based on through keywords.

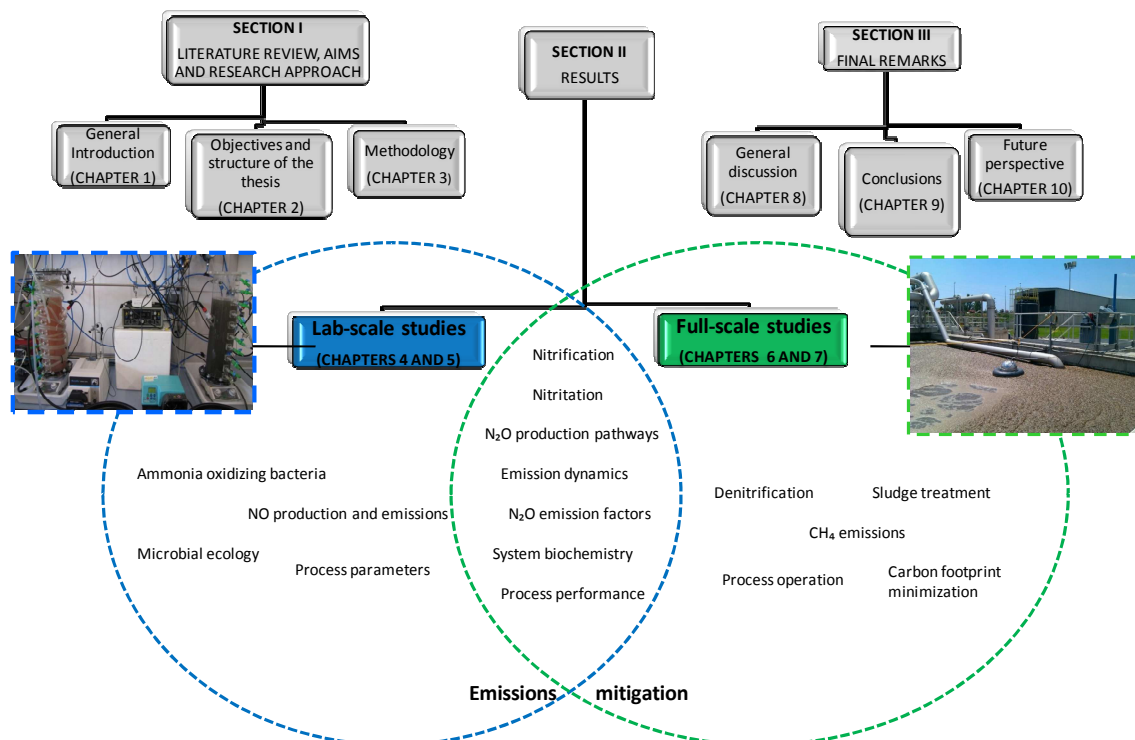


Figure 2.1 Graphical representation of the main contents of this thesis and the “keyword” interrelationships between chapters 4 and 5 (lab-scale studies) and 6 and 7 (full-scale studies).

The lab-scale experiments described in this thesis allowed unraveling some of the mechanisms involved in N₂O and NO production and emission during partial and full-nitrification, while the two full-scale monitoring campaigns related operational conditions with fugitive GHG emissions and mitigation strategies were suggested to process operators, as represented in Figure 2.1.

The research work (section II) of this thesis is described along four chapters:

- **CHAPTER 4:** *N₂O and NO emissions from a partial nitrification sequencing batch reactor: Exploring dynamics, sources and minimization mechanisms.*
- **CHAPTER 5:** *Nitritation versus full nitrification of ammonium-rich wastewater: Comparison in terms of nitrous oxides emissions.*
- **CHAPTER 6:** *Evaluation of process conditions triggering emissions of green-house gases from a biological wastewater treatment system.*
- **CHAPTER 7:** *Minimizing N₂O emissions and carbon footprint in a full-scale activated sludge sequencing batch reactor.*

Chapter 3

Methodology

As described in chapter 2, this thesis contains research work based on two well-defined approaches, namely lab and full-scale experimental studies. The methodology utilized for the elaboration of this thesis is summarized below. Further details on the materials and methods of each study can be found in the corresponding chapters describing the research work carried out (Section II).

3.1 Lab-scale studies

3.1.1 Experimental set-up

The **lab-scale experiments (chapters 4 and 5)** were performed in two cylindrical 8L SBRs (SBR1 and SBR2) which were inoculated with activated sludge from a domestic WWTP located in Girona (Spain). In SBR1, the enrichment of AOB was promoted and nitritation was achieved. This SBR was subjected to a set of experiments that focused on nitritation of NH_4^+ -rich synthetic wastewater, simulating the conditions in which bioreactors treating reject wastewater operate in full-scale WWTPs, as described in **chapter 4**. Additionally, SBR2 was utilized to enhance the growth of NOB and the nitratation reaction was established. SBR2 was utilized together with SBR1 in the set of experiments described in **chapter 5**. Only for the second set of experiments (**chapter 5**), SBR1 was transformed into a bioreactor performing full nitrification, as follows: Four litres of the mixed liquor enriched with AOB were removed from SBR1 (~ 0.8 gVSS/L) and substituted with the same amount of mixed liquor withdrawn from SBR2 (enriched with NOB), resulting in an equally mixed AOB/NOB community (~ 0.8 gVSS/L) (SBR1 MIX). Full nitrification was achieved during the following 30 d of the study. The experimental set-up installed for each of the lab-scale SBRs of this thesis is shown in Figure 3.1.

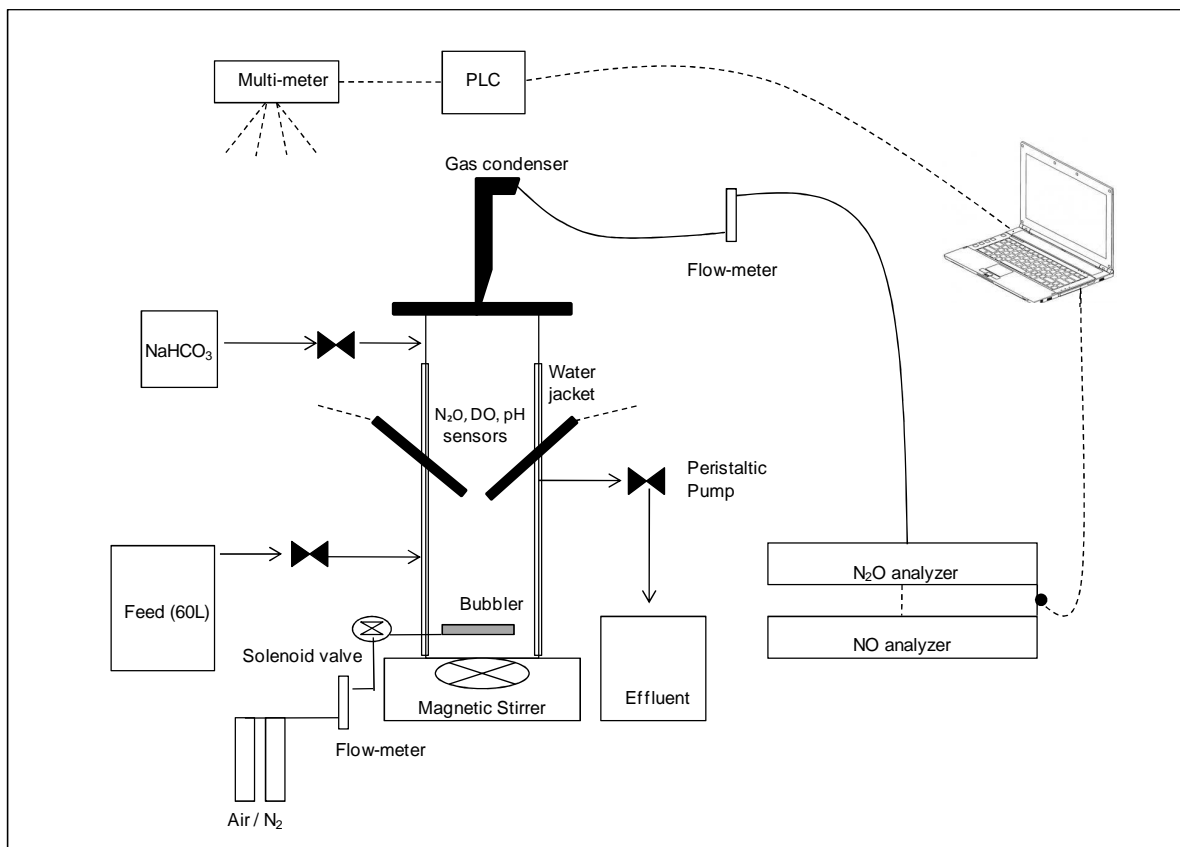


Figure 3.1 Scheme of the experimental set-up designed for the studies presented in chapters 4 and 5.

3.1.2 Microbial characterization

Different analyses of the bacterial populations were performed in each of the studies. Fluorescence in situ hybridization (FISH) was carried out in both cases, in order to evaluate the enrichment of the different targeted bacterial populations. Additionally, in the study described in chapter 5, bacterial DNA analyses by means of pyrosequencing were performed for the complete characterization of the bacterial diversity found in the lab-scale systems.

3.1.3 Chemical analyses, on-line N_2O and NO monitoring and emissions calculations

The concentration of NH_4^+ -N, mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were analyzed according to standard methods (APHA, 1998). Differently, NO_2^- -N and NO_3^- -N were analyzed via ion chromatography (ICS5000, DIONEX). Alkalinity was measured via a robotic titrosampler (Metrohm 855) and total organic carbon (TOC) was analyzed with a TOC analyzer (Shimadzu, TOC-V CSH) for samples of the synthetic wastewater.

Gaseous N_2O and NO were continuously analyzed using commercial analyzers. N_2O was analyzed with the infrared gas analyzer Servomex 4900 (Servomex Group Ltd. East Sussex, UK) and data was logged every 5 seconds. NO was analyzed via the chemiluminescence gas analyzer CLD64 (detection limit 2 ppb; Eco Physics. Dürnten, Switzerland). Only for the study described in chapter 4, N_2O patterns in the liquid phase were followed using a microsensor with data logging (model N_2O -R, detection limits 0.1-500 μ M; UNISENSE, Denmark). A two-point calibration of the microsensor was done before and after each measurement.

The total N_2O emitted was calculated using equation 1:

$$N_2O \text{ emitted} = \sum (C_{N_2O-N_{gas}} \times Q_{gas} \times \Delta t) \quad (1)$$

Where,

- C_{N_2O} (g N_2O -N/L) = C_{N_2O} (ppm v) * 10^{-6} * N_2O molar volume (0.041 at 25°C and 1atm) * 28.
- Q_{gas} = the gas flow rate going into the reactor.
- Δt = time interval by which the off-gas N_2O concentration was recorded.

The time interval utilized for the emission calculations was, in each cycle, the time in which air or N_2 stripping was given. A homologous calculation was done for the NO emission. The emission factor of each gas was calculated dividing the total amount of N_2O or NO emitted in a particular time (equation 1) by the total NH_4^+ converted at that time.

3.2 Full-scale studies

3.2.1 Monitoring sites

An in-depth description of the WWTPs of Granollers and La Roca del Vallès, which were chosen as subjects of the full-scale monitoring campaigns of this thesis, as well as the characteristics of the process operation in each case can be found in chapters 6 and 7. In general, the facilities where the monitoring campaigns were carried out can be considered as conventional municipal treatment plants. However, the biological treatment basins at Granollers WWTP consisted of two parallel plug-flow activated sludge bioreactors (Chapter 6), while La Roca del Vallès WWTP had two SBRs (Chapter 7).

3.2.2 Chemical characterization

Grab samples were regularly taken both manually and with automatic refrigerated samplers from each monitored site for ammonium (NH_4^+), NO_2^- and nitrate (NO_3^-) analyses. The analyses of these samples were performed according to standard methods (APHA, 1998) in the case of NH_4^+ , or via ion chromatography (ICS5000, DIONEX) for NO_2^- and NO_3^- . Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were analysed weekly according to standard methods (APHA, 1998). The influent and effluent water characteristics (COD, TKN and NH_4^+ , TSS and VSS) of each plant were provided weekly by plant operators. On-line data of the aeration flow as well as the influent wastewater flow were acquired from the supervisory control and data acquisition (SCADA) system of the WWTPs. During the monitoring campaigns, DO and temperature were continuously recorded with a portable DO sensor with an integrated thermometer (YSI Inc. USA). Dissolved N_2O was continuously measured via an N_2O microsensor (model $\text{N}_2\text{O-R}$, detection limits 0.1-500 μM ; UNISENSE, Denmark) connected to an in-situ amplifier box. In the monitoring campaign described in chapter 6, dissolved CH_4 in grab samples was analysed with a gas chromatograph (Thermofisher Scientific Inc. USA) equipped with a flame ionization detector (FID).

3.2.3 On-line N_2O and CH_4 monitoring and emissions calculations

Measurements of CH_4 and N_2O gases were performed using a commercial gas collection hood (AC'SCENT® Flux Hood) connected via gas tubing to a commercial gas analyser (VA-3000, Horiba, Japan) equipped with a sample conditioning system (series CSS, M&C Tech group). Oxygen concentration was also analysed using the same analyser for estimation of the flow of gas (Q_{gas}) coming out of the reactor in the anoxic (non-aerated) zones as explained below. Off gas was collected continuously (at 0.5L/min) from the reactor headspace and concentration data was logged every 15 seconds.

In the first monitoring campaign (chapter 6), gas emissions in aerated and non-aerated zones of the activated sludge basin were calculated utilizing equations 2, 3, 4 and 5 (adapted from Aboobakar et al., 2013). The emission factor for N_2O was calculated as a percentage of the average influent TKN load of the bioreactor lane being emitted as N_2O . A similar approach was employed for the calculation of the CH_4 emission factors, where influent COD load was used instead of TKN.

$$\text{Gas emitted (g)} = \left[\left(\sum (C_{\text{gas}} \times \Delta t \times Q_{\text{gas}}) \right) \times \left(\frac{A_{\text{site}}}{A_{\text{total}}} \right) \right] \quad (2)$$

Where,

- C_{gas} (g $\text{N}_2\text{O-N}$ or CH_4/L) = C_{gas} (ppm v) * 10^{-6} * gas molar volume⁻¹ (0.041 mol/L at 25°C and 1atm) * 28 (N_2O) or 16 (CH_4).
- Δt (min) = time interval by which the off-gas concentration was recorded.
- $A_{\text{site}}/A_{\text{total}}$ = ratio of surface area (m^2) of each sampling site (A_{site}) per surface area of the reactor lane (A_{total}).
- Q_{gas} (L/min) = the gas flow rate coming out from the reactor. In aerated zones, Q_{gas} was assumed to be the aeration flow rate. In anoxic zones or when aeration stopped Q_{gas} was calculated as follows:

$$Q_{\text{gas}} = \sum (Q_{\text{in}} - Q_{\text{sweep}}) \quad (3)$$

Where,

- Q_{in} (L/min) = the flow at which the sample conditioning system pumps gas out of the hood and into the analyser (0.5 L/min)
- Q_{sweep} (L/min) = the air flow rate being pumped into the hood by the conditioning system. To calculate " Q_{sweep} " the following equations are used:

$$Q_{\text{sweep}} \text{ (L/min)} * C_{\text{oxygen outside hood}} (\approx 21\%) = [Q_{\text{gas}} \text{ (L/min)} * C_{\text{oxygen gas}} (0\%)] + [Q_{\text{in}} \text{ (L/min)} * C_{\text{oxygen inside hood}} (\%, \text{ measured by the analyser})] \quad (4)$$

Then, assuming that the oxygen concentration of the gas being stripped out of the tank under anoxic conditions is zero:

$$Q_{\text{sweep}} \text{ (L/min)} = [Q_{\text{in}} \text{ (L/min)} * C_{\text{oxygen inside hood}} (\%)] / C_{\text{oxygen outside hood}} (\approx 21\%) \quad (5)$$

During the second monitoring campaign (chapter 7), gas emissions during aerated phases at the SBR were calculated utilizing equation 1. In this case however, the factor $A_{\text{site}} / A_{\text{total}}$ equals 1. During anoxic phases (no gas flow going through the SBR), Q_{gas} was calculated applying equations 3, 4 and 5. During this study, N_2O emission factors were calculated as a percentage of the average influent NH_4^+ -N load being emitted as N_2O .

Besides some common generalities, each of the monitoring campaigns was designed with a high degree of specificity due to the fact that the bioreactors and the general configuration of the WWTPs under study were significantly different. For further details on how the different monitoring campaigns were performed see materials and methods sections in chapter 6 and 7.

SECTION II

RESULTS

Chapter 4

N₂O and NO emissions from a partial nitrification sequencing batch reactor: Exploring dynamics, sources and minimization mechanisms

The contents of this chapter have been published as:

Rodriguez-Caballero, A. and Pijuan, M., 2013. N₂O and NO emissions from a partial nitrification sequencing batch reactor: Exploring dynamics, sources and minimization mechanisms. *Water Research* 47 (9), 3131-3140.

4.1 Preliminary remarks

This study provides for the first time a combined assessment of the N₂O and NO emission dynamics of a partial nitrification SBR enriched with AOBs, treating synthetic reject wastewater (1g NH₄⁺-N/L) with a 98% conversion rate to NO₂⁻. Four different cycle configurations were tested without affecting the ammonia (NH₃) oxidation performance of the system. The influence of these configurations on total N₂O and NO emissions was determined. Additionally, the contribution of the settling period to the overall cycle N₂O production is unraveled in this study. The effects of key compounds (i.e. NH₄⁺, NO₂⁻ and NH₂OH) on N₂O production during settling, which are shown to be the main contributors to the overall N₂O emissions in this study, are explored.

4.2 Materials and methods

4.2.1 SBR operation for partial nitrification of reject wastewater

A cylindrical 8L SBR was inoculated with activated sludge from a local domestic WWTP located in Girona (Spain). The mixed liquor temperature was controlled at 30°C using a water jacket, to mimic the common temperature conditions of reactors treating reject wastewater. The SBR was operated in cycles of 6h, consisting of feed-1 (2 min), aeration-1 (120 min), feed-2 (2 min), aeration-2 (120 min), settling (101 min) and decanting (15 min). 1L of synthetic reject wastewater was added in each feeding period, resulting in a hydraulic retention time (HRT) of 24h. 100 mL of biomass was removed at the end of the second aeration period providing a sludge retention time (SRT) of 20 d. Dissolved oxygen (DO) was controlled with a programmable logic controller (PLC) between 1-1.5 mg O₂/L by supplying air or nitrogen gas at 5 L/min. With this strategy, a constant gas flow rate of 5L/min was applied during all the reaction phases. The feed had a pH of 8 and a molar ratio of ammonium to bicarbonate of 1:1. After feeding, the pH of the reactor rose to 7.5 and decreased afterwards due to the nitrification reaction. When pH reduced to 6.7, it was automatically controlled by adding 1M NaHCO₃ solution. Cycle studies were performed on a weekly basis to monitor the nitrification activity, where samples for the analyses of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N were taken along the cycle and immediately filtered through disposable Millipore filter units (0.22 µm pore size). In each cycle, mixed liquor samples were taken after feed-1 (3 min), at the middle of aeration-1 (60 min), at the end of aeration-1 (119 min), after feed-2 (123 min), at the middle of aeration-2 (180 min), at the end of the aeration-2 (240 min) and at the end of the cycle (359 min). A schematic representation of the experimental set-up is shown in figure 3.1 (chapter 3).

The synthetic wastewater had the characteristics of anaerobic digester liquor. The wastewater composition was modified from Kuai and Verstraete (1998): 5.63 g/L of NH₄HCO₃ (1 g NH₄⁺/L), 0.064 g/L of each KH₂PO₄ and K₂HPO₄ and 2 mL of trace element stock solution per litre of feed. The trace element solution included (g/L): 1.25 EDTA, 0.55 ZnSO₄*7H₂O, 0.4 CoCl₂*6H₂O, 1.27 MnCl₂*4H₂O, 0.40 CuSO₄*5H₂O, 0.05 Na₂Mo₄*2H₂O, 1.37 CaCl₂*2H₂O, 1.25 FeCl₃*6H₂O and 44.40 MgSO₄*7H₂O. The alkalinity and total organic carbon (TOC) of the synthetic wastewater were 3469.63 mg CaCO₃/L and 4.89 mg C/L respectively.

4.2.2 Study of different cycle configurations

Experiments started once the AOB-enriched SBR reached stable operation conditions. Four cycle configurations were tested in order to identify if the cycle configuration had an effect on the overall N₂O and NO emissions as shown in figure 4.1. The cycle length and wastewater loading was kept the same as described previously. Cycle A corresponded to the cycle used in normal operation. Cycles B, C and D consisted of a modification of cycle A via implementing short anoxic or aerobic phases (N₂/Air

stripping) as described in figure 4.1. The settling phases lasted for 81 min in cycles B and D, while cycle C had a 61 min settling. In all cases, the monitoring was done after 24h of operation at that particular cycle configuration. Three different cycles were monitored for each of the configurations. Liquid-phase N_2O dynamics were followed together with gas emission measurements in each cycle type, in order to complete the information provided by the gas analyzers which were used during phases with gas stripping (air and/or N_2).

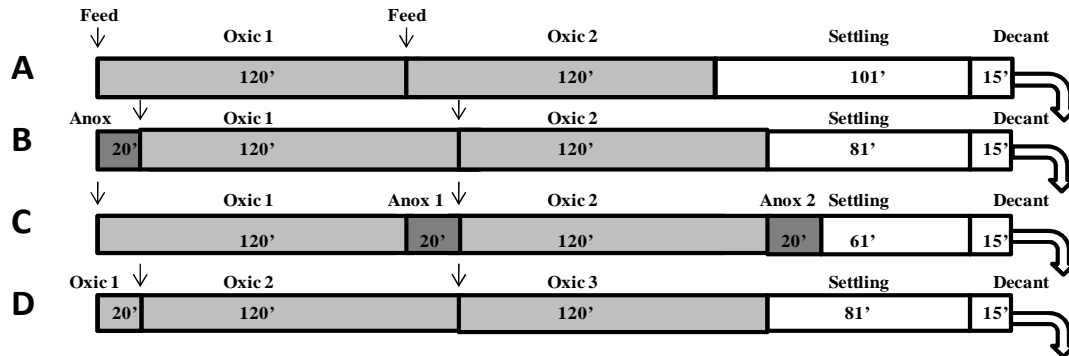


Figure 4.1 Cycle configurations used in this study.

4.2.3 Exploring the factors affecting initial N_2O peaks

Eight tests (T1-T8) were conducted in triplicate to assess the effect of several compounds to the initial N_2O peaks:

- T1: NH_4^+ (~100 mg N/L) and NO_2^- (~850 mg N/L) present in the bulk liquid before settling phase started.
- T2: No NH_4^+ (<0.2 mg N/L) present in the bulk liquid before settling phase started. NO_2^- concentration between 920-940 mg N/L.
- T3: Presence of NH_4^+ (~100 mg N/L) only.
- T4: Presence of NH_2OH (0.1 mg N/L) only.
- T5: Presence of NH_4^+ (~100 mg N/L), NO_2^- (~500 mg N/L), and NO_3^- (~300 mg N/L)
- T6: Presence of NO_3^- (940 mg N/L) only. No NH_4^+ , no NO_2^- (< 0.2 mg N/L in both cases) present in the bulk liquid before settling phase started.
- T7: No NH_4^+ , no NO_2^- and no NO_3^- present in the bulk liquid before settling phase started. Biomass washed before settling started.
- T8: NH_4^+ (~100 mg N/L) and NO_2^- (850 mg N/L) present in the bulk liquid before settling phase started. Biomass removed after 5 min of settling.

In each test, N_2O was monitored during the first 5 minutes of the aerated phase implemented after the decanting period, without feed entering the reactor. Changes were imposed in the cycle previous to the phase being monitored as described below:

Six of the tests (T1, T2, T3, T4, T7, T8) were done in the AOB-enriched SBR and T5 and T6 were done in another SBR. In the case of T1, the cycle previous to the monitoring consisted of 3h with a feeding phase (1 min) in which 0.5L synthetic wastewater was added, an aerated phase (63 min), settling (101 min) and decanting (15 min). For T2, the cycle was modified to 8h with the following phases: Feed (1 min) where 0.5L was added, aeration phase (363 min), settling (101 min) and decanting (15 min). In T3, the biomass was subjected to a washing process with phosphate buffer until the NO_2^- level was undetectable. Then, before starting the settling phase, a pulse of NH_4^+ (~100 mg N/L) was introduced in the bioreactor. T4 was performed identically to T3 but instead of NH_4^+ , a pulse of NH_2OH was added given a concentration of 0.1 mg NH_2OH -N/L inside the SBR before settling

started.

T5 and T6 were conducted in a separated SBR. 0.5L of mixed liquor was withdrawn from the AOB-enriched reactor and mixed with 0.5L of mixed liquor withdrawn from another SBR enriched with nitrite oxidizing bacteria (NOB) (not detailed in this study). This new SBR was operated for 1 week before the monitoring. For T5, the cycle time was 6h consisting of feed-1 (2 min), aeration-1 (120 min), feed-2 (2 min), aeration-2 (120 min), settling (101 min) and decanting (15 min). Under this configuration NH_4^+ was partially oxidized to NO_2^- and NO_3^- . In T6, the cycle configuration used for T5 was modified and consisted of feed (1 min) where 0.5L of synthetic reject wastewater was added, aeration phase (363 min), settling (101 min) and decanting (15 min). In this test, full nitrification conditions were reached: No NH_4^+ or NO_2^- was detected in the effluent. Instead, NO_3^- was present at a high concentration (around 1 g N/L). In T7 all the supernatant was removed after biomass settled (after the first 5 min of settling) and the biomass was washed and resuspended with phosphate buffer. With this test, any compounds present in the supernatant as polymeric substances, NH_4^+ , NH_2OH , NOH and NO_2^- were assumed to be removed. In T8 no changes in the cycle previous to the monitoring were done, maintaining configuration A as described in section 4.2.2. The only change made was removing the biomass from the SBR once settled (after the first 5 min of the settling period). Then, the supernatant was filtered through a 0.45 μm pore filter in order to completely remove bacterial cells and left it in the SBR for 101 min to mimic the settling time. This test was carried out to determine if chemical processes producing N_2O were also occurring during settling.

4.2.4 Online N_2O and NO monitoring

Gaseous N_2O and NO were continuously analyzed using commercial analyzers. N_2O was analyzed with the infrared gas analyzer Servomex 4900 (Servomex Group Ltd. East Sussex, UK) and data was logged every 5 seconds. NO was analyzed via the chemiluminescence gas analyzer CLD64 (Eco Physics. Dürnten, Switzerland). N_2O patterns in the liquid phase were followed using a microsensor with data logging (Unisense A/S. Aarhus, Denmark). A two-point calibration of the microsensor was done before and after each measurement.

4.2.5 Microbial and chemical analyses

Fluorescence in situ hybridization (FISH) was performed as described in Nielsen et al. (2009) with Cy5-labelled EUBMIX probes (for most bacteria), Cy3-labelled AOBMIX probes (for AOBs, comprising equal amounts of probes Nso1225), NEU, NmV, Cluster6a192 and Cy3-labelled Nso190. Cy3-labelled Ntspa662 and NIT3 were utilized for detection of NOB cells. FISH preparations were visualized with a Nikon CS1 confocal laser-scanning microscope (CLSM) using Plan-Apochromat 63x oil (NA1.4) objective. Thirty images were taken from each sample for quantification. The area containing Cy3-labelled specific probe (AOBMIX + NSO190) cells was quantified as a percentage of the area of Cy5-labelled bacteria probe (EUBMIX) within each image using pixel counting program.

$\text{NH}_4^+\text{-N}$, mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were analyzed according to the standard methods (APHA, 1998). $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were analyzed via ion chromatography (ICS5000, DIONEX.). Alkalinity was measured via a robotic titrosampler (Metrohm 855). TOC was analyzed with a TOC analyzer (Shimadzu, TOC-V CSH).

4.2.6 Calculations

The total N_2O and NO emitted was calculated using equation 1, as described in chapter 3.

4.3 Results

4.3.1 Reactor performance and N_2O and NO emission dynamics

The SBR achieved stable operation 4 months after the start-up, with a 98 % of NH_4^+ -N converted to NO_2^- -N and no NO_3^- -N detected in the effluent. A stable biomass concentration of 1.24 g MLVSS/L was reached. Characterization of the biomass by FISH showed an $80.5 \pm 1.6\%$ of the total bacteria being AOB. No NOB cells were detected in the SBR. The reactor was performing complete nitritation with around 98% of the $1\text{g } NH_4^+$ -N/L of the feed converted to NO_2^- and no NO_3^- was detected in the effluent. The effluent value for alkalinity was $222.12\text{ mg } CaCO_3/L$ during normal operation. Under the same conditions, TOC in effluent samples was $5.1\text{ mg } C/L$. The monitoring of the different cycle configurations on the SBR was done during the 5th month of operation. Four different cycle configurations were monitored in terms of nutrient removal performance and N_2O/NO emissions. Figure 4.2 shows the experimental profiles of NH_4^+ -N and NO_2^- -N, nitrogen oxides, pH and DO measured during the cycle types A (normal operation), B, C and D. In all the cases, NH_4^+ was

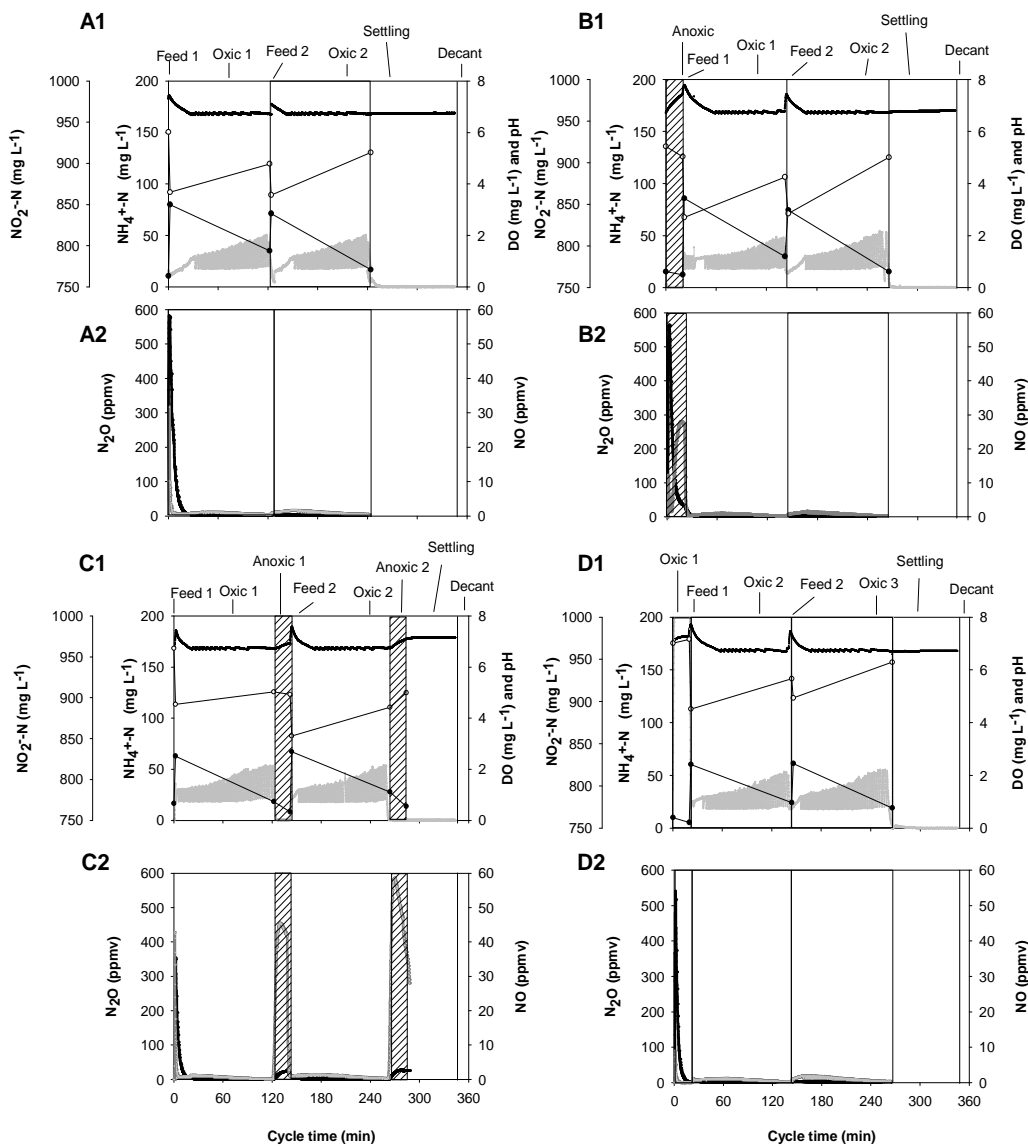


Figure 4.2 Experimental profiles of NH_4^+ -N (●), NO_2^- -N (○), pH (black line) and DO (grey line) in A1, B1, C1 and D1. N_2O (black line), NO (grey line) emission profiles in A2, B2, C2 and D2 measured in the SBR under the four different cycle configurations tested in this study.

oxidized and NO_2^- accumulated at similar rates. DO oscillated between 0.8 and 2 mg/L and pH increased during feeding phases (up to 7.5) and decreased to 6.7 as NH_4^+ was oxidized.

Under normal operational conditions (Cycle A, Fig. 4.2.A1 & A2) the majority of N_2O and NO emitted were detected during the first feeding period. Peaks of N_2O and NO were detected during the first 5 minutes of the cycle, decreasing very fast afterwards till reaching residual concentrations of 0.5-2 ppm v for both N_2O and NO respectively. In order to explore if these initial N_2O and NO peaks could be minimized, the normal cycle (A) was slightly modified introducing short anoxic or aerobic periods as detailed in figure 4.1. Figure 4.2B shows the emission profiles obtained in cycle B, where a 20 min anoxic period, with N_2 stripping, was introduced at the beginning of the cycle before the 1st feeding occurred. In this case, an initial peak for N_2O and NO very similar to the one detected in Cycle A was measured during the first 5 min of the cycle, decreasing very fast afterwards. In contrast to cycle A, NO emissions in cycle B started to increase again, producing a second NO peak under anoxic conditions. NO decreased sharply as soon as the feeding phase started together with aeration. From this point until the end of the cycle, N_2O and NO concentrations remained very low at around 0.5-2 ppm v respectively.

Figure 4.2C shows the effect of the addition of 20 min anoxic periods after each aerobic phase (Cycle C). During these periods, NO production increased substantially. N_2O also increased in each anoxic phase, but this increase was small if compared to the initial peak (after settling). Finally, a cycle with 20 min aerobic phase before the first feeding started was implemented (Cycle D, Fig.4.2D). Similar N_2O and NO peaks to the ones detected during normal operation (cycle A) were measured, which decreased sharply after a few minutes. After that, N_2O and NO emissions remained to the minimum for the rest of the cycle, and were hardly affected by the next feeding.

4.3.2 Comparing NO and N_2O emissions

Total NO and N_2O emissions are shown in figures 4.3A and B respectively. Under normal operation (Cycle A), NO emissions were kept below 0.1 mg NO-N/g VSS. As anoxic periods were implemented in cycles B and C, NO emissions substantially increased reaching 0.1 mg NO-N/g VSS and 0.3 mg NO-N/g VSS respectively. Cycle D presented similar NO emissions than cycle A. Only between 1% (cycle C) and 8% (cycle B) of NO emitted was registered during the first 5 min of the cycle.

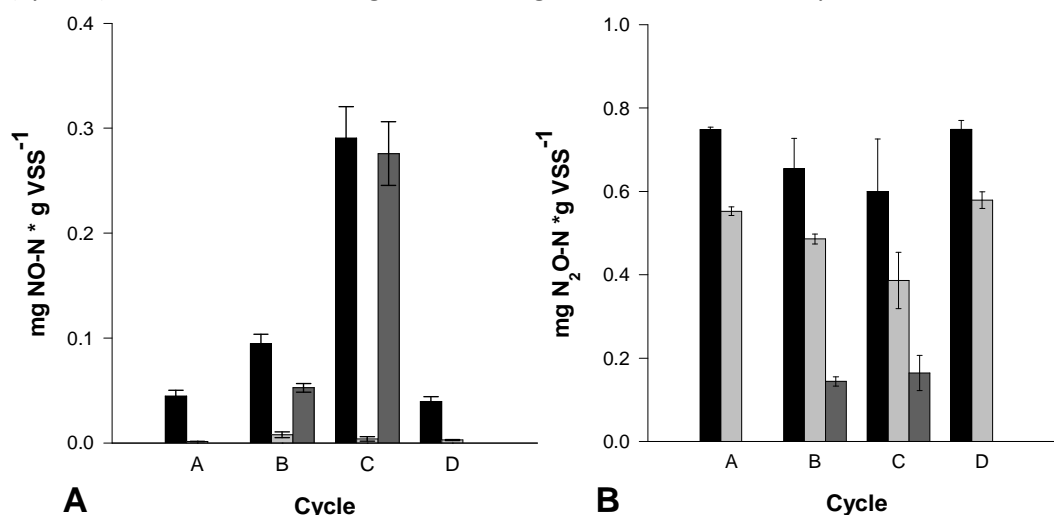


Figure 4.3 (A) Total NO emissions (black bars), NO emissions during the first 5 min of each cycle (light grey bars) and NO emissions during anoxic conditions (dark grey bars) during each cycle. (B) Total N_2O emissions (black bars), N_2O emissions during the first 5 min of each cycle (light grey bars) and N_2O emissions during anoxic conditions (dark grey bars) during each cycle. Error bars correspond to the standard error of the means.

Table 4.1 summarizes the specific ammonium oxidized as well as the NO and N₂O emission factors obtained for each type of cycle. NO emission factor was 0.049±0.002% for normal operational conditions, similar to the one detected in Cycle D (0.042±0.004%). This factor increased to 0.101±0.006 and 0.301±0.025% in cycles B and C respectively due to an increase of the anoxic time fraction of the cycle.

Table 4.1 Specific ammonium oxidized and N₂O-NO emission factors calculated for each cycle type.

Cycle	NH ₄ ⁺ oxidized (mg N/g VSS)	N ₂ O-N/converted-N (%)	NO-N/converted-N (%)
A	90.83±6.92	0.83±0.07	0.049±0.002
B	92.82±3.12	0.70±0.05	0.101±0.006
C	96.95±7.64	0.60±0.09	0.301±0.025
D	93.73±7.86	0.80±0.04	0.042±0.004

Normal operation (cycle A) and cycle D were very similar in terms of total N₂O emissions, reaching around 0.8 mg N₂O-N/g VSS in each case (Fig. 4.3B). As opposite to the case of NO, up to 80% of these N₂O emissions corresponded to the first 5 min of the cycle. On the other hand, although cycles B and C also presented the majority of the total N₂O emissions during the first 5 min, slightly lower N₂O emissions were measured compared to cycle A and D (Fig. 4.3B).

As shown in table 4.1, the N₂O emission factor was 0.83±0.07% of the ammonium converted under normal operational conditions (Cycle A). This emission was reduced to 0.70±0.05% when an anoxic phase was introduced at the beginning of the cycle (cycle B) and to 0.60±0.06% when two anoxic periods were introduced after the aerobic phases in cycle C. This decrease in N₂O was linked to an increase on NO emissions shown in figure 4.3A. In fact, the total emission of both NO and N₂O together tend to be equal (around 0.8% N emitted (N₂O+NO/N converted) in all cycles (Fig. 4.4). N₂O was also emitted during anoxic periods (cycle C) but the quantity of these emissions was small when compared with initial peak emissions.

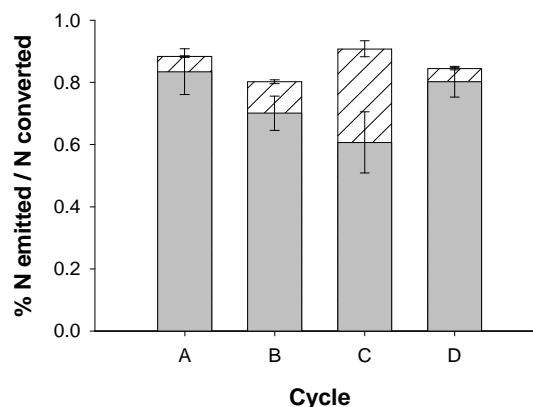


Figure 4.4 Accumulated N_xO-N emission factors (N₂O-N in grey and NO-N with black pattern) measured in each cycle configuration. Error bars correspond to the standard error of the means.

4.3.3 Factors affecting N₂O production during settling

Liquid-phase N₂O dynamics were followed together with gas emission measurements in each cycle type, in order to complete the information provided by the gas analyzer which could not be used during settling and decanting phases. Figure 4.5 shows the concentration of dissolved N₂O during each cycle type.

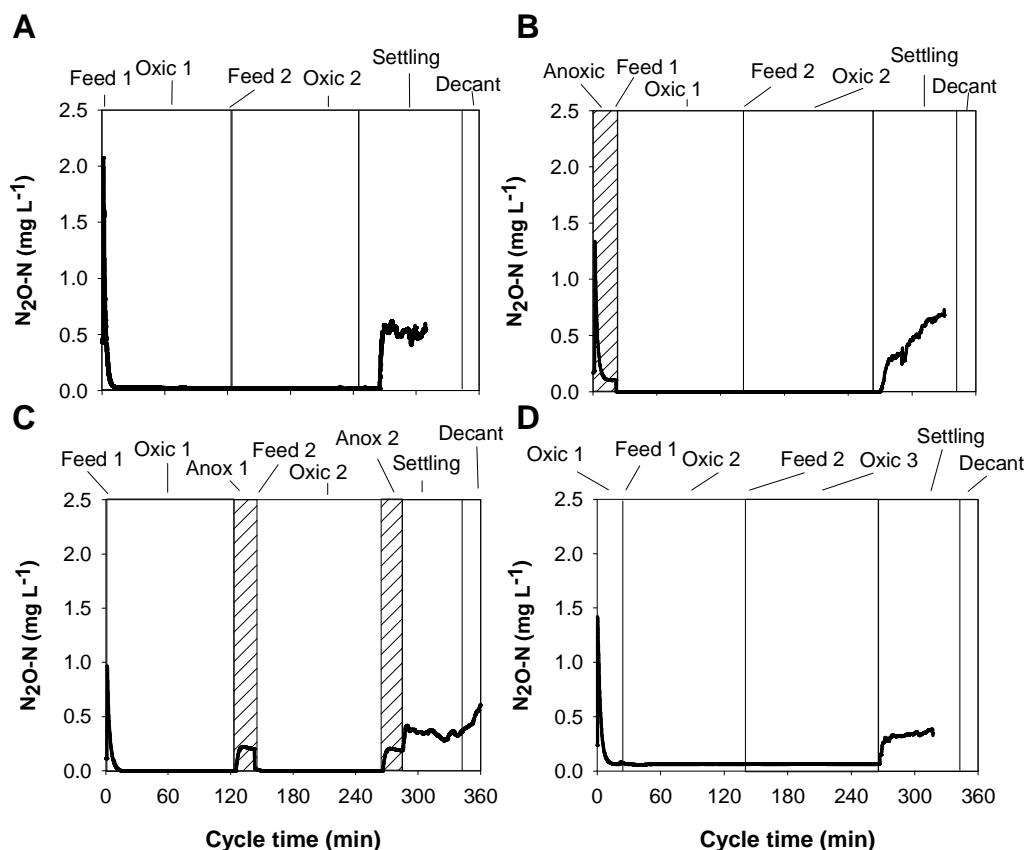


Figure 4.5 Experimental profile of liquid-phase N_2O (black line) measured in the SBR under the four different cycle configurations (A, B, C and D) tested in this study.

The measurements were prolonged between 60 and 90 minutes in the settling period, showing a similar increase of N_2O production, as soon as gas stripping and mixing stopped. During the settling phases, N_2O fluctuated around 0.5 mg N/L in all cases, as shown in figure 4.5 although higher or lower concentrations might have been present in the reactor due to unmixed conditions. An initial dissolved N_2O peak was also detected during the first minutes of each cycle configuration when stirring and gas stripping started, mimicking the N_2O profile obtained in the gas measurements. Cycle A presented the largest dissolved N_2O peak reaching 2.1 mg N_2O-N/L . Cycles B and D had similar initial liquid-phase N_2O peaks of around 1.4 mg N_2O-N/L and cycle C (two anoxic phases) presented the smallest initial peak (0.9 mg N_2O-N/L) (Fig. 4.5). This trend was similar to the one shown with the emission dynamics of cycle C, in which there was a minimization of the initial N_2O peak (Fig. 4.2C) if compared with the peaks measured in cycles A, B and D.

In order to investigate the factors contributing to N_2O production during settling, eight tests were performed as described in section 4.2.3. N_2O peaks registered during these experiments are shown in figure 4.6.

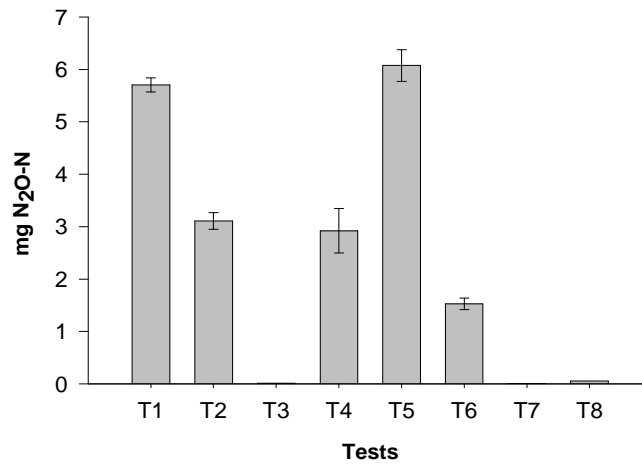


Figure 4.6 Total N₂O emissions during 5 min after settling, under the conditions of the different tests. Error bars correspond to the standard error of the means.

The first test (T1) was designed to mimic the conditions of a normal cycle achieving partial nitrification of NH₄⁺-rich wastewater in a SBR, where a certain amount of NH₄⁺ is converted to NO₂⁻. Under these conditions, effluent water consisted of high NO₂⁻ concentrations with some remnant NH₄⁺ concentration (~100 mg N/L). In T1, 5.7±0.1 mg N₂O-N were emitted during the first 5 min after settling at the conditions mentioned above. These emissions were taken as a reference to be compared with the emissions given under the conditions of the rest of the tests. Complete NH₄⁺ oxidation (due to an extension of the aeration period) in T2 led to a nearly 55% decrease in N₂O emissions during the first 5 min of aeration after settling when compared with those from T1. In this experiment, it was assumed that any intermediate products of NH₄⁺ oxidation such as NH₂OH or NOH were also not present, since NH₄⁺ was depleted for more than 60 min before aeration finished. As expected, high concentrations of NO₂⁻ were found in the effluent. The presence of NH₄⁺ only was tested in T3, where biomass had to be washed to remove the NO₂⁻ and NH₄⁺ was added before settling started. Negligible N₂O emissions were detected in this test. Same test was repeated but adding 0.1 mg NH₂OH-N/L instead of NH₄⁺ (T4). Interestingly, the presence of NH₂OH during settling originated in 3 mg N₂O-N being produced during the first 5 min of aeration of the subsequent cycle. When the same amount of NH₂OH was added on water, no N₂O production was detected indicating that the N₂O detected in T4 was biologically produced (data not shown).

The simultaneous presence of NH₄⁺, NO₂⁻ and NO₃⁻ during settling was tested in T5, carried out in another SBR where AOB and NOB were combined. T5 resulted in a production of 6 mg N₂O-N similar to T1. In order to explore the effect of NO₂⁻ in the initial N₂O peak, T6 was carried out. In this test, NH₄⁺ was completely oxidized to NO₃⁻ as a result of the combination of AOB and NOB populations. This had a direct effect on the N₂O peak of the subsequent cycle which was substantially minimized, with a reduction on N₂O emissions around 75% when compared with those in T1 and T5 respectively.

In T7, the biomass was washed and resuspended in phosphate buffer and thus nearly no chemical by-products of AOB metabolism could be found in the SBR. As expected, in this case the emission of N₂O after settling of was negligible.

When the biomass was removed from the bioreactor after the first 5 min of settling in T8, N₂O emissions were reduced 99% compared with the emissions found in T1. This test indicated that most of the N₂O production was biologically mediated, and only a minimal amount (< 1%) could come from chemical reactions.

4.4 Discussion

4.4.1 Comparing NO and N₂O emissions under different cycle configurations: possible interrelation and pathways

The NO and N₂O emission dynamics measured along the different cycle configurations differ not only on the quantity of each gas being emitted but also on the periods where each of the gases is emitted as has been shown in figure 4.3. During normal operation of the SBR (cycle A) and under the conditions implemented in cycle D (20 min aeration before feed-1), the NO emission factors were around 0.05%, similar to others reported in the literature for partial nitrification systems (0.07%, Gustavsson et al. 2011) and full nitrification reactors (0.03%, Kampschreur et al., 2008b). However, NO emissions increased one order of magnitude in cycles B and C respectively and were mainly produced during the anoxic periods present in these cycle configurations. Strict anoxic conditions have been suggested to cause an over expression of the NO₂⁻ reductase gene, whereas genes encoding for NH₃ and NH₂OH oxidation and NO reduction are under-expressed, thus causing an accumulation of NO (Kester et al., 1997; Kampschreur et al. 2008b; Yu et al., 2010). A possible explanation for this was given by Schmidt et al. (2008) who suggested that the over production of NO by AOB under anaerobic conditions was a mechanism for a fast recovery of NH₃ oxidation upon aeration. They speculated that in oxygen depleted environments NO is produced and serves, together with nitrogen dioxide (NO₂), as additional oxidant of NH₃. They suggested a novel NH₃ oxidation pathway in which NO₂ (product of NO oxidation) could be used as oxidizing agent in NH₃ oxidation under anaerobic conditions. However, for this pathway to occur, NO₂ needs to be produced in previous aerobic phases. Indeed, in cycle C, as soon as anoxia began, a peak of NO production was detected, which started to decrease after a few minutes, thus indicating that a compound (maybe NO₂) necessary for NO production in anoxia, was being consumed.

In contrast to NO, N₂O was mainly emitted during the first minutes of the cycle in each configuration. Normal operation (cycle A) and cycle D were also very similar in terms of total N₂O emissions (around 0.8% of the converted-N being emitted as N₂O). These emission factors were in the same order of magnitude as others found in similar partial nitrification systems (3.4% found by Kampschreur et al. (2008a); 1% found by Law et al. (2011)). On the contrary, slightly lower N₂O emission factors were measured in cycles B and C, where anoxic periods were introduced. The reasons behind this reduction in N₂O emissions remain unknown. However, the introduction of short anoxic phases in partial nitrification systems enriched with AOB cannot be postulated as a good minimization strategy due to the increase on NO production under these conditions. Also it is important to consider that in full-scale systems, where no gas stripping is applied during anoxic conditions, an increase on the concentration of dissolved NO can have a detrimental effect on the overall microbial population, due to its toxicity, affecting the performance of the plant.

4.4.2 N₂O production mechanisms during settling

In general, the results of the tests carried out suggest that a combination of different N₂O production pathways is responsible for the N₂O emissions detected after settling. Yu et al. (2010) found that transition from anoxic to aerobic conditions in the presence of NH₄⁺ favored the production of N₂O which, together with the stripping of previously accumulated gas, could result in large N₂O emission (peak emissions). A shift in the metabolism of AOB from a low specific activity toward the maximum specific activity was suggested by these authors as the reason behind N₂O production. This possibility could partially explain the results from T1, as remnant NH₄⁺ together with high NO₂⁻ concentrations were found in the SBR during the settling phase. Interestingly, when only NH₄⁺ was present (T3), N₂O emissions were almost negligible. Therefore, the presence of NO₂⁻ in

combination with NH_4^+ , had a clear influence on the subsequent N_2O emissions. In this case, the production of N_2O during settling could have been caused by the reduction of NO_2^- and/or NO by an electron donor such as NH_4^+ (Poeth and Focht, 1985; Ritchie and Nicholas, 1972). Indeed, denitrification by AOB could be a possible pathway for N_2O accumulation during settling, as high levels of NO_2^- were available. In this study the presence of both NH_4^+ and NO_2^- (T1, T5) resulted in larger N_2O peak emissions than in the cases of the presence of NO_2^- alone (T2) or NO_3^- (T6). The presence of NO_2^- in partial nitrification bioreactors under anoxic and sub-oxic conditions has been suggested by other authors as an important precursor of N_2O production due to the activation of the nitrifier denitrification pathway (Kampschreur et al., 2008a; Kampschreur et al., 2008b; Kim et al., 2010). However, a reduction on NO_2^- concentration during settling could not be accurately quantified and liquid-phase NO was not measured. Therefore, this hypothesis could not be fully confirmed.

Another hypothesis is the occurrence of the NH_2OH oxidation pathway as a possibility for N_2O production during settling. When only NH_2OH was present (T4) an N_2O peak was detected in the subsequent aeration period, which was around 50% of the amount of N_2O detected in T1, with NH_4^+ and NO_2^- . These results clearly suggest that NH_2OH rather than NH_4^+ is the compound regulating at least part of the N_2O emissions detected in this study. Chemically produced N_2O from NH_2OH (chemodenitrification) is believed to be the result of the chemical decomposition of NOH (produced by AOB) during NH_2OH oxidation or by chemical oxidation of NH_2OH with NO_2^- (Ritchie and Nicholas, 1972). Since NO_2^- was not present, the latest can be neglected. According to Igarashi et al. (1997), the oxidation of NH_2OH involves two separated reactions. First NH_2OH is converted to the nitrosyl radical NOH , and then NOH is converted to NO_2^- . The unstable decomposition of NOH may lead to chemical N_2O production on one side. Also, NO can be generated during the conversion of NOH to NO_2^- , and could be further reduced to N_2O (for a review, see Law et al. 2012a). Further research needs to be done over this issue, with especial focus on the relevance of this pathway in real-scale nitrification systems.

When only NO_3^- was present during settling (T6), N_2O emissions were reduced by 75% when comparing to the tests where NO_2^- and NH_4^+ were present (T1 and T5) and by 50% when comparing with tests with only NO_2^- (T2) or NH_2OH (T4). Since NO_3^- is not related with the metabolism of AOB, this small production of N_2O suggests that might be originating from the heterotrophic denitrification pathway. Although the biomass was highly enriched with AOB (>80%) we cannot exclude the presence of a small heterotrophic denitrifying community that could be contributing to the formation of a small fraction of N_2O under certain conditions.

The provision of sufficient aeration time to achieve full ammonia oxidation in systems performing partial nitrification of reject wastewater could be useful to reduce their overall N_2O emissions, as shown by the emission minimization given by T2 when compared with T1 (section 4.3). Extending the aeration time would however, increase the energy demand of the treatment system. Since the energy demand has a severe impact on the carbon footprint of a wastewater treatment system, an evaluation of the benefits of reducing direct green-house gas emissions by increasing aeration would be needed. Finally, as proven by this study, idle/settling phases will increase significantly the N_2O production in partial nitrification systems where AOB are dominant. Treatment processes that avoid those phases (i.e. continuous treatment) could present advantages in terms of reduced emissions.

Chapter 5

Nitritation versus full nitrification of ammonium-rich wastewater: Comparison in terms of nitrous and nitric oxides emissions

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5.1 Preliminary remarks

This study explores the different N₂O and NO emission profiles measured in lab-scale sequencing batch reactors (SBRs) performing nitritation, nitrataion, and full nitrification of synthetic reject wastewater (1g NH₄⁺-N/L). A characterization of the different bacterial populations based on fluorescence *in situ* hybridization (FISH) and 16S rRNA tag pyrosequencing was performed. A rapid shift from nitritation to full nitrification as a result of the combination of two different enriched populations (AOB and NOB) followed by a change in the SBR cycle configuration allowed a precise comparison between both processes in terms of gas emissions. We verified the hypothesis that N₂O and NO emissions would be minimized during full nitrification due to NO₂⁻ consumption by NOB. Moreover, the effect of wastewater N loading on total N₂O and NO emissions was further studied along individual experiments in the full nitrification system in order to evaluate its importance.

5.2 Materials and methods

5.2.1 Enrichment of AOB and NOB populations

Two cylindrical 8L SBRs (SBR1 and SBR2) were inoculated with activated sludge from a domestic wastewater treatment plant (WWTP) located in Girona (Spain). In SBR1, the enrichment of AOB was promoted and nitritation was achieved. SBR2 was utilized to enhance the growth of NOB and the nitrataion reaction was established. Both bioreactors were operated in cycles of 6h, consisting of feed-1 (2 min), aeration-1 (120 min), feed-2 (2 min), aeration-2 (120 min), settling (101 min) and decanting (15 min).

SBR1 was fed with synthetic reject wastewater (adapted from Kuai and Verstraete, 1998), containing 5.63 g/L of NH₄HCO₃ (1 g NH₄⁺-N/L), 0.064 g/L of each KH₂PO₄ and K₂HPO₄ and 2 mL of trace element stock solution. The trace element stock solution contained (per litre): 1.25g EDTA, 0.55g ZnSO₄·7H₂O, 0.40g CoCl₂·6H₂O, 1.275g MnCl₂·4H₂O, 0.40g CuSO₄·5H₂O, 0.05g Na₂MoO₄·2H₂O, 1.375g CaCl₂·2H₂O, 1.25g FeCl₃·6H₂O and 44.4g MgSO₄·7H₂O. The feed had a pH of 8 and a molar ratio of ammonium to bicarbonate of 1:1. After feeding, the pH of the reactor rose to 7.8 and decreased afterwards due to the nitritation reaction. When pH reached 6.7, it was automatically controlled with addition of 1M NaHCO₃ solution. The mixed liquor temperature in SBR1 was controlled at 30°C using a water jacket. 1L of synthetic wastewater was added in each feeding period, providing a hydraulic retention time (HRT) of 24h. 100 mL of biomass was removed at the end of the second aeration period providing a sludge retention time (SRT) of 20 d. Dissolved oxygen (DO) was controlled with a programmable logic controller (PLC) between 0.8-1.5 mg O₂/L by adding air or nitrogen gas at 5 L/min. After 4 months of operation, SBR1 achieved stable nitritation, with a 98 % of NH₄⁺ converted to NO₂⁻ and no NO₃⁻ detected in the effluent.

SBR2 was fed with synthetic wastewater which comprised the following composition per litre (adapted from Kuai and Verstraete, 1998): 4.93g of NaNO₂ (1g NO₂⁻-N/L), 0.4g of NaHCO₃, 1g of each KH₂PO₄ and K₂HPO₄ and 2 mL of a stock solution containing the same trace elements as for SBR1. 1L of synthetic wastewater was added in each feeding period, providing a HRT of 24h. During the start-up (~3 month) of SBR2 no biomass was wasted due to the slow bacterial growth. Then, small volumes of biomass were intermittently removed following bacterial growth observations keeping the SRT over 20 d during the experimental period. This bioreactor was operated at room temperature (~23-25°C). DO was controlled similarly to SBR1. There was no need for pH control as this parameter was around 7.5 and presented no significant changes during the operation of the reactor. SBR2 achieved stable nitrataion after 9 months of operation, with a 99 % of NO₂⁻ converted to NO₃⁻.

5.2.2 SBR operation for full nitrification

Four litres of the mixed liquor enriched with AOB were removed from SBR1 (~0.8 g MLVSS/L) and substituted with the same amount of mixed liquor withdrawn from SBR2 (enriched with NOB), resulting in an equally mixed AOB/NOB community (~0.8 g MLVSS/L) (SBR1 MIX). After a first settling period, the supernatant was extracted and the biomass was washed with phosphate buffer solution (1M) before the start of the next cycle. SBR1 MIX was then operated as SBR1 during 4 d (transition period). After this transition period, the cycle configuration was modified in order to achieve full nitrification. The feed volume was reduced to 0.5 L/cycle and the aeration time was extended. The new cycle configuration (480 min) consisted of feed (0.5 min), aeration (360 min), settling (104.5 min) and decanting (15 min). The composition of the synthetic wastewater used, the mixed liquor temperature as well as the pH and DO control were maintained as for normal operation of SBR1 (see section 5.2.1). Mixed liquor was manually wasted at a rate of 100 mL/d to maintain a stable MLVSS concentration. Full nitrification was achieved during the following 30 d of the study.

5.2.3 Bacterial composition analyses

Mixed liquor samples were taken from SBR1 (during nitritation) and SBR2, along with a sample of the mixed bacterial population (SBR1 MIX) during the full nitrification period. Samples were then subjected to FISH analysis for evaluation of the AOB and NOB communities' enrichment, and to pyrosequencing of bacterial DNA in order to characterize each one of the bacterial populations.

FISH was performed as described in Nielsen et al. (2009) with Cy5-labelled EUBMIX probes and Cy3-labelled AOBMIX probes (for AOBs, comprising equal amounts of probes Nso1225, NEU, NmV, Cluster6a192) and Cy3-labelled Nso190. Cy3-labelled Ntspa662 and NIT3 were utilized for NOB cells. FISH preparations were visualized with a Nikon CS1 confocal laser-scanning microscope (CLSM) using Plan-Apochromat 63x oil (NA1.4) objective. Thirty images were taken from each sample for quantification. The area containing Cy3-labelled specific probe (AOBMIX + NSO190 for AOB and Ntspa662 + NIT3 for NOB) cells was quantified proportionally to the area of Cy5-labelled bacteria probe (EUBMIX) within each image using the daime software package (Daims et al., 2006).

Bacterial DNA was extracted with the FastDNA[®] SPIN Kit for Soil (MP Biomedicals) from each biomass sample (SBR1, SBR2 and SBR1 MIX) and amplified with primers 27F (3'-GAG TTT GAT CNT GGC TCAG-5') and 519R (3'-GTN TTA CNG CGG CKG CTG-5'). The amplicons were then sequenced using Roche 454 GS FLX Titanium technology. All pyrosequencing reads were initially screened for quality and length of the sequences using the MOTHUR software package (Schloss et al., 2009). Sequences were trimmed to 250 bp and then aligned using the SILVA reference database (Pruesse et al., 2007). Potential chimeric sequences were detected and removed by using chimera.uchime incorporated into MOTHUR. The Ribosomal Database Project (RDP) pipeline and Classifier function (Wang et al., 2007) were used to align and provide taxonomic classification at a confidence threshold of 50%. The sequences from this study have been deposited in the NCBI Short Read Archive under accession number SRA064072.

5.2.4 Chemical analyses and online N₂O and NO monitoring

The concentration of NH₄⁺-N, mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were analyzed according to standard methods (APHA, 1998). NO₂⁻-N and NO₃⁻-N were analyzed via ion chromatography (ICS5000, DIONEX.).

Gaseous N₂O and NO were continuously monitored using commercial analyzers. N₂O was analyzed with the infrared gas analyzer Servomex 4900 (Servomex Group Ltd. East Sussex, UK) and data was

logged every 5 seconds. NO was analyzed via the chemiluminescence gas analyzer CLD64 (Eco Physics, Dürnten, Switzerland).

5.2.5 Calculations

The total N₂O and NO emitted was calculated using equation 1, as described in chapter 3.

5.2.6 Comparing nitrification and full nitrification systems

Total N₂O and NO emissions from SBR1 were compared under nitrification and full nitrification conditions. The bioreactor was operated during a number of cycles (triplicates) with a feed loading of 2 L/cycle and 0.5 L/cycle for nitrification and full nitrification conditions, respectively. Only one feed (1 L) and its correspondent aerobic phase (120 min) were taken into account when measuring and calculating N₂O and NO emissions under nitrification conditions. Similarly, during full nitrification N₂O and NO emissions were recorded during the first 120 min of the aeration after feeding. This allowed a direct comparison between both processes.

5.2.7 Testing the effect of NH₄⁺ loading on N₂O and NO emissions

Emissions of N₂O and NO were compared when different NH₄⁺ loadings were applied to SBR1 MIX under full nitrification conditions. During this experiment, SBR1 MIX was operated with a unique feed of 0.5 L (~1g NH₄⁺-N/L) which was completely converted to NO₃⁻ without accumulation of NO₂⁻. When stable conditions were achieved, different feeding regimes were applied (0.5, 1 or 1.5 L of synthetic reject wastewater per cycle) and analyses were carried out during 120 min of an aerated phase. These tests were conducted in triplicate.

5.3 Results and discussion

5.3.1 Bacterial community composition

Quantification of the biomass by FISH revealed high AOB/NOB populations in SBR1 (83.7±3.6% AOB) and SBR2 (74.8±1.8% NOB), respectively. When the biomass from SBR1 and SBR2 was mixed, 46.9±3.7% of the population corresponded to AOB and 45.7±3.7% to NOB. In the case of SBR2, only NIT3 probe, targeting *Nitrobacter* sp. could be used for quantification, as Ntspa662 (targeting *Nitrospira* sp.) showed negative results.

Pyrosequencing analysis of 16S rRNA genes was used to investigate the bacterial community composition and the results of the relative abundance of different bacterial families in each system (SBR 1, SBR2 and SBR1 MIX) are shown in figure 5.1.

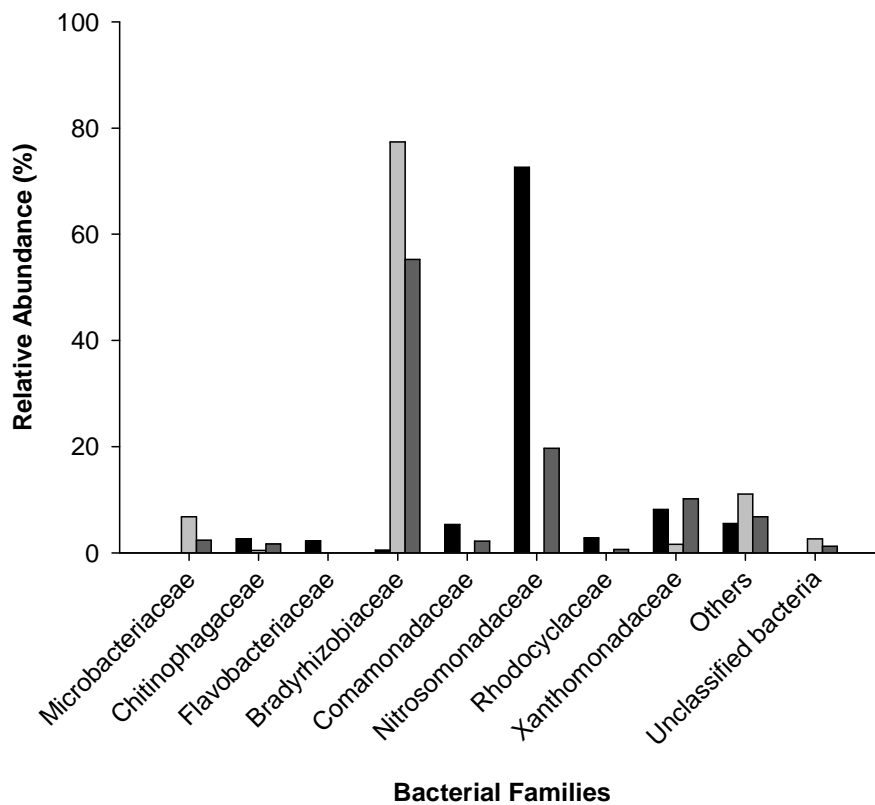


Figure 5.1 Relative abundances of each bacterial family (most dominant accounted only) found in SBR1 (black bars), SBR2 (light-grey bars) and SBR1 after biomass mixing (SBR1 MIX, dark-grey bars). Relative abundance is defined as the number of sequences related to a particular taxon divided by the total number of sequences per sample (%).

In SBR1, 72% of all the sequence reads generated corresponded to the family *Nitrosomonadaceae* (*Betaproteobacteria*), all of them being identified as *Nitrosomonas* sp. (data not shown). The dominant family in SBR2 was *Bradyrhizobiaceae* (*Alphaproteobacteria*) with 76% relative abundance, and within this group all the sequences belonged to *Nitrobacter* sp. (data not shown). In this system, none of the sequences could be related with *Nitrospira* sp., in agreement with the results obtained through FISH.

The relative abundances of the different bacterial groups calculated through sequence analyses were highly similar to the results found by means of FISH, and proved the successful enrichment of AOB and NOB in SBR1 and SBR2, respectively.

5.3.2 Process dynamics of the experimental bioreactors

Figure 5.2 shows the experimental profiles of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, nitrogen oxides and pH and DO measured during one cycle in SBR1 (under nitrification), SBR1 MIX (during transition and full nitrification) and SBR2 (nitrification).

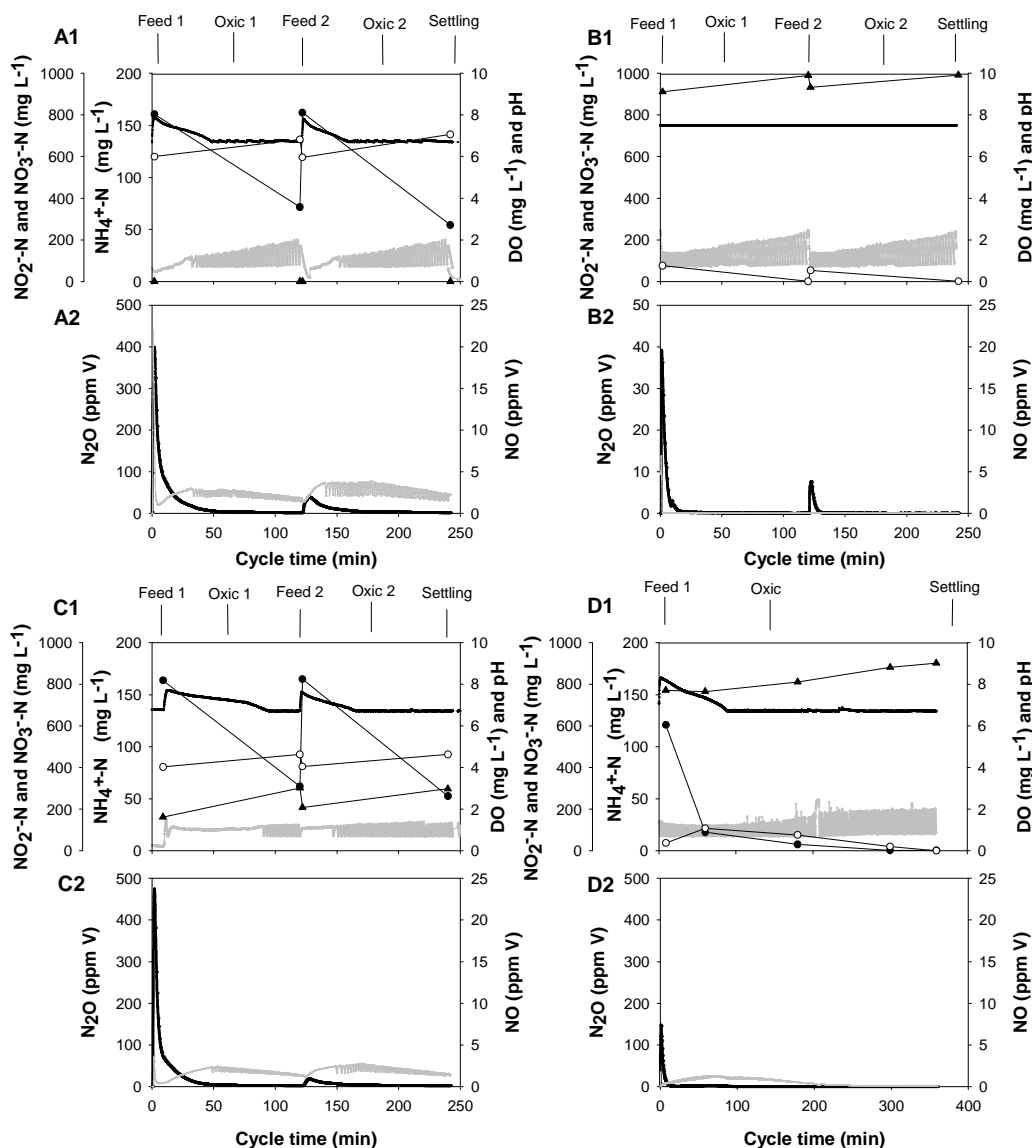


Figure 5.2 Experimental profiles of $\text{NH}_4^+\text{-N}$ (\bullet), $\text{NO}_2^-\text{-N}$ (\circ), $\text{NO}_3^-\text{-N}$ (\blacktriangle), pH (black line) and DO (grey line) (A1, B1, C1 and D1) and N_2O (black line) and NO (grey line) emission profiles (A2, B2, C2 and D2) measured during nitritation in SBR1 (A1 and A2), nitrification in SBR2 (B1 and B2), transition to full nitrification in SBR1 MIX (C1 and C2) and full nitrification in SBR1 MIX (D1 and D2).

In SBR1 (Figure 5.2A), AOB growth was enhanced and nitritation was achieved with 93-98% NH_4^+ being oxidized to NO_2^- after four months of operation. DO oscillated between 0.5 and 2 mg/L and pH increased during feeding phases (up to 7.8) and decreased to 6.7 as NH_4^+ was oxidized (Figure 5.2 A1). Approximately half of the total N_2O emitted was detected during the 1st feeding period. This has been reported in recent studies also conducting partial or full nitritation from reject wastewater (Kong et al., 2013; Law et al., 2011; Rodriguez-Caballero et al., 2013). Some of those have attributed this peak on N_2O emissions to the N_2O production and accumulation during settling, which is then stripped from the liquid phase as soon as air enters the reactor (Law et al., 2011; Rodriguez-Caballero et al., 2013). After the first peak of N_2O , the concentration of this gas decreased very fast till reaching residual concentrations of 0.5-2 ppm v during the rest of the first aerated phase. Then, a second smaller N_2O peak could be registered after the second feed, decreasing to residual concentrations until the end of the second aeration period (Figure 5.2 A2). During the nitritation process, while the first N_2O peak could be partly related to production during settling, the second one was probably influenced by a shift in the metabolism of the AOB population. Yu et al. (2010) suggested metabolic

changes suffered in nitrifying bacteria from a low specific activity towards the maximum specific activity as the reason behind N_2O production. According to this, a fast increase (pulse feed) of NH_4^+ concentration in SBR1 under nitritation conditions could be related with the second N_2O peak. NO emissions during operation in SBR1 also presented an initial peak. The relative importance of this peak was however low, as NO emissions decreased sharply after 1-2 min reaching concentrations of around 2 ppm v during the rest of the cycle.

Nitratation was steadily performed in SBR2 by a NOB enrichment at the time of the study. Nearly 100% of the influent NO_2^- was converted to NO_3^- during normal operation in SBR2 (pH of 7.5 and DO oscillating around 2 mg/L), as shown in figure 5.2 B1. During NO_2^- oxidation to NO_3^- , a small amount of N_2O was released as feed entered the reactor (Figure 5.2 B2). In general, nitrogen oxides emissions from SBR2 were of limited significance. Although there is a report suggesting that N_2O may be a terminal product of NO_3^- respiration in *Nitrobacter vulgaris* under anaerobic conditions, quantitative data were not presented (Freitag et al., 1987). Interestingly, the genomes of some *Nitrobacter* species lack the gene responsible for NO reduction to N_2O while some others possess the genomic potential to reduce NO to N_2O (Ward et al., 2011). Despite this possibility, it is widely accepted that NOB does not contribute to N_2O production in wastewater treatment systems (Law et al., 2012a).

Once the AOB and NOB populations were mixed (SBR1 MIX), there was a transition period of 4 d. NO_2^- accumulated as a result of NH_4^+ oxidation, and this NO_2^- was partially converted to NO_3^- (Figure 5.2 C1). In terms of gas emissions, the profiles described by both N_2O and NO were similar to those found during the nitritation period in SBR1. N_2O presented peaks in each feeding pulse, and NO was released at low concentrations (around 2 ppm v) along each aerated phase (Figure 5.2 C2). After 4 d of transition operation, SBR1 MIX showed an increasing accumulation of NO_2^- . The feed loading was lowered to 0.5 L/cycle and the aeration extended to allow more time for NOBs to nitrify the nitrite formed by AOBs. Following this approach, full nitrification conditions were rapidly reached, and over 95% of the NH_4^+ was converted to NO_3^- , as shown in figure 5.2 D1. N_2O and NO emission profiles registered during full nitrification conditions were different from those presented during nitritation, with the initial peak being significantly smaller for both N_2O and NO (Figure 5.2 D2). Similar results were reported by Ahn et al. (2011), suggesting larger N_2O production from a system performing partial nitrification.

5.3.3 Comparing N_2O and NO emissions from SBR1 under nitritation, transition and full nitrification conditions

The different N_2O and NO emission factors calculated during nitritation, transition and full nitrification are shown in table 5.1.

Table 5.1 Specific converted-*N and N_2O -NO emission factors calculated for SBR1 and SBR1 MIX in each period.

Process conditions	mg converted-N/ g VSS	N_2O -N/converted-N (%)	NO-N/converted-N (%)
Nitritation (SBR1)	255.98±10.95	1.22±0.22	0.066±0.004
Transition (SBR1 MIX)	285.71±25.88	1.15±0.13	0.058±0.001
Full Nitrification (SBR1 MIX)	60.95±3.20	0.54±0.07	0.021±0.002

Converted-*N refers to NH_4^+ -N

During the nitritation and transition periods, N_2O and NO emission factors were highly similar, whereas during full nitrification a reduction of over 50% was found. The N_2O emission factors under

nitritation and transition conditions were $1.22 \pm 0.22\%$ and $1.15 \pm 0.13\%$ of the NH_4^+ converted respectively. These emission factors are comparable to others found in partial nitrification systems such as 1% reported by Law et al. (2011), 1.6% reported by Kong et al. (2013) or 3.4% found in a full-scale bioreactor treating reject wastewater (Kampschreur et al. 2008a). Emission factors found by other authors in full nitrification systems were somewhat higher than the one calculated in this study ($0.54 \pm 0.07\%$). For example, Wunderlin et al. (2012) reported factors between 1.3 and 3.8% of the calculated nitrogen conversion rate and Kampschreur et al. (2008b) found 2.8% of the converted-N being emitted as N_2O . Ahn et al. (2011) registered an N_2O emission of 0.13% of the influent NH_4^+ loading (500 mg N/L), with 98% conversion to nitrate during steady state full nitrification. Pure AOB culture studies showed emission factors ranging from 0.05 to 3.3% (Yoshida, 1988; Colliver and Stephenson, 2000).

NO emission factors were $0.066 \pm 0.004\%$ and $0.058 \pm 0.001\%$ for nitritation and transition conditions respectively, in this study. Ahn et al (2011) reported a NO emission factor of 0.18% of the influent NH_4^+ loading (500 mg N/L) for a partial nitrification system during a transition period from full nitrification, which went down to 0.07% when the system stabilized. Studies on pure AOB cultures showed higher results (0.87%) (Kester et al., 1997). In the present study, when full nitrification conditions were imposed, the NO emission factor decreased to $0.021 \pm 0.002\%$, which is highly similar to 0.03% found by Kampschreur et al. (2008b) for a SBR performing full nitrification (mixed nitrifying population) or to the value reported in Ahn et al. (2011).

In order to perform a comparison of the emissions registered during nitritation (SBR1), transition and full nitrification (SBR1 MIX), total N_2O and NO emissions were recorded during the first 120 min of each cycle. Only one feeding pulse was taken into account for calculations of the total gas emissions (1L during nitritation and 0.5L during full nitrification). Figure 5.3 shows the average N_2O and NO emissions from SBR1 under the different process configurations.

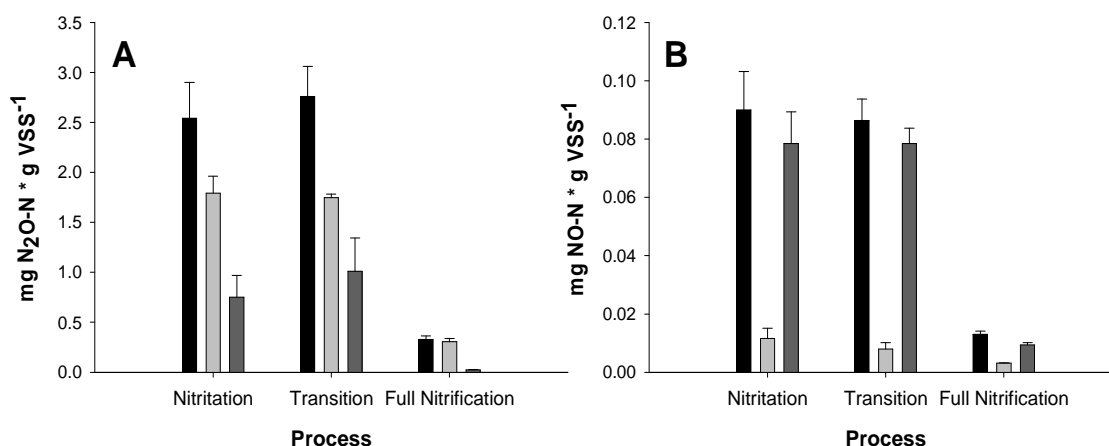


Figure 5.3 Total N_2O (A) and NO (B) emissions during the first 120 min (black bars), the first 20 min (light grey bars) of each cycle and emissions during the rest of the cycle (dark grey bars) during nitritation (SBR1), transition and full nitrification (SBR1 MIX). Error bars correspond to the standard error of the means of triplicate tests.

Similarly to the pattern shown by the calculated emission factors, there was a clear decrease in total emissions of both gases during full nitrification when compared with nitritation or transition conditions. These two processes presented emissions between 2.5 and 3 mg N_2O -N/g VSS, and 0.08 and 0.1 mg NO -N/g VSS. On the other hand, emissions during full nitrification were as low as 0.4 mg N_2O -N/g VSS and 0.015 mg NO -N/g VSS.

5.3.4 Factors and mechanisms involved in N₂O and NO emissions

Factors such as pH, DO, NO₂⁻ or NH₄⁺ concentrations could influence the different gas emission patterns described above for each one of the systems. Both pH and DO were automatically controlled during this study, and presented highly similar dynamics during the nitritation, transition and full nitrification periods. On the other hand, the content of NO₂⁻ and NH₄⁺ showed significant variations due to the different process conditions. Each one of the configurations compared presented different effluent composition, in terms of NH₄⁺, NO₂⁻ and NO₃⁻ concentrations, as shown in table 5.2.

Table 5.2 Influent and effluent characteristics during SBR1 and SBR1 MIX operation*.

Compound	Influent Wastewater	Effluent characteristics (mg N/L)		
		Nitritation period (SBR1, only AOB present)	Transition period (SBR1 MIX, AOB/NOB mixture)	Full Nitrification (SBR1 MIX, AOB/NOB mixture)
NH ₄ ⁺	850.57±11.47	44.20±7.26	51.35	3.34±0.42
NO ₂ ⁻	0	768.68±35.78	455.54	0
NO ₃ ⁻	0	0.31±0.14	297.47	811.51±61.71

*Except for the transition period, values presented are the average of 4 different cycles monitoring.

During transition from nitritation to full nitrification, NO₂⁻ concentrations in the mixed liquor decreased but emissions remained the same as under nitritation conditions. The difference of NO₂⁻ concentrations between the nitritation (768.68 ±35.78 mg/L) and the transition (455.54 mg/L) periods did not minimize nitrogen oxides emissions. However, when all NO₂⁻ was consumed during full nitrification, emissions of both N₂O and NO decreased substantially as previously shown (Figure 5.3). NO₂⁻ has been suggested as one of the main factors affecting nitrogen oxides emissions (Colliver and Stephenson, 2000; Kampschreur et al., 2008a; Kim et al., 2010). In this study, the high NO₂⁻ concentrations found in SBR1 during the nitritation and transition periods seem to be having an effect on the N₂O and NO emission. The consumption of NO₂⁻ during the full nitrification period by NOB (*Nitrobacter sp.*) could have helped to minimise the N₂O and NO emission. These results indicate that NOB might play an indirect but important role on minimizing these emissions from nitrifying systems by avoiding NO₂⁻ accumulation.

Along with the NO₂⁻ concentration, the content of NH₄⁺ was also considered. Under nitritation and transition conditions, effluent NH₄⁺ levels remained highly similar at around 50 mg N/L. As mentioned above, during these two periods nitrogen oxides emissions were similar even when the NO₂⁻ concentration decreased. The system performing full nitrification was the only one in which an effluent with very low NH₄⁺ was obtained, due to the extension of the aeration period. This factor needs to be accounted when considering the minimization of the overall emissions during the full nitrification period. Recovery from anoxic to aerobic conditions, together with NH₄⁺ being accumulated from previous cycles, has been shown to promote N₂O formation (Yu et al., 2010). Moreover, a recent study suggested the complete consumption of NH₄⁺ as a potential minimization strategy for N₂O and NO emissions from a partial nitrification system (Rodriguez-Caballero et al., 2013). However, the effect of variations on NH₄⁺ concentration on the N₂O emissions has not been reported to date. Thus, not only the presence of high NO₂⁻ concentrations but also remnant NH₄⁺ in the liquid phase of the bioreactor might be playing a crucial role on nitrogen oxides emissions.

Initial N₂O peak emissions (first 20 min) accounted for 60-70% of the total emissions during the

nitritation and transition periods, and for more than 95% of the total emissions for the full nitrification system (Figure 5.3A). This pattern was different from the NO emissions, as these were given during the entire aeration period and initial peaks were not large (Figure 5.3B). This fact indicated that most of the N₂O emitted from SBR1 and SBR1 MIX under the different conditions was produced during the settling phase of each cycle. In a recent study, Rodriguez-Caballero (2013) found that the majority of the N₂O emissions detected in a partial nitrification SBR were produced during the settling phase and were affected by the presence of NH₄⁺ (or most likely NH₂OH) and NO₂⁻. They suggested that a reduction of NO₂⁻ and/or NO, with NH₄⁺ acting as electron donor could be occurring during settling through a process called nitrifier denitrification (Kampschreur et al., 2008a; Kampschreur et al., 2008b; Kim et al., 2010). Also, the presence of NH₂OH, the intermediate product of NH₄⁺ oxidation, led to N₂O production during settling in their SBR, suggesting the involvement of the NH₂OH oxidation pathway on N₂O production.

From the results given above, the utilization of SBR technology might need further evaluation due to the N₂O production occurring during settling.

5.3.5 Effect of NH₄⁺ loading on N₂O and NO emissions under full nitrification

To achieve full nitrification, wastewater load in SBR1 MIX was reduced to 0.5L per cycle. In order to explore if this could have an effect on nitrogen oxides emission, wastewater load was increased during some cycles in SBR MIX under full nitrification conditions. The results obtained are shown in figure 5.4.

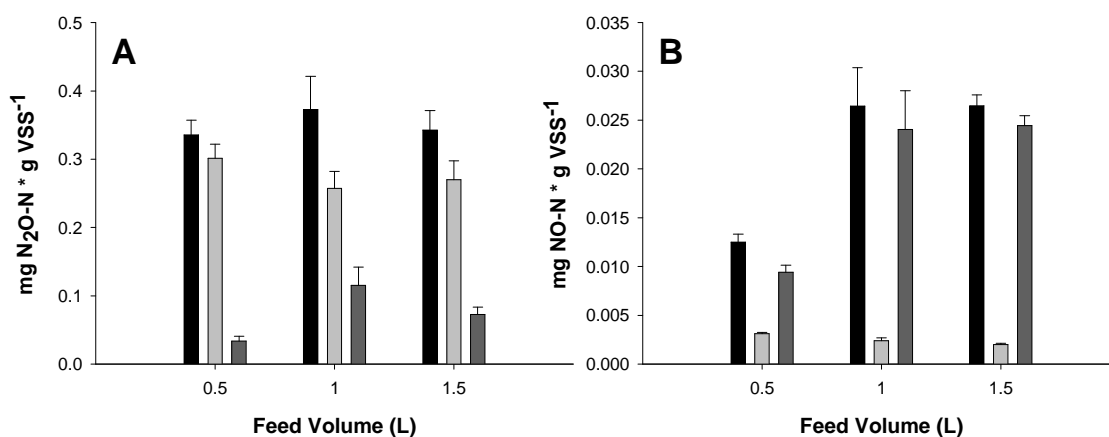


Figure 5.4 N₂O (A) and NO (B) emissions during the first 120 min of each cycle (black bars), first 20 min (light grey bars) and the rest of the cycle (dark grey bars) when different ammonium loadings were applied to SBR1 MIX (full nitrification period). Error bars correspond to the standard error of the means of triplicate tests.

When the feed volume was increased from 0.5 to 1 and 1.5L, N₂O emissions remained on similar levels, between 0.3 and 0.4 mg N₂O-N/g VSS (Figure 5.4A). There was however, a slight increase in NO emissions as feed loading increased from 0.5 to 1 (Figure 5.4B). Most of these emissions were measured along an aerated period, with initial peaks being of a lesser importance. The reasons behind this increase in NO emissions between the lowest feed loading test and the two other tests remains uncertain. Little attention has been paid to NO emissions from nitrification processes. The production of NO (and NO₂) by AOB under anoxic conditions was postulated as a mechanism for recovery of ammonia oxidation upon aeration (Schmidt et al. 2001a). In this study, anoxic periods were not imposed, therefore this source of NO was assumed not to be involved. Furthermore, NO can

be aerobically produced by AOB as a product of denitrification. In a different study by Schmidt et al. (2001b), a model of ammonia oxidation in *Nitrosomonas eutropha* was developed based on a hypothetical NO_x cycle in which not only NH_2OH but also NO would be produced under aerobic conditions. In the presence of oxygen, NO could then be oxidized to N_2O_4 , which is an oxidizing agent of NH_4^+ . Therefore, upon higher NH_4^+ loading, increased NO production could be expected, although further research on this issue is needed.

5.3.6 Applicability of nitritation in real-scale systems from a sustainable perspective

When compared to full nitrification, the implementation of nitritation for the treatment of reject wastewater offers a number of benefits due to lower operational costs. As described in this study however, the presence of high NH_4^+ and/or NO_2^- concentrations cannot be avoided in nitritation systems, especially those dealing with nitrogen rich wastewater, with the consequent increase in the production of the green-house gas N_2O , which presents a 265-fold stronger effect than CO_2 . Thus, attention needs to be paid when evaluating the applicability of this technology from an environmental perspective. Yang et al. (2009) suggested two possible solutions to reduce N_2O emissions from nitritation systems treating domestic wastewater: i) to reduce substrate concentrations (NO_2^- and/or NH_4^+) and ii) to enhance anoxic denitrification via carbon source addition in order to decrease NO_2^- levels. In some cases, high substrate concentrations cannot be avoided (i.e. reject wastewater) and the addition of an external carbon source would further increase operational costs. Classical nitritation-denitrification systems for reject wastewater treatment could be substituted by alternative configurations such as the nitritation-anammox process. Partial nitrification has been shown to be able to produce suitable effluents for anammox treatment (Zhang et al. 2011). Nevertheless, Kampschreur et al. (2008a), reported high N_2O emissions from a two-reactor nitritation-anammox system. A promising balance between low operational costs and greenhouse gas emissions may be found in systems implementing nitritation and anammox in one reactor (Joss et al., 2009), or simultaneous partial nitrification, anammox and denitrification (Lan et al., 2011).

Chapter 6

Evaluation of process conditions triggering emissions of green-house gases from a biological wastewater treatment system

The contents of this chapter have been published as:

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6.1 Preliminary remarks

This study presents the CH_4 and N_2O gas emission dynamics at different stages of an activated sludge plug-flow reactor of a municipal WWTP conducting biological nutrient removal. The main goal was to identify and evaluate the key operational conditions implemented in the plant that originated the majority of the uncontrolled CH_4 and N_2O emissions during the monitoring period.

6.2 Materials and Methods

6.2.1 Wastewater treatment process description

The monitoring site chosen in this study was the WWTP of the city of Granollers, near Barcelona (Spain). This plant treats the domestic wastewater of 112,000 population equivalents (P.E). After primary treatment and settling, the wastewater is treated biologically in two parallel and identical plug-flow reactors where chemical oxygen demand (COD) and nitrogen removal is performed. Then, the wastewater flows into a secondary settler before being released into the environment. Excess sludge is anaerobically digested in order to produce biogas for electricity generation. A flow of reject wastewater (produced in the anaerobic digester sludge dewatering process) is regularly released at the inlet of the plant for its treatment. The configuration layout of Granollers WWTP is shown in Figure 6.1.

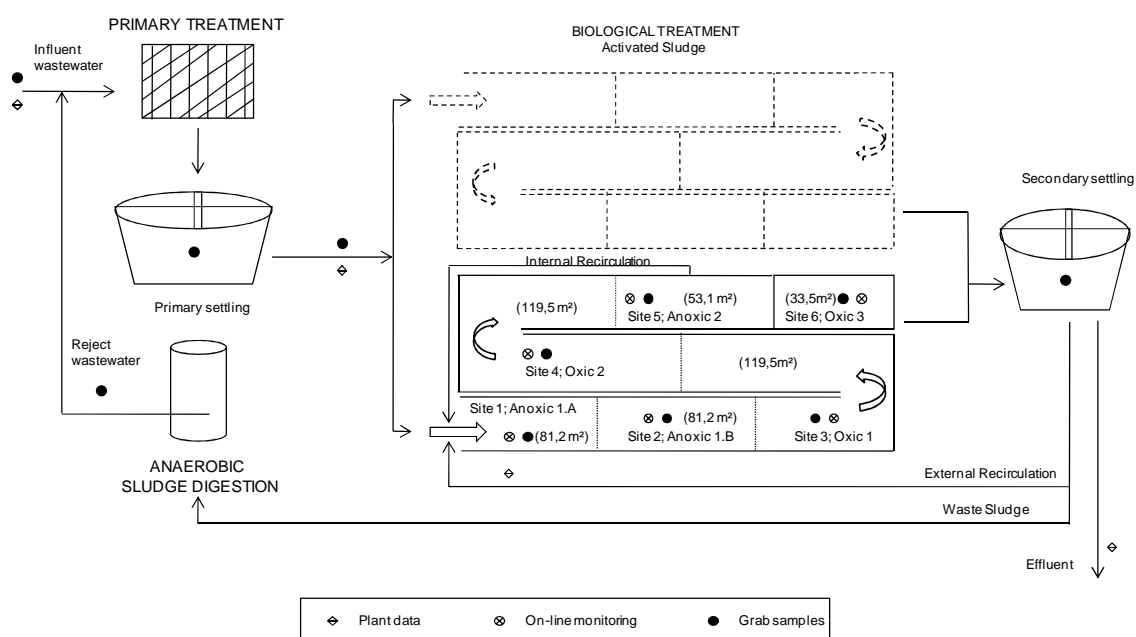


Figure 6.1 Scheme of Granollers WWTP, including points at which monitoring of gas emissions was performed, and locations where grab samples were taken and where online sensors connected to the supervisory control and data acquisition system (SCADA) of the plant were placed.

6.2.2 CH₄ and N₂O monitoring and calculations

Gas emissions and emission factors

CH₄ and N₂O emissions were monitored on-line during 48-72 hours per week during ten weeks between June and October, 2013. All the gas emission data recorded during this study was registered in aerated and non-aerated zones of one of the activated sludge lanes at Granollers WWTP. Wastewater is entering the first anoxic zone and it is transported across the reactor passing through an aerated zone, followed by a short anoxic zone (where mixed liquor is recirculated to the first anoxic zone) to finally end in another aerated zone before exiting the treatment lane. For the purposes of this study, the first anoxic zone is divided into two sites (Site 1 and Site 2) with the first one being more turbulent due to the flow of wastewater entering the bioreactor. The main aerated zone is also divided into 2 sites (Site 3 and Site 4) corresponding to the zones where two different air diffusers are present and independently controlled. The two last monitoring sites correspond to the second anoxic zone (from where mixed liquor is internally recirculated, Site 5) and the final aerated part of the bioreactor (Site 6). See Figure 6.1 for locations of the monitoring sites.

Liquid-phase CH₄ and N₂O measurements

Grab samples were taken in order to determine dissolved CH₄ concentrations at different points of the plant, from the influent wastewater to the settled water before it exited the treatment system. In addition, samples of reject wastewater were collected after the dewatering process for CH₄ analysis. On the other hand, liquid-phase N₂O patterns were followed along the WWTP (from the primary settler to the secondary settler) for periods of up to 24 hours with an N₂O microsensors with data logging (In situ amplifier system, Unisense A/S, Aarhus, Denmark).

6.2.3 Chemical analyses and WWTP data acquisition

In each sampling site of the bioreactor (Figure 6.1), DO and temperature were continuously recorded with a portable DO sensor with an integrated thermometer (YSI Inc. USA). The DO sensor was placed 1-2 m from the gas collection hood in each of the reactor sites. Grab samples were regularly taken both manually and with automatic refrigerated samplers from each monitored site for ammonium (NH₄⁺), NO₂⁻ and nitrate (NO₃⁻) analyses. During the monitoring period, the sampling time intervals were 30 to 60 min, for 2-3 days each week. These samples were first filtered through disposable Millipore filter units (0.22 μm pore size), and then processed for analysis according to standard methods (APHA, 1998) in the case of NH₄⁺, or via ion chromatography (ICS5000, DIONEX) for NO₂⁻ and NO₃⁻. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were analysed weekly according to standard methods (APHA, 1998). For dissolved methane analysis, 5 mL of sample was filtered through disposable Millipore filter units (0.22 μm pore size) and immediately injected into a vacuumed glass tube using a hypodermic needle attached to a plastic syringe. The tubes were allowed to reach the gas-liquid equilibrium overnight. The gas phase was measured with a gas chromatograph (ThermoFisher Scientific Inc. USA) equipped with a flame ionization detector (FID). Concentrations of methane in these samples were calculated using mass balance and Henry's law (Guisasola et al., 2008). Dissolved N₂O was continuously measured via an N₂O microsensors (N2O-R, UNISENSE, Denmark) connected to an in-situ amplifier box.

The influent and effluent water characteristics (COD, TKN and NH₄⁺, TSS and VSS) of the plant were provided weekly by plant operators. On-line data of the aeration flow from each one of the air diffusers located in each aerated sampling site as well as the influent wastewater flow were acquired from the SCADA system of the WWTP. Mean values of some of the parameters measured in this study were calculated when needed. The standard error of the means was calculated dividing the standard deviation by the square root of the number of samples in each case.

6.3 Results

6.3.1 Process performance

The WWTP presented over 94 and 91% removal rates for COD and TKN respectively, during the measurement campaign. Some of the process parameters and influent and effluent characteristics of the WWTP are presented in Table 6.1. The operational mode of the bioreactor was the typical of a plug-flow system, creating a DO gradient across the aerated zones as detailed in Table 6.1.

Table 6.1 Operational parameters, influent and effluent characteristics of the WWTP during the experimental campaign.

Process parameter	Average value \pm standard deviation
<i>Influent wastewater</i>	
Flow (m ³ /d) ^a	21811.5 \pm 399.5
Reject wastewater flow (m ³ /d) ^a	301.5 \pm 32.04
COD (mg/L) ^a	728.4 \pm 160.1
TKN (mg N/L) ^a	61.6 \pm 7.8
NH ₄ ⁺ (mg N/L) ^a	47.9 \pm 2.3
TSS(mg/L) ^a	167.3 \pm 0.001
VSS(mg/L) ^a	24.6 \pm 0.004
pH ^a	7.7 \pm 0.01
<i>Bioreactor</i>	
MLSS(mg/L) ^a	2639.8 \pm 328.5
HRT (d) ^a	~0.6
SRT (d) ^a	~10
Temperature(°C) ^b	25.0 \pm 1.3
Aeration flow in site 3, Oxid 1 (m ³ /h) ^a	713.1 \pm 91.1
Aeration flow in site 4, Oxid 2 (m ³ /h) ^a	1077.5 \pm 56.4
Aeration flow in site 6, Oxid 3 (m ³ /h) ^a	102.6 \pm 21.3
DO in site 3, Oxid 1 (mg/L) ^b	2.1 \pm 0.02
DO in site 4, Oxid 2 (mg/L) ^b	1.4 \pm 0.5
DO in site 6, Oxid 3 (mg/L) ^b	0.7 \pm 0.1
<i>Effluent water</i>	
COD (mg/L) ^a	40.8 \pm 6.3
TKN (mg N/L) ^a	5.08 \pm 3.8

^a Data provided by plant operators, based on average daily values calculated from online data (influent flow, reject wastewater flow and aeration flow) or daily samples analysis integrated on a weekly basis (TSS, VSS, MLSS, COD and TKN).

^b Data from sampling campaigns based on online sensors (DO, temperature).

6.3.2 CH₄ and N₂O emissions

Average CH₄ and N₂O gas emissions measured during the monitoring period are shown in Table 6.2. The anoxic zones of the bioreactor presented very low emissions of both gases. The largest emission of CH₄ was registered in the first aerated area (site 3) of the bioreactor (1.15 Kg CH₄/d) sharply decreasing in the following sites to levels well below 0.02 Kg CH₄/d. N₂O emissions from each of the aerated zones presented differences also, ranging from 0.45 (site 3) to 0.14 Kg N/d (site 6), as shown in Table 6.2. Additionally, the emissions of N₂O in sites 3 and 6 presented large variations, even between different days during the same week, as shown by the large standard deviation resulted from the calculation of mean values from triplicates. This is attributed to N₂O peak events registered for a period of some hours that increased significantly the total amount of N₂O emitted from these sites. These N₂O peak events are further described in section 6.3.3.

Table 6.2 CH₄ and N₂O gas emissions calculated for each sampling site of the bioreactor lane under study. Standard error values calculated when possible (at least three days monitoring).

Location	CH ₄ (Kg/d)	N ₂ O-N (Kg/d)
Site 1, Anox 1.A	1.009e-6	1.952e-7
Site 2, Anox 1.B	1.3e-8 ± 2.2e-9	1.84e-9 ± 5.5e-10
Site 3, Oxic 1	1.15 ± 0.05	0.45 ± 0.42
Site 4, Oxic 2	0.18 ± 0.01	0.18 ± 0.04
Site 5, Anox 2	2.4e-8	2.82e-9
Site 6, Oxic 3	0.02 ± 9.7e-3	0.14 ± 0.09
Total emissions from the bioreactor lane	1.35 ± 0.05 (0.016 % of the inf. COD)	0.78 ± 0.43 (0.116 % of the inf. TKN)

The total gas emissions from the bioreactor lane under study were 1.35 Kg CH₄/d and 0.78 Kg N₂O-N/d which corresponded to 0.016 % and 0.116 % of the influent COD or influent TKN respectively.

6.3.3 Process perturbations influencing gas emission patterns

During the measurement campaign, three different process perturbations occurred at the WWTP and were subjected to in-depth research. These consisted of periods of reject wastewater influxes, highly irregular aeration flow leading to aeration drop in some cases and nitrification instability (Table 6.3).

Table 6.3 Process perturbations under study during the measuring campaign.

Process perturbations	Parameters affected	Description
(1) Influent and Reject wastewater fluxes	Dissolved CH ₄ concentrations	Influent wastewater flow mixed with reject wastewater carrying high dissolved CH ₄ concentrations
(2) Irregular aeration	Aeration flow and DO	Aeration subjected to modifications. DO ranges varied from 0.5-1.5 to 1.5-2.5. Periods of no aeration (~ 2h) also occurred.
(3) Nitrification instability	NH ₄ ⁺ and NO ₂ ⁻ concentrations in bioreactor	Nitrification was partially disrupted.

Examples of CH₄ and N₂O patterns measured under the different process perturbations are presented below (Figures 6.2, 6.3 and 6.4).

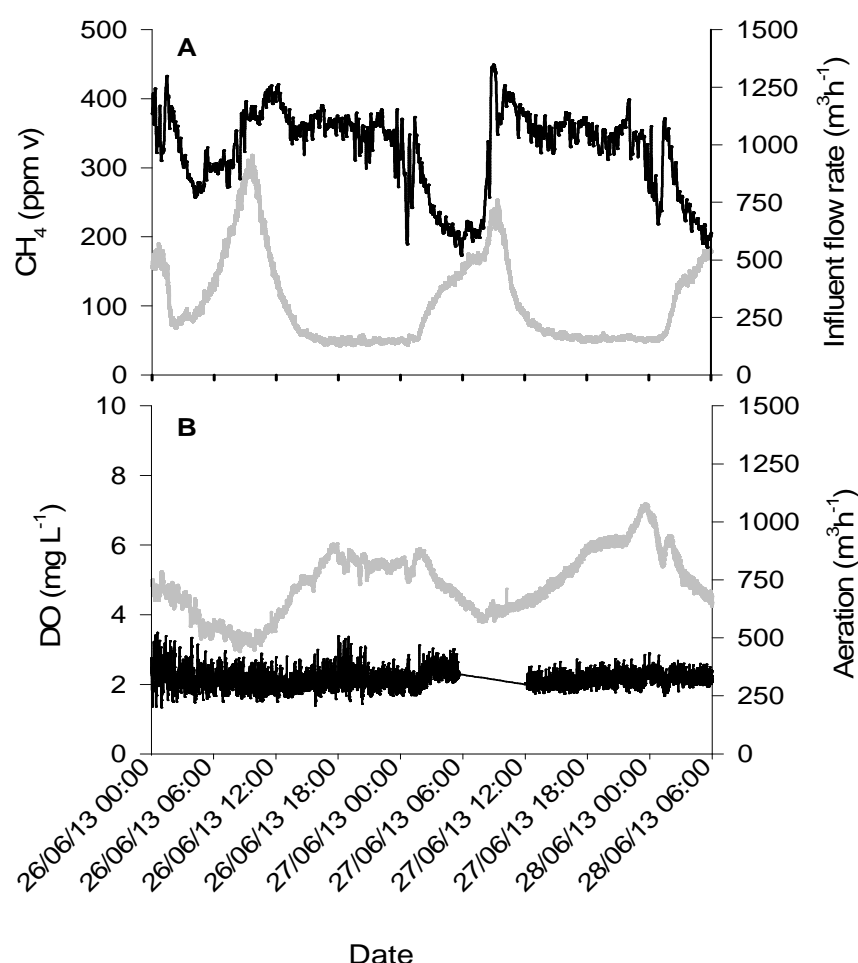


Figure 6.2 Dynamics of (A) CH₄ emissions (grey line) and influent wastewater flow going into the bioreactor (black line), and (B) aeration flow (grey line) and DO (black line) during a 54h-period measured in sampling site 3.

CH₄ emissions and influent wastewater influxes

The influent wastewater flow rate at Granollers followed a common pattern for municipal WWTPs, with an increasing trend from 05:00 to 12:00, steady values from 12:00 to 00:00, and a decreasing trend from 00:00 to 05:00 (Figure 6.2A). On the other hand, CH₄ presented also a regular periodic emission pattern in the aerated zones of the bioreactor (data shown corresponds to site 3 in Figure 6.2A), but the trend increased during night hours until approximately 08:00-10:00. The curve described by the CH₄ emission profile seemed not to be related with the aeration flow or DO patterns, which are shown in Figure 6.2B.

N₂O emissions under irregular aeration

Figure 6.3 shows the N₂O emission pattern together with the concentration of NH₄⁺, NO₂⁻ and NO₃⁻ (Figure 6.3A) during three consecutive days in which the aeration flow and the DO levels were heavily modified (Figure 6.3B). Generally, in terms of aeration and DO, there were two differentiated periods. During the first 36 h, the aeration flow ranged from around 500 to 1000 m³/h and the DO was kept at levels between 0.5 to 1.5 mg/L. Then, the aeration flow increased sharply and reached values up to 1700 m³/h. At the same time the DO also increased and was maintained at ~2 mg/L. During these two periods, N₂O emissions presented a very low and stable profile (nearly 0 ppm v).

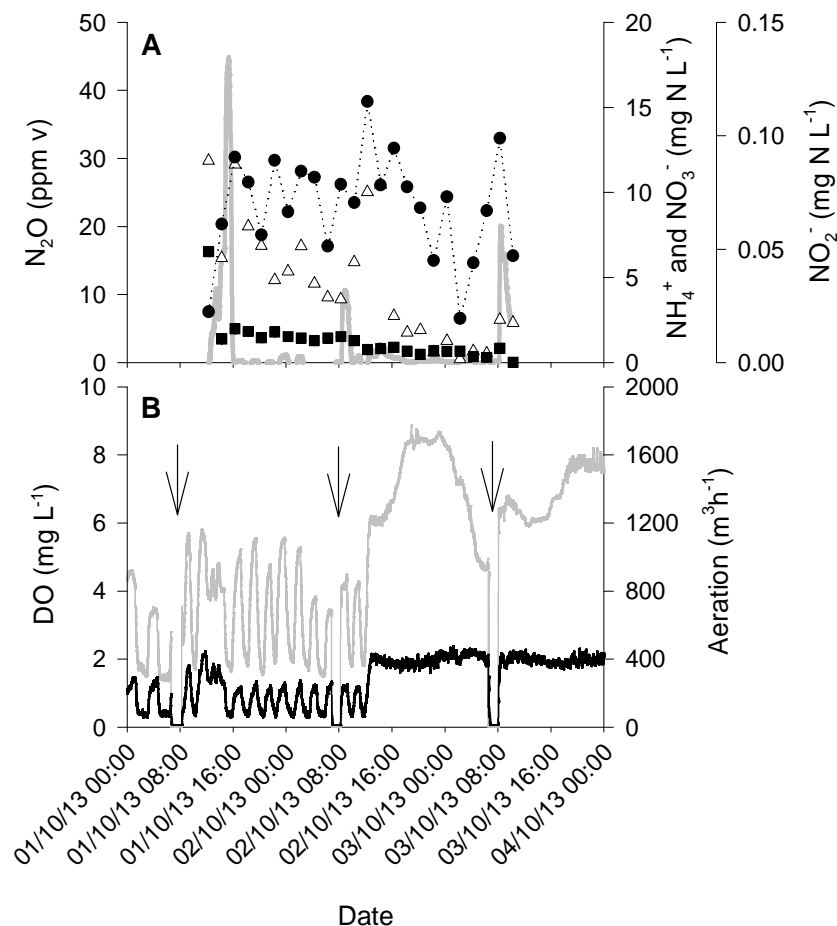


Figure 6.3 Dynamics of (A) N₂O (grey line), NH₄⁺ (Δ), NO₂⁻ (•) and NO₃⁻ (■) and (B) aeration flow (grey line) and DO (black line) during a period of different DO levels and aeration drops (marked with arrows) measured in sampling site 3.

Additionally, there were three isolated N₂O peaks ranging from around 10 to over 40 ppm v (Figure 6.3A). These were registered right after three episodes of ~2h aeration drop occurring along

the experimental profiling (Figure 6.3B). When aeration was re-started after each 2h-period and DO was recovered, N₂O peak emissions occurred. In general terms, both NH₄⁺ and NO₃⁻ concentrations showed a decreasing trend along this three-day period of irregular aeration and DO. However, NH₄⁺ peaks of different level (2.5-12 ppm v) were observed in parallel with the N₂O peaks mentioned before and small NO₂⁻ concentrations were also detected.

N₂O emission during nitrification instability

Figure 6.4 represents a 28 h-period in which the concentration of NH₄⁺ tended to accumulate in sampling site 3 of the bioreactor, showing an increasing trend from around 5-10 mg N/L in the beginning of the period to 15 mg N/L in the end. NO₃⁻ values were in general steady and remained at levels between 0 and 3 mg N/L while NO₂⁻ was also present ranging from 0.1 to 0.6 mg N/L along the period (Figure 6.4A). N₂O emissions described a large peak (around 900 ppm v), which was simultaneous to the moment in which NH₄⁺ started to accumulate and the NO₂⁻ concentration was at its peak. This N₂O peak lasted for around 3h before it decreased to lower levels (~1-2 ppm v for the rest of the period). The curve described by the aeration flow presented a valley (decreasing from 800 to 500 m³/h) during the same time in which N₂O emissions peaked, but the DO level was regular during the whole period, always around 2 mg/L as shown in Figure 6.4B.

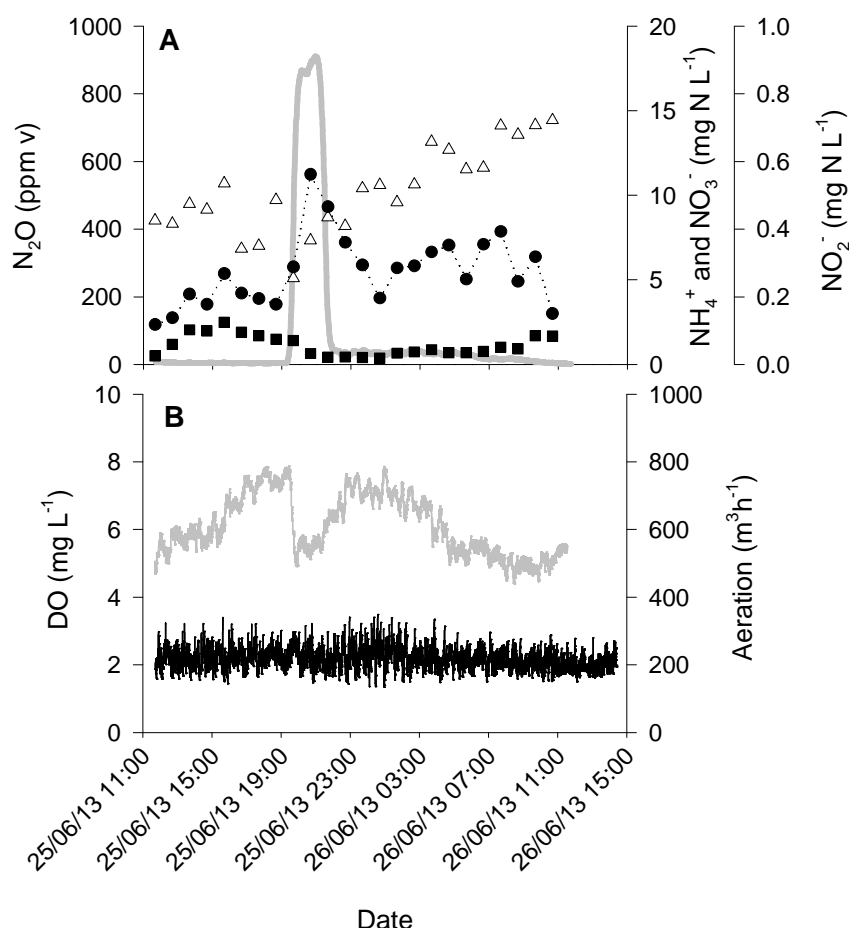


Figure 6.4 Dynamics of (A) N₂O (grey line), NH₄⁺ (Δ), NO₂⁻ (•) and NO₃⁻ (■) and (B) aeration flow (grey line) and DO (black line) during a period of nitrification instability measured in sampling site 3.

Dissolved CH₄ and N₂O concentrations along the WWTP

Grab samples for dissolved CH₄ analysis were taken to determine its presence not only in the bioreactor but also at different locations of the plant. Results are shown in Figure 6.5. Over 0.05 mg

CH_4/L was measured at the inlet of the plant, in samples of influent wastewater before it entered the system. Reject wastewater flowing from the sludge digester dewatering process was also measured and contained around $0.7 \text{ mg CH}_4/\text{L}$, and dissolved CH_4 in the primary settler reached levels of around $0.12 \text{ mg CH}_4/\text{L}$. In the first anoxic zone of the bioreactor lane under study $0.02 \text{ mg CH}_4/\text{L}$ could still be detected, but the rest of the samples taken along the bioreactor or at the secondary settler contained non-detectable dissolved CH_4 .

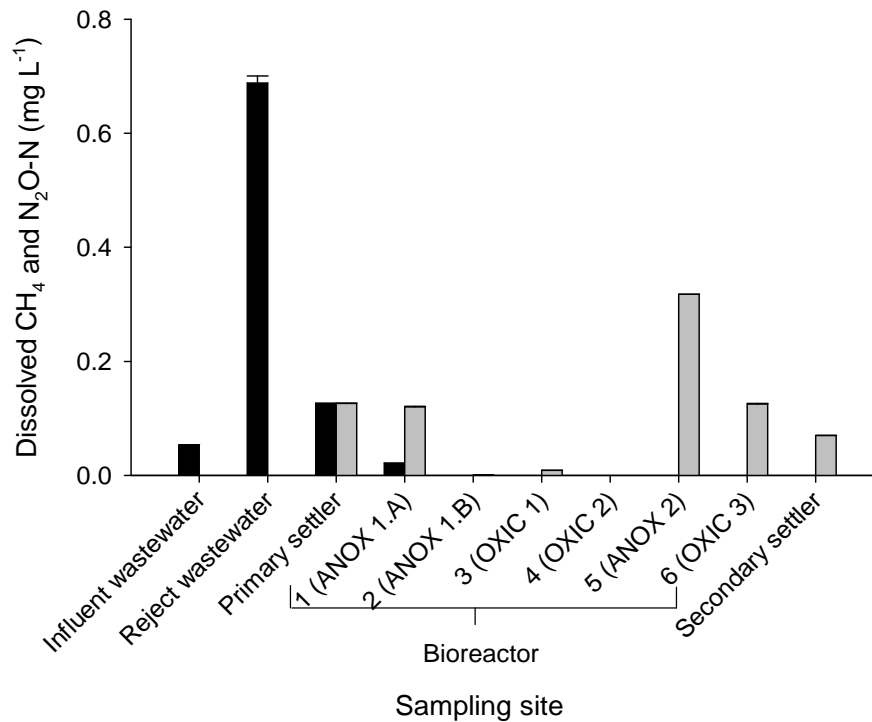


Figure 6.5 Spatial distribution of average dissolved CH_4 (Black bars) and N_2O (grey bars) in different sites along the WWTP of Granollers. Error bars correspond to the standard error of the mean values from triplicate samples and 6-12h monitoring for CH_4 and N_2O respectively.

In terms of dissolved N_2O , both primary and secondary settlers, as well as the first anoxic zone (site 1) of the bioreactor, presented similar average values ($\sim 0.1 \text{ mg N}_2\text{O-N/L}$). The largest concentration of N_2O was found in the second anoxic zone (site 5) with over $0.3 \text{ mg N}_2\text{O-N/L}$. Aerated parts of the bioreactor presented nearly undetectable levels of dissolved N_2O , with the exception of the third aerated area (site 6) in which these levels exceeded $0.15 \text{ mg N}_2\text{O-N/L}$ (Figure 6.5).

6.4 Discussion

6.4.1 Process performance: Regular operation and process perturbations

During the majority of the experimental campaign carried out at Granollers WWTP, the treatment system performed correctly with high COD and N removal rates, as described before. Process parameters (Table 6.1) given under this study could be considered normal for a municipal WWTP of these characteristics and configuration. The variations in aeration flow and the reject wastewater influxes were part of the regular operation of the plant. The period of nitrification instability occurred

accidentally. These events altered or disrupted the functioning of the bioreactor and were recorded in order to study their impact on CH₄ and N₂O emissions.

6.4.2 CH₄ emissions

During the experimental campaign of this study, a complete screening of dissolved CH₄ concentrations and emission dynamics was performed at the WWTP of Granollers. This allowed a better understanding of the spatial allocation and transportation of CH₄ along the WWTP, with a special focus on the activated sludge bioreactor. A fraction of the emitted CH₄ was arriving dissolved in the wastewater and most likely originated during the transport of this wastewater through the anaerobic sewer pipes as has been previously reported (Guisasola et al., 2008). Another fraction was coming from the reject wastewater stream being produced in situ during the anaerobic digestion of the waste sludge from the WWTP. During the monitoring period 1.35 Kg CH₄/d were emitted from the bioreactor which is much lower than the CH₄ emissions reported by Daelman et al. (2012) from a study on a WWTP in the Netherlands. In their WWTP, the CH₄ emissions from the plug-flow and carousel reactors were 48 Kg CH₄/d and 264 Kg CH₄/d respectively. This was due to the higher dissolved CH₄ flowing at the headworks level (influent and reject wastewater streams) in Daelman's study (192 Kg CH₄/d).

In this study, calculations taking into account the hydraulic residence time (HRT) of the different sites of the bioreactor and measurements of the dissolved CH₄ in the influent wastewater stream indicated that the daily CH₄ peak emissions detected at the bioreactor were related with the normal influent wastewater flow dynamics, although some deviations could be observed. In some of the cases recorded such as the one shown in Figure 6.2, CH₄ emissions continued increasing during night-time, even though the flow of influent wastewater decreased. This is probably due to the fact that reject water was released into the influent stream during night-time also, increasing the concentration of CH₄ when the volume of wastewater was smaller and modifying the CH₄ emissions pattern from the reactor. In terms of concentration, the CH₄ content of the reject wastewater was over 90% larger than the concentration measured in influent wastewater. However, the flow of reject wastewater was around 99% smaller than the average influent wastewater flow of the plant diminishing its contribution on the overall CH₄ emissions detected.

Mitigation strategies could be implemented from the design to the operational level of a wastewater treatment system in order to favour the conversion of CH₄ before it is stripped due to aeration. As reported, a large portion of the CH₄ that is emitted from WWTPs enters the system dissolved in the influent wastewater streams and is previously generated in the sewer pipes by methanogenic organisms. Thus, any measures taken in order to minimize or control the activity of methanogens may contribute to the overall minimization of CH₄ emissions. Research on this topic has successfully been carried out in recent years, mostly focusing on the addition of chemicals that inhibit methanogenic activity in the sewer networks (Mohanakrishnan et al., 2008; Gutierrez et al., 2009; Sudarjanto et al., 2014).

6.4.3 N₂O emissions

The total N₂O emissions presented in this study for the bioreactor lane under investigation (0.116% of the influent TKN) are similar to values reported in full-scale campaigns found in literature, where continuous, on-line gaseous emissions monitoring was also performed. For example, Ahn et al. (2010a) presented emission factors ranging from 0.18 to 0.62% of the influent TKN, depending on the type of reactor configuration. Similarly, Lotito et al. (2012) reported N₂O emissions ranging from 0.12 to 0.356% of the influent TN depending on reactor configurations in a pilot scale study. More recently however, Aboobakar et al. (2013) reported lower emissions in a bioreactor similar to the

one described in this paper (0.036% of the influent TN being emitted as N₂O). For a complete review of the different N₂O emission factors reported in literature see Law et al. (2012a).

The high variability of N₂O emissions contributes to the hypothesis of N₂O production being strongly bounded to process configurations, performance or events. Indeed, during the monitoring campaign described in this report, emissions were not constant and presented peaks of more or less magnitude even within the same day, increasing substantially the overall account of emissions. For example, the average N₂O emissions from site 3 (aerated zone 1) would decrease from approximately 0.45±0.42 Kg N₂O-N/d (~58% of the total N₂O emissions), to 0.11±0.08 Kg N₂O-N/d (~25% of the total N₂O emissions), if the N₂O peak event described in Figure 6.4 is not taken into consideration. Similarly, the N₂O emission factor of the bioreactor would decrease from 0.116 to 0.064% of the influent TKN. From this perspective, even isolated peak emissions at any of the aerated zones in a bioreactor can have a significant impact on the global emissions of a WWTP which highlights the importance of online monitoring. The design of mitigation strategies should aim at avoiding the main process scenarios that lead to peak N₂O emissions.

The N₂O peak emissions registered during the monitoring campaign at Granollers WWTP were found to be correlated with different process perturbations occurring along the experimental period, implying different N₂O production pathways and sources, as discussed below.

Effect of DO dynamics and transient anoxic conditions on N₂O peaks

The aeration flow of the bioreactor at the WWTP of Granollers suffered continuous variations during the measurements. The three aerated zones of the bioreactor presented highly different aeration flows (Table 6.1), ranging from around 100 m³/h in site 6, to over 1000 m³/h in site 3. Furthermore, within the same aerated areas, the aeration flow suffered heavy modifications as shown in the example of Figure 6.3. Unexpectedly, these large variations in the aeration flow did not seem to affect the N₂O emission dynamics, even when the DO level suffered changes of over 1.5 mg/L of difference (Figure 6.3). This was probably due to the fact that, in general, high levels of nitrification efficiency were maintained along the campaign (Butler et al. 2009; Ahn et al. 2010a). Differently, an increase in N₂O emissions from a nitrifying culture was reported in another study, under dynamic DO conditions in a lab-scale bioreactor (Kampschreur et al., 2008). It could be argued whether the biomass of the bioreactor at Granollers WWTP was adapted to the continuous changes in aeration and DO. Adaptation of nitrifying biomass to suboptimal levels of different process parameters has been reported in lab-scale studies. For example, the AOB community of a lab-scale reactor was shown to maintain high nitrification efficiency at DO levels as low as 0.5 mg/L (Belucci et al. 2011). In their study, Belucci et al. (2011) assumed the N₂O emissions to be low because of the absence of other known factors triggering N₂O release (i.e. ammonia or nitrite accumulation). The activated sludge bacterial community at Granollers could have built a certain degree of tolerance to different DO levels, although this hypothesis would need proper testing on a long-term basis.

Periods with no aeration were also implemented in the nitrifying/oxic areas, with the aim of reducing operational costs at Granollers WWTP. During these periods, the DO level dropped to 0 mg/L. Upon recovery of the aeration, DO increased and N₂O peaks could be measured (Figure 6.3), suggesting that transition from anoxic to aerobic conditions was triggering N₂O production in full-scale systems, as reported earlier by Yu *et al.* (2010) for a pure culture of *Nitrosomonas europaea*. Oxygen limitation has been traditionally suggested as precursor of the denitrification pathway in nitrifiers, leading to N₂O production (Bock *et al.* 1995). The incomplete oxidation of ammonia and the subsequent accumulation of hydroxylamine have also been postulated as a mechanism of N₂O production in ammonia oxidizers (Ritchie and Nicholas, 1972), although the importance of this pathway in full-scale systems remains unknown. Another possible explanation could be the production of this N₂O from heterotrophic denitrification that would occur under anoxic conditions

and would remain dissolved until aeration restarts, causing an N_2O peak. In general, it could be asserted that intermittent aeration with periods of no aeration favours the production of N_2O , even if the nitrogen removal efficiency remains stable, increasing substantially the total N_2O emissions of the system. Thus, it is advisable to avoid transient anoxic periods in nitrifying areas, due to potential N_2O peak events that could contribute to raise the green-house gas emission account of the WWTP. This is in agreement with the simulation study performed by Flores-Alsina et al. (2014) which highlighted the potential of increasing N_2O emissions when carrying out local energy optimization in WWTPs.

Effect of nitrification instability on N_2O peak events

When shock loads of toxic compounds are encountered, ammonia oxidizing bacteria can activate their denitrification pathway as a response to imbalances in their metabolism resulting in N_2O production (Butler et al., 2009). The Granollers WWTP mainly treats domestic wastewater, but is placed in a highly industrialized area. Industrial effluents of varied origin are occasionally released into the inlet of the plant, and may contain toxic compounds which can disrupt nitrification. This could be a valid explanation on the source of the N_2O peak event registered at site 3 of the bioreactor (Figure 6.4) although more data on the influent wastewater composition would have been needed in order to further develop this hypothesis. Whether the N_2O peak event registered at Granollers was originated by a toxic shock or not remains unclear. However, there was indeed a period of nitrification instability, as proven by the maintenance of the same DO level with a lower requirement of aeration (Figure 6.4B), suggesting a decrease of the biological activity of the biomass. This nitrification imbalance probably provoked the NH_4^+ accumulation and the raise in NO_2^- concentration measured in the bioreactor. Both NH_4^+ and NO_2^- (together with DO) are the most important parameters that have been reported to influence N_2O emissions in full-scale facilities (Foley et al, 2011). The presence of NH_4^+ has been proven to be correlated with N_2O production, under increasing DO concentrations (Yu et al. 2010). In a full-scale study in France, NH_4^+ and DO concentrations seemed to be positively correlated with N_2O production, as reported by Foley et al. (2011). Similarly, during the N_2O peak event of this study, not only NH_4^+ concentrations increased but also there was a peak in NO_2^- concentration (from 0.2 to 0.6 mg NO_2^- -N/L) in parallel with the maximum N_2O emission level. In a study by Foley et al. (2009), a threshold value at 0.3-0.5 mg NO_2^- -N/L was found to trigger N_2O generation. Several reports have also suggested a correlation between NO_2^- accumulation and N_2O production in full-scale systems (Kampschreur et al., 2009; Desloover et al., 2011) in agreement with the results of this study.

Chapter 7

Minimizing N₂O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor

The contents of this chapter have been published as:

Rodriguez-Caballero, A., Aymerich, I., Marques, R., Poch, M., Pijuan, M. 2015. Minimizing N₂O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor. *Water Research* 71, 1-10.

7.1 Preliminary remarks

The intermittent aeration and dynamic conditions typically found in SBRs may translate into increasing N_2O being emitted from these systems. As mentioned in chapter 1, transient conditions in terms of DO (anoxic/oxic transition) have been correlated with increasing N_2O production in nitrifying cultures. Similarly, transient conditions (settling, oxic and anoxic phases combined) in nitrifying laboratory SBRs have been reported to release relatively large amounts of N_2O (Chapter 4). Moreover, in a monitoring campaign covering different treatment configurations implemented in 12 full-scale WWTPs, Ahn et al. (2010a) reported lower N_2O emission factors from systems that avoided transient concentrations of NH_4^+ , NO_2^- or DO. In this study, the different operational cycles being applied in a municipal SBR and their effects on the reactor's N_2O production were investigated. The electricity consumption of the plant was also recorded under the different SBR process scenarios. This approach allowed estimating the carbon footprint of the system, taking into consideration not only the N_2O emissions but also the indirect carbon dioxide (CO_2) being emitted as a consequence of the plant's electricity consumption. The ultimate goal was to identify potential operational strategies that could lead to a simultaneous minimization of the N_2O emissions and the overall C-footprint.

7.2 Materials and methods

7.2.1 Description of the monitoring site

The monitoring site under investigation was the municipal WWTP of La Roca del Vallès (Barcelona, Spain) which treats the domestic wastewater of 48.000 population equivalents (P.E). A schematic representation of La Roca del Vallès WWTP is shown in Figure 7.1.

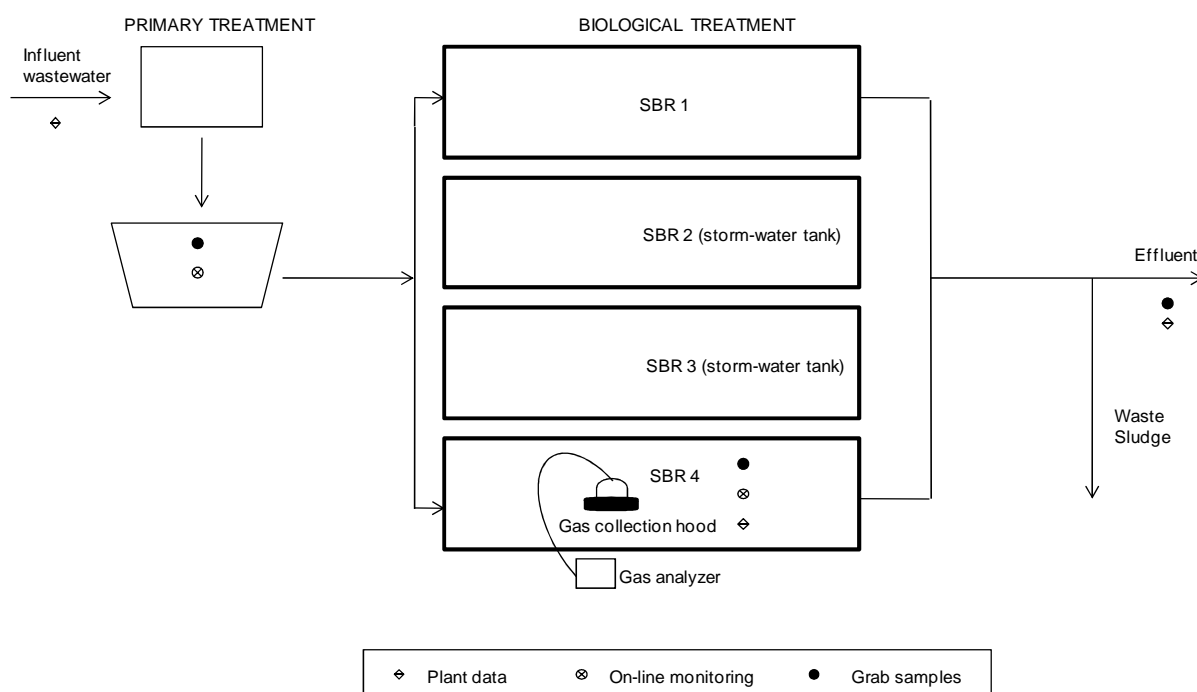


Figure 7.1 Scheme of the WWTP of La Roca del Vallès, including monitoring and sampling points.

The facility consists of a primary treatment section followed by a set of four identical SBRs with an operational volume of 4684.2 m^3 each, for the biological COD and N removal. After decanting, the

treated water flows to the outlet of the plant where it is released to the receiving river. Waste sludge is extracted for external treatment on a variable temporal basis.

7.2.2 Reactors operation and cycle configurations

Only two of the SBRs (SBR-1 and SBR-4) were functioning during the monitoring period while the two others served as storm-water storage tanks (Figure 7.1). The two reactors were equally operated in cycles of approximately 260 min, following the NH_4^+ set-point-regulated automatic control system or the process operator commands. Each cycle can be divided in a reaction phase (~130 min), a settling phase (~65 min) and a decanting phase (~65 min). The length of each phase could present variations of 10-20 min per cycle depending on the operational mode and/or operator's strategies. When SBR-1 was in reaction phase, SBR-4 was in settling/decanting period and vice versa. This configuration allowed the continuous treatment of wastewater. During the reaction phases, wastewater flowed into the reactor at a constant rate and it was permanently stirred and subjected to alternating aerobic and anoxic phases with different duration depending on the cycle configuration.

During the experimental campaign, three markedly different cycle configurations (A, B and C) were implemented and were part of the normal SBRs operation (Fig. 7.2).

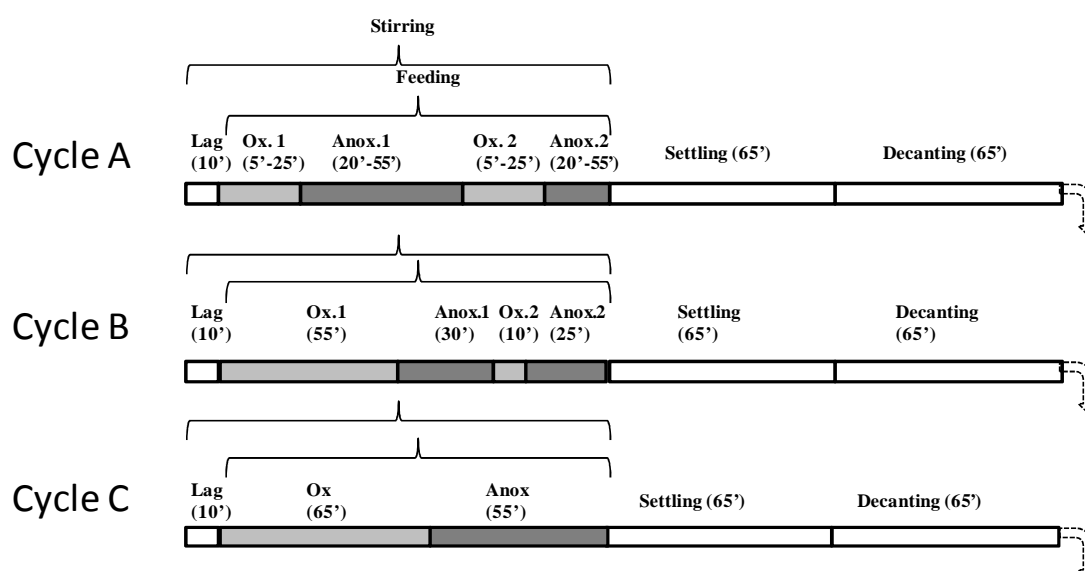


Figure 7.2 Schemes of the different cycle configurations used in La Roca del Vallès WWTP.

All cycle types started with a lag-phase in which the mixed liquor was stirred before feeding started. After the lag-phase, the wastewater was fed while aeration started. Type-A cycles consisted of a sequence of two short (5-25 min) aerated phases alternated by two non-aerated phases of 20-55 min, before settling and decanting of effluent. The reaction stage of cycles following the B configuration consisted of a sequence of a long (55 min) aerated phase followed by an anoxic phase (30 min) and a short aerated phase (10 min), to finish without aeration during 25 min before settling/decanting started. Finally, type-C cycles were built on a single sequence of a long aerated phase (65 min) followed by a non-aerated phase of 55 min.

7.2.3 Online N_2O monitoring

N_2O emissions were continuously monitored at SBR-4 for 33 days between February and March, 2014. A total number of 143 cycles were monitored during the experimental period. In addition,

dissolved N₂O patterns were followed at SBR-4 during the monitoring period with an N₂O micro-sensor with data logging (In situ amplifier system, Unisense A/S. Aarhus, Denmark).

7.2.4 Chemical characterization

Composite samples from the influent wastewater and effluent treated water were periodically analyzed for chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total phosphorous (TP) and NH₄⁺. Also, MLSS and MLVSS were monitored at the effluent. To analyze the nutrient dynamics (in terms of NH₄⁺, NO₂⁻ and NO₃⁻) in some of the cycles of the SBR, grab samples were taken with automatic refrigerated samplers from the mixed liquor at the SBR, near the site where the gas collection hood was placed with a sampling frequency of 20-120 min depending on the cycle. All samples were first filtered through disposable Millipore filter units (0.22 μm pore size), and then processed for analyses according to standard methods (APHA, 1998) or via ion chromatography (for NO₂⁻ and NO₃⁻ only). Additionally, the NH₄⁺ concentration at the inlet of the bioreactor and in the SBR was continuously monitored utilizing two on-line ion-selective electrodes (ammo::lyser™) coupled to a monitoring station (S::CAN Messtechnik GmbH, Austria). DO and temperature were recorded with a portable DO sensor with an integrated thermometer (YSI Inc. USA) during the monitoring campaign at SBR-4.

7.2.5 Data acquisition for emissions and carbon footprint calculations

On-line data of the aeration flow from the air diffusers at SBR-4 as well as the influent wastewater flow were acquired from the data acquisition system of the WWTP, for emission calculations. N₂O emissions (Kg N₂O-N) were calculated utilizing equations 2, 3, 4 and 5 as described in chapter 3.

The plant's electricity consumption was logged every 15 min during the monitoring period, and was provided by plant operators. The electricity consumption was multiplied with the amount of carbon dioxide (CO₂) that is typically emitted during energy generation in Spain (0.287 Kg CO₂/KWh) according to the International Energy Agency (IEA, 2012), in order to calculate the plant's C-footprint in indirect-CO₂ equivalents (CO₂ e) at different moments. For the calculation of the total direct-CO₂ equivalent emissions of the plant (the ones linked to N₂O), it was considered that the emissions detected in SBR-4 for each type of cycle would be the same as in the cycles occurring in SBR-1. This assumption was based on the fact that both SBRs presented the same cycle configurations and were treating the same volume of wastewater with similar N-load according to plant operators. N₂O emissions from the SBRs were included in the plant's C-footprint account by multiplying the mass of N₂O emitted with 298 CO₂ e following the guidelines of the International Panel for Climate Change (IPCC, 2013). Other sources of C-footprint such as chemical usage, sludge incineration or methane (CH₄) emissions were not taken into consideration in the calculations.

7.2.6 Testing a modified cycle configuration for N₂O mitigation

An experimental cycle configuration (cycle D) was implemented at La Roca del Vallès WWTP in order to further study N₂O emissions and C-footprint in SBR-4. This test cycle was a modification of a type-A cycle, where three short (30 min) aerated phases were alternated with three non-aerated stages of 20 min each (total reaction time of 150 min). Settling and decanting times were increased in correlation with the reaction stage, to reach a total cycle time of 300 min.

7.3 Results and discussion

7.3.1 Plant's overall performance

During the experimental campaign, the WWTP of La Roca del Vallès presented over 89% removal rates for both COD and TN, according to data provided by plant operators. In general, the bioreactors operation followed regular patterns and no significant perturbations of the functioning of the wastewater treatment process occurred. Some of the process parameters and influent and effluent characteristics of the WWTP during the monitoring period are presented in Table 7.1.

Table 7.1 Operational parameters, influent and effluent characteristics of the WWTP during the experimental campaign.

Process parameter	Average value \pm standard deviation
<i>Influent wastewater</i>	
Flow (m ³ /d) ^a	6553 \pm 227.6
COD (mg/L) ^a	604.1 \pm 37.2
TN (mg N/L) ^a	69 \pm 4.5
NH ₄ ⁺ (mg N/L) ^a	38.7 \pm 2.1
TP (mg P/L) ^a	7.5 \pm 0.5
pH ^a	7.7 \pm 0.04
<i>Bioreactor</i>	
NH ₄ ⁺ (mg N/L) ^a	7.9 \pm 5.1
NO ₂ ⁻ (mg N/L) ^b	0.47 \pm 0.03
NO ₃ ⁻ (mg N/L) ^b	< 0.06
MLSS (mg/L) ^a	3100 \pm 58.2
MLVSS/MLSS (%) ^a	80.7 \pm 0.5
HRT (d) ^a	1.53 \pm 0.02
SRT (d) ^a	26.3 \pm 2.2
Temperature (°C) ^a	15.4 \pm 0.5
<i>Effluent water</i>	
COD (mg/L) ^a	66 \pm 8.06
TN (mg N/L) ^a	7.5 \pm 0.8
NH ₄ ⁺ (mg N/L) ^a	3.3 \pm 0.6
TP (mg P/L) ^a	1.4 \pm 0.1

^aData provided by plant operators, from lab analyses (2-3 samples/week) or the plant's automatic control and data acquisition system and on-line sensors.

^bData corresponds to average values obtained from chemical analyses of samples grabbed with a frequency ranging from 20 to 120 min in 20 different cycles at the SBR.

7.3.2 Effect of operational mode on N₂O emissions

Two representative N₂O cycle profiles for each of the cycle configurations are given in Figure 7.3, together with the DO, NH₄⁺, NO₂⁻ and NO₃⁻ concentration profiles. For the cycle configuration A, an additional example is shown in Figure S.I.1 (supplementary information) in which a more intensive data set for NH₄⁺, NO₂⁻ and NO₃⁻ concentrations in the SBR is represented.

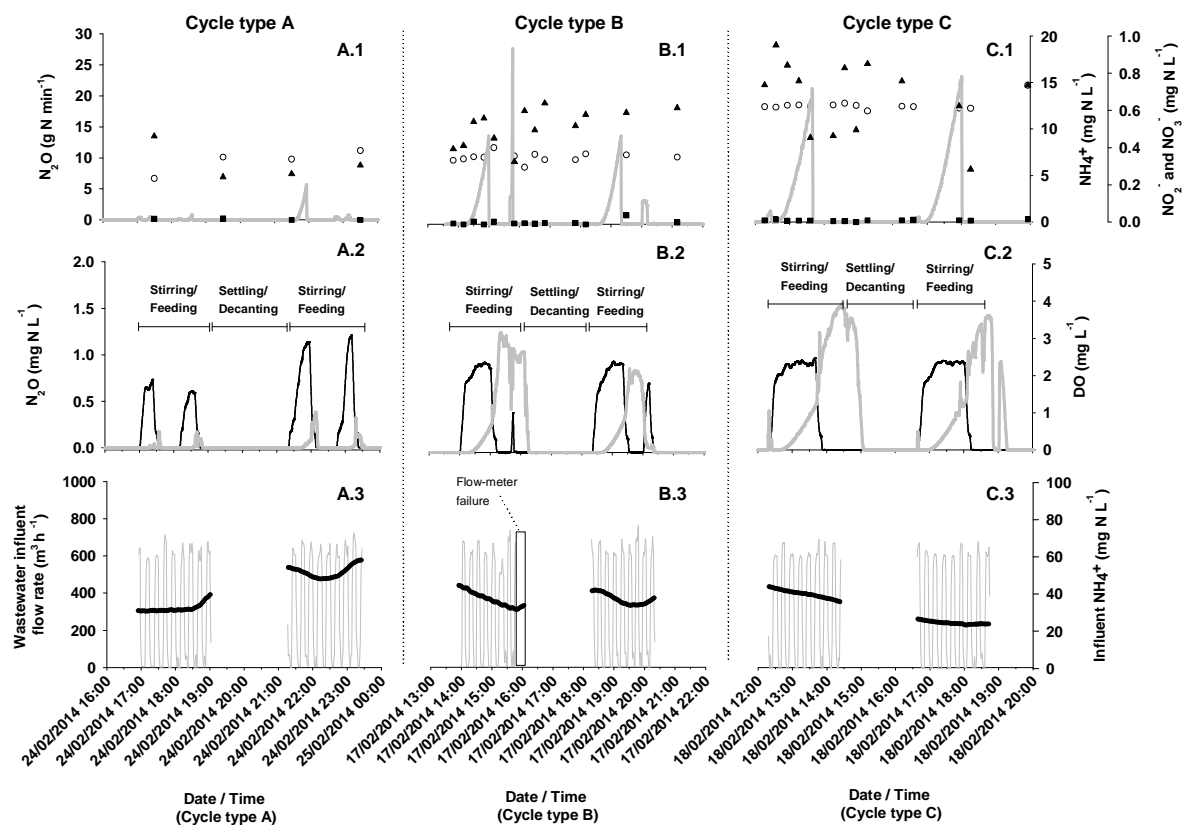


Figure 7.3 Representative profiles of N₂O emission rates (grey line), NH₄⁺-N (○), NO₂⁻-N (▲) and NO₃⁻-N (■) (A1, B1 and C1), dissolved N₂O (grey line) and DO (black line) (A2, B2 and C2) and influent wastewater flow rate and NH₄⁺-N concentration (A3, B3 and C3) measured in the SBR at La Roca del Vallès WWTP under the three different cycle configurations identified during this study.

The patterns described by the nutrient (NH₄⁺, NO₂⁻ and NO₃⁻) concentrations were similar in all cases, with small variations along each cycle and no significant differences between cycle types. The average concentration of NH₄⁺ in the bioreactor was highly similar under the occurrence of the three types of cycle (8.45 ± 0.6 mg NH₄⁺-N/L in cycles A, 8.48 ± 0.8 mg NH₄⁺-N/L in cycles B and 7.63 ± 0.6 mg NH₄⁺-N/L in cycles C), although in the cycle example C represented in Figure 7.3C.1 the NH₄⁺ concentration was slightly larger than in the other examples. NO₃⁻ didn't accumulate in the bioreactor (with nearly 0 mg N/L in all cases), while NO₂⁻ concentrations ranged from 0.2 to 1 mg N/L (Fig.7.3 A.1, B.1 and C.1). In every case, N₂O was emitted upon aeration, although the emissions presented significant differences between cycle types. Type-A cycles showed the lowest emission levels, peaking at 2-5 g N/min, as shown in Figure 7.3 A.1. The peaks observed under B and C configurations were however much larger, reaching values of around 25-30 g N/min in some cases (Fig. 7.3 B.1 and C.1). In all three cases, N₂O emissions sharply decreased when aeration stopped.

N₂O emissions correlated with the presence of dissolved N₂O in the mixed liquor (Fig. 7.3 A.2, B.2 and C.2). Under aerobic conditions dissolved N₂O accumulated in the bioreactor (mainly in cycles type B and C) indicating that the N₂O produced was higher than the one removed due to stripping. This accumulation increased for around 30-60 min when aeration stopped and the anoxic phase started, indicating that production of N₂O was continuing under decreasing DO and anoxic conditions, with levels ranging from 0.1-0.4 mg N₂O-N/L in cycles with configuration A, to over 1-1.5 mg N₂O-N/L when types B and C cycles were ongoing. Then, dissolved N₂O kept constant values

before sharply decreasing exactly at the time when stirring stopped and the settling phase started. A possible explanation to this sharp decrease on the dissolved N_2O signal could be that the N_2O bubbles produced were trapped in the sludge blanket during settling and were moved to the bottom of the reactor. Since the N_2O sensor was placed 0.5-0.75m below the water surface of the SBR, as soon as the sludge moved below that point, the N_2O signal decreased. The fact that no N_2O peaks are detected as soon as the new cycle starts with sludge mixing suggests that this N_2O could be reduced to nitrogen gas due to heterotrophic denitrification. Several authors have investigated the possibilities of utilising denitrification in N_2O mitigation strategies (Park et al., 2000; Lu and Chandran, 2010; Desloover et al., 2012). At the SBR of this study, allowing the system to consume N_2O through denitrification would contribute to emissions minimization.

According to the data observed in this study, N_2O probably followed a combination of different production pathways. On one side, the reactor's operation was based on sequencing cycles, implying transient anoxic/oxic conditions. Moreover, during the first minutes of the oxic phases NH_4^+ accumulated temporally in the reactor due to the inflow of wastewater. A correlation between transient anoxic/oxic conditions with N_2O production in a pure culture of *Nitrosomonas europaea* was established by Yu et al. (2010). According to Yu et al. (2010), recovery from anoxia under the presence of NH_4^+ triggers N_2O production due to specific enzymatic and metabolic responses in AOBs, in agreement with the results presented in this study. On the other hand, systems in which a certain accumulation of NO_2^- is given (as seems to be the case at the SBR of this study) have been proven to favor N_2O emissions as a result of the activation of the nitrifier denitrification pathway in AOB (Hynes and Knowles, 1984; Bock et al., 1995; Kampschreur et al., 2008a, 2008b; Foley et al. 2010; Kim et al., 2010; Rodriguez-Caballero and Pijuan, 2013).

Similarly in each cycle, when aeration stopped N_2O continued to be generated. Upon decreasing aeration, the N_2O accumulation rate continued to increase (as shown by the steeper slope described by the dissolved N_2O curves in Figure 7.3A.2, B.2 and C.2). In those periods, N_2O could also have been produced through the nitrifier denitrification pathway, which has been reported to be dependent on the oxygenation level (Tallec et al., 2006). Also, it cannot be excluded the contribution of heterotrophic denitrification on the N_2O detected under anoxic conditions. It is very difficult however, in a process such as the one of this study, to unravel whether the production of N_2O through denitrification was given by the activity of AOB or by heterotrophic bacteria, and this issue remains unclear.

N_2O emissions didn't correlate with the influent wastewater flow or NH_4^+ concentration, as these two parameters were similar during the occurrence of the different cycle types, as shown in the examples represented in Figure 7.3 (A.3, B.3 and C.3). The average N_2O emissions (normalized for NH_4^+ loading and NH_4^+ consumed) from the monitored SBR at La Roca del Vallès WWTP are shown in Figure 7.4. The lowest N_2O emissions were found under the conditions given by type-A cycles, with an average of ~ 3 g N_2O -N/Kg NH_4^+ -N load or ~ 5 g N_2O -N/Kg NH_4^+ -N consumed. Cycle types B and C showed higher emission factors, with ~ 39 and ~ 46 g N_2O -N/Kg NH_4^+ -N load, and ~ 53 and ~ 66 g N_2O -N/Kg NH_4^+ -N consumed, respectively (Figure 7.4).

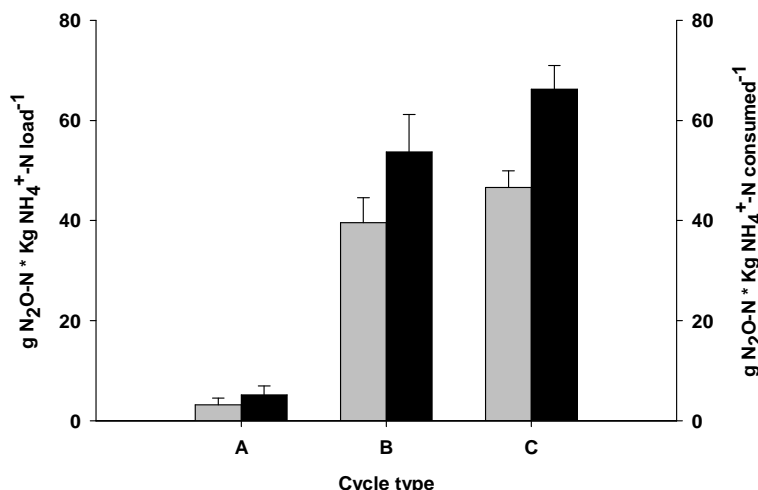


Figure 7.4 Average N₂O emitted per N load (grey bars) or N consumed (black bars) during the monitoring campaign, under each cycle configuration at the SBR. Error bars correspond to the standard error of the means.

A possible contribution of the different stripping conditions on the N₂O emissions detected during each cycle type cannot be completely excluded. However, it seems that the changes detected in N₂O emissions are mainly influenced by the length of the aeration phases in each cycle. Figure S.I.2 (supplementary information) shows the same three cycles previously shown in Figure 7.3, where the aeration flow rate is depicted together with the N₂O emission rates. Aeration flow rates under the different cycle configurations reached similar levels in general. Only one of the cycles with configuration A represented in Figure S.I.2 showed higher levels of aeration rate, but the higher aeration didn't correlate with higher emission rates in the SBR of this study.

7.3.3 Overall carbon footprint of the plant

The electricity consumption of the plant, when operating the SBRs under the different cycle configurations, ranged from 0.63 (type-A cycles) to 0.9 KWh/m³ of treated wastewater (type-C cycles) on average (Fig. 7.5A). Most of the cycles with type A configuration had shorter total aeration time

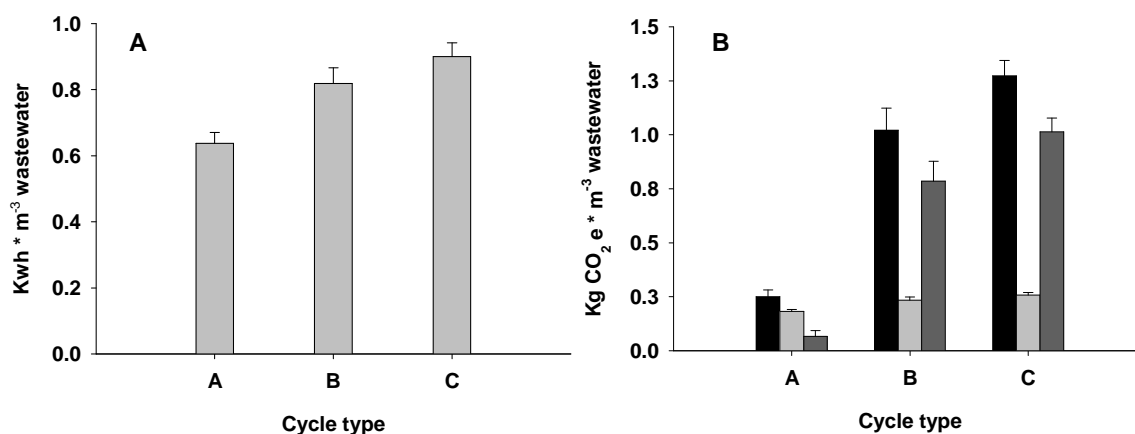


Figure 7.5 (A) Average plant's electricity consumption and (B) total average C-footprint (black bars), average electricity consumption-related C-footprint (light-grey bars) and average N₂O-related C-footprint (dark-grey bars) during each cycle configuration. Error bars correspond to the standard error of the means.

than the B or C-type cycles. Since aeration accounts for up to 50 % of the total electricity consumption of a conventional WWTP (Tchobanoglous et al., 2003), it could be assumed that the differences in electricity consumption were related with the differences in aeration time employed under each cycle configuration.

Significant differences were also observed when calculating the C-footprint generated under each cycle configuration, not only because of the electricity consumption but also due to the inclusion of N₂O emissions in the C-footprint calculations. Figure 7.5B shows the total average C-footprint, the average electricity-related C-footprint and the N₂O emissions-related C-footprints of La Roca del Vallès under each cycle type. The average C-footprint of cycles with configuration A was 0.25 ± 0.03 Kg CO₂ e/m³ wastewater. When operating the bioreactors with configurations B and C, the average C-footprint increased to 1.02 ± 0.1 and 1.27 ± 0.07 Kg CO₂ e/m³ wastewater, respectively. The C-footprint related with N₂O emissions was 0.06 ± 0.02 , 0.78 ± 0.09 and 1.01 ± 0.06 Kg CO₂ e/m³ wastewater for configurations A, B and C, respectively, while the C-footprint related with the electricity consumption was similar in all three cases, ranging from 0.18 to 0.25 Kg CO₂ e/m³ wastewater (Fig. 7.5B).

The overall average N₂O emissions from the plant were 97.1 ± 6.9 g N₂O-N/Kg NH₄⁺-N consumed or 6.8% of the influent NH₄⁺-N load. This emission factor is somewhat higher than the majority of emission values reported to date for full-scale systems treating domestic wastewater, which range from 0.003 to 2.8% of the influent N (Law et al. 2012; Daelman et al. 2013). For comparative purposes, data from studies employing on-line quantification systems is most suitable, and releases more accurate results. For example, Ahn et al. (2010) presented emission factors ranging from 0.01 to 1.8% of the influent TKN, depending on the type of reactor configuration and process characteristics in a set of 12 WWTP's in USA. Lower emissions were recently reported by Aboobakar et al. (2013) (0.036% of the influent TN) or Rodriguez-Caballero et al. (2014) (0.116% of the influent TKN) for plug-flow reactors. The high emission values presented at the SBR of this study could be attributed to the specificities of the operation itself, with continuous fluctuations of parameters such as DO, NH₄⁺ or NO₂⁻ concentrations, probably activating different mechanisms responsible for N₂O production.

N₂O emissions from the SBR had a large impact on the overall C-footprint of La Roca del Vallès WWTP. The plant's total average C-footprint was 0.96 ± 0.07 Kg CO₂ e/m³ wastewater. On average, 60.6 ± 2.3 % of the total C-footprint was related with the N₂O emitted from the bioreactors, and only 39.3 ± 2.4 % of the total C-footprint was generated as a result of the plant's electricity consumption. This calculation was done assuming that the N₂O emissions in SBR-1 were also related to the type of cycle applied. To date, the only studies that took into consideration N₂O emissions when evaluating the C-footprint from full-scale WWTPs were performed in The Netherlands. As reported in previous studies, between 2 and 90% of the C-footprint calculated in three different WWTPs in the Netherlands was related with the N₂O emissions (GWRC, 2011). The lowest share of C-footprint was shown in the facilities with low N-loading. However, these observations were based on data from monitoring campaigns lasting for one week. Whether the emissions given during one week are representative of the plant's emissions or not remains uncertain. Long-term data presented by Daelman et al. (2013) showed that the contribution of the N₂O emissions from full-scale WWTP was over 78% of the C-footprint. In that same investigation, the rest of the C-footprint was related with the plant's electricity consumption and the CH₄ emissions.

CH₄ emissions were not taken into account in the C-footprint calculations at La Roca del Vallès. Preliminary analyses at the SBR of this study showed low CH₄ emission values with no significant weight on the overall C-footprint of the WWTP (see supplementary information for additional data) and all efforts were invested into performing an in-depth study of the N₂O emission dynamics. CH₄ emissions share in the C-footprint of wastewater treatment facilities ranges from 5 to 40% (GWRC,

2011), with plants with no anaerobic sludge digestion for biogas production situated in the lower end of this range. WWTPs coupled with anaerobic sludge digestion for electricity generation have been shown to release large amounts of CH_4 (Daelman et al., 2012; Rodriguez-Caballero et al., 2014) and in those cases CH_4 emissions need to be evaluated and controlled. But even in those facilities, the contribution of CH_4 to the overall C-footprint may be limited (13.5% reported by Daelman et al. (2013)). It should be noted that La Roca del Vallès had no anaerobic sludge digestion facility, and excess sludge from the SBRs was disposed to external receivers. The large impact of N_2O emissions on the C-footprint account was confirmed when operating the SBR under each of the cycle configurations. However, the C-footprint of the plant severely decreased under type-A cycles, which presented much less N_2O emissions than cycles B or C.

7.3.4 Aeration intermittency: Key parameter for N_2O mitigation at La Roca del Vallès SBR

Differences in aeration patterns as a result of different cycle configurations were previously shown to deliver different N_2O emission dynamics during the sampling campaign (Fig. 7.3). As mentioned, cycles with configuration A presented the lowest N_2O emissions, with no substantial increase of the electricity consumption and thus, generating the lowest C-footprint. However, it was unclear whether it was the shorter aeration time or the actual aeration phase structure (short aeration pulses), the main factor contributing to the smaller N_2O production. In order to further investigate this issue, the bioreactor was forced to operate with a modified configuration (D) during three consecutive cycles (Fig. 7.6).

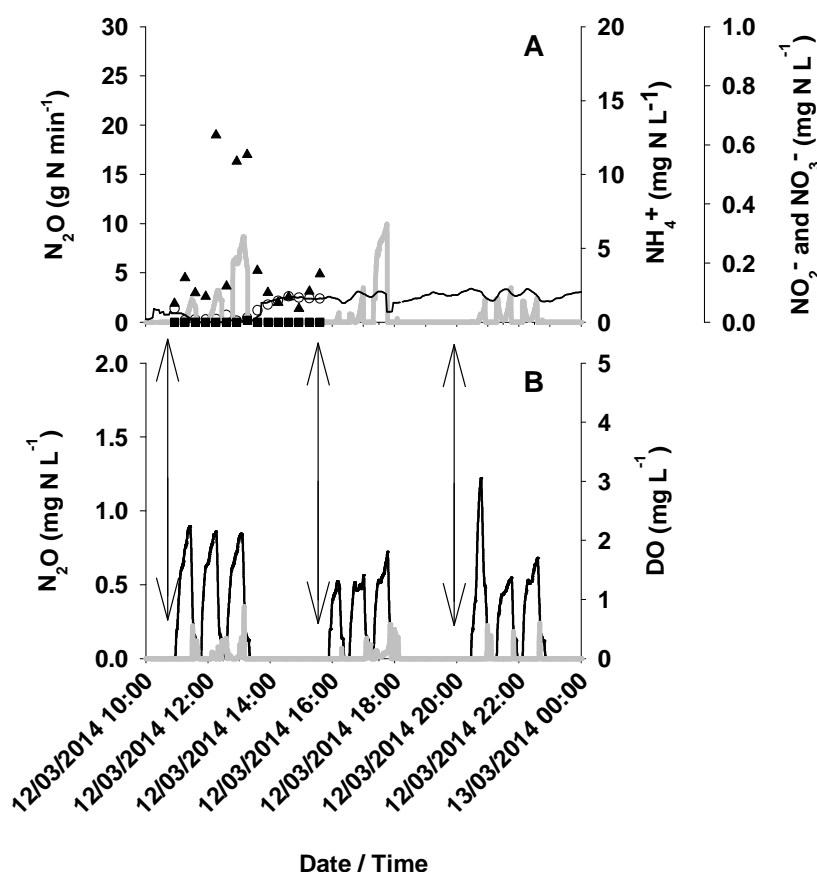


Figure 7.6 (A) Profiles of N_2O emission rate (grey line), NH_4^+ -N (black line for continuous measurement and (○) for grab samples), NO_2^- -N (▲) and NO_3^- -N (■) and (B) dissolved N_2O (grey line) and DO (black line), measured in the SBR at La Roca del Vallès WWTP under the experimental cycle configuration D, performed in triplicate. Arrows mark the moment at which each cycle starts.

The aeration pattern of cycle D (described in section 7.2.6) was similar to the one of type-A cycles, but with an overall longer aeration time length (similar to the aeration time of type-C cycles).

During the three experimental cycles that followed D-configuration, N_2O emissions increased upon aeration, with peaks up to 10 g N/min. The NH_4^+ concentration in the bioreactor was low, with values up to 2.5 mg NH_4^+ -N/L. NO_3^- didn't accumulate in the SBR and NO_2^- concentration ranged from 0.2 to 0.7 mg N/L (Fig. 7.6.A). Dissolved N_2O concentrations in the mixed liquor never exceeded 0.5 mg N/L, and followed the same pattern described for type-A cycles.

Under cycle configuration D, the SBR presented an emission factor of 7.83 ± 2.5 g N_2O -N/Kg NH_4^+ -N load on average, or 0.78% of the NH_4^+ -N load. These emissions are similar to the average emission given by the bioreactor under operation with cycle configuration A, and significantly lower than the emissions shown when implementing cycles B and C. Similarly, the plant's average electricity consumption when experimenting with type-D cycles was 0.59 ± 0.02 KWh/ m^3 wastewater, which is highly similar to the consumption presented when operating the SBR under cycle configuration A.

All cycles during the monitoring campaign were subjected to an average NH_4^+ influent loading ranging from 20 to 25 Kg NH_4^+ -N/cycle, as shown in Figure 7.7, including the experimental cycle D. Furthermore, the performance of both A and D-configuration cycles was similar to that shown under the cycle types B or C in terms of average NH_4^+ consumption (Fig. 7.7).

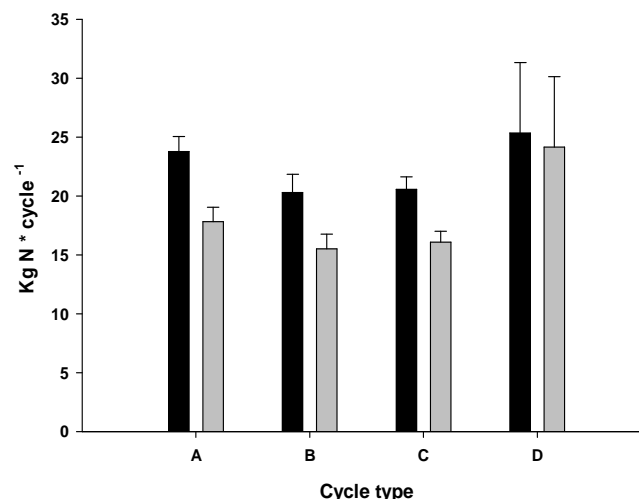


Figure 7.7 Total average NH_4^+ -N load (black bars) and average NH_4^+ -N consumed (grey bars) under each cycle configuration during the monitoring campaign. Error bars correspond to the standard error of the means.

According to these results, the aeration pattern could be considered as a control strategy for minimization of both the overall N_2O emissions from the SBR and the C-footprint of the plant by adopting a cycle configuration in which short aerated periods are implemented. Laboratory investigations demonstrated that reducing N_2O emissions from activated sludge treating swine wastewater was feasible when applying intermittent instead of continuous aeration (Osada et al., 1995; Beline and Martinez, 2002). Similarly, Kimochi et al. (1998) identified the aeration pattern as the critical factor to be considered for reducing N_2O emissions from a full-scale N-removal system. Beline and Martinez, (2002) attributed the emissions minimization to the consumption of N_2O and N_2O precursors (NO , NO_2^-) during heterotrophic denitrification between aeration periods. When implementing cycles with aeration intermittency (types A or D) at the SBR in La Roca del Vallès WWTP, lower NO_2^- concentrations than in cycles B or C were observed (Fig. 7.3 and 7.6). The higher NO_2^- concentration accumulated in cycles B and C resulting from the oxidation of NH_4^+ may have

affected heterotrophic denitrification performance (Alinsafi et al., 2008) resulting in a lower degree of NO_2^- reduction (during anoxic phases), with the consequent further accumulation of NO_2^- being converted to N_2O by nitrifiers in the following aeration phase. This hypothesis would need additional tests with on-line NO_2^- concentration dynamics in order to be confirmed.

Supplementary information

(SI) Materials and methods

CH₄ emissions were recorded online at SBR-4 in La Roca del Vallès WWTP during one week in February 2014. As performed for the N₂O monitoring, a commercial hood (AC'SCENT® Flux Hood) was utilized to collect the gas from the reactor. The gas collection hood was connected to a commercial gas analyzer (VA-3000, Horiba, Japan) equipped with a sample conditioning system (series CSS, M&C Tech group). Off gas was collected continuously (at 0.5L/min) from the reactor headspace and concentration data was logged every 15 seconds.

CH₄ emissions were calculated as follows (equation S.I.1):

$$\text{Gas emitted (g)} = \left[\left(\sum (C_{gas} \times \Delta t \times Q_{gas}) \right) \right] \quad (\text{S.I.1})$$

Where,

- C_{gas} (g CH₄/L) = C_{gas} (ppm v) * 10⁻⁶ * gas molar volume⁻¹ (0.041 mol/L at 25°C and 1atm) * 16
- Δt (min) = time interval by which the off-gas concentration was recorded.
- Q_{gas} (L/min) = the gas flow rate coming out from the reactor. In aerated zones, Q_{gas} was assumed to be the aeration flow rate (provided by plant operators). When aeration was off (anoxic phases) Q_{gas} was calculated as described for N₂O emissions calculations (section 2.5).

CH₄ was assumed to be emitted in the same quantities from SBR-1 and SBR-4. The overall CH₄ emissions were included in the plant's C-footprint account by multiplying the mass of CH₄ emitted with 34 CO₂ e following the guidelines of the International Panel for Climate Change (IPCC, 2013).

(SI) Results

Table S.I.1 Average CH₄ emissions, CH₄-related C-footprint, total C-footprint and CH₄ share on the total C-footprint during the first week of the monitoring campaign at SBR-4 in La Roca del Vallès WWTP.

Parameter	Average ± Error
CH ₄ emissions (Kg CH ₄ / d)	0.38±0.006
CH ₄ emissions (% of influent COD)	0.02±0.002
CH ₄ emissions (Kg CH ₄ / m ³ wastewater)	0.0001±1.4 x10 ⁻⁵
CH ₄ -related C-footprint (Kg CO ₂ e / m ³ wastewater)	0.0034±0.0004
*Total C-footprint (Kg CO ₂ e / m ³ wastewater)	0.66±0.05
CH ₄ -related C-footprint (% of the Total C-footprint)	0.35±0.1

* C-footprint calculated taking into account CH₄, N₂O (from SBR-1 and SBR-4) and indirect CO₂ emissions.

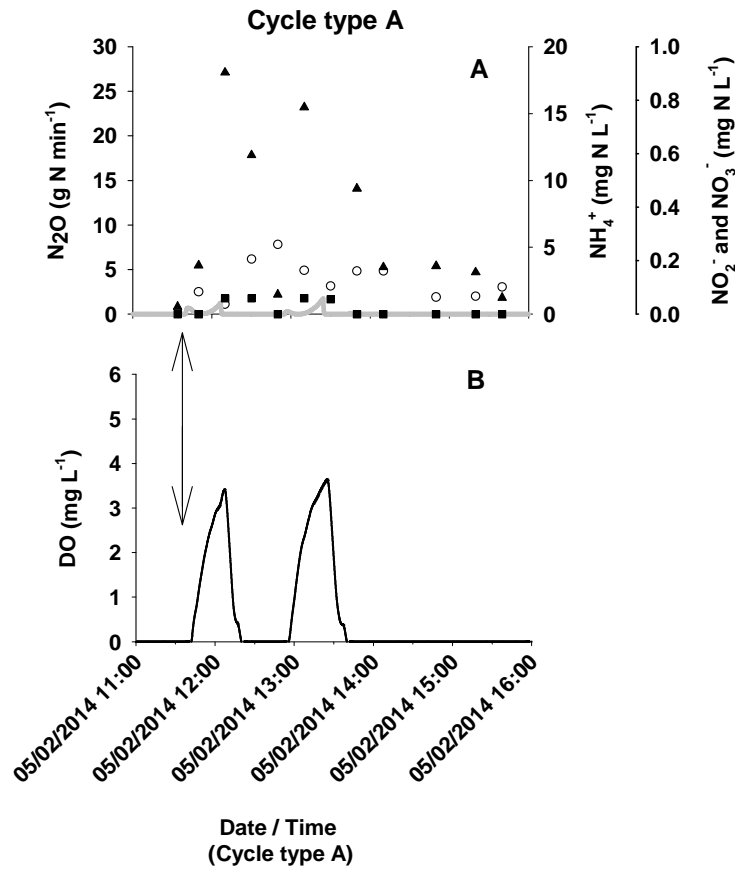


Figure S.I.1 Representative profiles of N_2O emission rate (grey line), NH_4^+-N (\circ), $NO_2^- -N$ (\blacktriangle) and $NO_3^- -N$ (\blacksquare) (A) and DO (black line) (B), measured in the SBR at La Roca del Vallès WWTP during the occurrence of a cycle configuration type A. The arrows mark the moment at which the cycle starts.

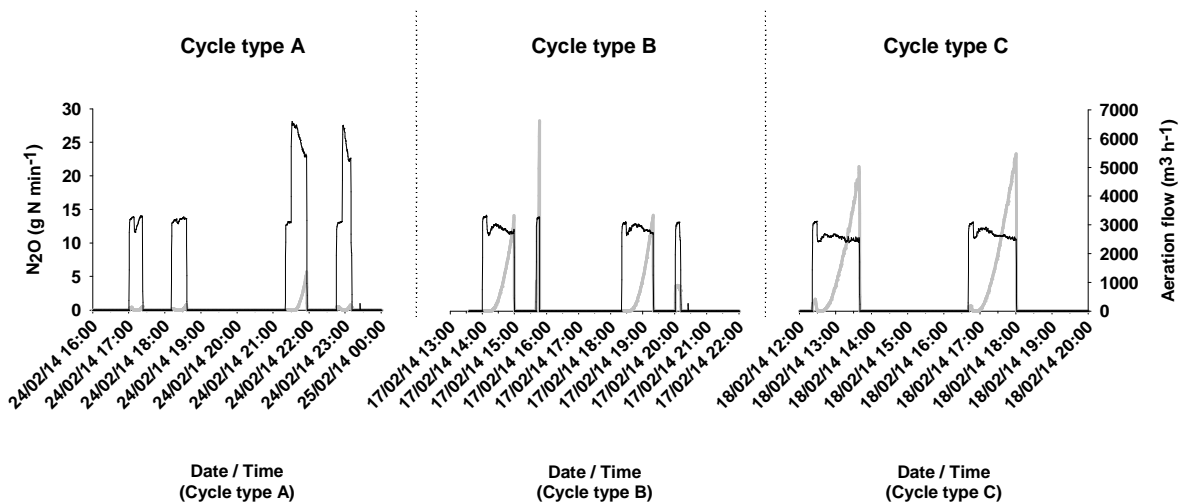


Figure S.I.2 Representative profiles of N_2O emission (grey line) and aeration flow rates (black line), measured in the SBR at La Roca del Vallès WWTP measured in the SBR at La Roca del Vallès WWTP under the three different cycle configurations identified during this study.

SECTION III

FINAL REMARKS

Chapter 8

General discussion

During the last decade, the amount of research studies focusing on fugitive non-CO₂ green-house gas emissions from wastewater treatment systems has severely increased. Most of the work performed to date has been directed towards the investigation of different production and emission pathways, with a special attention on N₂O dynamics, which were highly unknown. However, there is still a high degree of uncertainty around the most important factors and parameters that affect N₂O production in complex bacterial populations. Furthermore, research on full-scale wastewater treatment systems employing on-line technology has been performed in few occasions, and there is a lack of comprehensive full-scale data on gas emissions and dynamics in WWTPs.

The work carried out during the elaboration of this thesis can be divided into two main sections. On one hand, laboratory experiments were carried out on bioreactors with enriched nitrifying bacterial populations. These experiments allowed the identification of some of the most important factors triggering production of N₂O (and NO) during partial and full nitrification of NH₄⁺-rich wastewater, simulating processes that are commonly applied in full-scale wastewater treatment. On the other hand, research was scaled up to real conditions, and two on-line monitoring campaigns targeting N₂O (and CH₄) emissions were performed in municipal WWTPs of different characteristics. The most important outcomes of the studies presented in this thesis are summarized below:

8.1 Exploring N₂O emission factors, dynamics and mitigation potential in lab-scale partial and full nitrification reactors

The performance of experiments on a lab-scale nitrification reactor treating high NH₄⁺ concentration synthetic wastewater allowed the identification of some of the most important factors affecting N₂O and NO production (**Chapter 4**). Nitrification or partial nitrification systems are commonly applied for the treatment of high NH₄⁺-loaded wastewater (i.e. for the treatment of anaerobic digesters reject wastewater). It was shown that different parameters and conditions could activate different N₂O production pathways in these nitrifying bacterial populations. On one hand, high concentration of both NH₄⁺ and NO₂⁻ in the bulk liquid of the nitrification bioreactor utilized during the experimental work of the study was shown to promote N₂O formation, mostly during settling phases. As previously suggested by other authors (Kampschreur et al., 2008a and 2008b and Kim et al., 2010) NH₄⁺ could act as electron donor in the reduction of NO₂⁻ through the pathway known as *nitrifier denitrification* in AOB. It has also been suggested that the transition from anoxic (low activity) to oxic (high activity) conditions can lead to N₂O production from nitrifiers, in the presence of NH₄⁺ (Yu et al. 2010). It was therefore logical to establish a hypothesis over the fact that supplying sufficient aeration time for the AOB to completely oxidize NH₄⁺ to NO₂⁻ could minimize N₂O production. This hypothesis was tested and confirmed leading to the proposal of an N₂O mitigation strategy for partial nitrification systems, namely the provision of sufficient aeration time in order to completely consume NH₄⁺ before letting the bioreactor enter into a settling phase. Longer aeration times however imply higher carbon footprint due to increased electricity consumption, so the application of this strategy on a full-scale bioreactor would need to follow a complete assessment of the direct and indirect green-house gases budget.

Another N₂O mitigation strategy was also proposed as a result of different tests performed on the experimental lab-scale nitrification bioreactor mentioned above. This consisted of the addition of short anoxic phases to the cycle configuration of such reactor. The N₂O emission factor of the bioreactor under these conditions decreased from 0.83 to 0.6%. However, this reduction was given simultaneously an increase of the NO emissions, leading to the same overall N-gas emissions. NO contributes to ozone depletion, and it is a precursor of N₂O, so emissions of this gas are also undesired. Moreover, NO can be toxic for the bacterial population of the bioreactor itself, leading to malfunctioning of the nutrient removal process. Production of N₂O and NO in the experimental lab-scale reactor of this thesis clearly followed different trends. While up to 60-80% of the N₂O was

emitted during the first minutes of aeration after a settling phase (and demonstrated to be produced during settling), NO was found to be emitted under anoxic conditions, in cycle configurations with non-aerated phases. As suggested in other studies, anoxic conditions may cause an over expression of the NO_2^- reductase gene in AOB, while other genes encoding the reduction of NO or the oxidation of NH_3 might be suppressed (Kester et al. 1997; Kampschreur et al. 2008b and Yu et al. 2010).

In another set of experiments, the processes of nitrification and full nitrification of synthetic reject wastewater were compared in terms of N_2O and NO emissions (**Chapter 5**). In this case, two lab-scale SBRs (SBR1 and SBR2) were enriched with *Nitrosomonas* (AOB) and *Nitrobacter* (NOB). Stable conversion of NH_4^+ to NO_2^- and NO_2^- to NO_3^- was achieved in SBR1 and SBR2, respectively. Under nitrification conditions in SBR1, 1.22% of the converted-N was emitted as N_2O , and 0.066% as NO. Then, biomass from SBR2 was added in SBR1 in order to achieve full nitrification. During the transition from nitrification to full nitrification, effluent NO_2^- concentrations decreased but nitrogen oxides were emitted at levels similar to the nitrification period (1.15% of the converted-N was emitted as N_2O). N_2O emissions decreased sharply after the reactor achieved full nitrification conditions (0.54% N_2O -N/converted-N; 0.021% NO-N/converted-N), as a result of the combined effect of very low NO_2^- and NH_4^+ concentrations in the effluent from the bioreactor, minimizing N_2O and NO production during settling. Results from this study show that nitrification systems are expected to release larger amounts of both N_2O and NO than reactors performing full nitrification. Therefore, when taking into account green-house gases or carbon footprint calculations, this type of systems may need to be subjected to further research in order to reach lower emission levels.

The results presented above were originated through experimenting with lab-scale (8L) bioreactors mimicking nitrification or nitrification used in the treatment of reject wastewater. These reactors were highly enriched with nitrifying bacterial populations (AOB or AOB+NOB) which allowed unraveling some of the factors affecting N_2O and NO emissions in these microbial systems. The second block of experimentation focused on the monitoring of the N_2O emission dynamics in two full-scale domestic wastewater treatment plants (WWTP). These monitoring campaigns constituted the first of this kind conducted in the Spanish context. The main outcomes are summarized below.

8.2 Monitoring fugitive greenhouse gas (N_2O and CH_4) emissions from full-scale WWTP

A large part of the research work of this thesis consisted of carrying out full-scale on-line monitoring campaigns to identify the N_2O and also CH_4 emission dynamics from municipal WWTPs. Two campaigns were carried out in municipal WWTPs with different configurations and characteristics removing COD and nitrogen compounds from wastewater. Results from these studies allowed the identification of key process perturbations or conditions that lead to peak N_2O and CH_4 emissions, and mitigation strategies were proposed.

In the first campaign (**Chapter 6**), N_2O and CH_4 emission dynamics of a plug-flow bioreactor located in the municipal WWTP of the city of Granollers (Spain) were monitored during a period of 10 weeks. In general, CH_4 and N_2O gas emissions from the bioreactor accounted for 0.016% of the influent COD and 0.116% of the influent TKN respectively. These emissions are similar to other emission factors reported for other plug-flow reactors. In order to identify the emission patterns in the different zones of the plug-flow bioreactor, this was divided in six different sampling sites and a gas collection hood was placed for a period of 2-3 days in each of these sites. This sampling strategy also allowed the identification of different process perturbations leading to N_2O and CH_4 peak emissions. N_2O emissions were given along all the aerated parts of the bioreactor and were strongly dependent on the occurrence of process disturbances such as periods of no aeration or nitrification instability. Indeed, N_2O peaks were detected in the aerobic zones after periods when aeration was stopped. On the other hand, CH_4 emissions mainly occurred in the first aerated site due to the

stripping of the dissolved CH_4 present in the mixed liquor, and were mostly related with the influent and reject wastewater flows entering the bioreactor. Dissolved CH_4 and N_2O concentrations were monitored in the bioreactor and in other parts of the plant, as a contribution for the better understanding of the production and transport of these greenhouse gases across the different stages of the treatment system.

CH_4 emissions were shown to be a relevant contributor to the overall green-house gas emissions account of a WWTP, when anaerobic sludge digestion for electricity generation is carried out. In the case of Granollers WWTP, the regular release of reject wastewater from the digester into the bioreactor had a severe impact on the overall CH_4 emissions, generating large peak emission events. The benefits of performing anaerobic sludge digestion for biogas production and electricity generation could be counteracted by an increase in uncontrolled CH_4 emissions and carbon footprint in some cases. In this same study, the sewer system was also found to be an important source of dissolved CH_4 that was emitted as a result of stripping in aerated activated sludge basins. Strategies aiming at reducing CH_4 production in sewer pipes, or a more efficient utilization of the CH_4 in the internal biogas facilities could importantly contribute to decrease uncontrolled CH_4 emissions.

Experience from the long-term monitoring of N_2O emissions at Granollers WWTP demonstrated that, in general, process disturbances that impaired the correct functioning of nitrification could eventually be a source of large peak emissions from the activated sludge basins and could contribute to increase the overall emissions account of a WWTP. From this perspective, any mitigation strategy targeting the minimization of these peak emission events may be successful in decreasing the carbon footprint of a treatment facility.

The second monitoring campaign was performed at the municipal WWTP of La Roca del Vallès (**Chapter 7**), where a continuous, on-line quantification of the N_2O emissions from a real-scale SBR was performed. In this second monitoring campaign, different cycle configurations were implemented in the SBR aiming at reaching acceptable effluent values. Each cycle configuration consisted of sequences of aerated and non-aerated phases of different time length being controlled by the NH_4^+ set-point fixed. Cycles with long aerated phases showed the largest N_2O emissions, with the consequent increase in carbon footprint. In general, N_2O emissions from the biological wastewater treatment system (two parallel SBRs) were 97.1 ± 6.9 g $\text{N}_2\text{O-N/Kg NH}_4^+\text{-N}$ consumed or 6.8% of the influent $\text{NH}_4^+\text{-N}$ load. In the WWTP of La Roca del Vallès, N_2O emissions accounted for up to 60% of the total carbon footprint of the facility and less than 40% of the carbon footprint was related with the indirect CO_2 emissions attributed to the plant's electricity consumption. CH_4 emissions were not taken into account in the carbon footprint calculations at La Roca del Vallès. Preliminary analyses at the SBR of this study showed low CH_4 emission values with no significant weight on the overall carbon footprint of the WWTP. This confirmed the results presented in previous studies, asserting that plants with no anaerobic sludge digestion for biogas production present low fugitive emissions of CH_4 .

In order to evaluate the possibilities for a substantial minimization of N_2O emissions through a process operation strategy, a modified cycle configuration was tested during the monitoring campaign at La Roca del Vallès WWTP. This cycle consisted of three aerated phases (up to 20-30 min) followed by short anoxic phases and it was proven to effectively reduce N_2O emissions, without compromising nitrification performance or increasing electricity consumption. According to these results, the aeration pattern could be considered as a control strategy for minimization of both the overall N_2O emissions from the SBR and the carbon footprint of the plant. When implementing cycles with aeration intermittency at the SBR in La Roca del Vallès WWTP, lower NO_2^- concentrations were observed. The higher NO_2^- concentration accumulated in cycles with longer aeration phases resulting from the oxidation of NH_4^+ , may have affected heterotrophic denitrification performance leading to a lower degree of NO_2^- reduction (during anoxic phases), with the consequent further accumulation

of NO_2^- being converted to N_2O . This result represents the first operational strategy for N_2O mitigation being successfully tested and applied in a full-scale WWTP.

N_2O emissions from the SBR at La Roca del Vallès were higher than those reported by other authors in recent full-scale campaigns. The common operation of SBRs is based on sequencing cycles, implying transient anoxic/oxic conditions. Moreover, in the case of the SBR at La Roca del Vallès WWTP, during the first minutes of the oxic phases NH_4^+ accumulated temporally in the reactor due to the inflow of wastewater. Transient anoxic/oxic conditions have previously been proven to lead to N_2O production by nitrifiers. Some authors have also pointed at recovery from anoxia under the presence of NH_4^+ to trigger N_2O production in AOBs, in agreement with the results presented in this thesis. On the other hand, systems in which a certain accumulation of NO_2^- is given (as seems to be the case at the SBR of La Roca del Vallès) have been proven to favor N_2O emissions as a result of the activation of the nitrifier denitrification pathway in AOB. The high variability of the results from all the full-scale monitoring campaigns performed to date show that N_2O emission factors can be considered case-specific. However, the intrinsic characteristics usually linked to the process operation of an SBR may indicate that this type of bioreactor can lead to higher N_2O emissions, as in La Roca del Vallès case. From this perspective, SBR technology should be subjected to further research and undergo additional gas emissions minimization protocols.

Chapter 9

Conclusions

The main conclusions of the research work carried out for this thesis are divided in two blocks: (9.1) outcomes from the studies conducted in the lab-scale SBRs treating reject wastewater and (9.2) outcomes from the monitoring campaigns conducted in the two full-scale domestic WWTPs. Through lab-scale experiments, the main factors with a relevant effect on N_2O and NO emissions during partial and full nitrification of NH_4^+ -rich wastewater were identified and understood. The full-scale monitoring campaigns led to a deeper understanding of the different process conditions that can cause increasing fugitive GHG emissions during municipal wastewater treatment. As a result of these full-scale studies, emissions mitigation strategies were suggested. A summary of the conclusions of each section of the thesis is presented below:

9.1 Research outcomes from lab-scale SBRs treating reject wastewater

- Under nitritation conditions, larger N_2O and NO emissions were registered in the SBR of this study when compared with full nitrification conditions, during the treatment of synthetic reject wastewater. The presence of remnant NH_4^+ and high NO_2^- concentrations combined, strongly triggered nitrogen oxides emissions during nitritation. Therefore, the potential for $\text{N}_2\text{O}/\text{NO}$ emissions needs to be accounted when implementing nitritation in wastewater treatment systems.
- The majority of the N_2O production in partial nitrification SBRs treating reject-like wastewater occurred under settling conditions and was emitted during the first minutes of the cycle, as soon as aeration started. This production was biologically mediated and probably occurred due to the enhancement of the nitrifier denitrification pathway in AOBs during settling, due to the decrease of DO concentration in the system. N_2O production was directly linked to the presence of NO_2^- and $\text{NH}_4^+/\text{NH}_2\text{OH}$ in the reactor when the settling phase started.
- The introduction of short anoxic phases in the cycle configuration of an SBR treating reject like wastewater allowed a reduction of the N_2O emissions from the reactor. However, these short anoxic phases triggered the production of another detrimental gas, NO , which increased one order of magnitude (from 0.04 to 0.3% of the N treated). The overall combined NO and N_2O emissions resulted equal, with and without the application of anoxic phases. This finding highlights the importance of monitoring NO emissions together with N_2O in those reactors where AOB are the dominant microbial community.
- Partial nitrification leads to larger N_2O and NO emissions than full nitrification of NH_4^+ -rich wastewater. Increasing the aeration phase in the SBR to ensure complete NH_4^+ oxidation to NO_3^- substantially minimize these emissions. However, this would also increase the energy demand, resulting in higher indirect CO_2 emissions and operational costs. An evaluation of the benefits of reducing GHG emissions by increasing aeration costs would be needed for each case study.
- No correlation was found between feed loading and N_2O emissions during full nitrification of NH_4^+ -rich wastewater. However, NO emissions increased slightly as feed loading raised. More research is needed to unravel the mechanisms behind $\text{N}_2\text{O}/\text{NO}$ production in nitrifying cultures.

9.2 Research outcomes from the two full-scale monitoring campaigns

- N₂O emission dynamics were highly variable in both WWTPs and occurred during aeration in the form of peak emissions. This highlights the importance of conducting continuous (on-line) monitoring across several weeks when possible.
- N₂O emissions in the plug-flow reactor monitored (Granollers WWTP) were in general low. However peaks of N₂O were detected during the 2 months monitoring campaign and were mainly related to instability of the nitrification process. N₂O peak emission events can significantly increase the overall emission account of a WWTP. Sudden aeration stops should be avoided in the nitrifying zones when possible, in order to minimize N₂O peak emissions.
- The production of N₂O outside bioreactors (primary or secondary settlers) could be relevant, as suggested by the data recorded at Granollers WWTP, and needs specific additional research.
- N₂O emissions from the full-scale SBR monitored (La Roca del Vallès WWTP) were larger than the emissions reported from systems with other bioreactor configurations. Transient NH₄⁺ and NO₂⁻ accumulation in this type of reactors as well as transition from anoxic to aerobic conditions could be responsible of the larger emissions detected.
- N₂O emissions contribute heavily to the overall carbon footprint account of a WWTP. An overall carbon footprint calculation was conducted in La Roca del Vallès WWTP taken into account the energy consumption of the plant and the indirect CO₂-emissions related to this energy consumption. Based on this calculation, 60% of the carbon footprint of this facility was attributed to the N₂O emissions.
- N₂O emissions from the SBR at La Roca del Vallès WWTP were reduced through applying intermittent aeration with short oxic and anoxic phases, achieving the desired effluent quality and without increasing electricity consumption.
- CH₄ emissions from the plug-flow reactor at Granollers WWTP presented a pattern which was related with the regular inflow of influent and reject wastewater containing dissolved CH₄ into the bioreactor. In WWTPs with anaerobic sludge digestion, CH₄ emissions may be relevant and need attention, as the environmental and economical benefits of biogas production for electricity generation could be counteracted.

Chapter 10

Future perspective

10.1 Recommendations for future research

10.1.1 Exploring CH_4 mitigation strategies

While CH_4 production processes have been extensively described, some of the mechanisms responsible for N_2O production and emissions are still under debate and need further characterization. From this perspective, much effort should be put into lab-scale studies in order to fill the knowledge gaps related with the different N_2O production pathways (section 10.1.2). On the other hand, the potential mitigation of CH_4 and the different strategies for its consumption in wastewater treatment systems needs to be further explored by the scientific community. There is potential for implementing CH_4 consumption in activated sludge systems as proven by modelling and simulation of aerobic CH_4 oxidation, and future research should be directed towards testing this option in full-scale experiments. Alternatively, the continuation of scientific investigations on the different possibilities for CH_4 mitigation on its sources (chemical dosing in sewer systems, optimization of sludge storing in order to avoid leaking, etc) should also be granted. Some of the CH_4 mitigation strategies suggested by the scientific community are ready to be tested in field experiments. Therefore, a closer collaboration between administrations, plant operators and researchers would lead to the minimization of CH_4 emissions in WWTPs in the near future.

10.1.2 Unravelling N_2O production and consumption pathways

During the last years, evidence of different N_2O producing mechanisms has been given as a result of different research studies. Both nitrifier denitrification by AOB, the chemical or biologically-mediated decomposition of nitrification-related compounds (NH_2OH , NOH , etc.) and heterotrophic denitrification have been shown to be responsible of N_2O production under different conditions. The contribution of each of the N_2O -producing pathways is however uncertain, and this issue should be addressed by future research. Another important niche of further research is the potential adaptation of bacterial nitrifying communities to particular non-optimal conditions (i.e. high NO_2^- or NH_4^+ levels, dynamic DO conditions or low temperatures among others) which could lead to lower N_2O emissions. If the factors that promote the adaptation of a certain bacterial community are known, mitigation strategies could be based on the control of these factors. This type of research however, needs the combination of different areas of science such as molecular microbiology, chemistry and engineering for the better understanding of the biochemical processes behind N_2O production.

Additionally, N_2O consumption pathways need also further attention. As shown in this thesis, a potential minimization of N_2O emissions could be based on providing the conditions for heterotrophic denitrifiers to reduce the previously accumulated N_2O in a bioreactor. However, there is a high degree of uncertainty within this area of study. For example, the relative importance of NO_2^- or free nitrous acid (HNO_2) in the inhibition of N_2O reduction during heterotrophic denitrification remains unclear. In addition, the inhibitory effect of transient aerobic or anaerobic conditions on the N_2O reductase in denitrifying bacteria should be object of further research. The better understanding of N_2O reduction through heterotrophic denitrification by different bacterial populations and its control in engineered systems could represent a way to mitigate N_2O emissions to a certain extent. Although the results found in laboratory investigations can't be directly translated to full-scale scenarios, knowledge gained in lab-scale studies may support the understanding of the results released through full-scale monitoring campaigns, and the design of gas mitigation strategies. Lab-scale studies are best suited for unravelling N_2O production and consumption pathways, as full-scale data is too complex and subjected to the cascade effect of interrelated factors. Lab-scale experiments with isolated, enriched or mixed bacterial communities in combination with modern

materials and methods (i.e. isotope tracing, N₂O, NO and NO₂⁻ on-line sensors, etc.) may represent the way forward to clarify some of the concepts that are still under controversy.

10.1.3 GHG emissions quantification and mitigation in full-scale WWTPs

In general, the emission dynamics of both CH₄ and N₂O and their contribution to the C-footprint of real WWTPs is still largely unknown and need to be studied extensively. As proven in this thesis, there is a high potential for GHG emissions minimization during wastewater treatment, although more full-scale applied research is still needed. GHG emission factors reported in scientific literature are highly variable and respond to the specificities of the different processes. Moreover, differences in the monitoring protocols applied are also a source of variability and uncertainty. For example, the application of grab or on-line sampling strategies, or the utilization of one or multiple interconnected gas collection hoods are crucial factors with significant effects on the estimation of gas emissions from a certain system. From this perspective, a unified protocol for full-scale monitoring of fugitive gas emissions should be designed by the scientific community for the water authorities to implement it on a regular basis, in each major WWTP. This would represent a useful tool for future studies on full-scale gas emissions, and would support the implementation of mitigation strategies. Furthermore, default emission factors suggested by the Intergovernmental Panel on Climate Change (IPCC, 2006) are far from being representative of the real emissions from wastewater treatment systems. The performance of standardized systematic full-scale gas monitoring campaigns in each WWTP would also be useful for the calculation of accurate average gas emissions. In many cases, gas emissions may be lower than the standardized factor applied by default. But even in those cases where the emissions are large, monitoring campaigns will lead to the identification of gas production sources, and emissions minimization strategies will be able to be designed and implemented on a case-specific level.

Future full-scale monitoring studies should also aim at identifying new sources of CH₄ and N₂O emissions, such as the settlers, and those would need to be accounted when quantifying gas emissions. To date, not all type of processes have been subjected to research. Gas production and emissions of biofilm-based bioreactors, anaerobic lagoons, constructed wetlands among others, would need future attention. In particular, activated sludge processes performing biological phosphorous removal could be an interesting area of future research, as lab-scale studies with the bacterial communities inhabiting these systems show accumulation of N₂O during the anoxic removal of phosphorus.

Another aspect to be explored in relation with GHG emissions during wastewater treatment is its potential use within the automatic control mechanisms of a WWTP. In particular, N₂O production has been linked with instability in the process of nitrification, as previously reported in this thesis. This could be utilized as an automatic control tool for the warning of nitrification failure in wastewater treatment systems, as described in an exploratory study by Butler et al. (2009). The further development of reliable, durable and affordable N₂O sensors or analysers designed for the continuous measurement of emissions in full-scale facilities and connected to their automatic control systems would support this possibility.

10.2 Global outlook

With an exponentially growing human population, the need for wastewater treatment will tend to increase, together with the consequent increase of green-house gas emissions from WWTPs. The potential emissions of green-house gases should be interpreted by no means as a drawback of wastewater treatment. Wastewater treatment is essential for the environment and the society. The public authorities must provide sufficient financial support for public or private organizations to carry

out wastewater treatment in order to obtain effluents of the highest quality, not only under the legal pollution limits but also ensuring the lowest impact on the natural water recipients. However, green-house gas emissions need to be considered when designing and/or operating wastewater treatment systems, and minimization mechanisms should be implemented. Future imposition of environmental taxes targeting green-house gas emissions during wastewater treatment can be considered as a powerful motivation for plant managers and process operators at wastewater treatment facilities to perform monitoring campaigns and identify the potential gas mitigation strategies in each case-scenario. In the long term, the high carbon footprint (high electricity consumption and green-house gas emissions) exerted by conventional wastewater treatment systems may indicate that wastewater treatment needs to be redesigned in order to meet future sustainability requirements. Concepts such as the decentralization of wastewater treatment networks, ecological sanitation with source separation and nutrient recovery should be considered by scientists and policy makers as possibilities for a sustainable future society.

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