Universitat Autònoma de Barcelona

Departament d'Enginyeria Química
Escola d'Enginyeria

ELIMINACIÓ BIOLÒGICA DE NITROGEN VIA NITRIT D'UN CORRENT AMB ALTA CÀRREGA D'AMONI

(NITRITE PATHWAY BIOLOGICAL NITROGEN REMOVAL OF A HIGH STRENGTH AMMONIUM WASTEWATER)

Memòria de tesi doctoral

Sota la direcció de

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Bellaterra, gener de 2012

Títol: Eliminació biològica de nitrogen via nitrit d'un corrent amb alta càrrega d'amoni (Nitrite pathway biological nitrogen removal of a high strength ammonium wastewater)

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JUAN ANTONIO BAEZA LABAT i JULIÁN CARRERA MUYO, professors agregats del Departament d'Enginyeria Química de la Universitat Autònoma de Barcelona,

CERTIFIQUEM:

Que l'enginyer químic JOSEP ANTON TORÀ SUÁREZ ha dut a terme sota la nostra direcció el treball que, amb títol "Eliminació biològica de nitrogen via nitrit d'un corrent amb alta càrrega d'amoni (Nitrite pathway biological nitrogen removal of a high strength ammonium wastewater)", es presenta en aquesta memòria, i que constitueix la seva Tesi per a optar al Grau de Doctor per la Universitat Autònoma de Barcelona.

I perquè se'n prengui coneixement i consti als efectes oportuns, presentem a l'Escola d'Enginyeria de la Universitat Autònoma de Barcelona l'esmentada Tesi, signant el present certificat a

Bellaterra, gener de 2012

Dr. Juan Antonio Baeza Labat

Dr. Julián Carrera Muyo

als meus pares,

a la meva germana

i a l'Ari

"L'èxit és aprendre a anar de fracàs en fracàs sense perdre l'entusiasme."

Winston Churchill

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Índex

Llista de publicacions

I Torà, J. A., Lafuente, J., Carrera, J. and Baeza, J. A.
Fast start-up and controlled operation during a long term period of a high-rate partial nitrification activated sludge system
[Environmental Technology. 2011; doi: 10.1080/09593330.2011.626802]

II Torà, J. A., Baeza, J. A., Carrera, J. and Oleszkiewicz, J. A.
Denitritation of a high-strength nitrite wastewater in a sequencing batch reactor using different organic carbon sources
Chemical Engineering Journal. 2011; Volume 172, Issues (2-3), Pages 994-998

III Torà, J. A., Lafuente, J., Baeza, J. A. and Carrera, J.
Long-term starvation and subsequent reactivation of a high-rate partial nitrification activated sludge pilot plant
Bioresource Technology. 2011; Volume 102, Issue 21, Pages 9870-9875

IV Torà, J. A., Lafuente, J., Baeza, J. A. and Carrera, J.
Combined effect of inorganic carbon limitation and inhibition by free ammonia and free nitrous acid on ammonia oxidizing bacteria
Bioresource Technology. 2010; Volume 101, Issue 15, Pages 6051-6058

Resum

En aquesta tesi s'ha estudiat l'eliminació biològica de nitrogen en aigües residuals amb alta càrrega d'amoni. Aplicant certes condicions d'operació en un sistema de llots actius es pot aconseguir la nitrificació parcial o nitritació, que és l'oxidació de l'amoni a nitrit, evitant la conseqüent oxidació d'aquest nitrit a nitrat. Aquesta reducció en el procés de nitrificació aporta un seguit d'avantatges en front de la nitrificació convencional, tals com la reducció de les necessitats d'oxigen en la nitrificació i de la matèria orgànica en la desnitrificació, l'increment de la velocitat de desnitrificació i la reducció de la producció de biomassa.

Aquesta tesi s'ha presentat com a compendi de publicacions. A continuació es presenta un breu resum dels articles:

En l'**Article I** es presenta un sistema de nitrificació parcial format per una planta pilot amb tres reactors de fangs actius en sèrie. Aquesta planta pilot es va inocular a partir de llots d'una EDAR urbana i es va operar amb un llaç de control de la càrrega de nitrogen aplicada que consisteix en la modificació del cabal d'entrada segons els valors d'OUR mesurat als reactors. Utilitzant aquest llaç de control i treballant amb un temps de residència cel·lular baix es va aconseguir rentar els bacteris nitrit-oxidants del sistema. Simultàniament, també es va aconseguir tractar una elevada càrrega d'amoni i es va demostrar la viabilitat del procés a llarg termini tot obtenint una nitrificació parcial amb pràcticament només nitrit.

Posteriorment, en l'**Article II**, es presenta la desnitrificació del corrent de nitrit obtingut amb el sistema desenvolupat en l'Article I. Aquesta desnitrificació es va realitzar utilitzant diferents fonts de carboni tals com etanol, glicerol, lixiviats d'abocador i finalment llots primaris i secundaris fermentats. Aquest estudi es va dur a terme en reactors discontinus seqüencials (SBR) i es van obtenir bones velocitats de desnitrificació per totes les fonts de carboni estudiades excepte pels llots secundaris fermentats.

En l'**Article III**, es presenta l'estudi de l'efecte d'una llarga aturada del sistema de nitrificació parcial tot deixant d'alimentar durant 30 dies. Aquest estudi es va realitzar en quatre reactors discontinus que es van operar en diferents condicions d'aeració. Durant aquest període de temps es va fer un seguiment de l'activitat dels bacteris amoni-oxidants mitjançant respirometries i l'anàlisi FISH. Es va observar que és millor aturar el sistema de nitrificació parcial en condicions anòxiques i, a poder ser, no més de dues setmanes. Finalment, es va obtenir una recuperació ràpida del sistema utilitzant el llaç de control per OUR emprat en la planta pilot de l'Article I.

Finalment, en l'**Article IV** es presenta l'estudi de l'efecte de les inhibicions per amoníac i per àcid nitrós en els bacteris amoni-oxidants, a més de l'efecte d'aquestes inhibicions en condicions de limitació per carboni inorgànic. Es va comprovar que la inhibició per amoníac es pot descriure amb precisió amb al model cinètic Haldane i la inhibició per àcid nitrós a un model d'inhibició no competitiva. Es va observar que l'efecte d'aquestes inhibicions s'incrementa en condicions de limitació per carboni inorgànic, sent molt més gran en el cas de l'àcid nitrós.

Summary

Biological nitrogen removal of high-strength ammonium wastewater was studied in this thesis. Applying specific operational conditions in an activated sludge system, partial nitrification or nitritation (oxidation of ammonium to nitrite) was achieved, avoiding the consequently oxidation of this nitrite to nitrate. This reduction in the nitrification process provides some advantages in comparison to the complete nitrification process, such as the reduction of the oxygen requirements during nitrification and the organic matter during denitrification, the increasing of the denitrification rates and the reduction of the biomass production.

This thesis was presented as a compendium of publications and a brief summary of the papers are presented:

In **Paper I**, a partial nitrification system consisting of a pilot plant with three continuous stirred tank reactors in series is presented. This pilot plant was inoculated with sludge from a municipal WWTP and it was operated with a control loop of the nitrogen loading rate which was applied modifying the inflow rate depending on the OUR values measured in the reactors. With this control loop and low sludge retention time the washout of the nitrite-oxidizing bacteria was performed. Simultaneously, almost a complete partial nitrification to nitrite with a high nitrogen loading rate was achieved during a long term operation.

Subsequently, in **Paper II**, the heterotrophic denitrification of the nitrite obtained with the pilot plant developed in Paper I was presented. Different organic carbon sources such as ethanol, glycerol, landfill leachate, fermented primary sludge centrate and fermented secondary sludge centrate were used in the heterotrophic denitrification. This study was carried out in sequential batch reactors (SBR) and a complete denitritation of a high-strength nitrite wastewater was achieved using these organic carbon sources with the exception of fermented secondary sludge centrate.

In **Paper III** the study of the effect of a long-term starvation of a partial nitrification system during 30 days was presented. Four ammonium-starved reactors under different

conditions of aeration were used. During this period the ammonia-oxidizing bacteria activity was evaluated using respirometric tests and the FISH analysis. It was observed that is better to shut-down a partial nitrification system under anoxic conditions and, if it is possible, no more than two weeks. Finally, a fast recovery of the system was achieved using the OUR control loop used in the pilot plant of Paper I.

Finally, in **Paper IV** a study of the inhibitory effect by free ammonia and free nitrous acid on the ammonia-oxidizing bacteria under total inorganic carbon limitations and without total inorganic carbon limitations was presented. It was observed that the inhibition by free ammonia can be described accurately using the Haldane model and the inhibition by free nitrous acid using a non-competitive inhibition model. The effect of these inhibitions increased under total inorganic carbon limitation, being much higher in the case of the free nitrous acid.

Resumen

En esta tesis se estudia la eliminación biológica de nitrógeno en aguas residuales con una alta carga de amonio. Aplicando ciertas condiciones de operación en un sistema de lodos activos se puede conseguir la nitrificación parcial o nitritación, que es la oxidación del amonio a nitrito, evitando la consecuente oxidación de este nitrito a nitrato. Esta reducción en el proceso de nitrificación aporta unas ventajas frente a la nitrificación convencional, tales como la reducción de les necesidades de oxigeno en la nitrificación y de la materia orgánica en la desnitrificación, el incremento de la velocidad de desnitrificación y la reducción de la producción de biomasa.

Esta tesis se presenta como compendio de publicaciones. A continuación se presenta un breve resumen de los artículos:

En el **Artículo I** se presenta un sistema de nitrificación parcial formado por una planta piloto con tres reactores de lodos activos en serie. Esta planta piloto se inoculó a partir de lodos de una EDAR urbana y se operó con un lazo de control de la carga de nitrógeno aplicada que consiste en la modificación del caudal de entrada según los valores de OUR medidos en los reactores. Utilizando este lazo de control y trabajando con un tiempo de residencia celular bajo se consiguió lavar las bacterias nitrito-oxidantes del sistema. Simultáneamente, también se consiguió tratar una elevada carga de amonio y se demostró la viabilidad del proceso a largo plazo obteniendo una nitrificación parcial con prácticamente solo nitrito.

Posteriormente, en el **Artículo II**, se presenta la desnitrificación de la corriente de nitrito obtenida con el sistema desarrollado en el Artículo I. Esta desnitrificación se realizó utilizando distintas fuentes de carbono tales como etanol, glicerol, lixiviados de vertedero y finalmente lodos primarios y secundarios fermentados. En este estudio se utilizaron reactores discontinuos secuenciales (SBR) y se obtuvieron buenas velocidades de desnitrificación para todas las fuentes de carbono estudiadas excepto para los lodos secundarios fermentados.

En el **Artículo III**, se presenta el estudio del efecto de una larga parada del sistema de nitrificación parcial dejando de alimentar durante 30 días. Este estudio se realizó en cuatro reactores discontinuos que se operaron en distintas condiciones de aireación. Durante este período de tiempo se hizo un seguimiento de la actividad de las bacterias amonio-oxidantes utilizando técnicas respirométricas y el análisis FISH. Se observó que es mejor parar el sistema de nitrificación parcial en condiciones anóxicas y, a poder ser, no más de dos semanas. Finalmente, se consiguió una recuperación rápida del sistema utilizando el lazo de control por OUR utilizado en la planta piloto del Artículo I.

Finalmente, en el **Artículo IV** se presenta el estudio del efecto de las inhibiciones por amoníaco y por ácido nitroso en las bacterias amonio-oxidantes, además del efecto de estas inhibiciones en condiciones de limitación por carbono inorgánico. Se comprobó que la inhibición por amoníaco se puede ajustar con precisión utilizando el modelo cinético Haldane y la inhibición por ácido nitroso utilizando el modelo de inhibición no competitiva. Se observó que el efecto de estas inhibiciones se incrementa en condiciones de limitación por carbono inorgánico, siendo mucho mayor en el caso del ácido nitroso.

Capítol 1

Introducció

1 Introducció

1.1 Visió general

Els compostos nitrogenats es troben entre els compostos contaminants més importants de les aigües residuals, sobretot degut a la seva importància en l'eutrofització, al seu efecte en la concentració d'oxigen de les masses aquàtiques i a la seva toxicitat sobre els éssers vius (Dodds et al. 2009). Per tal de protegir el medi ambient, la Unió Europea exigeix l'acompliment d'unes estrictes condicions per l'abocament de nutrients en les aigües residuals (Directiva 91/271/CEE). Així els nivells de matèria orgànica biodegradable dels rius europeus s'han reduït entre un 20 i un 30% des de la dècada dels 90, les concentracions de fòsfor han disminuït entre un 30 i un 40% i les de nitrogen amoniacal al voltant del 40% (EEA 2002). Tot i així, els nivells de nitrogen en els rius europeus s'han mantingut elevats, resultat de la incidència de l'agricultura i de l'encara insuficient eliminació de nitrogen en les estacions depuradores d'aigües residuals (EDARs). Moltes d'aquestes EDARs van ser dissenyades anteriorment a aquesta directiva i només estaven pensades per eliminar matèria orgànica, per tant, s'han hagut de modificar posteriorment per eliminar també els compostos nitrogenats (Henze et al. 2008).

Una de les fonts més importants de contaminació per nitrogen són les aigües residuals que contenen una alta concentració d'aquests compostos, per tant una forma de reduir la contaminació és realitzant un tractament específic d'aquests tipus d'aigua residual.

1.2 Aigües residuals amb alta càrrega de nitrogen

S'anomenen així les aigües residuals que contenen una alta concentració de compostos nitrogenats (generalment amoni). Aquestes aigües es poden classificar en dos grans tipus:

- Aigües residuals urbanes
- Aigües residuals industrials

1.2.1 Aigües residuals urbanes

Les aigües residuals urbanes generalment no tenen una gran concentració de compostos nitrogenats, tot i així en les EDARs hi ha un corrent que prové de l'assecament dels fangs digerits en el digestor anaerobi que conté una alta concentració d'amoni. Aquesta aigua, anomenada aigua de rebuig, és un corrent intern de l'EDAR que pot contenir fins a un percentatge entre el 15 i el 30% del nitrogen total d'entrada a l'EDAR i, d'altra banda, només representa entre l'1 i el 2% del cabal total. Actualment, aquesta aigua de rebuig es recircula a la capçalera de l'EDAR, tot augmentant la quantitat de nitrogen a eliminar. Degut a les característiques específiques (concentració d'amoníac, demanda química d'oxigen (DQO), alcalinitat, pH i temperatura), es pot estudiar la realització d'un tractament específic de l'aigua de rebuig, el qual permetria eliminar una gran part de la càrrega total de nitrogen a l'EDAR. A la Figura 1 es pot observar l'esquema d'una EDAR i el corrent de l'aigua de rebuig en el qual s'hauria d'aplicar el tractament específic.

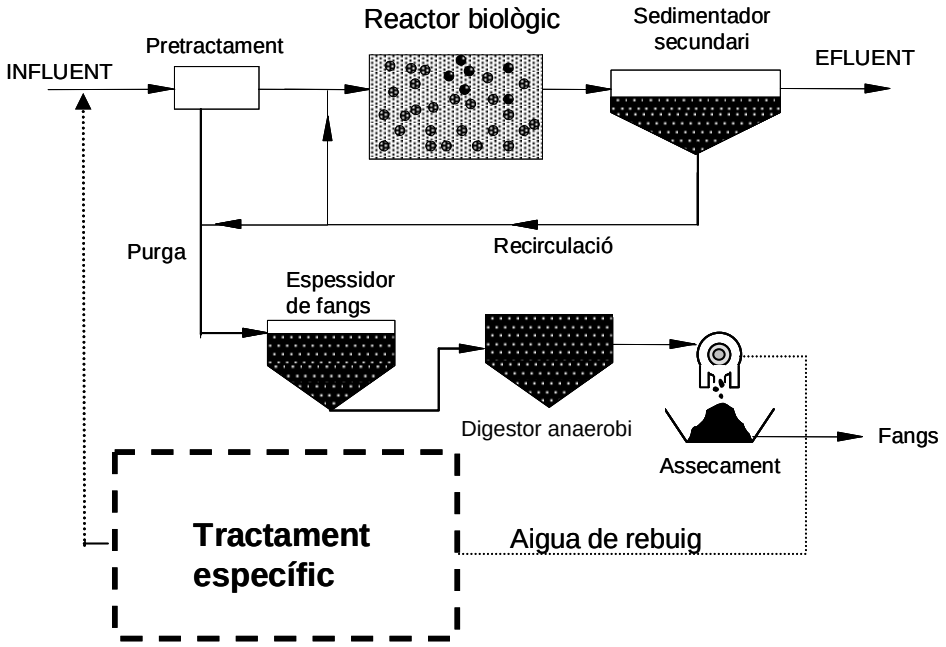

Figura 1. Esquema d'una EDAR convencional i situació del possible tractament específic de l'aigua de rebuig.

Diversos estudis han demostrat que en molts casos és més avantatjós incrementar la capacitat d'eliminació de nitrogen en una EDAR urbana mitjançant l'optimització del procés existent i la instal·lació de tècniques complementàries (tals com el tractament específic de l'aigua de rebuig) que no pas ampliar el procés existent (Janus and Van Der

Roest 1997; Wett et al. 1998; Fux and Siegrist 2004; Wyffels et al. 2004; Carrera et al. 2011).

1.2.2 Aigües residuals industrials

Hi ha diverses activitats industrials que generen aigües residuals amb un alt contingut d'amoni: petroquímica, farmacèutica, indústria de fertilitzants, alimentària, etc. Aquestes aigües residuals, que també han de ser tractades prèviament al seu abocament, tenen una problemàtica afegida, i és que sovint experimenten canvis en el cabal o en les seves característiques (Wun Jern 2006; Sipma et al. 2010). Aquests canvis en les aigües residuals sovint estan relacionats amb períodes de baixa activitat industrial o fins i tot amb l'aturada total de les indústries, ja sigui per tal de realitzar operacions de manteniment o per períodes de vacances. Aquestes variacions en la producció poden perjudicar seriosament als sistemes de tractament biològic, sobretot pel que fa a la seva capacitat, podent arribar a la pèrdua total d'activitat en períodes prolongats d'aturada.

1.3 Tractaments per a l'eliminació del nitrogen de les aigües residuals

L'eliminació dels compostos nitrogenats de les aigües residuals pot realitzar-se mitjançant una gran varietat de processos, que poden ser tan biològics com fisicoquímics. No obstant, com que l'eliminació biològica és més eficaç i té uns costos menors, és el tractament més utilitzat (US-EPA 1993; Teichgräber and Stein 1994).

Les principals tecnologies per a l'eliminació biològica del nitrogen són les següents:

- Nitrificació i desnitrificació convencional
- Nitrificació parcial i desnitrificació via nitrit
- Nitrificació parcial i anammox
- Nitrificació i desnitrificació autòtrofa

1.3.1 Nitrificació i desnitrificació convencional

Aquesta és la tecnologia més desenvolupada i aplicada arreu del món per a l'eliminació biològica de nitrogen. Aquest procés es divideix en dues parts, que són la nitrificació i la posterior desnitrificació.

La nitrificació és l'oxidació biològica de l'amoni fins a nitrat i es produeix en condicions estrictament aeròbies. A més a més, la nitrificació succeeix en dues fases d'oxidació consecutives: l'oxidació de l'amoni fins a nitrit (nitritació) i la posterior oxidació del nitrit fins a nitrat (nitratació) (Wiesmann 1994). Cadascuna d'aquestes fases d'oxidació està realitzada per un tipus de bacteri diferent, la nitritació pels bacteris amoni oxidants (*ammonia oxidizing bacteria*, AOB) i la nitratació pels bacteris nitrit oxidants (*nitrite oxidizing bacteria*, NOB). Aquests bacteris utilitzen l'amoni o el nitrit com a font d'energia, l'oxigen com a acceptor d'electrons i el diòxid de carboni com a font de carboni. El procés de nitrificació és un procés que consumeix alcalinitat, i en alguns casos, quan les aigües residuals no contenen l'alcalinitat necessària per oxidar tot l'amoni, aquesta alcalinitat s'ha de subministrar externament.

El segon pas, la desnitrificació, implica la reducció biològica del nitrat produït anteriorment fins a nitrogen gas (N_2). Igual que la nitrificació, aquest pas es realitza en diferents fases, que són la reducció del nitrat fins a nitrit, la posterior reducció del nitrit a òxid nítric, posteriorment a òxid nitrós, i finalment la reducció de l'òxid nitrós a nitrogen gas (Gujer et al. 1999). El procés de desnitrificació és realitzat per bacteris heteròtrofs en condicions anòxiques, que utilitzen el nitrat enlloc de l'oxigen com a acceptor d'electrons i matèria orgànica com a font de carboni i d'energia. Normalment, en aigües industrials que contenen una baixa relació DQO/N la desnitrificació està limitada per la manca de font de carboni i aquesta s'ha de subministrar externament. Aquesta font de carboni ha de ser biodegradable i es pot utilitzar un ampli rang de productes, tals com àcid acètic, metanol, etanol, glucosa, etc, o fins i tot altres fonts de carboni que presentin una alta relació DQO/N que poden provenir d'altres aigües residuals (Ahn 2006). Per elegir la millor font de carboni s'ha de tenir en compte la velocitat de desnitrificació que es pot assolir amb cadascuna d'elles, el cost i la seva disponibilitat (Carrera et al. 2003). L'addició d'aquesta font de carboni suposa un increment important en els costos de tractament de l'aigua residual.

En general, la nitrificació i desnitrificació convencional consumeixen una considerable quantitat de recursos, ja que es necessiten 4.57 kg d'O_2 i entre 2 i 4 kg de DQO per cada kg de nitrogen en forma d'amoni eliminat. Aquestes quantitats inclouen l'oxigen que es necessita durant la nitrificació i l'addició de la font de carboni externa durant la desnitrificació (Paredes et al. 2007).

Les reaccions de nitrificació i desnitrificació convencional sense considerar la formació de biomassa són les següents (Wiesmann 1994):

- Nitrificació

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \quad (1)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (2)$$

- Desnitrificació utilitzant acetat com a font de carboni

$$8\ NO_3^- + 5\ CH_3COOH \rightarrow 4\ N_2 + 10\ CO_2 + 6\ H_2O + 8\ OH^- \quad (3)$$

Aquests dos processos biològics es poden dur a terme en reactors de biomassa en suspensió o en reactors de biomassa immobilitzada o biopel·lícules. A més, tots aquests reactors poden treballar en continu o en discontinuo (tipus *Sequencing Batch Reactor, SBR*).

Aquesta tesi es centra en l'estudi d'un sistema de biomassa en suspensió en continu (fangs actius). En fangs actius, la tecnologia de nitrificació i desnitrificació convencional es pot implementar en dos tipus de sistemes: el sistema d'un llot (*single-sludge*) o el sistema de dos llots (*two-sludge*). En el sistema d'un llot (Figura 2a) hi ha dos reactors, un anòxic i un aerobi els quals contenen la mateixa biomassa. Al final del sistema hi ha un sedimentador que permet recircular part de la biomassa al reactor anòxic. Els principals inconvenients d'aquest sistema són: (a) que part de la biomassa no es troba en condicions òptimes d'operació (els microorganismes heteròtrofs es troben sense substrat en el reactor aerobi i els nitrificants sense oxigen en el reactor anòxic), (b) la presència d'oxigen procedent de la recirculació interna en el reactor anòxic i finalment (c) la presència de nitrat en l'efluent. D'altra banda, si l'aigua a tractar conté la suficient DQO, aquest sistema no requereix l'addició d'una font de carboni externa ja que en el reactor anòxic entra DQO procedent de l'aliment. D'altra banda, també permet l'estalvi d'una gran quantitat d'alcalinitat externa ja que en el reactor anòxic es produeix la meitat de l'alcalinitat que és consumida posteriorment en el reactor aerobi.

D'altra banda, el sistema de dos llots manté els dos processos separats (Figura 2b), cadascun amb el seu propi sedimentador i la seva recirculació independent. Aquest sistema és més eficaç per al tractament d'aigües residuals amb alta càrrega de nitrogen que el sistema d'un llot ja que pot assolir velocitats de nitrificació i desnitrificació més elevades i, per tant, requereix menor volum dels reactors (Carrera et al. 2003; Carrera et al. 2004a). No obstant, aquest sistema de dos llots només es pot aplicar a aigües residuals amb una baixa relació DQO/N i requereix de l'adició d'una font externa d'alcalinitat en el reactor aerobi i d'una font externa de carboni en el reactor anòxic.

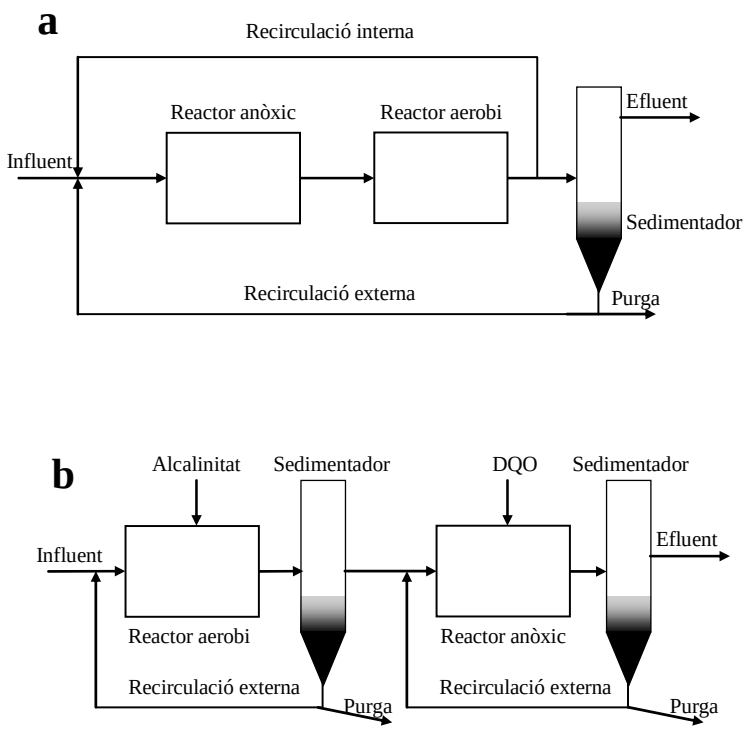

Figura 2. a) Esquema d'un sistema d'un llot. b) Esquema d'un sistema de dos llots.

1.3.2 Eliminació de nitrogen via nitrit

Tal i com s'ha comentat anteriorment en la nitrificació i desnitrificació convencional, el nitrit és un producte intermedi, tant del procés de nitrificació com del procés de desnitrificació. Per tant aquesta tecnologia consisteix en reduir el procés de nitrificació d'una reacció de dos passos (nitritació i nitratació) a tan sols un pas, el de nitritació. Aquest nou procés rep el nom de nitrificació parcial. Posteriorment aquest nitrit és reduït a nitrogen gas mitjançant la desnitrificació des de nitrit o desnitritació. El procés global de nitritació i desnitritació rep el nom d'eliminació de nitrogen via nitrit.

Les reaccions de la nitrificació i desnitrificació via nitrit sense considerar la formació de biomassa són les següents (Henze et al. 2008):

- Nitritació

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \quad (1)$$

- Desnitritació utilitzant acetat com a font de carboni

$$8\ NO_2^- + 3\ CH_3COOH \rightarrow 4\ N_2 + 6\ CO_2 + 2\ H_2O + 8\ OH^- \quad (4)$$

Comparant les reaccions anteriors de nitrificació i desnitrificació convencional (1)-(3) i les de nitritació i desnitritació (1) i (4) s'observa que el fet de modificar el procés convencional i reduir l'oxidació de l'amoni a nitrit, sense arribar a nitrat, comporta: (a) una reducció del 25% dels requeriments d'oxigen necessaris per a la nitrificació, (b) una reducció entre el 30 i el 40% de la matèria orgànica necessària per a la desnitrificació i a més (c) com a resultat d'evitar la formació de nitrat, es produeix un 40% menys de biomassa (Turk and Mavinic 1987; Van Hulle et al. 2010). A més, les velocitats de desnitrificació des de nitrit són entre 1.5 i 2 vegades més ràpides que les velocitats de desnitrificació des de nitrat (Peng and Zhu 2006; Aslan and Dahab 2008).

No obstant això, no és fàcil suprimir la nitratació, i s'ha de fer afectant el menys possible a la nitritació. D'altra banda, s'ha reportat que tot i aconseguir evitar la nitratació durant un cert període de temps, és difícil de mantenir aquesta supressió durant un llarg període de temps (Villaverde et al. 2000; Yun and Kim 2003; Fux et al. 2004; Ma et al. 2009). Els mètodes de selecció de la nitritació en front de la nitratació es fonamenten en el fet que els AOB i NOB tenen diferents característiques de resposta a factors ambientals (Ruiz et al. 2003; Jubany et al. 2009a). Els factors que tenen major influència per aconseguir la nitrificació parcial són els següents:

- Temperatura

Els AOB i els NOB presenten diferent variació de la velocitat de creixement en funció de la temperatura, així doncs, a temperatures per sota dels 20ºC els NOB creixen a major velocitat que els AOB, però aquesta tendència s'inverteix per temperatures superiors als 20ºC (Hunik et al. 1994). El procés SHARON es fonamenta en aquesta diferent influència de la temperatura sobre les velocitats de creixement. La velocitat de creixement dels AOB és aproximadament el doble que la dels NOB a 35ºC (Bougard et

al. 2006). Així doncs, aquest sistema treballa a 35ºC amb un reactor de mescla completa sense retenció de biomassa. D'aquesta manera, el temps de residència cel·lular (TRC) és igual al temps de residència hidràulic (TRH), i fixant un TRH adequat es pot aconseguir rentar els NOB del sistema tot mantenint els AOB en el reactor (Hellinga et al. 1998; Mosquera-Corral et al. 2005). No obstant això, aquest procés només és viable si es treballa a temperatures entre 30-35ºC.

- Concentració d'oxigen dissolt

S'ha trobat que els NOB tenen una menor afinitat per l'oxigen que els AOB. Això es reflecteix en el valor del coeficient d'afinitat per l'oxigen que és menor pels AOB que pels NOB (Guisasola et al. 2005). Aquest fet pot ser aprofitat per aconseguir la nitrificació parcial limitant l'activitat dels NOB amb baixes concentracions d'oxigen dissolt (OD). Malgrat això, en sistemes de biomassa en suspensió és pràcticament impossible eliminar completament l'activitat NOB només amb una disminució de la concentració d'OD (Ruiz et al. 2003; Wang and Yang 2004; Wyffels et al. 2004). En canvi, en sistemes de biopel·lícula s'ha demostrat que el factor clau per aconseguir una nitrificació parcial estable i completa és la limitació per oxigen dels NOB (Bartroli et al. 2010).

- Inhibicions

Els bacteris AOB i NOB poden ser inhibits per les formes no ionitzades dels seus propis substrats (amoníac i àcid nitrós) tal com es mostra a l'esquema de la Figura 3 (Anthonisen et al. 1976).

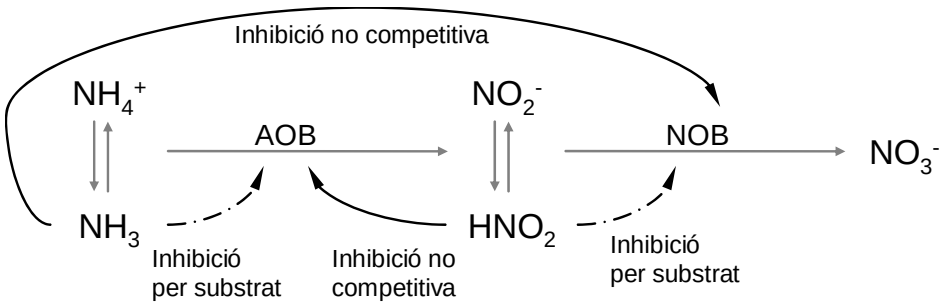

Figura 3. Diagrama de les inhibicions dels AOB i dels NOB per amoníac i àcid nitrós.

Les inhibicions dels AOB per amoníac i dels NOB per àcid nitrós són inhibicions per substrat i normalment es modelitzen mitjançant l'equació de Haldane (Equació 5).

D'altra banda les inhibicions dels AOB per àcid nitrós i dels NOB per amoníac són inhibicions no competitives i normalment es modelitzen amb l'Equació 6.

$$r = \frac{r_{\max} \cdot S}{K_S + S + \frac{S^2}{K_I}} \quad (5)$$

$$r = r_{\max} \cdot \frac{K_I}{K_I + I} \quad (6)$$

On r és la velocitat de consum del substrat, r_{max} és la màxima velocitat de consum del substrat, S és la concentració de substrat, K_S és la constant d'afinitat pel substrat, K_I és la constant d'inhibició del compost inhibitori i I és la concentració del compost inhibitori.

Anthonisen et al. (1976) van demostrar que els NOB s'inhibeixen a concentracions d'amoníac entre 0.1 i 1.0 mg L^{-1}, i els AOB entre 10 i 150 mg L^{-1}. Per tant, els NOB es veuran molt més afectats per concentracions altes d'amoníac. Tot i així, el fet d'aconseguir la nitrificació parcial utilitzant la major inhibició dels NOB té possibles inconvenients, com ara la possibilitat de que els AOB també s'inhibeixin per una gran acumulació d'amoníac o d'àcid nitrós (Carrera et al. 2004b) i la possibilitat de que els NOB, amb el temps, s'aclimatin a l'amoníac tot deixant d'estar inhibits (Turk and Mavinic 1989). No obstant això, s'ha demostrat experimentalment que es pot aconseguir una nitrificació parcial estable en un sistema de fangs actius afavorint la inhibició per amoníac a un pH elevat utilitzant més d'un reactor en sèrie (Jubany et al. 2009a).

1.3.3 Nitrificació parcial i Anammox

Aquest procés és el més innovador de tots els presentats a l'inici del capítol. El primer pas consisteix en oxidar només el 50% de l'amoni d'entrada fins a nitrit, tot mantenint el 50% restant sense oxidar. Aquest pas té certes diferències respecte la nitrificació parcial comentada anteriorment ja que en el sistema anterior es pretén oxidar el 100% de l'amoni fins a nitrit. Per tal d'obtenir un corrent amb una relació amoni-nitrit al 50% es pot utilitzar un sistema de nitrificació parcial on es tracti la meitat del cabal d'aigua residual oxidant tot l'amoni fins a nitrit. L'altra meitat del cabal seria derivat i mesclat amb l'efluent del sistema nitrificant. Una altra alternativa és obtenir directament el 50% de nitrificació parcial en un reactor nitrificant. Això s'aconsegueix, generalment,

limitant la capacitat nitrificant del sistema per manca d'alcalinitat. Si l'aigua d'entrada només conté la meitat de l'alcalinitat necessària per compensar tots els protons produïts en l'oxidació de l'amoni, el pH baixarà a partir d'haver oxidat la meitat de l'amoni present, fent cinèticament impossible l'oxidació de la resta d'amoni (Van Dongen et al. 2001; Okabe et al. 2011; Zhang et al. 2011). D'altra banda, és conegut que la nitrificació és un procés que es troba limitat per carboni inorgànic (Wett and Rauch 2003; Guisasola et al. 2007) i, per tant, aquestes condicions d'operació podrien fer disminuir la velocitat de nitrificació. A més, s'hauria d'estudiar l'efecte de les inhibicions d'amoníac i d'àcid nitrós en condicions de limitació per carboni inorgànic.

El segon pas d'aquesta tecnologia (procés Anammox) és realitzat per uns bacteris autòtrofs capaços d'oxidar l'amoni a nitrogen gas en condicions anòxiques utilitzant el nitrit com a acceptor d'electrons. En aquest procés no es necessita afegir una font de carboni orgànic i aproximadament un 10% del nitrogen es transforma a nitrat. L'estequiometria del procés Anammox és la següent (Strous et al. 1998):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (7)$$

Aquest procés té una sèrie d'avantatges importants (Strous et al. 1998; Liu et al. 2008):

- Al requerir només l'oxidació del 50% de l'amoni de l'aigua residual, la demanda d'oxigen es redueix considerablement.
- Al ser una desnitrificació autòtrofa no es necessita afegir una font de carboni externa per aigües amb baixa relació DQO/N.
- La producció de biomassa és baixa ja que el rendiment biomassa/substrat dels bacteris Anammox és molt baix. Això disminueix considerablement els costos de tractament de fangs.

Tot i els avantatges comentats anteriorment, el creixement extremadament lent d'aquests bacteris (tenen un temps de duplicació d'11 dies (Strous et al. 1998)) i la dificultat de trobar un inòcul amb prou presència d'aquests microorganismes, fan que la posada en marxa d'un reactor Anammox sigui lenta. En el primer reactor Anammox que es va construir a escala industrial a Rotterdam (Holanda), tot i que es va inocular amb biomassa Anammox procedent d'una planta pilot, la posada en marxa va durar entre 3 i

4 anys (van der Star et al. 2007). Tot i així, actualment hi ha onze reactors Anammox operant a escala industrial arreu del món (Paques 2011). Una altra problemàtica que presenta aquest procés és la inhibició per substrat dels bacteris Anammox a baixes concentracions de nitrit. La inhibició s'inicia a partir de 20-30 mg $N\text{-}NO_2^-$ L^{-1} i és pràcticament total a 70 mg $N\text{-}NO_2$ L^{-1} (Schmidt et al. 2003; Li et al. 2004). A més a més, també s'ha observat inhibició per oxigen a concentracions per sobre de 0.042 mg L^{-1} (Schmidt et al. 2003; Kuenen 2008).

Tot i així, s'han desenvolupat un cert nombre de processos que combinen la nitritació amb el procés Anammox i que es poden agrupar en sistemes d'una i dues biomasses.

- Sistema de dues biomasses

El procés Anammox necessita un influent amb una relació amoni/nitrit de 1:1.3, per tant es necessita un reactor de nitrificació parcial capaç de produir un efluent amb aquestes característiques. Actualment el més utilitzat, sobretot a escala industrial, és el procés SHARON/Anammox (Van Dongen et al. 2001; Hwang et al. 2005). No obstant això, s'ha reportat que el procés SHARON/Anammox està limitat per la màxima càrrega que pot tractar-se en el reactor SHARON (Jaroszynski and Oleszkiewicz 2011). Per tant, s'han de dissenyar sistemes de nitrificació parcial que redueixin aquesta limitació amb un augment de la càrrega tractada, com per exemple amb reactors continus de tanc agitat amb sedimentador (Ciudad et al. 2005), reactors discontinus (Ganigue et al. 2009), reactors airlift amb biomassa granular (Bartroli et al. 2010), reactors airlift amb biopel·lícula (Furukawa et al. 2009) o reactors de llit fluïditzat (Qiao et al. 2010).

- Sistema d'una biomassa

Aquest sistema consisteix en un sol reactor granular airejat que és capaç de fer la nitritació simultàniament amb procés Anammox. Això s'aconsegueix perquè la part interna dels grànuls està ocupada per bacteris Anammox i l'externa per bacteris AOB. Els sistemes més utilitzats són: CANON (eliminació autòtrofa completa del nitrogen via nitrit) (Sliekers et al. 2002; Third et al. 2005) i OLAND (nitrificació i desnitrificació autòtrofa per limitació d'oxigen) (Kuai and Verstraete 1998; Windey et al. 2005). Aquests sistemes es fonamenten en subministrar oxigen al procés per tal que l'oxidació de l'amoni només arribi a nitrit. Llavors, degut a la falta d'un acceptor d'electrons, el nitrit és consumit pels bacteris Anammox amb la resta d'amoni.

Els principals inconvenients i avantatges de cadascun dels dos sistemes es troben resumits en diferents treballs de recopilació (Van Hulle et al. 2010; Jaroszynski and Oleszkiewicz 2011).

1.3.4 Nitrificació i desnitrificació autòtrofa

El primer pas d'aquesta tecnologia és la nitrificació convencional comentada anteriorment, on l'amoni és oxidat a nitrat. Posteriorment aquest nitrat s'ha de reduir a nitrogen gas tot utilitzant com a font d'energia hidrogen (Lee and Rittmann 2002; Mansell and Schroeder 2002; Rezania et al. 2007) o compostos derivats del sofre, tals com H_2S, S, $S_2O_3^{2-}$, $S_4O_6^{2-}$ o SO_3^{2-} (Trouve et al. 1998; Kimura et al. 2002; Kleerebezem and Mendez 2002; Soares 2002). Al tractar-se de bacteris autòtrofs utilitzen CO_2 com a font de carboni. Les equacions estequiomètriques de desnitrificació autòtrofa utilitzant hidrogen (Lee and Rittmann 2002) i els diferents compostos derivats del sofre (Campos et al. 2008) es presenten a continuació:

$$NO_3^- + 3.03\ H_2 + H^+ + 0.229\ CO_2 \rightarrow 0.0458\ C_5H_7O_2N + 0.477\ N_2 + 3.37\ H_2O \quad (8)$$

$$0.844\ S_2O_3^{2-} + NO_3^- + 0.347\ CO_2 + 0.086\ HCO_3^- + 0.086\ NH_4^+ + 0.434\ H_2O \rightarrow 1.689\ SO_4^{2-} + 0.500\ N_2 + 0.086\ C_5H_7O_2N + 0.697\ H^+ \quad (9)$$

$$0.421\ H_2S + 0.421\ HS^- + NO_3^- + 0.346\ CO_2 + 0.086\ HCO_3^- + 0.086\ NH_4^+ \rightarrow 0.842\ SO_4^{2-} + 0.500\ N_2 + 0.086\ C_5H_7O_2N + 0.434\ H_2O + 0.262\ H^+ \quad (10)$$

$$1.10\ S^0 + NO_3^- + 0.76\ H_2O + 0.40\ CO_2 + 0.08\ NH_4^+ \rightarrow 1.10\ SO_4^{2-} + 0.50\ N_2 + 0.08\ C_5H_7O_2N + 1.28\ H^+ \quad (11)$$

Comparada amb la desnitrificació heteròtrofa convencional, la desnitrificació autòtrofa té dos grans avantatges: (1) no requereix d'una font externa de carboni orgànic com per exemple metanol o etanol, que incrementarien els costos d'operació; i (2) es produeix una menor quantitat de llots (Claus and Kutzner 1985; Zhang and Lampe 1999). Últimament s'ha donat més importància a la desnitrificació autòtrofa amb productes derivats del sofre que a la que utilitza hidrogen, degut al preu i a la dificultat de la utilització d'aquest últim. D'altra banda, com es pot observar en les equacions (9)-(11), la desnitrificació autòtrofa amb derivats del sofre incrementa la concentració de sulfats en l'aigua residual i consumeix alcalinitat.

Com que una possible aplicació d'aquests processos és el tractament d'una aigua residual amb una alta concentració de nitrogen amoniacal, durant el primer pas de nitrificació es produirà una gran quantitat de nitrat. Posteriorment, el procés de desnitrificació autòtrofa amb productes derivats del sofre, aquesta alta concentració de nitrat produirà una important quantitat de sulfats que poden esdevenir un problema de producció de sulfit en condicions anaeròbies.

A més, el consum d'alcalinitat, tant en el procés de nitrificació, com en el procés de desnitrificació autòtrofa, provoca que en aigües residuals amb un baix contingut d'alcalinitat aquesta s'hagi d'addicionar externament per tal d'evitar un important descens del pH. Aquesta possible addició d'alcalinitat comporta un important incrementant en els costos d'operació del sistema. En molts casos s'ha utilitzat pedra calcària com a font d'alcalinitat i de carboni inorgànic (Flere and Zhang 1999; Zhang and Lampe 1999; Liu and Koenig 2002). Tot i que la utilització de pedra calcària sembla ser una metodologia econòmica i efectiva per compensar el consum d'alcalinitat, també té alguns inconvenients com l'increment de la duresa de l'aigua, l'increment de sòlids totals en l'efluent i la baixa solubilitat del $CaCO_3$, que dificulta el subministrament de l'alcalinitat suficient (Oh et al. 2001) quan es tracta una aigua residual amb una alta concentració de nitrat.

Recentment, en alguns estudis s'ha desenvolupat un sistema de dos passos on es combina la desnitrificació heteròtrofa i l'autòtrofa (Kim and Bae 2000; Lee et al. 2001; Liu et al. 2009). En aquest sistema, inicialment es redueix una part del nitrat utilitzant la desnitrificació heteròtrofa, posteriorment la part restant del nitrat es redueix utilitzant la desnitrificació autòtrofa amb compostos derivats del sofre. D'aquesta manera, en la desnitrificació heteròtrofa es produeix alcalinitat i hidroxils (Equació 3) que posteriorment es consumeixen en la desnitrificació autòtrofa (Equacions 9-11). Així s'aconsegueix mantenir el pH constant i s'obté una bona eliminació de nitrat.

1.4 Motivacions de la recerca i presentació de la tesi

Aquesta tesi es troba emmarcada en una de les línies del grup de recerca GENOCOV (Grup de tractament biològic d'efluents líquids i gasosos: Eliminació de Nutrients, Olors i Compostos Orgànics Volàtils) del Departament d'Enginyeria Química de la Universitat Autònoma de Barcelona. Aquest grup és un grup de recerca consolidat de la Generalitat de Catalunya (referència 2009 SGR 815).

Aquesta tesi és una continuació de la recerca iniciada per la Dra. Irene Jubany, que va desenvolupar l'operació, la modelització i el control mitjançant velocitats de consum d'oxigen (*oxygen uptake rate*, OUR) d'un sistema de nitrificació total (a nitrat) i de nitrificació parcial (a nitrit) en llots actius tot tractant un corrent d'alta concentració d'amoni (Jubany 2007). En la seva tesi es va definir un sistema de control de la càrrega basat en la mesura de l'activitat nitrificant amb l'OUR tot modificant el cabal d'entrada (Jubany et al. 2008). Es va desenvolupar un sistema de nitrificació parcial estable amb llots actius treballant en continu, demostrant que la causa del rentat total dels NOB era la combinació de diversos factors d'operació (inhibició per amoníac, inhibició per àcid nitrós, temperatura i limitació per oxigen). Malgrat aquesta combinació es va observar que el factor més important era la inhibició per amoníac (Jubany et al. 2009a). A més, es va desenvolupar, calibrar i validar un model matemàtic per aquest procés (Jubany et al. 2005; Jubany et al. 2009b). No obstant això, van quedar diversos temes per estudiar. Es va treballar amb una molt elevada concentració d'amoni a l'aigua d'entrada (3000 mg $N\text{-}NH_4^+$ L^{-1}) que afavoreix aconseguir la nitrificació parcial per l'efecte de les inhibicions per amoníac i àcid nitrós, però no es va provar a una concentració menor, típica de l'aigua de rebuig (1000 mg $N\text{-}NH_4^+$ L^{-1}). A més, es va demostrar l'estabilitat del sistema amb 100 dies d'operació però alguns autors han reportat la dificultat de mantenir la nitrificació parcial a més llarg termini (Villaverde et al. 2000; Yun and Kim 2003; Fux et al. 2004; Ma et al. 2009). No es va determinar el possible efecte combinat de la inhibició per amoníac i àcid nitrós amb la limitació per carboni inorgànic, ni l'efecte d'un llarg procés d'aturada sense alimentació.

En la present tesi, per tal de demostrar l'estabilitat d'aquest sistema durant un llarg període de temps, es va operar durant 800 dies, obtenint els resultats presentats en l'Article I d'aquest treball. Un cop aconseguida i optimitzada la nitrificació parcial, el

pas següent va ser l'estudi del posterior tractament, la desnitrificació heteròtrofa des de nitrit, amb els resultats presentats en l'Article II d'aquesta tesi. Tal com s'ha comentat anteriorment, el principal cost del procés de desnitrificació és l'elevat preu de la font de carboni que s'ha d'afegir. Per aquest motiu, en aquesta tesi s'han provat diverses fonts de carboni més econòmiques a les normalment utilitzades (metanol i etanol). Per tal de reduir costos d'operació s'han utilitzat com a font de carboni residus d'altres processos, tals com llots primaris i secundaris d'EDAR, els quals s'han fermentat per obtenir una major concentració de DQO soluble, glicerol (subproducte de les plantes de producció de biodiesel) i lixiviats d'abocador. Anteriorment, s'havia estudiat la viabilitat d'alguna d'aquestes fonts de carboni en processos de desnitrificació des de nitrat (ÆsØy et al. 1998; Fernández-Nava et al. 2010), però no des de nitrit. Part d'aquests resultats s'han realitzat en una estada de 4 mesos a la University of Manitoba (Winnipeg, Canadà).

Tal com s'ha comentat anteriorment, al tractar-se d'un procés d'eliminació biològica, els sistemes de nitrificació parcial en aigües residuals industrials presenten una problemàtica addicional, ja que aquestes aigües no presenten un cabal amb unes condicions constants durant tot l'any. Moltes indústries paren la seva producció, i per tant també la producció de l'aigua residual a tractar, durant algunes setmanes o inclús un mes. Això significa que, durant aquest període, el sistema de tractament biològic no rebrà aliment. En l'Article III es van estudiar les millors condicions d'aturada d'un sistema de nitrificació parcial (anòxic, aerobi, o diferents combinacions d'aquests), així com la seva posterior recuperació després de 30 dies d'aturada.

S'ha observat que molts sistemes de nitrificació parcial obtenen un efluent idoni per un reactor Anammox aprofitant que algunes aigües com ara l'aigua de rebuig contenen l'alcalinitat justa per tal d'oxidar només la meitat de l'amoni d'entrada. Això significa que aquests sistemes treballen, durant certs períodes, en condicions limitants de carboni inorgànic i, a la vegada, amb altes concentracions d'amoníac i àcid nitrós. Per tant, es va estudiar l'efecte de les inhibicions per amoníac i per àcid nitrós dels AOB en condicions limitants de carboni inorgànic per establir les possibles interaccions entre aquests processos. Aquests resultats es presenten en l'Article IV.

Capítol 2

Objectius

2 Objectius

L'objectiu principal d'aquesta tesi és l'obtenció d'un sistema d'eliminació biològica de nitrogen via nitrit a escala pilot pel tractament d'aigües residuals amb elevada concentració d'amoni i poc contingut en matèria orgànica, tals com l'aigua de rebuig de l'assecatge de fangs d'EDAR o altres aigües industrials. El sistema a estudiar estarà basat en configuracions de dos llots amb una primera etapa de nitritació autotròfica i una segona etapa de desnitritació heterotròfica.

El primer pas per a assolir l'objectiu principal és l'obtenció d'un sistema de nitrificació parcial de fangs actius que pugui operar de forma estable i robusta a llarg termini.

Posteriorment es tractarà l'efluent del sistema de nitrificació parcial en un sistema de desnitrificació de fangs actius per tal de poder tancar el cicle d'eliminació biològica de nitrogen per la via del nitrit. A més, es buscaran alternatives a les típiques fonts externes de carboni orgànic (metanol i etanol) per tal de poder-ne abaratir els costos d'operació.

Un cop demostrada la viabilitat del sistema d'eliminació biològica de nitrogen via nitrit s'estudiarà la problemàtica que pot aparèixer en algunes indústries degut a les possibles aturades temporals en el sistema de producció que poden tenir conseqüències en l'activitat dels microorganismes. Per tant, es desenvoluparà i aplicarà una estratègia d'aturada del sistema de nitrificació parcial, així com la seva posterior recuperació després d'un llarg període sense estar alimentat.

Finalment, es vol estudiar l'efecte de les inhibicions, tant d'amoníac com d'àcid nitrós, en sistemes que es troben en condicions limitants de carboni inorgànic, ja que poden aparèixer aquestes condicions d'operació en el sistema de nitritació. A més, es vol calcular les constants d'inhibició tant en condicions limitants com en condicions no limitants de carboni inorgànic.

Capítol 3

Resultats i discussió

3 Resultats i discussió

En aquesta secció es presenta un breu resum dels resultats obtinguts en aquesta tesi i la seva discussió. Una descripció més detallada amb un major anàlisi d'aquests resultats es pot trobar en el recull dels articles que es presenta a l'Apèndix I.

3.1 Eliminació de nitrogen via nitrit

Tal com s'ha comentat anteriorment, en el nostre grup de recerca s'havia desenvolupat un sistema de nitrificació parcial amb un llaç de control basat en la mesura d'OUR. Inicialment es va partir d'un sistema de nitrificació total i amb el canvi d'alguns paràmetres d'operació tals com el pH i la concentració d'OD es va assolir un sistema de nitrificació parcial. En aquest cas s'ha utilitzat la mateixa planta pilot que s'havia utilitzat prèviament, formada per tres reactors en sèrie de 26 L cadascun, juntament amb un sedimentador de 25 L, però s'ha inoculat directament amb el fang d'una EDAR i s'ha operat la planta pilot per tal d'obtenir una ràpida posada en marxa d'un sistema de nitrificació parcial. A la Figura 4 es presenta un esquema de la planta pilot utilitzada amb els llaços de control corresponents.

L'aliment utilitzat ha estat una aigua sintètica que simula la concentració típica de l'aigua de rebuig. Així doncs, aquest corrent d'entrada contenia una alta concentració d'amoni (1150±150 mg $N\text{-}NH_4^+$ L^{-1}) amb una baixa concentració de demanda química d'oxigen biodegradable (DQOb) en forma d'acetat (30-35 mg O_2 L^{-1}).

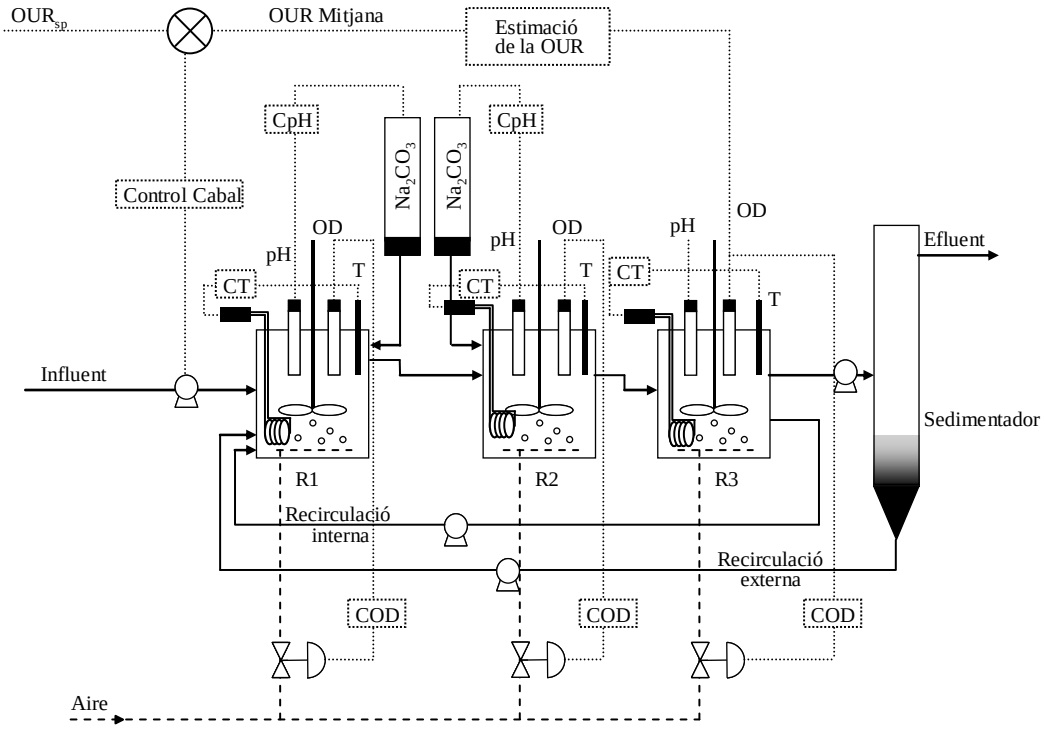

Figura 4. Esquema de la planta pilot on es mostren els llaços de control. CT és el control de temperatura, COD és el control d'oxigen dissolt i CpH és el control de pH.

Per tal d'afavorir la nitrificació parcial s'han fixat els següents paràmetres d'operació:

- Temperatura de 30ºC. A part d'afavorir el creixement dels AOB en front dels NOB a temperatures superiors a 20ºC (Hunik et al. 1994) l'objectiu d'aquest treball ha estat simular el tractament de l'aigua de rebuig, i aquesta aigua es troba a una temperatura al voltant dels 30ºC.
- pH de 8.3. La configuració de tres reactors en sèrie permet obtenir un gradient de concentracions d'amoni entre el primer i el tercer reactor. Això permet obtenir una concentració elevada d'amoni en els dos primers reactors, que combinada amb el pH elevat de 8.3, fa que la concentració d'amoníac sigui inhibitòria pels NOB tot afectant el menys possible als AOB. En el tercer reactor s'aplica el llaç de control per OUR amb un punt de consigna fixat del 40% de la OUR en el primer reactor. Aquest punt de consigna permet que la concentració d'amoni en el tercer reactor, i també a la sortida, sigui menor de 1 mg N L^{-1} degut a la baixa constant d'afinitat dels AOB per l'amoni.
- Concentració d'OD de 2.0 mg L^{-1}. Aquesta concentració relativament baixa d'oxigen permet que els NOB es trobin al voltant de la seva constant

d'afinitat per oxigen (1.75 mg L^{-1}, (Guisasola et al. 2005)) i per tant estiguin limitats per oxigen. D'altra banda, els AOB es troben menys afectats per la concentració d'OD que els NOB perquè tenen una constant d'afinitat menor (0.74 mg L^{-1}; Guisasola et al. (2005)).

Apart dels paràmetres comentats, s'ha fixat un TRC de 8±3 dies. Aquest TRC baix permet que els NOB es rentin del sistema al trobar-se clarament desfavorits en front dels AOB per les condicions d'operació aplicades (Ahn et al. 2008; Jubany et al. 2009a).

Mantenint aquests paràmetres d'operació es va assolir una posada en marxa molt ràpida. En tan sols 30 dies es va aconseguir tractar un cabal d'entrada més de 10 vegades superior al cabal inicial i la població d'AOB va passar de 2±0.5% a 72±10% del total de bacteris. D'altra banda, pel que fa als NOB se'n va assolir el 2±1% al final de la posada en marxa, la qual cosa demostra que s'han vist molt desafavorits en front dels AOB. Aquest baix percentatge de NOB va veure's reflectit en un important percentatge de nitrit a la sortida (85%).

Hi ha sistemes de nitrificació parcial que funcionen correctament durant un cert període de temps però després perden la capacitat d'acumular nitrit (Villaverde et al. 2000; Fux et al. 2004). Per tant, una vegada finalitzada la posada en marxa del sistema, aquest es va operar durant 800 dies per tal de demostrar que el sistema format per tres reactors en sèrie treballant amb un TRC baix i amb un llaç de control de la càrrega aplicada, és capaç de mantenir la nitrificació parcial a llarg termini de forma estable. Durant tot aquest període s'ha obtingut un efluent amb pràcticament un 100% de nitrogen en forma de nitrit i només en tres períodes curts el percentatge de nitrit va disminuir al 70%, sent la resta nitrat. Precisament aquests períodes on va disminuir el percentatge d'acumulació de nitrit van correspondre a períodes on el TRC havia augmentat per una millora en les propietats de sedimentació de la biomassa. Per compensar la menor pèrdua de sòlids del sistema es va purgar més i per tant es va disminuir el TRC de nou, tot recuperant l'acumulació de pràcticament el 100% de nitrit. Per tant, s'ha pogut observar la importància de treballar amb un TRC baix per tal d'obtenir un sistema de nitrificació parcial de forma estable. Pel que fa als percentatges d'AOB i NOB, aquests es van mantenir a 80±7% i menor al 1%, respectivament.

D'altra banda la càrrega volumètrica mitjana tractada pel sistema durant els 800 dies d'operació va ser de 2.0±0.4 g N L^{-1} d^{-1} a 30ºC. Aquesta càrrega va ser molt superior a la de 0.72 g N L^{-1} d^{-1} obtinguda anteriorment per Jubany et al. (2009a) a 25ºC. El motiu principal és perquè en aquest sistema s'ha treballat amb un TRC de 8±3 dies, molt menor als 30 dies amb els que van treballar Jubany et al. (2009a). Treballar amb un TRC menor implica que la concentració de biomassa en el sistema també és menor, però a la vegada aquesta biomassa té una major capacitat específica d'oxidació d'amoni (Pollice et al. 2002; Munz et al. 2010).

Després d'obtenir, de forma estable, un efluent amb una concentració aproximadament del 100% de nitrit, s'ha investigat la posterior desnitrificació heteròtrofa d'aquest nitrit (desnitritació). Tal com s'ha comentat anteriorment, la desnitrificació heteròtrofa requereix d'una font de carboni que pot ser interna, present a la pròpia aigua residual, o externa, addicionada externament. Normalment, les fonts de carboni externes més utilitzades són l'etanol i el metanol (Christensson et al. 1994; Carrera et al. 2003), perquè es poden trobar de forma abundant i relativament econòmica. A més, proporcionen unes bones velocitats de desnitrificació (Christensson et al. 1994; Carrera et al. 2003). Tot i ser productes relativament barats, aquests augmenten significativament els costos d'operació en el procés d'eliminació del nitrogen. Per tant, en aquesta tesi s'han estudiat diverses fonts de carboni econòmicament més viables i més sostenibles des d'un punt de vista ambiental tals com els llots primaris i secundaris fermentats, lixiviats d'abocador i una solució de glicerol que simula un residu de la indústria del biodiesel. S'han avaluat les velocitats de desnitritació amb aquestes fonts alternatives de carboni orgànic en un reactor SBR i s'han comparat amb l'obtinguda amb l'etanol.

S'han obtingut uns bons resultats de desnitritació amb glicerol, etanol, llots primaris fermentats i lixiviats utilitzant un reactor SBR sense control de pH i a una temperatura de 23ºC. La càrrega específica més elevada s'ha obtingut amb el glicerol (0.25±0.05 g N g^{-1} SSV d^{-1}) mentre que amb l'etanol, lixiviats d'abocador i llots primaris fermentats s'han obtingut càrregues més baixes (0.17, 0.16 i 0.13 g N g^{-1}SSV d^{-1}, respectivament). D'altra banda, pel que fa als llots secundaris fermentats, s'ha obtingut una velocitat de desnitritació molt baixa degut, probablement, a la baixa DQO biodegradable obtinguda amb la fermentació d'aquest residu (Gavala et al. 2003; Arnaiz et al. 2006).

D'altra banda, utilitzant els lixiviats d'abocador com a font externa de carboni s'ha observat un increment important de la quantitat de sòlids inorgànics en el sistema, amb una relació SSV/SST de 0.42 quan amb la resta de fonts de carboni, aquesta relació s'ha mantingut constant al voltant de 0.80. Aquesta part important de sòlids inorgànics prové de la precipitació de les sals que es formen a partir de l'alt contingut d'ions Ca^{2+} i Mg^{2+} i el bicarbonat dels lixiviats (Lozecznik et al. 2010).

Com que les necessitats de DQO per al procés de desnitritació depenen específicament del tipus de matèria orgànica utilitzada, s'han mesurat els requeriments de cadascuna de les fonts utilitzades, obtenint unes necessitats molt menors per l'etanol i el glicerol (3.0 i 3.8 mg DQO/mg N, respectivament) que pels llots primaris fermentats (5.5 mg DQO/mg N) o pels lixiviats d'abocador (8.8 mg DQO/mg N). Aquest consum s'ha de tenir molt en compte quan la font de carboni s'ha de comprar a preu de mercat. Tot i així, una relació elevada no ha de ser necessàriament dolenta ja que, per exemple, l'alt contingut de DQO que tenen els lixiviats d'abocador s'ha d'eliminar igualment ja que és un residu que ha de ser tractat.

3.2 Estudi de diferents condicions d'aturada d'un sistema de nitrificació parcial i posterior recuperació

Una de les problemàtiques que poden presentar els sistemes de nitrificació parcial aplicats al tractament d'aigües industrials és l'aturada durant un llarg període de temps per requeriments de producció. L'objectiu d'aquest treball era obtenir les millors condicions d'aturada del sistema (ja sigui en condicions aeròbies, anòxiques o alternant les dues) i comprovar si és possible la posterior recuperació de la planta pilot (Figura 4) després d'haver estat aturada sense aliment durant 30 dies.

Per a estudiar l'efecte d'aquesta aturada s'han omplert quatre reactors de 25 L cadascun amb biomassa nitrificant i s'han deixat d'alimentar en condicions diferents. Un s'ha mantingut en condicions anòxiques, un altre en condicions aeròbies i finalment dos

reactors en els quals s'han alternat les condicions anòxiques i aeròbies (un 1h aerobi i 5h anòxic i un altre 1h aerobi i 23h anòxic). Durant el mes en el qual no s'han alimentat els reactors, s'ha fet un seguiment de la pèrdua d'activitat mitjançant tècniques respiromètriques i també s'ha fet un seguiment de la pèrdua de biomassa nitrificant mitjançant l'anàlisi FISH (Fluorescence *in situ* hybridization). Finalment s'han mesclat les biomasses dels quatre reactors en la planta pilot i s'ha tornat a posar en marxa.

Mitjançant el seguiment de l'activitat (mesurat amb OUR) que s'ha realitzat durant els 30 dies d'aturada del sistema, s'han pogut calcular les diferents velocitats de mort dels AOB en les diferents condicions d'operació. Aquesta velocitat de mort ha estat més baixa en condicions anòxiques (0.11 ± 0.01 d^{-1}) i més elevada a mesura que la fracció aeròbia s'anava incrementant (0.14 ± 0.02 d^{-1} pel reactor que estava 1 h aerobi i 23 h anòxic i 0.19 ± 0.02 d^{-1} pel que va operar a 1 h aerobi i 5 h anòxic). Finalment, pel reactor completament aerobi la velocitat de mort va ser de 0.24 ± 0.02 d^{-1}. Aquest augment de la velocitat de mort en condicions aeròbies front les condicions anòxiques també va ser observat per altres autors (Siegrist et al. 1999; Lee and Oleszkiewicz 2003).

Mitjançant l'anàlisi FISH s'ha determinat la disminució dels AOB al llarg dels 30 dies d'aturada del sistema. Igual que en el cas de les activitats per OUR, s'ha observat que la disminució dels AOB ha estat més accentuada a mesura que la fracció aeròbia era major. Tot i així, a partir de 20 dies d'aturada de l'alimentació, el percentatge dels AOB mesurat amb FISH era pràcticament zero en els quatre reactors, determinant que en cas d'haver d'aturar un sistema nitrificant la millor opció és que aquesta aturada no sobrepassi els 15 dies. D'altra banda també s'han quantificat els NOB en els quatre reactors. Pel que fa aquests bacteris, s'ha observat un increment del percentatge en els reactors aerobis, degut a que en aquests reactors tenen oxigen per poder oxidar el nitrit present en el medi en el moment d'aturar el sistema i, conseqüentment, poden créixer i augmentar el percentatge. D'altra banda en el reactor anòxic no s'ha observat cap increment dels NOB degut a que en aquest reactor no tenen oxigen per a poder oxidar el nitrit. Aquest creixement dels NOB en els reactors aerobis dificultaria la posterior recuperació de la nitrificació parcial, i per tant és un motiu més que confirma que si s'ha d'aturar un sistema de nitrificació parcial és millor fer-ho en condicions anòxiques.

Finalment, per tal de recuperar el sistema de nitrificació parcial es va mesclar la biomassa dels quatre reactors. La fracció d'AOB en aquests quatre reactors era molt semblant i es va mesclar per tal de tenir una major concentració de biomassa i així poder obtenir una recuperació del sistema més ràpida. Des del moment inicial es va connectar el llaç de control de la càrrega d'entrada amb l'OUR com a variable mesurada, el qual permetia anar augmentant la càrrega d'entrada de tal manera que la quantitat d'amoni que entra al sistema és la quantitat d'amoni que aquest pot consumir, tot evitant les possibles acumulacions d'amoni. La càrrega d'entrada va augmentar ràpidament dels 0.05 g N L^{-1} d^{-1} inicials a 1.2 g N L^{-1} d^{-1} a 30ºC en tan sols 5 dies, tot obtenint un 98% de nitrit en l'efluent. D'aquesta manera es va demostrar que es pot recuperar un sistema de nitrificació parcial que ha estat aturat sense alimentació durant un llarg període de temps i que el llaç de control utilitzat és una bona forma de recuperar-lo ràpidament.

3.3 Estudi de les limitacions per carboni inorgànic

Molts sistemes de nitrificació parcial utilitzats pel tractament de l'aigua de rebuig de la digestió anaeròbia aprofiten, per tal d'obtenir un efluent adequat per a un reactor Anammox, el fet de que aquesta aigua conté aproximadament la meitat de carboni inorgànic necessari per oxidar tot el nitrogen amoniacal (Gali et al. 2007). Aquests sistemes treballen sense control de pH, deixant que pràcticament s'esgoti el carboni inorgànic. Per tant, aquests sistemes poden treballar en condicions limitants de carboni inorgànic i com a conseqüència la velocitat de nitritació es pot veure afectada (Wett and Rauch 2003; Guisasola et al. 2007). En aquesta tesis s'ha comprovat que pel sol fet de treballar en condicions limitants de carboni inorgànic, sense limitació ni inhibició de cap altre compost, la velocitat de nitritació es veu reduïda a un 75 % de la velocitat màxima.

A més, al tractar-se d'aigües residuals amb alta concentració d'amoni, les concentracions d'amoni i nitrit als reactors nitrificants poden ser elevades. És conegut que els AOB s'inhibeixen a altes concentracions d'amoníac i d'àcid nitrós (Anthonisen et al. 1976). El que no es coneix és si existeix un efecte sinèrgic entre les limitacions per carboni inorgànic i les inhibicions per amoníac i àcid nitrós. Per tant, s'ha estudiat l'efecte d'aquestes inhibicions en sistemes limitats per carboni inorgànic.

Pel que fa a la inhibició per amoníac dels AOB, s'ha comprovat que es pot modelitzar com una inhibició per substrat de tipus Haldane i s'ha observat un increment d'aquesta inhibició quan el sistema es troba en condicions limitants de carboni inorgànic. La constant d'inhibició va disminuir de 376±45 a 139±17 mg NH_3 L^{-1}. A més, la constant d'afinitat pel substrat va augmentar de 0.28±0.04 a 4.3±0.7 mg NH_3 L^{-1}, fet que indica que es necessita una concentració més elevada d'amoni per obtenir la mateixa velocitat de nitritació quan el sistema treballa amb limitació de carboni inorgànic. D'altra banda, la inhibició per àcid nitrós dels AOB es va ajustar al tipus d'inhibició no competitiva i es va comprovar que està fortament afectada quan es treballa en condicions limitants de carboni inorgànic. En aquest cas la constant d'inhibició va disminuir de 1.32±0.14 a 0.21±0.02 mg HNO_2 L^{-1} quan es treballa amb limitacions de carboni inorgànic. S'ha de considerar que els sistemes de nitrificació parcial que treballen en condicions de limitació per carboni inorgànic, també ho fan a un pH baix, normalment igual o inferior a 7 (Feng et al. 2007; Ganigue et al. 2007). La comprovació de que la inhibició per àcid nitrós dels AOB augmenta amb la limitació per carboni inorgànic té una gran rellevància en aquests sistemes ja que al treballar a pH lleugerament àcid, la concentració d'àcid nitrós augmenta i com a conseqüència, la inhibició dels AOB es veu incrementada per la suma dels dos efectes. Per exemple, una concentració d'àcid nitrós de 0.5 mg HNO_2 L^{-1} sense limitació per carboni inorgànic suposa una disminució de la velocitat màxima de nitritació del 28 %, però en canvi, en condicions limitants per carboni inorgànic aquesta disminució és del 70 %. Això té un efecte molt important sobre la càrrega tractada o sobre el volum de disseny del reactor.

Capítol 4

Conclusions

4 Conclusions

S'ha demostrat que és possible obtenir un sistema de nitrificació parcial de fangs actius que treballi en continu i que estigui format per tres reactors agitats en sèrie i un sedimentador. El sistema s'ha operat de forma estable durant un llarg període de temps tot utilitzant un control fonamentat en la mesura de l'OUR als reactors i tractant una aigua sintètica amb una concentració d'amoni de 1000 mg N L^{-1} que simula l'aigua de rebuig d'una EDAR. S'ha assolit una càrrega mitjana molt elevada, al voltant de 2.0 g N L^{-1} d^{-1}, a la temperatura de 30ºC. A més, s'ha aconseguit fer un rentat selectiu dels NOB tot combinant la inhibició per amoníac i les limitacions per oxigen dissolt juntament amb la selecció d'un temps de residència cel·lular adequat.

Un cop desenvolupat el sistema de nitrificació parcial, s'ha estudiat la posterior desnitritació en un reactor SBR sense control de pH. Al tractar-se d'una desnitrificació heteròtrofa s'han utilitzat diferents fonts de carboni tals com l'etanol, el glicerol, els llots primaris i secundaris fermentats i finalment els lixiviats d'abocador. S'han obtingut elevades velocitats de desnitrificació per tots els compostos menys pels llots secundaris fermentats, ja que són de difícil fermentació. Els reactors s'han inoculat amb biomassa convencional d'EDAR, i després de 20 dies de posada en marxa s'ha obtingut una càrrega volumètrica al voltant de 0.2 g N L^{-1} d^{-1} pel glicerol, etanol i lixiviats d'abocador. Les velocitats específiques d'eliminació de nitrit han estat, de major a menor: glicerol > etanol > lixiviats d'abocador > llots primaris fermentats. Tot i haver obtingut una elevada velocitat d'eliminació de nitrit utilitzant els lixiviats d'abocador s'ha observat que no és la millor font de carboni que es pot utilitzar degut a un increment important de la fracció inorgànica de la biomassa. D'altra banda s'han obtingut uns requeriments de DQO/N en la desnitrificació heteròtrofa que varien entre 3.0 per l'etanol i 8.8 pels lixiviats d'abocador.

En aquesta tesi s'ha demostrat que un llarg període d'aturada (30 dies) del sistema de nitrificació parcial de fangs actius no suposa un gran inconvenient per a la implementació del sistema a escala industrial ja que es pot aconseguir amb facilitat la seva recuperació després del període d'aturada. Després de l'aturada, el sistema s'ha recuperat en tan sols 5 dies d'operació tot obtenint la mateixa càrrega de 1.2 g N L^{-1} que

s'havia assolit anteriorment a la parada i amb una acumulació de nitrit del 98 %. S'ha demostrat que les millors condicions de conservació dels AOB i la seva activitat durant l'aturada són condicions anòxiques i que, a mesura que s'incrementa la fracció aeròbia en les condicions de conservació, la velocitat de mort dels AOB es veu incrementada. Amb l'anàlisi FISH s'ha corroborat aquest increment de la mort en incrementar la fase aeròbia i, a més, s'ha observat que la fracció d'AOB disminueix dràsticament a partir dels 17 dies d'aturada, el que significa que si s'ha d'aturar un sistema de nitrificació parcial és millor no fer-ho més de dues setmanes. També s'ha observat un increment de la fracció de NOB en condicions aeròbies, ja que aquests poden consumir l'oxigen i el nitrit present en el medi en el moment d'aturar el sistema.

S'ha demostrat que els AOB es poden inhibir per amoníac i per àcid nitrós i que aquestes dues inhibicions s'incrementen quan els AOB es troben limitats per carboni inorgànic. Pel que fa a la inhibició per amoníac, la seva cinètica s'ha ajustat a un model d'inhibició per substrat de tipus Haldane, tot obtenint unes constants d'inhibició de 139 i 376 mg NH_3 L^{-1} en condicions limitants i no limitants de carboni inorgànic, respectivament. A més, s'ha observat que la constant d'afinitat s'ha incrementat de 0.28 mg NH_3 L^{-1} sense limitacions per carboni inorgànic a 4.3 mg NH_3 L^{-1} en condicions limitants. D'altra banda, la cinètica d'inhibició dels AOB per àcid nitrós s'ha ajustat a un model d'inhibició no competitiva, obtenint també un increment considerable d'aquesta inhibició en condicions limitants de carboni inorgànic (passant d'una constant d'inhibició de 1.31 mg HNO_2 L^{-1} sense limitació per carboni inorgànic a 0.21 mg HNO_2 L^{-1} en condicions limitants). Finalment, també s'ha observat que només pel fet d'estar en condicions limitants de carboni inorgànic la velocitat màxima d'oxidació d'amoni es veu reduïda al 75 %.

Capítol 5

Bibliografia

5 Bibliografia

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Apèndix I

Articles acceptats

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Fast start-up and controlled operation during a long-term period of a high-rate partial nitrification activated sludge system

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Fast start-up and controlled operation during a long-term period of a high-rate partial nitrification activated sludge system

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Partial nitrification of a high-strength ammonium wastewater (1150 ± 150 mg N-NH_4^+ L^{-1}), mimicking reject water, was achieved in an activated sludge pilot plant with a configuration of three continuous reactors in series plus a settler. Stable and robust partial nitrification was maintained during 800 days of operation at 30°C with a sludge retention time (SRT) of 8 ± 3 days. A high volumetric ammonium oxidation rate (2.0 g N L^{-1} d^{-1}) was obtained with a [N-NO_2^-]/[N-NO_x^-] ratio of 1, i.e. full nitritation. The start-up of the partial nitrification system was quickly and successfully performed with an on-line control system using municipal wastewater treatment plant (WWTP) sludge as inoculum. An ammonia-oxidizing bacteria (AOB) fraction of $72 \pm 10\%$ was obtained after only 30 days of start-up. The applied SRT of 7–10 days with the combination of free ammonia inhibition and dissolved oxygen limitation provided the selective washout of nitrite-oxidizing bacteria (NOB) and an active nitrifying population with high ammonium oxidizing rates.

Keywords: control; nitritation; NOB washout; SRT; start-up

1. Introduction

Biological nitrogen removal (BNR) via nitrite has recently gained interest because of its associated economical savings compared with classical nitrogen removal technologies in which the ammonium is fully oxidized to nitrate [1,2]. This technology is especially interesting for treating high-strength ammonium wastewaters with a low organic matter content such as rejected water from sludge dewatering. The first step in BNR via nitrite is the achievement of a stable partial nitrification or nitritation (ammonium oxidation to nitrite), which can be obtained in biofilm [3–5] or activated sludge systems [6–8]. The second step to complete the BNR via nitrite is to reduce this nitrite to nitrogen gas, which can be performed autotrophically [9,10] or heterotrophically [11].

Several studies have reported problems in maintaining partial nitrification in long-term operation [12–14] due to nitrite-oxidizing bacteria (NOB) acclimation to the non-favouring conditions during long periods. Therefore, the total washout of NOB is fundamental for stable operation and the sludge retention time (SRT) is one of the key parameters to achieve it [7,15–17]. In this sense, when non-favouring conditions for NOB are applied, the specific growth rate of NOB is lower than the specific growth rate of ammonia-oxidizing bacteria (AOB) and, under these conditions, the lower the SRT of the system, the easier the NOB washout. The lowest value of SRT is when both hydraulic retention time (HRT) and sludge retention time are equal (HRT = SRT), as in the SHARON process [17]. Moreover, the lower the SRT, the higher are the specific nitrifying rates [18–20]. However, the activated sludge systems working at low SRT have a limited treatment capacity due to the low biomass concentration that can be achieved and it was reported that the SHARON process could be limited by this restriction [21]. The way to increase the biomass concentration and, consequently, the treatment capacity is working with biomass retention, i.e. with a settler and a high SRT. In these conditions, NOB washout is more difficult and the specific ammonium oxidation rate is lower than in systems operating at lower SRT. Nevertheless, once NOB washout is achieved, the system is more stable to possible SRT variations due to, for example, changes on settling properties [7,15].

In a previous study [7], it was demonstrated that a synthetic industrial wastewater with extremely high ammonium concentration (3000–4000 mg N L^{-1}) and low chemical oxygen demand (COD) concentration (250–350 mg L^{-1}, acetate) could be treated in an activated sludge pilot plant with a configuration of three continuous stirred-tank reactors (CSTR) in series plus a settler using a proper on-line control system. With this system, it was possible to achieve and maintain stable complete nitritation working at high SRT (30 d) with a significant nitrifying biomass concentration (1800 mg VSS L^{-1}). However, the nitrification rate was probably limited in that system by the high SRT used [18–20]. Moreover, the start-up of

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that partial nitrification system was carried out using an inoculum with high AOB percentage [7].

Considering the previous achievements obtained in the three-CSTRs configuration, the main issues raised in this study were:

i. Evaluating the feasibility of the system to treat synthetic wastewater with an ammonium content around 1100 mg N L^{-1} (mimicking reject water), maintaining full nitritation over the long term with the on-line control system.
ii. To achieve a fast start-up of this type of system using an inoculum with low AOB percentage coming from a municipal wastewater treatment plant (WWTP).
iii. To demonstrate that a high partial nitrification rate can be achieved in this type of system with a low SRT during a long-term period.

2. Materials and methods

2.1. Pilot plant description

The pilot-scale activated sludge system (Figure 1) consisted in three aerobic reactors (R1, R2 and R3), each with a working volume of 26 L, and followed by a 25 L settler. The reactors were connected in series and they worked under completely mixed conditions. A fraction of R3 effluent was recycled to R1 (internal recycle) with a flow rate of 380 L d^{-1}. Without this internal recycle the individual HRT of each reactor was 5 h, and with this recycle it was 1.2 h. This increased the dynamics of the system and improved the mixing between reactors and the control response. Furthermore, sludge was recycled from the settling tank to R1 in order to maintain the biomass concentration in the reactors (external recycle) with a flow rate of 90 L d^{-1}.

Each reactor was equipped with dissolved oxygen (DO), pH and temperature probes. pH controllers actuated in R1 and R2 as on–off controllers via the addition of solid sodium carbonate through solid dispensers. The pH in R1 and R2 was controlled at 8.3 ± 0.1 and remained at 8.2 ± 0.1 in R3. The DO concentration was controlled at 2.0 mg L^{-1} and the control was based on a proportional integral derivative (PID) algorithm. It operated by manipulating pneumatic control valves which modified the airflow supplied through air diffusers placed at the bottom of the reactors. The temperature was fixed at 30°C and on–off controllers were implemented in each reactor and operated by switching electrical heating devices.

Automatic on-line oxygen uptake rate (OUR) estimation was implemented in each reactor. The OUR measurement was performed every 10 min and was based on the DO decrease in the liquid phase with no air inlet.

The synthetic influent mimicked the reject water from the dewatering process of anaerobic digested sludge with high ammonium concentration (1150 ± 150 mg N-NH_4^+ L^{-1}) and low biodegradable COD concentration (30–35 mg COD L^{-1}). The following compounds were also supplied as micronutrients: 13.0 mg L^{-1} KH_2PO_4, 3.0 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 9.0 mg L^{-1} NaCl, 6.0 mg L^{-1} $MgCl_2 \cdot 7H_2O$, 0.13 mg L^{-1} $FeSO_4 \cdot 7H_2O$, 0.1 mg L^{-1} $MnSO_4 \cdot H_2O$, 0.13 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.07 mg L^{-1} $CuSO_4 \cdot 5H_2O$ and 0.007 mg L^{-1} H_3BO_3.

The average SRT was maintained at 8 ± 3 days throughout the whole operational period of 800 days. SRT was

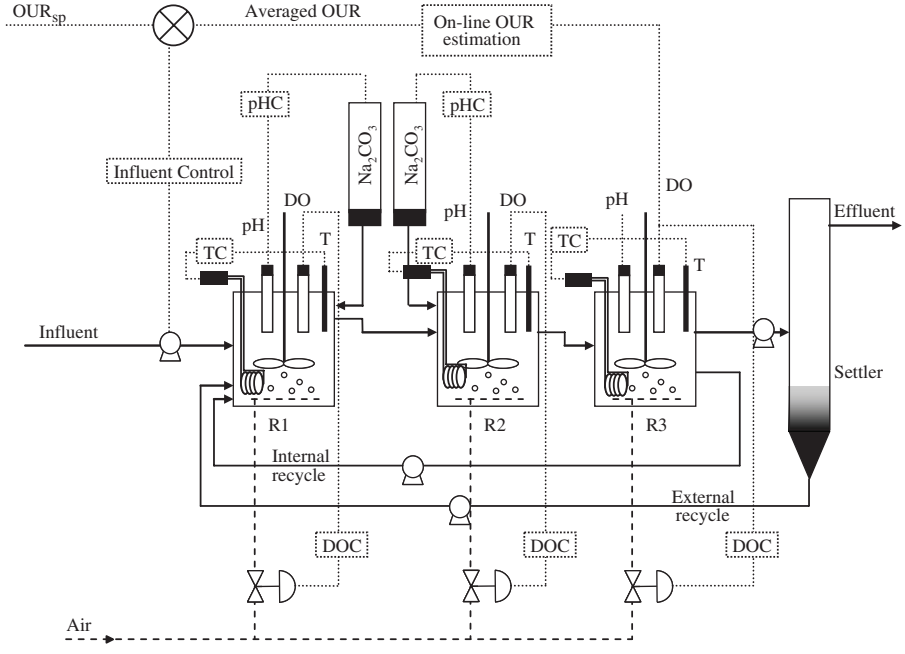

Figure 1. Diagram of activated sludge pilot plant where TC, pHC and DOC are the temperature, pH and DO automatic control loops, respectively.

calculated considering the sludge wastage and the solids concentration in the effluent.

2.2. Inflow control loop

An inflow control loop was implemented in a supervisory expert control system developed in Gensym G2© (version 4.1), running in a Sun workstation, where the information of the pilot plant was centralized. This control loop was based on a previous theoretical development designed by simulation [22]. The overall effect of this control loop was to adapt the inflow rate to maintain a stable concentration of nitrogen compounds in the effluent. The control loop consisted of a proportional–integral (PI) feedback controller where the measured variable was the OUR in R3. Every 10 min, the supervisory expert controller calculated an averaged OUR value with data from the last 30 min and compared it to an OUR setpoint (OUR_{SP}) selected to achieve low ammonium concentration in the effluent. The difference between these two OUR values was used by the controller algorithm to calculate a new inflow value and, as a result, the new nitrogen loading rate (NLR). Finally, the control action was transmitted to the process computer that changed the pump flow.

2.3. Microbial and chemical analyses

A fluorescence in situ hybridization (FISH) technique coupled with confocal microscopy was used to investigate the nitrifying population dynamics. A Leica TCS SP2 AOBS confocal laser scanning microscope at a magnification of ×63 (objective HCX PL APO ibd.B1 63 × 1.4 oil) equipped with two HeNe lasers with light emission at 561 and 633 nm was used for biomass quantification. Hybridizations were carried out using at the same time a Cy3-labelled specific probe and Cy5-labelled EUBmix probe (general probe). The specific probe used for AOB detection was Nso190 [23] and for NOB detection it was NIT3 [24]. The EUBmix probe consisted of a mix of probes EUB338, EUB338 II and EUB338 III [25,26]. Detailed information about FISH quantification can be found in Jubany *et al*. [27].

Total ammonia nitrogen ($TAN = N\text{-}NH_4^+ + N\text{-}NH_3$) was analysed using a continuous flow analyser based on potentiometric determination of ammonia [28]. Total nitrite nitrogen ($TNN = N\text{-}NO_2^- + N\text{-}HNO_2$) and nitrate were measured by ionic chromatography using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an autosampler AS40. Free ammonia and free nitrous acid concentrations were calculated from TAN and TNN respectively, using the acid–base equilibrium [29]. Volatile suspended solids (VSS) and total suspended solids (TSS) concentrations were determined according to standard methods [30].

3. Results and discussion

3.1. Start-up of the partial nitrification system

The pilot plant was inoculated with activated sludge from the municipal WWTP of Manresa (Barcelona, Spain), which works with a modified Ludzack–Ettinger configuration. The inoculum had a VSS/TSS ratio of 0.72 and was composed of 97 ± 2% of heterotrophs, 2 ± 0.5% AOB and <1% NOB.

The inflow control loop described in Section 2.2 requires a setpoint for the OUR in R3. This OUR_{SP} for R3 was automatically updated every 3 h by the supervisory control system and calculated as 0.4 times the average OUR in R1. This selected setpoint resulted in an effluent ammonium concentration around 1 mg $N\text{-}NH_4^+$ L^{-1}, near the affinity constant of ammonium, and it allowed working at the maximum NLR without ammonium accumulation. In addition, the pH setpoint of the reactors was set at 8.3 to increase the fraction of free ammonia for inducing inhibiting conditions on NOB. These operational conditions were applied to achieve the start-up of the partial nitrification system from an activated sludge with a low percentage of nitrifying bacteria in 30 d.

The inflow was initially set at 13 L d^{-1} but after only 30 d of operation it increased to 165 L d^{-1} due to the action of the inflow control loop (Figure 2(a)). In terms of treatment capacity, the volumetric nitrogen loading rate (NLR_v) increased from 0.16 g $N\text{-}NH_4^+$ L^{-1} d^{-1} (slightly higher than the NLR_v applied in the Manresa WWTP) to a high value of 2.0 g $N\text{-}NH_4^+$ L^{-1} d^{-1}. The fast NLR_v increase was directly related to a high increase in the AOB fraction (from 2 ± 0.5% to 72 ± 10% of the total bacteria) (Figure 3). However, the high increase in AOB did not lead to a significant change in NOB since its fraction increased only from <1 ± 0.1% to 2 ± 1% of the total bacteria.

The VSS/TSS ratio at the end of the start-up period was 0.80 and the total biomass concentration decreased from 2100 to 1200 mg VSS L^{-1}, probably due to the decay of heterotrophic bacteria because of the low COD concentration in the influent. However, in spite of the VSS decrease, a nitrifying system with a biomass concentration of 1200 mg VSS L^{-1} and an AOB fraction of 72 ± 10% can be considered a noteworthy result.

The large difference between AOB and NOB fractions meant that, at the end of the start-up, only 15% of the inlet TAN was oxidized to nitrate and 85% to TNN, with a low TAN concentration (<3 mg $N\text{-}NH_4^+$ L^{-1}) in the effluent (Figures 2(b) and 2(c)). All these results allow us to classify the start-up as a fast and successful process.

The growth rates of AOB and NOB populations are influenced by four factors: inhibition by free ammonia and free nitrous acid, limitation by DO and temperature. Using a combination of these factors it is possible to washout the NOB from the system to achieve a stable partial nitrification [7,15,16]. The DO concentration was fixed at 2.0 mg L^{-1}, which is quite limiting for NOB but not as much for AOB [31]. As Figure 2(b) shows, the configuration of the pilot plant with three CSTRs in series allowed an effluent without TAN to be achieved, but simultaneously there was a stable accumulation of TAN in R1 and R2 (150 ± 50 mg L^{-1} and 20 ± 10 mg L^{-1}, respectively). This TAN accumulation,

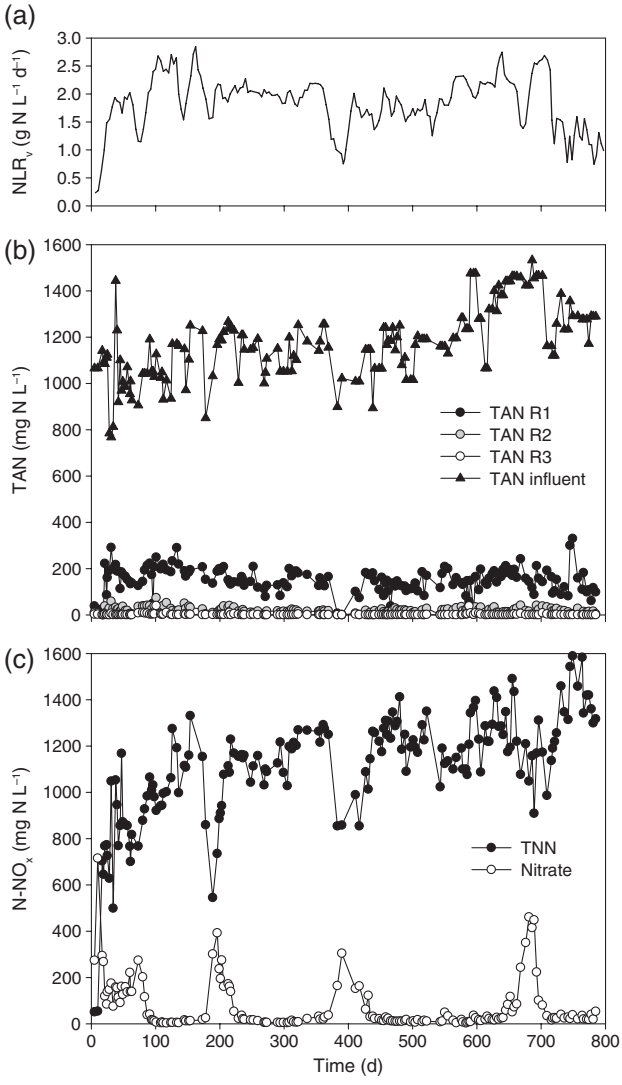

Figure 2. (a) Volumetric nitrogen loading rate (NLR_v) during the whole experimental period. (b) TAN concentration in each reactor (R1, R2 and R3). (c) TNN and nitrate concentration in the last reactor of the pilot plant (R3).

combined with the high setpoint of pH (8.3), favoured the formation of free ammonia (25 mg NH_3 L^{-1} in R1 and 3.4 mg NH_3 L^{-1} in R2). These free ammonia concentrations are extremely inhibitory for NOB but not for AOB [29] and they can be considered the main cause of NOB washout. Regarding the possible inhibitory effect of free nitrous acid, despite the measurement of TNN concentrations up to 1500 mg L^{-1}, the high pH setpoint caused a low free nitrous acid concentration (0.05 mg HNO_2 L^{-1}), which was not inhibitory for AOB or NOB [29].

3.2. *Long-term controlled operation at low SRT to achieve a high nitrification rate*

After the start-up, the pilot plant was successfully operated for 800 d (Figure 2). This time was enough to demonstrate

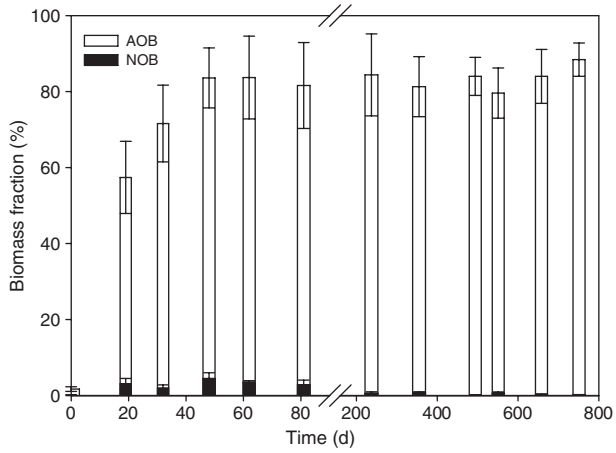

Figure 3. AOB and NOB fractions determined by FISH analysis.

the reliability and stability of the developed process treating mimicked reject water in a long-term operation. The inlet TAN was fully oxidized to TNN during the whole operational period, except for some short periods when TNN accumulation decreased to 70% (Figure 2(c)).

During the operational period after the start-up, the inflow ranged from 53 L d^{-1} to the highest value of 173 L d^{-1} due to the inflow control loop. The average inflow was 121 ± 30 L d^{-1}, which supposed an average HRT of 15 ± 4 hours. The main cause of the inflow fluctuations was the variation in the biomass concentration in the pilot plant (from 600 to 4300 mg VSS L^{-1}, with an average value of 1350 ± 840 mg VSS L^{-1}). This variation was mainly due to some operational problems in the settler and different biomass settling properties, which could be related to the accumulation of a high TNN concentration in the system [32].

From day 50 onwards, the AOB fraction was maintained around $80 \pm 7\%$ (Figure 3) while the NOB population was almost washed out of the system ($\leq 1 \pm 0.4\%$). This washout allowed a full and stable partial nitrification to be maintained during the long-term operation. However, three short periods with some nitrate accumulation were observed. In spite of nitrate accumulations, most of the inlet TAN was oxidized to TNN, i.e. the system was always working under conditions of partial nitrification. Nitrate accumulation was caused by an SRT increase in the pilot plant due to the unexpected improvement in the settling properties. As a consequence, the subsequent increase in the biomass concentration in the reactors allowed NOB growth in the system in those situations. In order to re-establish the full nitritation, i.e. 100% of TAN oxidation to TNN, the purge of biomass was increased to decrease the SRT. After few days, NOB were removed from the system and the full nitritation was recovered.

The average biomass concentration (1350 mg VSS L^{-1} at SRT = 8 d) was lower, as expected, than the one obtained by Jubany *et al*. [7] with a higher SRT (1800 mg VSS L^{-1}

Table 1. Overview of volumetric TAN oxidation rates for partial nitrification activated sludge systems composed of one or more CSTR in series plus a settler.

T (°C)	Operational period (d)	Volumetric TAN oxidation rate (g N L^{-1} d^{-1})	SRT (d)	Reference
30*	200	2.0	7	Ciudad *et al*. [34]
30	800	2.0	8	This study
30	70	2.2	10	Chen *et al*. [35]
30*	120	1.1	13	Yamamoto *et al*. [8]
30	60	0.7	16	Chen *et al*. [35]
30*	150	1.3	30	Jubany *et al*. [7]

*Volumetric TAN oxidation rate was calculated at 30°C using the following equation: $NLR_{30°C} = NLR_{25°C} \cdot 1.123^{(30-25)}$ [36].

at SRT = 30 d). However, the biomass concentration in this study is clearly higher than the reported for other partial nitrification activated sludge systems operated at lower SRTs [15,33]. On the other hand, the average specific nitrifying capacity in this study (1.5 ± 0.3 g N g^{-1} VSS d^{-1} at SRT = 8 d) was clearly higher than the one obtained by Jubany *et al*. [7] (0.7 ± 0.2 g N g^{-1} VSS d^{-1} at SRT = 30 d). This result agrees with those studies reporting an increase of the specific nitrifying capacity with a decrease of the SRT [18–20].

However, the main data for the design of a full-scale system is the treatment capacity or volumetric TAN oxidation rate (in g N L^{-1} d^{-1}), which is a combination of the biomass concentration (in g VSS L^{-1}) and its specific nitrifying capacity (in g N g^{-1} VSS d^{-1}). In this case, the average volumetric TAN oxidation rate achieved was 2.0 ± 0.4 g N L^{-1} d^{-1} while the average value in Jubany *et al*. [7] was 1.3 ± 0.4 g N L^{-1} d^{-1}. It means that, in spite of the higher biomass concentration obtained with a higher SRT, the big difference in the specific ammonium oxidation rates caused that the volumetric TAN oxidation rate was 35% higher with a lower SRT.

The volumetric TAN oxidation rate achieved with a SRT = 8 d (2.0 ± 0.4 g N L^{-1} d^{-1} at 30°C) allows us to classify the reported pilot plant as a high-rate partial nitrification activated sludge system. The volumetric TAN oxidation rate reported in this study is compared in Table 1 with the values achieved in other partial nitrification activated sludge systems at different SRTs. There is a clear difference between the volumetric TAN oxidation rates obtained in systems operated at SRTs between 7–10 d and the ones achieved in systems operated at SRTs between 13–30 d. This difference could be due to a more active fraction of AOB at lower SRT because the inert solids and dead biomass are washed out faster from the system. According to these results, a partial nitrification activated sludge system should be operated at a SRT around 7–10 d to increase its treatment capacity.

A specific feature of the proposed system compared with the other references of Table 1 is the controlled effluent composition. The high TAN oxidation rate is linked in our case to full ammonium oxidation to achieve a controlled effluent composition lower than 3 mg N-NH_4^+ L^{-1} during long-term operation.

4. Conclusions

A full nitritation activated sludge system with an automatic inflow control loop was stably operated for 800 d treating mimicked reject water. The start-up was successfully carried out from a municipal WWTP sludge, obtaining 72 ± 10% of AOB in only 30 d. The selective washout of NOB was achieved with the combination of free ammonia inhibition and DO limitation linked to a properly selected SRT. The operation at SRT = 8 ± 3 d provided a high volumetric TAN oxidation rate (2.0 g N L^{-1} d^{-1}), while the control system allowed the maintenance of a systematically ammonium concentration lower than 3 mg N-NH_4^+ L^{-1}.

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Denitritation of a high-strength nitrite wastewater in a sequencing batch reactor using different organic carbon sources

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ABSTRACT

The use of different carbon sources (ethanol, acid-fermented primary sludge centrate, acid-fermented secondary sludge centrate, glycerol and landfill leachate) in heterotrophic denitrification from nitrite (denitritation) was studied in a sequencing batch reactor, operated without pH control. Complete denitritation of a high-strength nitrite wastewater was achieved using these organic carbon sources with the exception of fermented secondary sludge centrate. Loading rates around 0.2 g N L^{-1} d^{-1} were obtained for glycerol, landfill leachate and ethanol after a short start-up period of 20 days. The maximum specific nitrite removal rate of 0.25 g N g^{-1} VSS d^{-1} was achieved for glycerol, while values between 0.13 and 0.17 g N g^{-1} VSS d^{-1} were obtained using ethanol, landfill leachate and fermented primary sludge centrate. The COD/N ratio consumed varied between 3.0 for ethanol and 8.8 for landfill leachate. The denitritation rates and the required COD/N ratio for each carbon source are reported for the first time – they can be used for the scale-up of the denitritation process.

1. Introduction

Biological nitrogen removal (BNR) via nitrite pathway has gained interest recently due to distinct advantages over the BNR via nitrate pathway [1,2]. The first step for the BNR via nitrite is the partial nitrification process (the oxidation of ammonium to nitrite or nitritation). Partial nitrification has been well studied using different configurations [3–6]. For a complete BNR via nitrite, partial nitrification must be followed by a second step that reduces, autotrophically or heterotrophically, nitrite to nitrogen gas (denitritation). The anaerobic ammonium oxidation (anammox) [7,8] and the denitrification using sulphur compounds [9] or hydrogen [10] are examples of autotrophic denitrification. The main difficulty in the implementation of the anammox process in full-scale is the slow growth rate of the anammox bacteria and their high sensitivity to some environmental parameters [11,12]. Heterotrophic denitrification on the other hand tends to be less sensitive to environmental parameters than autotrophic denitrification processes, although high nitrite concentrations can inhibit the process [13–15]. The availability of an organic carbon source is imperative for heterotrophic denitrification. Denitrification quite often suffers from the lack of available carbon – which needs to be added to complete the process [16]. Methanol and ethanol are two of the most commonly used external carbon sources because they are readily available and provide high denitrification rates [17,18]. The increased operational costs of nitrogen removal with purchased carbon lead to search for alternative more sustainable sources [19,20]. These carbon sources include digested sludge [21], many industrial wastes such as glycerol-containing waste from the biodiesel production or high-COD municipal landfill leachates [22]. These alternative carbon sources should have lower price as when compared to methanol or ethanol and be as effective.

Most studies of denitrification with alternative carbon sources in lab-scale reactors were carried out from nitrate [19,21], but no references have been found on denitrification from nitrite using a sequencing batch reactor (SBR) operation. The aim of this study was to compare the effectiveness of denitritation using different alternative organic carbon sources: ethanol, fermented primary sludge centrate, fermented secondary sludge centrate, glycerol and landfill leachate to perform denitrification, using SBR reactor without pH control.

2. Materials and methods

2.1. Experimental setup

The study was performed using two different lab-scale SBR. The first one, called SBR_A, had a working volume of 14 L and was operated at 23 ± 1 °C without pH control. The reactor was mechanically stirred and the feeding was provided by two membrane pumps: one

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for carbon source and the other for high-strength nitrite wastewater. The SBR_A influent was the result of mixing both solutions. SBR_A was controlled with a Siemens Logo PLC and the operation was established in cycles of 24 h divided into an anoxic reaction phase of 23 h (with two 24 min filling phases at $t = 0$ h and 11 h), a settling phase of 40 min, a draw phase of 13 min and an idle phase of 7 min. The exchange volume in each cycle was 54% of the total volume. Three different organic carbon sources were fed during the whole operational period: (i) ethanol during the first 54 days, followed by (ii) fermented secondary sludge centrate during 18 days and finally, (iii) fermented primary sludge centrate during the last 26 days.

The second lab-scale SBR, called SBR_B, had a working volume of 3 L and was operated at the same temperature than SBR_A (23 ± 1 °C) and also without pH control. The feeding and the effluent withdrawal were provided by peristaltic pumps and the mixing was done through a magnetic stirrer. The influent to SBR_B was the result of mixing a high-strength nitrite wastewater with the organic carbon source solution. SBR_B was controlled with timers and the operation was established in cycles of 24 h divided into an anoxic reaction phase of 23 h (with two 2.5 min filling phases at $t = 0$ h and 9 h), a settling phase of 40 min, a draw phase of 7 min and an idle phase of 13 min. The exchange volume in each cycle was 67% of the total volume. Two identical SBR_B were used in parallel, one was fed with glycerol and the other one was fed with landfill leachate as organic carbon source.

2.2. Characteristics of the wastewater and the external organic carbon sources

All denitrifying SBRs used in this study were fed with two different streams: a high-strength nitrite wastewater and a solution with the external organic carbon source. The effluent of a partial nitrifying pilot plant containing around 1000 mg $N–NO_2$ L^{-1} was used as high-strength nitrite wastewater in SBR_A. More details about the partial nitrifying system can be found in Jubany et al. [23]. Both SBR_B were fed with a synthetic high-strength nitrite wastewater prepared with a dilution of $NaNO_2$. The nitrite concentration in the influents of SBR_A and SBR_B was changed to increase or decrease the applied nitrogen loading rate depending on the denitrifying capacity of each system.

Five different organic carbon sources were used in this study: a representative commercial organic carbon source (ethanol) and other four alternative organic carbon sources: fermented primary sludge, fermented secondary sludge, glycerol and landfill leachate. The ethanol solution was prepared diluting ethanol 70% with tap water. This solution was fed to SBR_A with an average chemical oxygen demand (COD) concentration of 1700 ± 600 mg O_2 L^{-1}.

The two sludge solutions were prepared by batch-fermenting the primary and the secondary sludge from a full scale municipal wastewater treatment plant (WWTP) located in Manresa (Barcelona, Spain) for 3 days in a 37 °C room. During the fermentation period, both sludges were manually stirred twice a day. At the end of the fermentation, both sludges were centrifuged and only centrates were used in SBR_A as external organic carbon source. The COD concentration in the influent of SBR_A was 170 ± 90 mg O_2 L^{-1} using the fermented secondary sludge centrate and 750 ± 200 mg O_2 L^{-1} using the fermented primary sludge centrate. Ammonia in both centrates was: 70 mg N L^{-1} in the fermented secondary sludge and 240 mg N L^{-1} in the fermented primary sludge. Nitrate was not detected in any of the carbon sources.

The glycerol solution mimicked the waste produced by the biodiesel industry and was prepared using pure glycerol mixed with tap water. It was fed into SBR_B with the average influent COD of 1450 ± 200 mg O_2 L^{-1}.

Landfill leachate supplied into SBR_B was collected from the Brady Road municipal landfill in Winnipeg (Manitoba, Canada). It was collected once at the beginning of the study and it was stored in a refrigerator at 4 °C. The leachate had a COD of 2750 ± 250 mg O_2 L^{-1} and ammonium of 120 ± 20 mg N L^{-1}. No nitrate was detected in the leachate.

Influent COD concentration was modified depending on the denitrifying capacity of each system to avoid possible COD limitations. When the denitrifying capacity increased, both nitrite and COD concentrations in the influent were also increased to provide a higher nitrogen loading rate. Ethanol and glycerol solutions were synthetically prepared and the concentration was easily changed. The leachate had a high COD concentration and was therefore diluted as a function of the COD requirements. Fermented secondary and primary sludge centrates had low COD concentration. In case of these substrates, nitrite concentration in the influent had to be reduced to avoid possible nitrite accumulation due to COD deficiency.

The sludge retention time (SRT) was maintained around 50 d in all the reactors. However, when SBR_A was fed with fermented primary sludge centrate, SRT was only 15 days due to high solids concentration in the effluent.

2.3. Analytical methods

Volatile suspended solids (VSS), total suspended solids (TSS) and COD concentrations were determined according to Standard Methods [24]. Nitrite and nitrate concentration for SBR_A samples were measured with ion chromatography using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an auto-sampler AS40, whereas ammonium was analyzed using a continuous flow analyzer based on potentiometric determination. Ammonium, nitrite and nitrate concentrations for SBR_B samples were measured with a flow injection analysis (LACHAT Quick Chem 8500).

3. Results and discussion

3.1. Start-up

SBR_A was inoculated with activated sludge from the Manresa WWTP (Barcelona, Spain) and was fed with an ethanol solution as carbon source. Both SBR_B were inoculated with activated sludge from the Winnipeg-West biological nutrient (N and P) removal plant (Manitoba, Canada). Both SBR_B were operated in parallel, one fed with glycerol and the other one with landfill leachate. A volumetric nitrogen removal rate (NRR_V) of 0.2 g N L^{-1} d^{-1} was achieved after 20 days of operation during the start-up of the denitrifying SBR_A (Fig. 1). Similar NRR_V was achieved in both SBR_B also after 20 days (Fig. 1). It was concluded that the length of the start-up period of a denitrifying SBR with two alternative organic carbon sources (glycerol and landfill leachate) was similar to ethanol–the commercial carbon source.

3.2. Denitritation using alternative organic carbon sources

Denitrification from nitrite was found feasible with the selected alternative electron donors, except with fermented secondary sludge centrate – Figs. 1 and 2. With ethanol, fermented primary sludge centrate, glycerol and leachate, nitrite concentration in the influent was progressively increased to determine the maximum NRR of the system. Poor denitritation with the fermented secondary sludge centrate was due to very low COD concentration (170 ± 90 mg O_2 L^{-1}) and low COD biodegradability typical for such fermentates [25,26].

The specific nitrogen removal rate (NRR_S) obtained using the different carbon sources is presented in Table 1. The highest NRR_S was achieved with glycerol (0.25 ± 0.05 g N g^{-1} VSS d^{-1}) and was maintained for 60 days in one of the SBR_B (Fig. 1). This value was higher

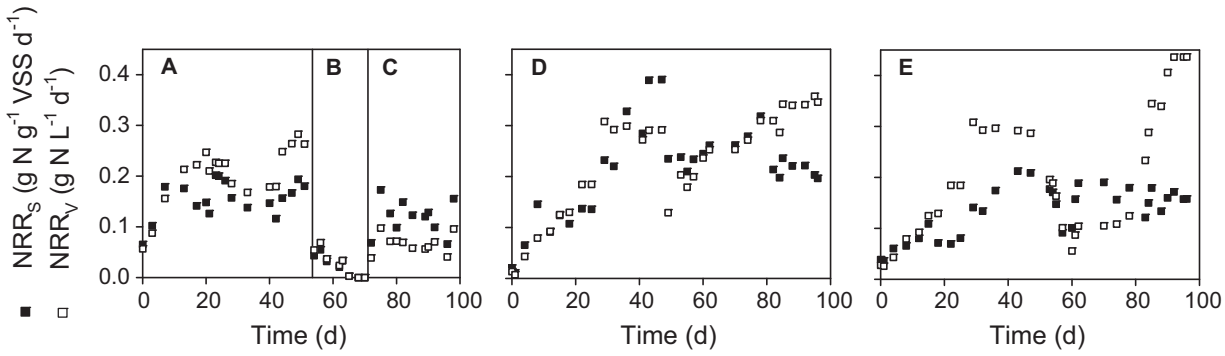

Fig. 1. Specific (■) and volumetric (□) nitrogen removal rate obtained using different carbon sources: (A) ethanol, (B) fermented secondary sludge centrate, (C) fermented primary sludge centrate, (D) glycerol and (E) leachate.

Table 1
Results of the denitritation with different organic carbon sources.

Organic carbon source	T (°C)	pH	Average NRR_S (g N g^{-1} VSS d^{-1})	$\frac{\text{mg COD consumed}}{\text{mg N–}NO_2^-\text{ eliminated}}$	$\frac{VSS}{TSS}$
Ethanol	23	9.1 ± 0.2	0.17 ± 0.03	3.0 ± 0.2	0.79 ± 0.07
Glycerol	23	8.9 ± 0.3	0.25 ± 0.05	3.8 ± 0.2	0.77 ± 0.08
Fermented primary sludge centrate	23	9.0 ± 0.2	0.13 ± 0.02	5.5 ± 0.3	0.79 ± 0.05
Landfill leachate	23	8.6 ± 0.3	0.16 ± 0.04	8.8 ± 0.5	0.42 ± 0.09

than the NRR_S of 0.124 g N g^{-1} VSS d^{-1}, calculated by adjusting the original value of 0.24 g N g^{-1} VSS d^{-1} reported by Akunna et al. [27] to the temperature of this study using an Arrhenius temperature coefficient of 1.10 [28]. It should be noted that Akunna et al. [27] used a denitrifying biomass that was not previously acclimated to glycerol and they determined the NRR_S in a batch test.

The maximum NRR_S achieved with ethanol, landfill leachate and fermented primary sludge centrate (Table 1) were also maintained in the denitrifying SBR for more than 30 days, demonstrating the stability of denitritation using these alternative organic carbon sources. The NRR_S obtained with ethanol, landfill leachate and fermented primary sludge centrate were very similar: 0.17, 0.16 and 0.13 g N g^{-1} VSS d^{-1}, respectively. These results demonstrate that some alternative organic carbon sources work as well as ethanol as electron donor for denitritation (landfill leachate and fermented primary sludge centrate) or even better (glycerol). No other NRR_S values were reported in literature for denitritation using these organic carbon sources. The NRR_S values reported in literature for nitrate denitrification using ethanol as organic carbon source were higher than the value obtained in this study [18,29,30]. This difference could be mainly related to the operational conditions used. All the cited studies were carried out in continuous denitrifying reactors working with an automatic pH control with set at 7.5, while this study was carried out in a denitrifying SBR without pH control.

Denitritation capacity was lost after two weeks of using the fermented secondary sludge centrate as organic carbon source in SBR_A (Figs. 1 and 2). Denitrifying capacity recovered when the organic carbon source was changed to fermented primary sludge centrate, which had a higher COD concentration and a higher biodegradable fraction [25]. In terms of volumetric removal rates, the highest NRR_V was achieved with landfill leachate at the end of the operational period when the biomass concentration increased significantly in the SBR_B fed with this organic carbon source (Fig. 3). The NRR_S achieved with landfill leachate was maintained relatively constant through the study but the NRR_V experienced significant changes due to high biomass concentration variations (Figs. 1 and 3). The changes of the biomass concentration (Fig. 3) were due to a high nitrite accumulation in the SBR_B fed with leachate (Fig. 2) causing the accumulation of nitrogen gas into the flocs during the settling phase which led to a considerable loss of settling properties [31]. A very similar situation was observed in the SBR_B fed with glycerol when the nitrite concentration in the effluent was around 50 mg L^{-1} (Figs. 2 and 3). Nitrate was not detected in the effluent of any of the denitrifying SBRs which confirmed that nitrate was not produced during these experiments.

The VSS/TSS ratio achieved in the denitrifying SBRs was very similar for all organic carbon sources (around 80%, Table 1), except for the SBR_B fed with landfill leachate in which the VSS/TSS ratio was only 0.42. A high percentage of inorganic solids was also reported in other biological reactors treating leachates, which was attributed to a combination of a high concentration of cations, such as Ca^{2+} and Mg^{2+} and a high carbonate content, which caused pre-

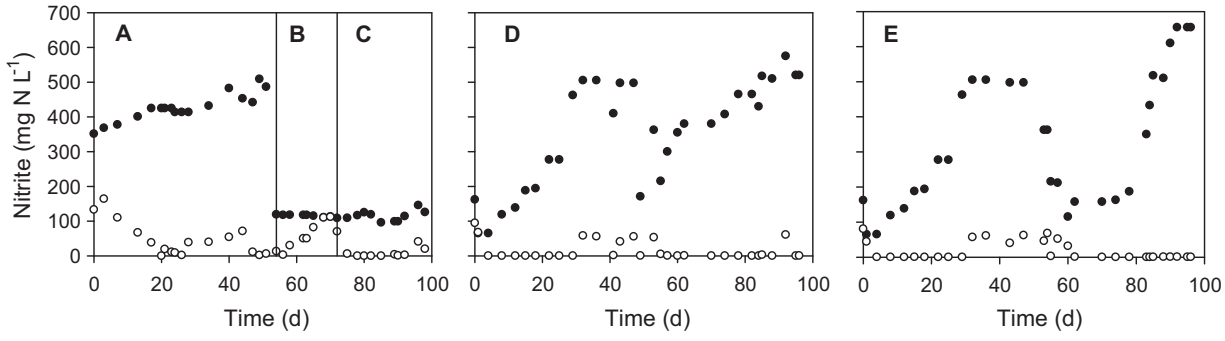

Fig. 2. Nitrite concentration in the influent (●) and the effluent (○) of the denitrifying SBRs using different carbon sources: (A) ethanol, (B) fermented secondary sludge centrate, (C) fermented primary sludge centrate, (D) glycerol and (E) leachate.

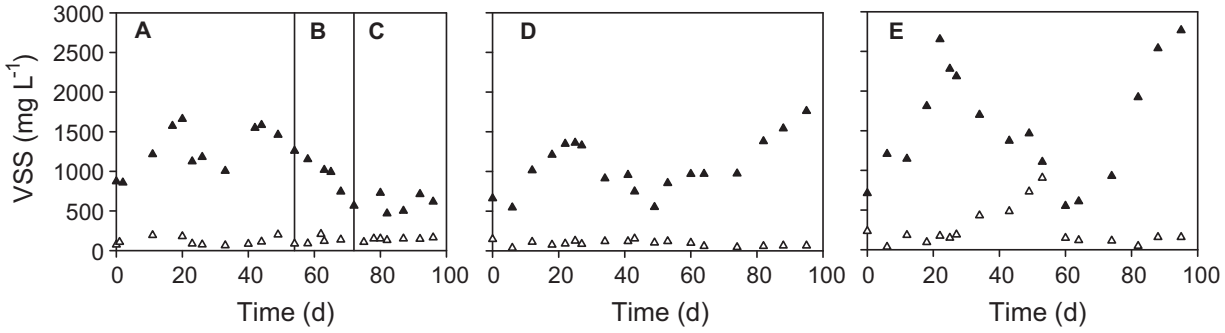

Fig. 3. Biomass concentration in the denitrifying SBRs (▲) and the effluent (△) using different carbon sources: (A) ethanol, (B) fermented secondary sludge centrate, (C) fermented primary sludge centrate, (D) glycerol and (E) leachate.

cipitation of carbonate salts [32]. The combination of the alkalinity production during the denitritation and lack of pH control in the reactors led to pH increase in all the SBRs (Table 1). Despite lack of an automatic control loop, the standard deviation of pH values in all denitrifying reactors was low, which indicated that pH values were similar during all experiments. The denitrification inhibition by free nitrous acid (FNA) was reported at concentrations around 0.01 mg HNO_2–N L^{-1} [13]. Due to high pH values in this study, the highest FNA concentration was only 0.003 mg HNO_2–N L^{-1}, which was lower than the inhibitory threshold.

3.3. Comparison of COD/N ratios

The COD/N ratio consumed during the denitritation process was different for each organic carbon source (Table 1), ranging from 3.0 obtained for ethanol to 8.8 for landfill leachate. For ethanol, the consumed COD/N ratio (3.0) was lower than one obtained during nitrate denitrification: 3.9 [33] and 4.3 [18], which agreed with the expected 30% decrease of the COD consumption when the denitrification was performed from nitrite instead of nitrate [1].

The COD/N ratio for glycerol was 3.8, very similar to a value of 4.1 reported in literature [27]. The highest COD/N ratio consumed (8.8) was obtained for the landfill leachate. The high COD consumption in this case is not necessarily a condition to discard the landfill leachate as an alternative organic carbon source because it is a waste that has to be treated, and its use as external carbon source for denitritation could be a good alternative to remove part of its organic matter.

4. Conclusions

Denitritation (or nitrite denitrification) of high-strength nitrite wastewater was successfully performed in SBR reactors without pH control, using different organic carbon sources. Nitrite removal rates obtained with different substrates can be ranked from the highest to the lowest as: glycerol > ethanol > landfill leachate > fermented primary sludge centrate.

Denitritation with landfill leachate as an organic carbon source was found feasible and proceeded at a high NRR_S, however it was not the best alternative due to the increase in the inorganic fraction of the biomass. Such an increase could lead to precipitation and subsequent clogging. Denitritation was not achieved using fermented secondary sludge centrate potentially due to the low biodegradable COD fraction in the centrate.

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Long-term starvation and subsequent reactivation of a high-rate partial nitrification activated sludge pilot plant

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ABSTRACT

The starvation process of a high-rate partial nitrification system during 30 days and its controlled recovery were studied in an activated sludge pilot plant. Four ammonium-starved reactors under anoxic, aerobic and two different alternating aerobic/anoxic conditions were evaluated. The highest and the lowest decay rates of ammonia oxidizing bacteria (AOB) were obtained under full aerobic (0.24 d^{-1}) and full anoxic (0.11 d^{-1}) conditions, respectively. The evolution of biomass activity correlated well with the AOB quantification using FISH technique. AOB fractions lower than 1% were measured in the four reactors after 23 days of starvation. The recovery of the system was achieved in only 5 days using a nitrogen loading rate (NLR) control loop, obtaining the same conditions than before the long-term starvation period with a NLR of 1.2 g N L^{-1} d^{-1} and 98% of nitrite accumulation in the effluent.

1. Introduction

There are many different industrial activities that generate high-strength ammonium wastewaters: petrochemical, pharmaceutical, fertilizer and food industries, leachates produced by urban solid waste disposal sites, waste from pig farms, anaerobic treatment or sludge dewatering processes. Currently, it is widely accepted that the biological nitrogen removal via nitrite is the cheapest and most recommended treatment for this kind of wastewater (Carrera et al., 2011; Van Hulle et al., 2010). However, the biological treatment of industrial wastewater is often challenged by its operation under transient states with respect to the flow pattern or variable influent characteristics (Sipma et al., 2010; Wun Jern, 2006). The fluctuation of the wastewater flow and periods with low activity or even the complete production shutdown (due to annual maintenance or vacation periods) during some weeks and even months are common in some of the abovementioned industries. During these periods, the biological treatment system can be found under starvation conditions and its removal capacity can be seriously affected. For this reason, a procedure to maintain the biological activity under starvation conditions as well as a fast start-up of the biological treatment capacity have to be designed.

The value of the decay rate for the nitrifying biomass is a key parameter in the design of a procedure for managing a long-term starvation period and the subsequent reactivation of a nitrifying activated sludge (Jaroszynski and Oleszkiewicz, 2011; Salem et al., 2006). Some researchers have investigated the decay rate of a nitrifying population (including ammonia and nitrite oxidizing bacteria) during a starvation period on different biological wastewater treatments. These studies demonstrated that the nitrifying decay rate is higher under aerobic conditions and the best strategy to maintain the activity of the nitrifiers should be keeping the sludge under anoxic or anaerobic conditions (Salem et al., 2006; Siegrist et al., 1999) and even alternating aerobic/non-aerobic conditions (Lee and Oleszkiewicz, 2003; Morgenroth et al., 2000). In addition, the separate identification of the decay rate for ammonia oxidizing bacteria (AOB) (Munz et al., 2011a) and nitrite oxidizing bacteria (NOB) (Jubany et al., 2005) is required to achieve a better model description of nitrification systems.

Some authors have studied the recovery of different biological wastewater treatments after some weeks of starvation: anaerobic digestion of a swine wastewater (Hwang et al., 2010), simultaneous nitrogen and phosphorus removal from a domestic wastewater (Pijuan et al., 2009; Yilmaz et al., 2007) and complete nitrification to nitrate of an industrial wastewater (Cabezas et al., 2009). However, no in-depth study about the evolution of biomass activity and composition under different long-term starvation conditions and its subsequent recovery has been reported in the literature for a high-rate partial nitrification system treating a high-strength ammonium wastewater.

The goal of this study is to determine the best conditions to deal with a long-term starvation period of a partial nitrification activated sludge system and its subsequent reactivation. The study includes: (i) the determination of the AOB decay rate under four

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different starvation conditions: full aerobic, full anoxic and two different alternating aerobic/anoxic, (ii) the evolution of the AOB activity and the microbial population by fluorescence in situ hybridization (FISH) analysis during a long-term starvation period (30 days) and (iii) the fast recovery of the partial nitrification system after a long-term starvation period through the use of an automatic control loop of the applied nitrogen loading rate (NLR).

2. Methods

2.1. Pilot plant configuration and nitrogen loading rate control loop

The pilot-scale activated sludge system consisted in three aerobic reactors (called R1, R2 and R3) with a working volume of 26 L each one and followed by a 25 L settler. The reactors were connected in series and they worked under completely mixed conditions. A fraction of R3 effluent was recycled to R1 (internal recycle) to increase the dynamics of the system and to improve the mixing between reactors and the control response. Detailed information about the pilot plant operation can be found in Jubany et al. (2009a).

Each reactor was equipped with dissolved oxygen (DO) (WTW Oxi 340i CellOx 325), pH (Crison pH 52-03) and temperature probes (Pt-100). The pH controller actuated in R1 and R2 as on–off controller by the addition of solid sodium carbonate through solid dispensers. The DO control was based on a PID algorithm and operated by manipulating pneumatic control valves which modified the airflow supplied through air diffusers placed at the bottom of the reactors. The temperature control, based on an on–off control, was implemented in each reactor and it was operated by switching an electrical heating device.

Automatic on-line oxygen uptake rate (OUR) estimation was implemented in each reactor. The OUR measurement was performed every 10 min and it was based on the DO decrease in the liquid phase with no air inlet. These OUR measurements were used by the control loop, which consisted of a proportional-integral (PI) feedback controller where the measured variable was the OUR in R3. Every 10 min, the supervisory expert controller calculated an averaged OUR value with data from the last 30 min and compared it to an OUR setpoint (OUR_{SP}) selected to achieve low ammonium concentration in the effluent. The difference among these two OUR values was used by the controller algorithm to calculate a new inflow value and, as a result, the new NLR. Finally, the control action was transmitted to the process computer that changed the pump flow. Detailed information about the control system and the selection of the OUR_{SP} can be found in Jubany et al. (2009a).

The synthetic influent mimics an industrial high-strength ammonium wastewater (1350 ± 25 mg N-NH_4^+ L^{-1}) with low biodegradable COD concentration (30–35 mg L^{-1}). The average sludge retention time (SRT) was maintained at 5.0 ± 0.7 days and it was calculated considering the purge of the system and the solids concentration in the effluent.

2.2. Starvation conditions

Some industries have periods around 1 month with low activity or even the complete production shutdown (due to annual maintenance or vacation periods). Hence, four reactors (26 L each one) were filled with the nitrifying biomass of the activated sludge pilot plant and were maintained under ammonium starvation conditions for 30 days to simulate a long shutdown period. The starvation conditions were different in each reactor. One of them was maintained under full aerobic conditions, another one was maintained under full anoxic conditions and two of them were maintained under alternating aerobic/anoxic conditions (one of them was aerated 1 h in every 6 h and the other one was aerated 1 h in every 24 h). During the aerobic phases the DO was maintained at 6.5 ± 0.4 mg L^{-1} and during the anoxic phases DO was lower than 0.1 mg L^{-1}. The average temperature in the four reactors during the long-term starvation period was 18 ± 2 °C. Although during normal plant operation the pH was controlled at 8.3, in order to simulate the easiest and cheapest shutdown procedure for an industry, the pH was not modified nor controlled during the starvation period. Consequently, the pH increased to 8.5–9.0 during the starvation due to the stripping of inorganic carbon. Finally, no mechanical mixing conditions were applied in the reactors and each of them contained an initial nitrite concentration of 1350 mg N-NO_2^- L^{-1} corresponding to the usual operational conditions of the pilot plant.

2.3. AOB activity measurement and decay rate determination

The AOB activity was determined through respirometric experiments using a LFS (liquid-flow-static) respirometer, where DO concentration is measured in the liquid phase, which is static and continuously aerated (Spanjers et al., 1998). The respirometer consisted of a magnetically stirred vessel of 1 L capacity. Aeration was supplied from the bottom through a microdiffuser, which ensured small air bubbles and good oxygen transfer from the gas phase to liquid phase. A mass flow controller (Bronckhorst HiTec 825) was used to provide the steady and specific air flow required for an accurate OUR calculation. The temperature of the vessel was controlled at 18 °C with a thermostatic bath. The pH was continuously measured with a pH probe (WTW-Sentix 81) and controlled at 8.3 by the automatic addition of acid or base by an automatic burette (Crison MultiBurette 2S). DO was measured with a WTW-CellOx 325 probe. The probes were connected to a WTW inoLab Level 3 terminal which was connected via RS232 to a PC allowing for data monitoring and storage. A detailed description of the procedure for OUR calculation using a LFS respirometer can be found elsewhere (Spanjers et al., 1998).

The AOB activity was measured as the maximum OUR obtained after the addition of an ammonium pulse of 25 mg N-NH_4^+ L^{-1} in the respirometer. A previous ammonium pulse was added 3 h before the AOB activity determination to avoid the overestimation of the decay rate due to enzyme deactivation (Vanrolleghem et al., 2004). OUR was divided by the volatile suspended solids (VSS) concentration in the respirometer to obtain the specific OUR (SOUR). The SOUR reported in this work corresponds to exogenous SOUR obtained subtracting the endogenous SOUR value to the total measured SOUR. Each SOUR value was corrected for possible oxygen limitation effects using the oxygen affinity coefficient for AOB ($K_{O,AOB}$ = 0.74 mg O_2 L^{-1}; (Guisasola et al., 2005)). The respirometric experiments were carried out with 1 L of biomass from one of the reactors under starvation conditions and biomass concentrations between 750 and 900 mg VSS L^{-1}. After performing the respirometric tests the biomass was not returned to the reactors to avoid any interference to the starved biomass.

The respirometric experiments were performed at days 3, 7, 10, 16, 22 and 29 of the starvation experiment for the full aerobic and 1 h aerobic/23 h anoxic reactors and at days 4, 8, 11, 17, 23 and 30 of the starvation experiment for full anoxic and 1 h aerobic/5 h anoxic reactors.

The mathematical model used to determine the AOB decay rate was an extension of the IWA Activated Sludge Model 1 (ASM1) (Henze et al., 2000) modified with two-step nitrification and denitrification processes. The kinetic and stoichiometric parameters of the model were obtained from Henze et al. (2000) and Jubany et al. (2008). The model was implemented in MATLAB® and integrated using *ode15s*. The AOB decay rate was optimized using *fminsearch*

to describe correctly the time course of SOUR in the respirometric experiments.

To model the DO dependency of AOB decay rate, the values obtained for each reactor using the previous model were fitted to Eq. (1) (Munz et al., 2011a).

$$b_{AOB} = b_{AOB,AER}\frac{S_O}{S_O + K_{O,AOB}} + b_{AOB,ANOX} \quad (1)$$

where b_{AOB} is the decay rate of the AOB, $b_{AOB,AER}$ is the decay rate that depends on the DO concentration, $b_{AOB,ANOX}$ is the decay rate under anoxic conditions and S_O is the DO concentration. The DO for alternating anoxic/aerobic reactors was considered as the average concentration for a cycle.

2.4. Microbial and chemical analysis

FISH technique coupled with confocal microscopy was used to determine the predominant nitrifying species. Hybridizations were carried out using at the same time a Cy3-labeled specific probe and Cy5-labeled EUBmix probe (general probe). Specific probe used for AOB detection was Nso190 (Mobarry et al., 1996) while for NOB detection was NIT3 (Wagner et al., 1996). EUBmix probe consisted of the mix of probes EUB338, EUB338 II and EUB338 III (Amann et al., 1990; Daims et al., 1999). A Leica TCS SP2 AOBS confocal laser scanning microscope (CLSM) at a magnification of 63× (objective HCX PL APO ibd.B1 63 × 1.4 oil) equipped with two HeNe lasers with light emission at 561 and 633 nm was used for biomass quantification. The area containing Cy3-labeled specific probe (Nso190 or NIT3) was quantified as a percentage of the area of Cy5-labeled general probe (EUBmix). This area was expressed as an average of the area for 40 images. Detailed information about FISH quantification can be found in Jubany et al. (2009b).

The ammonium concentration was analyzed using a continuous flow analyzer based on potentiometric determination (Baeza et al., 1999). Nitrite and nitrate were measured with ionic chromatography using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an auto-sampler AS40. Total suspended solids (TSS) and VSS were determined according to APHA (1995).

3. Results and discussion

3.1. Operation of the pilot plant before the starvation period

The partial nitrification activated sludge pilot plant was operated at steady-state conditions for several months before the starvation experiment. During this period, a stable and robust partial nitrification (98 ± 1% of nitrite accumulation in the effluent) was maintained. The ammonium in the effluent was lower than 1 mg $N\text{-}NH_4^+$ L^{-1}, indicating that ammonium depletion was almost complete (Fig. 1). The average NLR was 1.2 ± 0.2 g N L^{-1} d^{-1} with a SRT of 5.0 ± 0.7 days. The average biomass concentration and the VSS/TSS ratio were 800 ± 200 mg VSS L^{-1} and 0.78, respectively.

3.2. Decrease of the AOB activity during the starvation period

Fig. 2 shows the time course of the experimental and theoretical AOB activities during the starvation experiment in the four reactors under different starvation conditions. The lowest AOB decay rate was obtained in the starved reactor under full anoxic conditions (0.11 ± 0.01 d^{-1}). This value is into the wide range of bibliographic values: 0.015–0.16 d^{-1} (Lee and Oleszkiewicz, 2003; Manser et al., 2006; Munz et al., 2011b; Salem et al., 2006; Siegrist et al., 1999; Yilmaz et al., 2007). The highest AOB decay rate was determined in the starved reactor under full aerobic conditions (0.24 ± 0.02 d^{-1}) and is also in accordance to the range reported

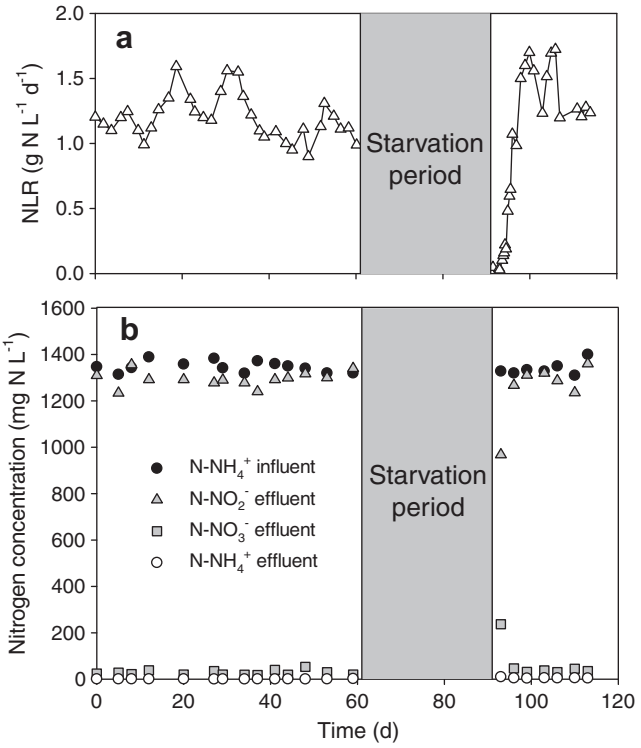

Fig. 1. Time course of NLR, ammonium, nitrite and nitrate concentrations before and after the starvation period of 30 days.

for aerated systems: 0.14–0.28 d^{-1} (Hao et al., 2009; Lee and Oleszkiewicz, 2003; Munz et al., 2011b; Salem et al., 2006; Siegrist et al., 1999). This wide range could be due to different experimental setups, operational parameters, composition of the microbial community and wastewater characteristics (Manser et al., 2006).

Intermediate values for decay rate were obtained for alternating aerobic/anoxic conditions: 0.14 ± 0.02 d^{-1} for the 1 h aerobic/23 h anoxic starved reactor and 0.19 ± 0.02 d^{-1} for the 1 h aerobic/5 h anoxic starved reactor.

The decay rates were fitted to Eq. (1) showing that the modeling approach proposed by Munz et al. (2011a) is able to describe the dependence on DO concentration of AOB decay rate (Fig. 3). AOB decay rate increases fast when oxygen is present during starvation; the model predicts a value of 0.22 d^{-1} for an oxygen concentration of 3.0 mg L^{-1}, which is close to the value obtained at 6.5 mg L^{-1}. Therefore, these results suggest that the best alternative to maintain AOB activity when a partial nitrifying system has to be shutdown is to avoid completely the aerobic conditions.

3.3. Microbial community evolution during starvation

AOB and NOB populations were quantified throughout the starvation experiment using FISH techniques (Fig. 4). AOB population decreased from the initial 77 ± 14% to <1% in any of the starvation experiments (Fig. 4a). Comparing the four different starvation conditions, the slowest decrease of the AOB fraction was observed under full anoxic conditions and, as well as the activity test, the fast decrease of the AOB fraction took place under full aerobic conditions. The results show that an important AOB fraction can be maintained under starvation conditions during 17 days even for aerobic conditions (around 10%). However, AOB fraction was almost negligible for any reactor after 23 days under starvation conditions. Consequently, if a partial nitrification activated sludge has to be shutdown, it would be better to restart it in a period which not exceeded 2 weeks in order to avoid a significant decrease of AOB biomass.

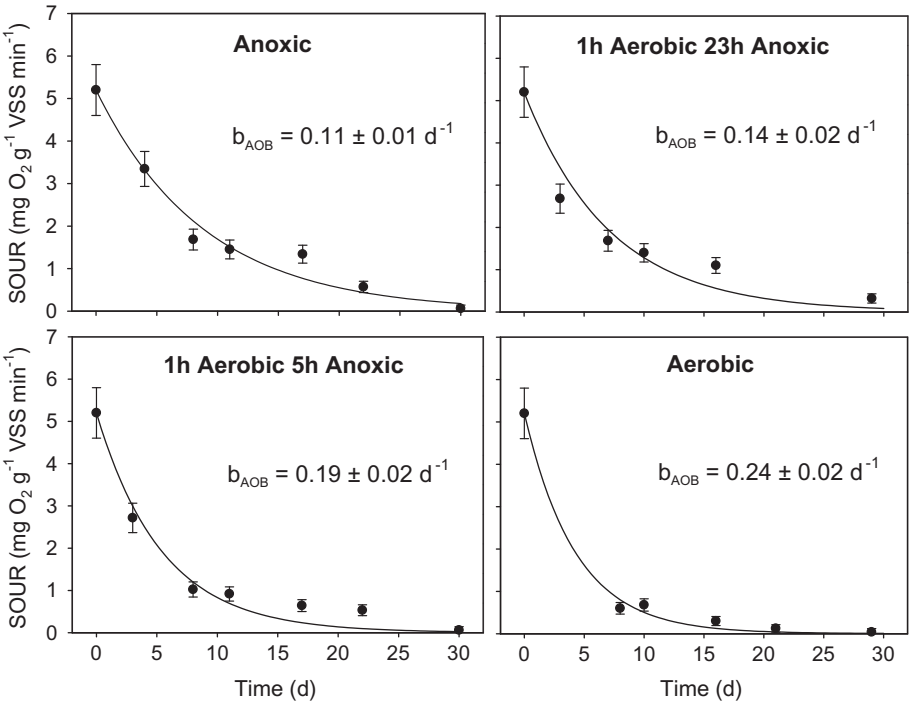

Fig. 2. Decrease of the experimental and theoretical AOB activities during the starvation period in the four reactors under different starvation conditions, measured as SOUR and predicted by the extended ASM1 model modified with two-step nitrification and denitrification processes. Error bars were calculated by propagation of error method.

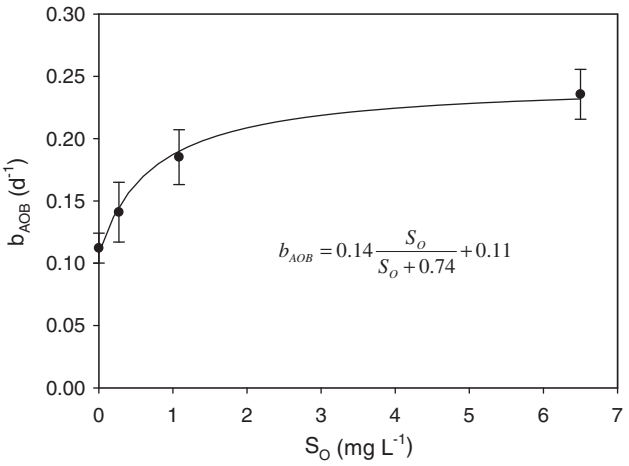

Fig. 3. AOB decay rates during the long-term starvation study as a function of the DO concentration. Continuous line: model fit from Eq. (1).

The time course of NOB population was also quantified using FISH technique (Fig. 4b). An increase of this population was observed under full aerobic and alternating aerobic/anoxic starvation conditions. It was a consequence of starting the starvation experiment with a high nitrite concentration (1350 mg N-NO_2^- L^{-1}) in the reactors. This nitrite was oxidized to nitrate under full aerobic and alternating aerobic/anoxic conditions and consequently, the NOB population percentage increased in these reactors. It reached a maximum value of 5 ± 2% at day 17 under full aerobic conditions but then this fraction decreased to less than 2% at the end of the starvation experiment. The reason for this decline was the absence of nitrite after day 17, which means that NOB population was also under nitrite starvation conditions and consequently, there was a decrease of its percentage. A similar situation was observed for the reactor that was alternating 1 h aerobic and 5 h anoxic conditions, but the NOB population reach the maximum value at day 23 because nitrite consumption was slower in this reactor. Finally, the maximum NOB percentage in the reactor working at 1 h aerobic and 23 h anoxic was reached at the end of the starvation experiment because nitrite was only marginally consumed. On the other hand, the oxidation of nitrite was not possible in the reactor under full anoxic conditions due to the lack of oxygen and consequently, NOB population was not able to grow and its fraction was always lower than 0.5%. The increase of the NOB fraction under full aerobic and alternating aerobic/anoxic conditions during the starvation period would represent an added difficulty to reach partial nitrification after the re-startup of the system, since NOB population must be washed out again.

The significant increase of NOB population and the high AOB decay rate measured under full aerobic conditions are the main factors to reject the maintenance of the partial nitrification system under aeration during a long-term starvation period. However, NOB growth could be easily avoided decreasing nitrite concentration in the reactor before the starvation. In any case, the best starvation conditions will be full anoxic because the AOB decay rate is the lowest, the decrease of the AOB population is the slowest and NOB population is not able to grow.

The time course of the solids concentration in the full aerobic and the full anoxic reactors during the starvation experiment is presented in Fig. 5. The trend was similar in both reactors, with a decrease from 900 to 600 mg VSS L^{-1}, while maintaining a constant VSS/TSS ratio around 0.80. In both conditions, the solids concentration was maintained relatively constant during the first week and then decreased. The nil variation during the first week of the starvation experiment contrasts with the high loss of AOB activity and biomass fraction in the same period (Figs. 2 and 4a). This stable solids concentration during the first days of starvation was also observed in other biological wastewater treatment systems (Yilmaz et al., 2007). The lower decrease of the solids concentration in contrast to the high decrease of AOB fraction at the end of the starvation period could be interpreted using the extended ASM1 model

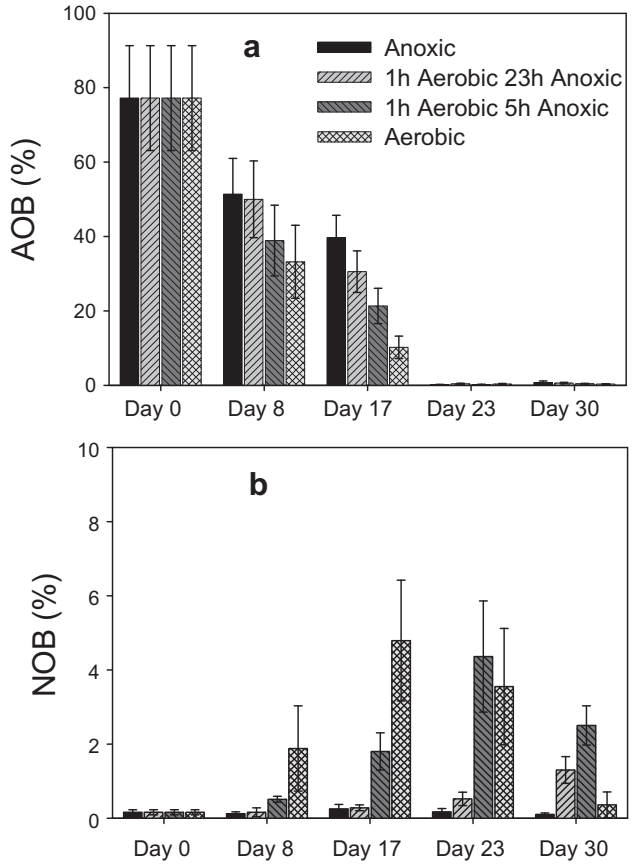

Fig. 4. AOB and NOB populations, expressed as percentage of the total biomass, determined by FISH analysis during the starvation period.

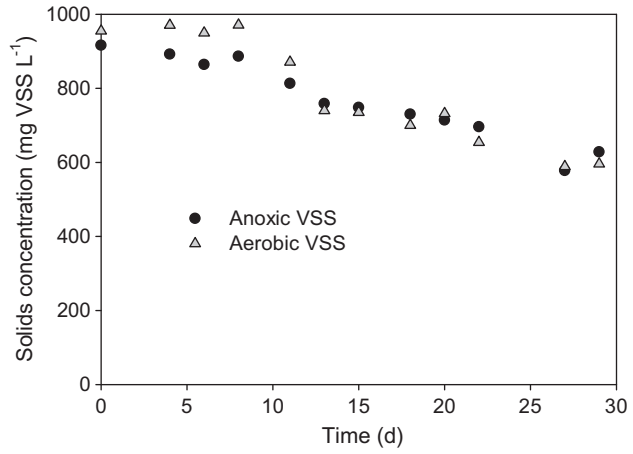

Fig. 5. Time course of solids concentration during starvation period under full anoxic and full aerobic conditions.

predictions. VSS concentration was mostly related to (i) the maintenance of an important concentration of heterotrophic biomass based on some growth on lyses products and (ii) the formation of particulate inert products from the decay of biomass.

3.4. Recovery of the partial nitrification activated sludge after a long-term starvation period

A fast recovery capacity of the partial nitrification system after a long-term starvation period is a key point to deal with the full-scale implementation of this process. To study the recovery of the partial nitrification system, the biomass of the four reactors used in the starvation experiment was mixed and introduced into the three reactors of the pilot plant. The AOB fraction in the four reactors at the end of the starvation experiment was very low (<1%, Fig. 4a), which seemed to suggest that the recovery of the AOB activity to the values achieved before the starvation would be slow. The recovery of the partial nitrification system was carried out connecting the automatic NLR control loop. The control loop modified the inflow adapting it to the progressive increase of the AOB activity, but avoiding any harmful accumulation or limitation of ammonium in the pilot plant (Fig. 1). The NLR quickly increased in 5 days from 0.05 g N L^{-1} d^{-1} to a value slightly higher than the achieved before the starvation period (1.2 g N L^{-1} d^{-1}).

Moreover, the percentage of nitrite accumulation after the first 5 days of the controlled recovery process was 98% whereas the ammonium concentration in the effluent was always below 1 mg N-NH_4^+ L^{-1}. These values of NLR and nitrite accumulation were maintained constant during several weeks, showing the stability of the recovered partial nitrification system. A small accumulation of nitrate (20%) was observed in the first 2 days of the recovery process but this nitrate probably came from that accumulated during the nitrite oxidation in the aerobic starvation experiment. The fractions of AOB and NOB populations were determined by FISH analysis after 15 days of the controlled recovery and their values (73 ± 10% of AOB and 0.6 ± 0.1% of NOB) were similar to the values measured before the starvation period.

There are few bibliographic references of recovery of biological wastewater treatments after a long-term starvation period. For example, Yilmaz et al. (2007) and Pijuan et al. (2009) recovered, after 1 month under starvation conditions, the organic matter, nitrogen and phosphorus removal in granular reactors in 4 and 21 days, respectively. Gali et al. (2007) studied the recovery of two lab-scale partial nitrification systems treating a high-strength ammonium wastewater after a short-term starvation period (only 5 days). Both recoveries were fast, around 2 and 4 days.

To the best of our knowledge, this work is the first description of the controlled recovery of a high-rate partial nitrification system at pilot-scale treating a high-strength ammonium wastewater after a long-term starvation period. The use of the automatic control loop modifying the applied NLR was demonstrated as a good tool to achieve a fast recovery of a long starved nitrifying biomass. The applied NLR and the fraction of AOB population can be increased from almost 0 to 1.2 g N L^{-1} d^{-1} and 72.5% respectively, in only 5 days. During the full anoxic starvation, a significant fraction of the AOB population can be maintained after 15 days (40% from an initial value of 80%) but this fraction decreased significantly after 21 days. These findings are useful to develop strategies to operate partial nitrification reactors during industrial production shutdown periods.

4. Conclusions

This study shows that a long-term starvation period (30 days) is not an unbeatable problem for the implementation of a high-rate partial nitrification activated sludge process for treating an industrial high-strength ammonium wastewater.

Full anoxic conditions are the best to maintain the AOB activity and the AOB population fraction under a starvation period because the AOB decay rate and the NOB growth on residual nitrite are lower than in full or partial aerobic conditions. Under anoxic starvation, the AOB fraction only decreased from 80% to 40% after 15 days, but it decreased significantly after 21 days.

The use of an automatic control loop modifying the applied NLR was demonstrated as a good tool to achieve a fast recovery of a long starved nitrifying biomass.

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Combined effect of inorganic carbon limitation and inhibition by free ammonia and free nitrous acid on ammonia oxidizing bacteria

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ABSTRACT

The inhibitory effect of free ammonia (NH_3 or FA) and free nitrous acid (HNO_2 or FNA) on the ammonia oxidizing bacteria (AOB) and the dependence of the AOB activity on the concentration of total inorganic carbon (TIC) are well-established. In contrast, less is know about the effect of high FA and FNA concentrations in combination with TIC limitation. Respirometric tests performed with an enriched AOB sludge (81% of AOB as measured with fluorescent in situ hybridization) established that AOB inhibition by FA under TIC limitation was higher than under non-limiting TIC conditions (Haldane inhibition coefficients of 139 and 376 mg NH_3 L^{-1}). AOB affinity for FA decreased under TIC limitation conditions (half-saturation coefficient of 0.28 mg NH_3 L^{-1} without TIC limitation and 4.3 mg NH_3 L^{-1} with TIC limitation). Higher inhibition by FNA was observed when TIC was limited since the non-competitive inhibition coefficient decreased from 1.31 mg HNO_2 L^{-1} (without TIC limitation) to 0.21 mg HNO_2 L^{-1} (with TIC limitation). This study demonstrates that AOB inhibitions by FA and FNA are amplified with TIC limitation and consequently, AOB dynamics are strongly modified under TIC limitation conditions.

1. Introduction

The process of partial nitrification (ammonium oxidation to nitrite) for treating high-strength wastewaters (reject water, landfill leachate and others) has many advantages with respect to complete nitrification (ammonium oxidation to nitrate) since it has lower oxygen requirements, reduced COD demand and CO_2 emissions during heterotrophic denitrification, lower biomass production during anoxic growth and the opportunity to use the autotrophic denitrification or Anammox process (Peng and Zhu, 2006; Turk and Mavinic, 1987; Van der Star et al., 2007).

Partial nitrification is achieved in continuous or batch reactors provided that growth of ammonia oxidizing bacteria (AOB) is favored and the growth of nitrite oxidizing bacteria (NOB) is limited or inhibited (Jubany et al., 2009a). Temperature, dissolved oxygen (DO) and pH are the main factors that affect AOB and NOB kinetics to a different degree and therefore are useful to achieve partial nitrification (Guisasola et al., 2005; Van Hulle et al., 2007). The pH also influences the ammonium/free ammonia (NH_4^+/NH_3 or FA) and nitrite/free nitrous acid (NO_2^-/HNO_2 or FNA) equilibria, and the unionized nitrogen forms (FA and FNA) can inhibit both AOB and NOB populations. NOB is affected by FA and FNA more than AOB (Anthonisen et al., 1976; Park and Bae, 2009; Vadivelu et al., 2006, 2007) and hence this characteristic can be used to washout NOB from the process obtaining an enriched AOB population (Jubany et al., 2009a).

The effluents of partial nitrification systems treating high-strength wastewaters typically contain as nitrogen discharge either only nitrite (Jubany et al., 2009a; Tokutomi, 2004) or ammonium/nitrite ratio of 50% (Xue et al., 2009; Yamamoto et al., 2008). Consequently, the enriched AOB population of these systems is subjected to high concentrations of total nitrite nitrogen (TNN = $NO_2^- + HNO_2$) or total ammonia nitrogen (TAN = $NH_4^+ + NH_3$) and TNN. Another feature of these systems is the possible existence of inorganic carbon limitations, which can decrease AOB activity (Guisasola et al., 2007). This limitation can be due to the lack of enough total inorganic carbon (TIC) in the influent (Parkes et al., 2007; Wett and Rauch, 2003; Whang et al., 2009) or due to the operation mode used for achieving partial nitrification (Feng et al., 2007; Ganigué et al., 2007). TNN and/or TAN accumulation, jointly to lack of TIC, are useful situations to eliminate NOB population or to obtain a suitable effluent for the Anammox process. In these circumstances, however, AOB population can be affected by FA and/or FNA inhibition, by TIC limitation or by the combination of these effects. In this sense, some authors point out the need for studies devoted to clarify these effects during partial nitrification process (Ganigué et al., 2009; Yamamoto et al., 2008).

The objective of this work was to evaluate the combined effect of TIC limitation and inhibition by FA or FNA on the kinetics of an enriched AOB population by respirometry and fitting of the data to a kinetic model.

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2. Methods

2.1. Partial nitrification activated sludge system

The biomass used in the experiments was taken from a partial nitrification pilot plant operated with a low influent COD/N ratio (around 0.1). The nitrifying activated sludge system consisted of three aerobic reactors connected in series (with a working volume of 26 L each one) followed by a settler (with a working volume of 25 L). The inflow was controlled with a closed control loop based on the in-line oxygen uptake rate (OUR) measurement. The pilot plant configuration with three reactors in series and the automatic inflow control produced a gradient of TAN concentration through the reactors. More details about the operation of the pilot plant and the inflow control loop can be found elsewhere (Jubany et al., 2009a,b). The activated sludge pilot plant was inoculated with sludge from a municipal WWTP and was fed with a synthetic high-strength ammonium wastewater (1000 mg N-TAN L^{-1}) during one year. Temperature and pH were controlled at 30 °C and 8.3, respectively, whereas sludge retention time was maintained around 10 days. During the whole operational period, 97% of the influent TAN was oxidized to TNN.

2.2. Microbial and chemical analysis

Fluorescence in situ hybridization (FISH) technique coupled with confocal microscopy was used to determine the predominant nitrifying species. A Leica TCS SP2 AOBS confocal laser scanning microscope (CLSM) at a magnification of ×63 (objective HCX PL APO ibd.B1 63 × 1.4 oil) equipped with two HeNe lasers with light emission at 561 and 633 nm was used for biomass quantification. Hybridizations were carried out using at the same time a Cy3-labeled specific probe and Cy5-labeled EUBmix probe (general probe). Specific probe used for AOB detection was Nso190 (Mobarry et al., 1996) while for NOB detection was NIT3 (Wagner et al., 1996). EUBmix probe consisted of the mix of probes EUB338, EUB338 II and EUB338 III (Amann et al., 1990; Daims et al., 1999). Detailed information about FISH quantification can be found elsewhere (Jubany et al., 2009c).

TAN was analyzed using Lange LCK302, LCK303 and LCK304 ammonium kits. TNN and nitrate were measured with ionic chromatography using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an auto-sampler AS40. Volatile suspended solids (VSS) and total suspended solids (TSS) concentrations were determined according to APHA (1995). TIC was measured using a 1020A O-I-Analytical TOC analyzer.

2.3. Respirometer and OUR estimation procedure

Batch experiments to determine the inhibitory effects of FA and FNA were carried out in a respirometer with the enriched AOB sludge withdrawn from the previously described pilot plant. The respirometer used in this work was a LFS (liquid-flow-static) type, where the DO concentration is measured in the liquid phase, which is static and continuously aerated (Spanjers et al., 1998). The respirometer consisted of a magnetically stirred vessel of 2 L capacity. Aeration was supplied from the bottom through a microdiffuser, which ensured small air bubbles and good oxygen transfer from the gas phase to liquid phase. A mass flow controller (Bronckhorst HiTec 825) was used to provide the steady and specific air flow (1 LN min^{-1}) required for an accurate OUR calculation. The temperature of the vessel was controlled at 30 °C with a thermostatic bath. The pH was continuously measured with a pH probe (WTW-Sentix 81) and controlled by the automatic addition of acid or base by an automatic burette (Crison MultiBurette 2S). DO was measured with a WTW-CellOx 325 probe. These pH and DO probes were connected to multiparametric equipment (WTW-Inolab 3), which was connected via RS232 to a PC that monitored the data and stored it in a Microsoft Excel sheet through Visual Basic 6.0 software. A detailed description of the procedure for OUR calculation using a LFS respirometer can be found elsewhere (Spanjers et al., 1998). The OUR reported in this work correspond to exogenous OUR obtained subtracting the endogenous OUR value to the total measured OUR. The obtained values of endogenous OUR were very low, both with standard aeration (0.09 mg O_2 L^{-1} min^{-1}) and CO_2-free synthetic aeration (0.04 mg O_2 L^{-1} min^{-1}). Each OUR value was corrected for possible oxygen limitation effects using the oxygen affinity coefficient for AOB ($K_{O,AOB}$ = 0.74 mg O_2 L^{-1}; Guisasola et al., 2005). The specific OUR (SOUR) was obtained dividing OUR by the VSS concentration in the respirometer. The respirometric experiments were carried out with a biomass concentration around 900 mg VSS L^{-1}.

2.4. Experimental determination of the AOB inhibition by FA and FNA

The study of AOB inhibition by FA and FNA was performed in experiments with and without TIC limitation. TIC limitation conditions for AOB were experimentally established in a previous study (Guisasola et al., 2007), where it was found that an enriched AOB population is limited by inorganic carbon at TIC concentrations lower than 36 mg C L^{-1}. To achieve these limiting TIC conditions for AOB biomass, CO_2-free aeration for several hours was used to increase TIC stripping efficiency (Guisasola et al., 2007). Therefore, two different sets of respirometric batch experiments were carried out: (i) one set with standard aeration using air containing CO_2, which guaranteed non-limiting TIC conditions for AOB (TIC concentration in the bulk liquid > 50 mg C L^{-1}) and (ii) another set using CO_2-free synthetic air (79% N_2, 21% O_2: Air Liquide, Spain) (TIC concentration in the bulk liquid < 3.0 mg C L^{-1} after 24 h of CO_2-free aeration).

The effect of each inhibitory compound (FA or FNA) was assessed in duplicate. For the FA inhibition experiments, TAN concentration was increased step by step to high concentrations (1500 or 4000 mg N L^{-1}, depending on the experiment). The pH was maintained at 8.3 to attain high FA concentration. For the FNA inhibition experiments, the TNN concentration was also increased step by step to high concentrations (900 or 3500 mg N L^{-1}, depending on the experiment). The pH was maintained at 7.0 to attain high FNA concentration whereas a non-limiting and non-inhibiting TAN concentration for AOB was kept in the respirometer. Eqs. (1)–(4), derived from acid–base equilibriums, were used for the calculation of the FA and the FNA concentrations in equilibrium with TAN and TNN, respectively.

$$FA = \frac{TAN \cdot 10^{pH}}{(K_b/K_w + 10^{pH})} \cdot \frac{17}{14} \quad (1)$$

$$FNA = \frac{TNN}{(K_a \cdot 10^{pH} + 1)} \cdot \frac{47}{14} \quad (2)$$

$$\frac{K_b}{K_w} = \exp\left(\frac{6344}{(273 + T)}\right) \quad (3)$$

$$K_a = \exp\left(-\frac{2300}{(273 + T)}\right) \quad (4)$$

where K_b is the ionization constant of the ammonia equilibrium, K_w is the ionization constant of water and K_a is the ionization constant of the nitrous acid equilibrium (Anthonisen et al., 1976).

2.5. Model development

FA is considered the main nitrogenous substrate for AOB instead of ammonium or TAN (Pambrun et al., 2006; Suzuki et al., 1974). Moreover, the substrate inhibition of AOB is also due to FA (Anthonisen et al., 1976). Frequently, the Haldane model has been used in modeling this type of inhibition (Jubany et al., 2009a; Pambrun et al., 2006; Park and Bae, 2009).

$$\mathrm{SOUR} = \mathrm{SOUR}_{\max} \frac{S_{\mathrm{FA}}}{K_{\mathrm{S,FA}} + S_{\mathrm{FA}} + \frac{S_{\mathrm{FA}}^2}{K_{\mathrm{I,FA}}}} \quad (5)$$

where SOUR is the specific OUR (mg O_2 g^{-1}VSS min^{-1}); $\mathrm{SOUR}_{\max}$ is the maximum specific OUR (mg O_2 g^{-1} VSS min^{-1}); S_{FA} is the FA concentration (mg NH_3 L^{-1}); $K_{\mathrm{S,FA}}$ is the half-saturation coefficient for FA (mg NH_3 L^{-1}) and $K_{\mathrm{I,FA}}$ is the inhibition coefficient for FA (mg NH_3 L^{-1}).

Further, two more parameters can be calculated for a substrate inhibition model (Lipczynska-Kochany and Kochany, 2008):

$$\mathrm{SOUR}_{\mathrm{CI}} = \frac{\mathrm{SOUR}_{\max}}{\left(1 + 2 \cdot \sqrt{\frac{K_{\mathrm{S,FA}}}{K_{\mathrm{I,FA}}}}\right)} \quad (6)$$

$$S_{\mathrm{CI}} = \sqrt{K_{\mathrm{S,FA}} \cdot K_{\mathrm{I,FA}}} \quad (7)$$

where $\mathrm{SOUR}_{\mathrm{CI}}$ is the critical rate constant or the practical maximum specific OUR (mg O_2 g^{-1} VSS min^{-1}); and S_{CI} is the FA concentration at which $\mathrm{SOUR}_{\mathrm{CI}}$ is attained (mg NH_3 L^{-1}).

AOB are also inhibited by its product (FNA) and frequently this type of inhibition is modeled using a non-competitive inhibition model (Jubany et al., 2009a; Pambrun et al., 2006; Park and Bae, 2009).

$$\mathrm{SOUR} = \mathrm{SOUR}_{\max} \cdot \frac{K_{\mathrm{I,FNA}}}{K_{\mathrm{I,FNA}} + S_{\mathrm{FNA}}} \quad (8)$$

where $K_{\mathrm{I,FNA}}$ is the inhibition coefficient for FNA (mg HNO_2 L^{-1}) and S_{FNA} is the FNA concentration (mg HNO_2 L^{-1}).

3. Results

3.1. Enriched AOB sludge

The biomass used in the batch experiments was withdrawn from the pilot plant, where the TAN oxidation was stopped at TNN with a negligible nitrate concentration in the effluent. According to FISH analysis, the AOB fraction, detected as *Nitrosomonas*-like bacteria, was 81 ± 8% whereas the NOB fraction, detected as *Nitrobacter*-like bacteria, was 0.75 ± 0.25%. The remaining 18% was unidentified biomass, probably heterotrophic bacteria.

3.2. TAN starvation of AOB

The determination of the time without TAN availability that caused a decrease of the AOB activity is required to assess one by one the effect of TIC limitation and TAN starvation on the AOB inhibition by FA. This time was determined with a specific respirometric experiment in which the AOB population was maintained without TAN availability during 3 days with standard aeration. Only one TAN pulse of 10 mg N L^{-1} was added every day and the SOUR obtained with each TAN addition was measured (Fig. 1).

No starvation effect was observed after 30 h because there was not difference in the maximum SOUR values at 0 and 30 h. However, a significant decay of the maximum SOUR value was observed after 54 h. Consequently, more than 30 h are required to decrease AOB activity under TAN starvation conditions. As a result, the experiments to determine the effect of TIC limitation should be performed after only 24 h of CO_2-free aeration for excluding the simultaneous effect of TAN starvation.

3.3. AOB inhibition by FA with and without TIC limitation

The combined effect of AOB inhibition by FA and TIC limitation was studied with three different respirometric experiments. The first two experiments consisted on measuring the maximum SOUR at different FA concentrations with and without TIC limitation conditions using a non-starved AOB population. Additionally, the simultaneous effect of TAN starvation, FA inhibition and TIC limitation was studied with a third experiment using an AOB population under TAN starvation conditions (N-starved biomass).

Fig. 2a and b shows the results of SOUR profiles obtained with non-starved biomass and standard aeration, i.e. without TIC limitation. Fig. 2c and d shows the SOUR profiles obtained with TIC limitation and non-starved biomass. Finally, Fig. 2e and f shows the SOUR profiles obtained after 96 h of TAN starvation with TIC limitation. All the experiments were repeated twice with very similar results. The figures also show the TAN concentration measured through off-line analysis. During these experiments TNN was measured (data not shown) in order to assure non-inhibiting concentrations of FNA. The concentration of nitrate (data not shown) did not increase during batch tests, which demonstrate that the obtained SOUR was only due to AOB activity.

A pulse with low TAN concentration was firstly carried out (Fig. 2a, c and e) in order to obtain values of SOUR around the half-saturation coefficient of FA ($K_{\mathrm{S,FA}}$). After that, the SOUR was measured with series of consecutive TAN additions (Fig. 2b, d and f).

Fig. 2a clearly shows that SOUR without TIC limitation was maintained at its maximum value when TAN concentration was

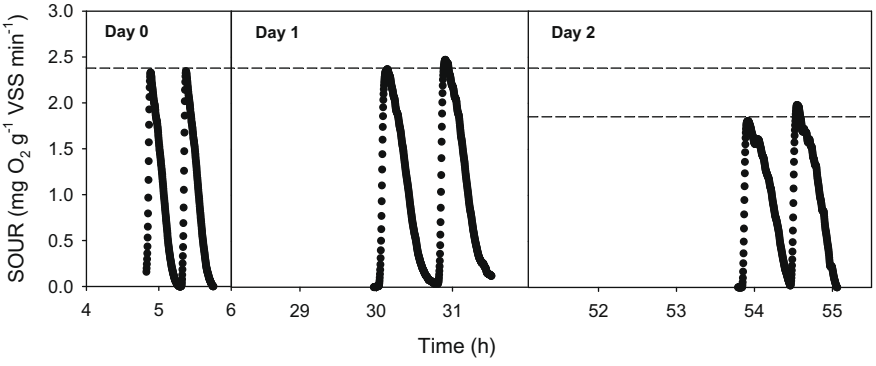

Fig. 1. SOUR obtained with two consecutive TAN pulses of 10 mg N L^{-1}. Day 0: initial response. Day 1: after 24 h of standard aeration without TAN addition. Day 2: after additional 24 h under the same conditions.

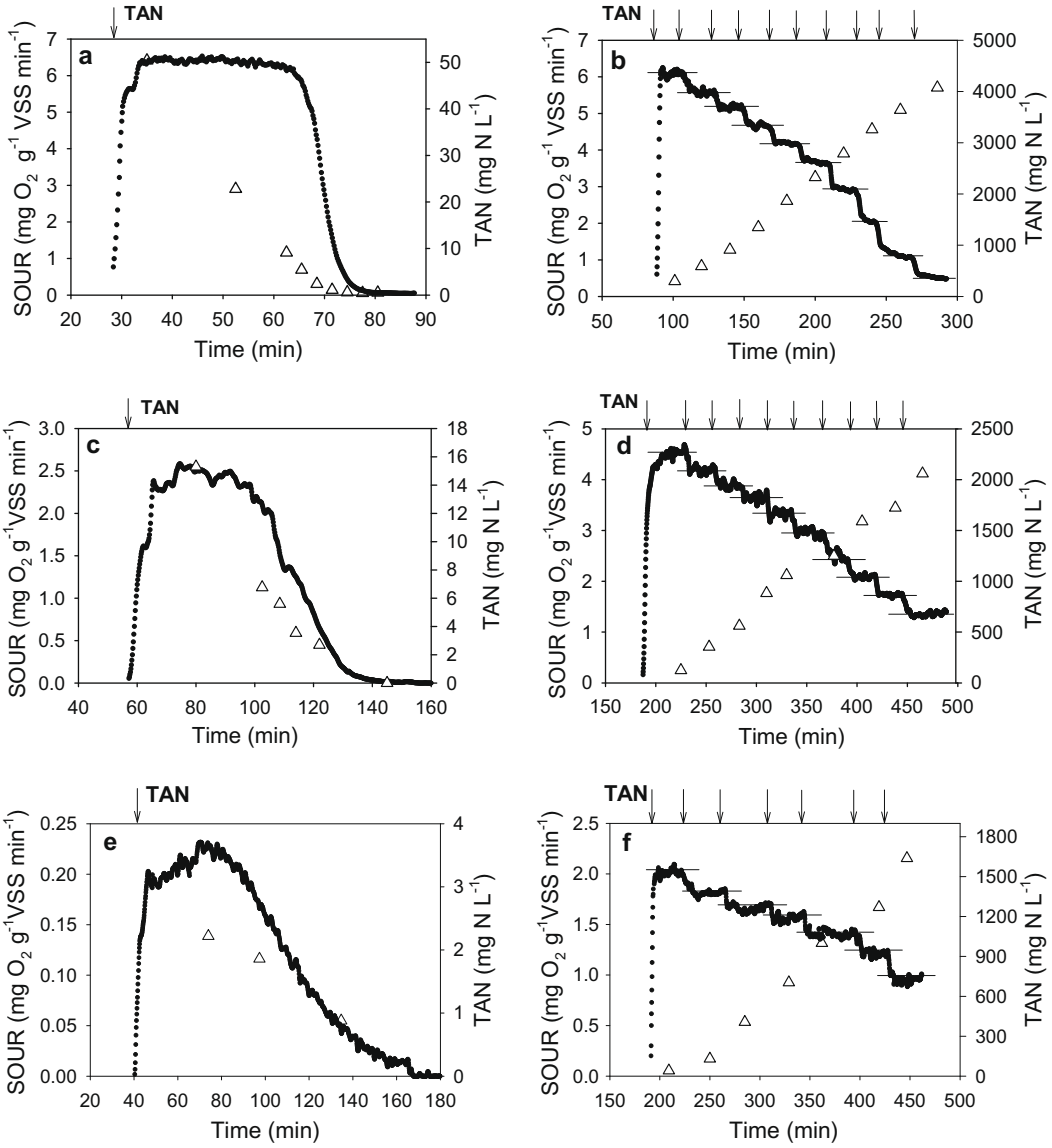

Fig. 2. SOUR (dots) and TAN concentration (open triangles) during batch respirometric experiments with standard aeration and non-starved biomass (Fig. 2a and 2b), with CO_2-free synthetic aeration and non-starved biomass (Fig. 2c and d) and with CO_2-free synthetic aeration and N-starved biomass (Fig. 2e and f). Arrows and solid lines indicate TAN additions and average SOUR values for each TAN concentration, respectively.

higher than 10 mg N L^{-1} and it quickly decreased when this concentration was lower. This maximum SOUR was maintained up to TAN concentration of approximately 100 mg N L^{-1} (Fig. 2b). A clear decrease on the SOUR was observed when the TAN concentration increased above 100 mg N L^{-1}. On the other hand, the maximum SOUR with TIC limitation was achieved when TAN concentration was around 150 mg N L^{-1} (Fig. 2d) and it decreased with the increase of TAN concentration. Additionally, substrate limitation could be observed when TAN concentration was lower than 10 mg N L^{-1} (Fig. 2c).

The SOUR corresponding to each FA concentration was calculated as an average of the values contained in the stable period of SOUR at each TAN concentration (solid lines in Fig. 2). The experimental SOUR values are plotted versus FA and TAN concentration in Figs. 3 and 4 for non-starved and N-starved biomasses, respectively. For an easy comparison between both experiments, all the SOUR values are represented as a fraction of the maximum SOUR obtained in the experiment without TIC limitation. The experimental SOUR values show a typical substrate-inhibition profile: low SOUR at low FA concentrations due to the substrate limitation and a decrease of the SOUR at high FA concentrations due to the substrate inhibition (Carrera et al., 2004; Pambrun et al., 2006). A Haldane kinetic model (Eq. (5)) was successfully fitted to the data obtained in the three experiments (Figs. 3 and 4). The kinetic parameters obtained for each experiment are presented in Table 1. $SOUR_{CI}$ and S_{CI} were obtained according to Eqs. (6) and (7).

For an easy understanding of each effect on AOB population, the prediction of the Haldane model obtained with the N-starved biomass under TIC limitation is presented in Fig. 4 together with the predictions of the Haldane model obtained with and without TIC limitation and non-starved biomass (from Fig. 3).

3.4. AOB inhibition by FNA with and without TIC limitation

The combined effect of AOB inhibition by FNA and TIC limitation was studied with two different respirometric experiments. These experiments consisted of measuring the maximum SOUR at different FNA concentrations with and without TIC limitation conditions. In both experiments, FA concentration was maintained around each obtained S_{CI} to guarantee that AOB population was not

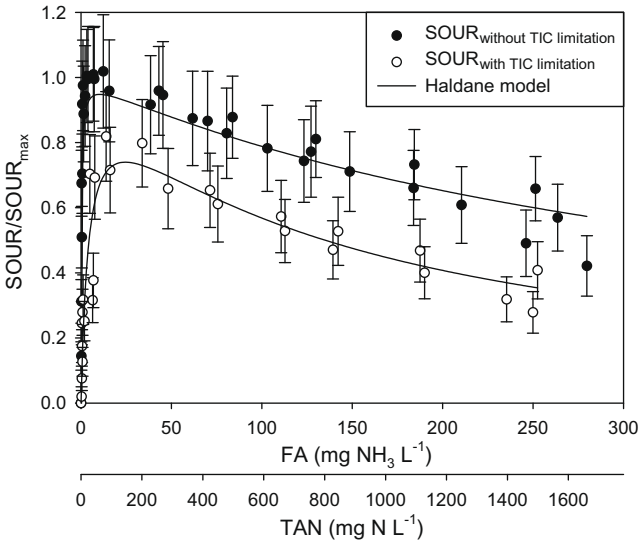

Fig. 3. SOUR values without TIC limitation (TIC concentration in the bulk liquid higher than 50 mg C L^{-1}) and SOUR values with TIC limitation (TIC concentration in the bulk liquid lower than 3 mg C L^{-1}) represented as a fraction of the maximum SOUR without TIC limitation versus TAN and FA concentrations (pH 8.3 and $T = 30$ °C). The $SOUR_{max}$ of the process without TIC limitation was 6.25 mg O_2 g^{-1} VSS min^{-1}. The solid lines are the prediction of the Haldane model for the experimental results. Error bars were calculated by propagation of error method.

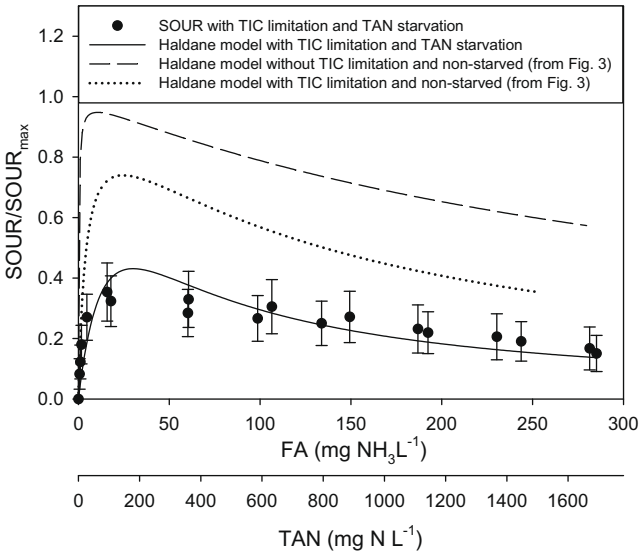

Fig. 4. SOUR with TIC limitation (TIC concentration in the bulk liquid lower than 3 mg C L^{-1}) and TAN starvation conditions after 4 days with CO_2-free aeration. SOUR is represented as a fraction of the maximum SOUR without TIC limitation versus FA and TAN concentrations (pH 8.3 and $T = 30$ °C). The $SOUR_{max}$ of the process without TIC limitation was 6.25 mg O_2 g^{-1}VSS min^{-1}. Error bars were calculated by propagation of error method.

limited and/or inhibited by FA. Fig. 5a shows the results of SOUR profile obtained with standard aeration, i.e. without TIC limitation, whereas Fig. 5b shows the SOUR profile obtained with TIC limitation. Both experiments were repeated twice without significant differences. A series of consecutive TNN additions were carried out in order to increase FNA concentration in both experiments and Fig. 5a and b shows the TNN concentrations measured through off-line analysis. Equally to the experiments of FA inhibition assessment, nitrate concentration did not increase during batch tests, confirming that the obtained SOUR was only due to AOB activity.

Table 1
Parameters obtained fitting the Haldane model to the experimental data of AOB inhibition by FA in different operational conditions.

Parameter	Without TIC limitation and non-starved AOB	With TIC limitation and non-starved AOB	With TIC limitation and N-starved AOB
$K_{S,FA}$ (mg NH_3 L^{-1})	0.28 ± 0.04	4.3 ± 0.7	20 ± 4
$K_{I,FA}$ (mg NH_3 L^{-1})	376 ± 45	139 ± 17	46 ± 5
$SOUR_{CI}/SOUR_{max}$	0.95	0.74	0.43
S_{CI} (mg NH_3 L^{-1})	10.3	24.5	30.3

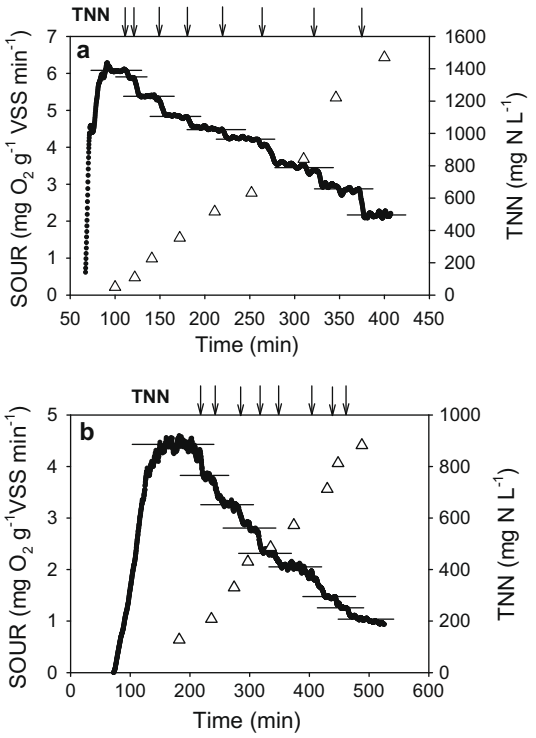

Fig. 5. SOUR (dots) and TNN concentration (open triangles) during batch respirometric experiments with standard aeration (a) and with CO_2-free synthetic aeration (b). Arrows and solid lines indicate TNN additions and average SOUR values for each TNN concentration, respectively.

The SOUR corresponding to each FNA concentration was calculated as an average of the values contained in the stable period of SOUR at each TNN concentration (solid lines in Fig. 5). The experimental SOUR values are plotted versus FNA and TNN concentration in Fig. 6. For an easy comparison between both experiments, the SOUR values are represented as a fraction of the maximum SOUR obtained in the experiment without TIC limitation. In both cases, with and without TIC limitation, a SOUR decrease was observed when FNA concentration was increased, proving a clear inhibition by FNA. A non-competitive inhibition kinetic model (Eq. (8)) is usually applied for this type of inhibition (Jubany et al., 2009a; Van Hulle et al., 2007). This model was satisfactorily fitted to the experimental data and the obtained non-competitive inhibition coefficients ($K_{I,FNA}$) are shown in Table 2.

4. Discussion

4.1. Assessment of the effect of TIC limitation and TAN starvation conditions on the AOB inhibition by FA

The accurate fitting of Haldane model in Figs. 3 and 4 confirmed that the type of kinetic model for an enriched AOB sludge inhibited

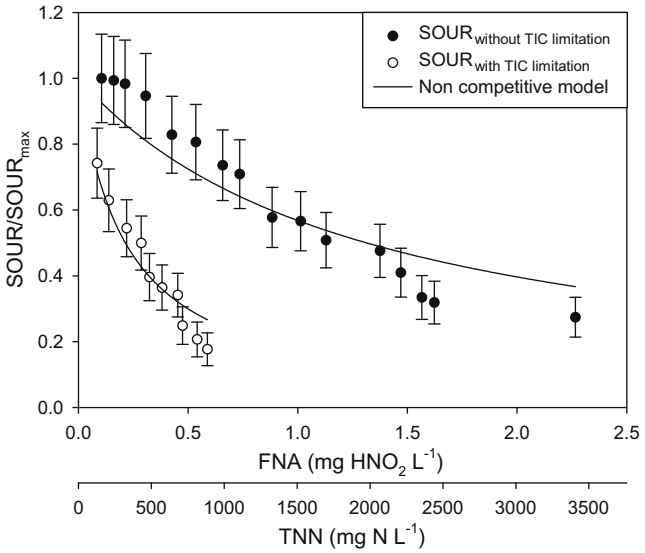

Fig. 6. SOUR values without TIC limitation (TIC concentration in the bulk liquid higher than 50 mg C L^{-1}) and SOUR values with TIC limitation (TIC concentration in the bulk liquid lower than 3 mg C L^{-1}) represented as a fraction of the maximum SOUR without TIC limitation versus FNA and TNN concentrations (pH 7.0 and T = 30 °C). The solid lines are the prediction of the non-competitive model for the experimental results with and without TIC limitation. Error bars were calculated by propagation of error method. The $SOUR_{max}$ of the process without TIC limitation was 6.15 mg O_2 g^{-1}VSS min^{-1}.

Table 2
AOB non-competitive inhibition coefficient by FNA obtained for experiments with and without TIC limitation and comparison with data from the literature.

$K_{I,FNA}$ (mg HNO_2 L^{-1}) without TIC limitation and non-starved AOB	$K_{I,FNA}$ (mg HNO_2 L^{-1}) with TIC limitation and non-starved AOB	Reference
1.31 ± 0.14	0.21 ± 0.02	This work
0.18	–	Pambrun et al. (2006)
0.57	–	Park and Bae (2009)
0.68	–	Hellinga et al. (1999)
1.48	–	Magrí et al. (2007)
1.65	–	Ganigué et al. (2007)
6.85	–	Van Hulle et al. (2007)

by FA did not depend of the TIC availability or the TAN starvation conditions. However, the kinetic parameters obtained in all the cases were significantly different. The half-saturation coefficients ($K_{S,FA}$ in Table 1) suggested that the AOB affinity for FA depended on the conditions of TIC availability. The $K_{S,FA}$ for non-limiting TIC conditions was in the range reported by others (Pambrun et al., 2006; Van Hulle et al., 2007) but was one order of magnitude higher for limiting TIC conditions and non-starved AOB. Furthermore, $K_{S,FA}$ increased significantly for the N-starved biomass with TIC limitation suggesting that the AOB affinity for FA also depended on the conditions of TAN starvation. Indeed, the $K_{S,FA}$ for N-starved AOB with limiting TIC conditions was at least two order of magnitude higher than those reported by Vadivelu et al. (2006) and Van Hulle et al. (2007).

In the same way, S_{CI} (the substrate concentration at which $SOUR_{CI}$ is attained) was higher for experiments with TIC limitation. This agreed with the trend of $K_{S,FA}$ since both parameters depend on the substrate concentration at which maximum process rate is achieved. However, S_{CI} is significant for a process with substrate inhibition because, from a practical point of view, it indicates the real substrate concentration for minimizing the effects of limitation and inhibition by substrate.

In contrast, Vadivelu et al. (2006) reported a $K_{S,FA}$ (0.44 mg NH_3 L^{-1}) that was independent of the presence or absence of TIC. The reason for the difference in outcomes is not known. According to our experimental results, the process rate in a nitrifying reactor under TIC limitation conditions could be increasingly limited by FA.

A decrease in $SOUR_{CI}$ was observed from non-limiting TIC conditions when the biomass was subjected to TIC limitation and a higher decrease was observed for N-starved biomass with TIC limitation (Table 1). These results indicate that the starvation conditions affected the rate of the partial nitrification process as it was observed by other authors (Tappe et al., 1999). Indeed, comparing all the values of $SOUR_{CI}$ in Table 1, the conclusion was that only a 78% of the maximum rate could be achieved with TIC limitation. This percentage is very similar to the one obtained by Vadivelu et al. (2006). However, this percentage decreased to only 40% when TAN starvation conditions were added to the TIC limitation.

With regard to the substrate inhibition coefficient ($K_{I,FA}$ in Table 1), the value obtained with non-starved biomass under TIC limitation was lower than that attained without TIC limitation. It indicated that inhibition by FA was higher when the AOB population was under TIC limitation. This difference was also not detected by Vadivelu et al. (2006). However, these authors did not find any inhibitory effect of FA with or without TIC limitations in their FA studied range (from 0 to 16 mg N NH_3 L^{-1}). Perhaps, a different result would have been obtained with higher FA concentrations.

In spite of the different inhibition coefficients obtained in the presented study, their values were higher than those previously reported (Antileo et al., 2002; Carrera et al., 2004; Park and Bae, 2009), i.e. the AOB population used in this work was adapted to high FA concentrations and the possibility of a noteworthy reduction of the process rate by FA inhibition was low with or without TIC limitation.

Moreover, the $K_{I,FA}$ for N-starved biomass (Table 1) was lower than that obtained for non-starved biomass and TIC limitation, suggesting that the inhibition by FA strongly affects the N-starved biomass. In a similar way, Gerards et al. (1998) found that an N-starved AOB population was more inhibited by FNA than a non-starved AOB population.

4.2. Assessment of the effect of TIC limitation on the AOB inhibition by FNA

The AOB inhibition by FNA was higher when the biomass was under TIC limitation conditions (Fig. 6). $SOUR_{CI}$ with TIC limitation was 75% of $SOUR_{CI}$ without TIC limitation, which was in accordance with the value obtained in the experiments with FA inhibition. Under non-limiting TIC conditions (black dots in Fig. 6), the enriched AOB sludge showed a high capacity to oxidize TAN to TNN even at high FNA concentrations (higher than 1.5 mg HNO_2 L^{-1}). When this value is compared with those in the literature, it can be concluded that the AOB population was adapted to high FNA concentrations (Tan et al., 2008).

Both accurate fittings of a non-competitive inhibition kinetic model in Fig. 6 confirmed that the type of kinetic model for an AOB population inhibited by FNA did not depend of the TIC availability. However, the inhibition coefficients obtained in both cases were significantly different (Table 2). The coefficient obtained under TIC limitation was lower than that attained without TIC limitation. Therefore, inhibition by FNA was higher when AOB were under TIC limitation in a similar way than the results obtained for the inhibition by FA. Again, these results only partially agree with the work of Vadivelu et al. (2006). These authors found that the OUR of AOB without TIC limitation decreased by 50% when the FNA increased to 1.34 mg HNO_2 L^{-1}, which is very similar to the OUR drop detected in this work without TIC limitation at the

same FNA concentration (see Fig. 6). However, Vadivelu et al. (2006) found that the OUR of AOB with a high TIC limitation (defined in that work as complete absence of TIC) was less inhibited by FNA than the OUR without TIC limitation. Moreover, these authors found that the OUR with TIC limitation at 1.7 mg HNO_2 L^{-1} was 50% of the maximum OUR without neither TIC limitation nor FNA inhibition. However, as can be observed in Fig. 6, the OUR obtained in this work with TIC limitation at 0.5 mg HNO_2 L^{-1} was only 20% of the maximum OUR without neither TIC limitation nor FNA inhibition. The reasons for this discrepancy are not known.

Both $K_{I,FNA}$ obtained in this work are included in the wide range of the bibliographic references for this type of inhibition (Table 2). This wide range of coefficients could be due to the different adaption of AOB to FNA in each study (Van Hulle et al., 2007). However, our results suggest that the determination of some of these coefficients under TIC limitations conditions could explain the wide range of bibliographic coefficients.

4.3. Practical considerations of the combined effect of FA or FNA inhibition, TIC limitation and TAN starvation on an enriched AOB sludge

The AOB inhibition by FA is somewhat increased under TIC limitation conditions but, as the previous results demonstrated, the inhibition by FA increases if AOB are simultaneously under TIC limitation and TAN starvation conditions. A partial nitrification system could be under TAN starvation due to different operational situations, i.e. an unplanned inflow stop or a seasonal closure of the wastewater production. Under these circumstances, if the start-up of the inflow is carried out without a well designed scheduling, the biomass could experience the combination of three different effects: TAN starvation, FA inhibition and TIC limitation, which can lead to an unexpected failure of the partial nitrification system.

The inhibition of AOB by FNA is especially amplified by the combination with TIC limitation conditions. For instance, the accumulation of a FNA concentration of 0.5 mg HNO_2 L^{-1} under non-limiting TIC conditions caused a decrease of 28% of the maximum AOB activity. Nevertheless, this percentage increases up to 70% if the system is under TIC limitation conditions.

The combination of a pH below 7.0 (which typically indicates TIC limitation) and a high TNN concentration (consequently high FNA concentration) can often be found in partial nitrification systems treating high-strength ammonium wastewaters (Feng et al., 2007; Gali et al., 2007; Magrí et al., 2007; Parkes et al., 2007; Yamamoto et al., 2008). Under these circumstances, the reactor performance worsens and this problem is sometimes attributed to the intrinsic effect of pH on the nitritation rate (Gali et al., 2007). However, the results of this study demonstrate that the decrease of the nitritation rate in these situations can be related to the increase of the AOB inhibition by FNA under TIC limitation conditions.

5. Conclusions

This study showed that AOB were inhibited by FA and FNA, and both inhibitions were higher under TIC limitation conditions.

AOB inhibition by FA was successfully fitted to a Haldane kinetic model for both limiting and non-limiting TIC conditions although a lower inhibition coefficient was obtained under TIC limitation. The values of the half-saturation coefficients showed that the AOB affinity for FA depends on the conditions of TIC availability. Moreover, this study demonstrated that both the AOB inhibition and the affinity by FA worsen after four days under TAN starvation conditions.

The non-competitive inhibition model provided a good fitting for inhibition by FNA under both limiting and non-limiting TIC conditions but this inhibition was especially amplified with TIC limitation.

The findings of this study can explain some of the problems that occur in the operation of partial nitrification systems. However, further work is needed to understand the consequences of the combined effect of FA and FNA inhibitions with TIC limitation for long operational periods of those systems.

Acknowledgements

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Apèndix II

Currículum Vitae

Josep Anton Torà Suárez

Aguilar de Segarra (Barcelona), 27 de Novembre de 1983
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Educació personal

2007–present: Estudiant de doctorat en Ciències Ambientals a la Universitat Autònoma de Barcelona.
Títol: Eliminació biològica de nitrogen via nitrit d'un corrent amb alta càrrega d'amoni (Nitrite pathway biological nitrogen removal of a high strength ammonium wastewater).

2006–2007 Màster en Estudis Ambientals. Especialitat Tecnologia Ambiental per la Universitat Autònoma de Barcelona.
Títol: Posada en marxa d'un sistema de nitrificació parcial a escala pilot pel tractament de l'aigua de rebuig.

2001 – 2006 Llicenciat en Enginyeria Química per la Universitat Autònoma de Barcelona amb l'obtenció del premi extraordinari.

Publicacions

Josep A. Torà, Javier Lafuente, Juan A. Baeza, Julián Carrera (2010). Combined effect of inorganic carbon limitation and inhibition by free ammonia and free nitrous acid on ammonia-oxidizing bacteria. Bioresource Technology. Volume 101, Issue 15, Pages 6051-6058.

Josep A. Torà, Javier Lafuente, Julián Carrera, Juan A. Baeza (2011). Fast start-up and controlled operation during a long term period of a high-rate partial nitrification activated sludge system. Environmental Technology. 2011; doi: 10.1080/09593330.2011.626802.

Josep A. Torà, Juan A. Baeza, Julián Carrera, Jan A. Oleszkiewicz (2011). Denitritation of a high-strength nitrite wastewater in a sequencing batch reactor using different organic carbon sources. Chemical Engineering Journal. Volume 172, Issues (2-3), Pages 994-998.

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Ana García, Lucia Delgado, **Josep A. Torà**, Eudald Casals, Edgar González, Víctor Puntes, Xavier Font, Julián Carrera, Antoni Sánchez (2011). Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. Journal of Hazardous Materials. doi:10.1016/j.jhazmat.2011.10.057

Capítols de llibre

Julián Carrera, Albert Bartrolí, Eduardo Isanta, Irene Jubany, **Josep A. Torà**, Juan A. Baeza, Julio Pérez. Advanced technology for the biological nitrogen removal of the reject water from the sludge dewatering systems. In: Reduction, Modification and Valorisation of Sludge (REMOVALS). Editors: Christophe Bengoa, Azael Fabregat, Josep Font, Frank Stüber. IWA Publishing. ISBN: 9781843393450 (2011). Chapter 7, Pages 101-116.

Participació en projectes

Finançats en convocatòries públiques

- REduction, MOdification and VALorisation of Sludge (REMOVALS).
  Entitat finançadora: Comissió Europea. Contracte No.018525
  Duració: Juliol 2006 – Juny 2009
  Investigador responsable: Julián Carrera Muyo
  Import al grup UAB: 274.000 €
  Import total del projecte: 3.053.000 €

- Automatic coNtrol for partial nitriFIcation to nitrite in BIOfilm reactors (ANFIBIO).

Entitat finançadora: Generalitat de Catalunya 2010 VALOR 0096

Duració: Gener 2011 – Desembre 2012

Investigador responsable: Julio Pérez Cañestro

Import del projecte: 76.970 €

Finançats per empreses

- Tractament i depuració biològica d'efluents.

Entitat finançadora: ECOIMSA S.A.

Duració: Febrer 2009 – Abril 2009

Investigador responsable: Julián Carrera Muyo i Javier Lafuente Sancho

Import del projecte: 42.000 €

- Investigació en matèria de bioreactors.

Entitat finançadora: Centro Tecnológico del Agua (CETaqua)

Duració: Setembre 2010 – Febrer 2011

Investigador responsable: Julián Carrera Muyo

Import del projecte: 18.000 €

Congressos

Congrés: Third International Meeting on Environmental Biotechnology and Engineering (3IMEBE)

Lloc de Celebració: Palma de Mallorca (Espanya)

Data: 21-25 Set. 2008

Autors: **Josep A. Torà**, Juan A. Baeza, Javier Lafuente, Julián Carrera

Títol: Kinetic models for ammonium-oxidising bacteria inhibition by free ammonia and free nitrous acid.

Tipus: Presentació oral

Estades

Estada a la University of Manitoba (Winnipeg, Canadà) del juliol de 2010 a l'octubre de 2010 sota la direcció del Prof. Jan A. Oleszkiewicz.