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DESARROLLO DE ESTRATEGIAS OPERACIONALES PARA LA ESTABILIDAD Y MEJORAMIENTO DEL PROCESO DE REMOCIÓN DE NITRÓGENO EN PRESENCIA DE CARBONO ORGÁNICO

OPERATIONAL STRATEGIES FOR THE IMPROVEMENT OF PARTIAL NITRITATION/ANAMMOX PROCESS IN PRESENCE OF ORGANIC MATTER

POR

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Preface

This thesis is submitted to fulfill the requirements for the Doctor of Philosophy (Ph.D.) degree at the Universidad de Concepción. The work presented has been carried out at the Bioengineering laboratory of the Chemical Engineering Department of the Universidad de Concepción (UdeC), Chile and at the Group of Environmental Biotechnology of the Chemical Engineering Department of the Universidade de Santiago de Compostela (USC), Spain. This research was developed under the guidance of Professor Marlene D. Roeckel (UdeC) as main supervisor and Professor José-Luis Campos (Universidad Adolfo Ibañez, Viña del Mar, Chile) as co-supervisor.

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My first approaches to waste treatment in 2008 were born from the idea of "saving the world" cultivated in childhood and that the passage through the university was modeling towards a concrete idea of "avoiding harm". That year I started operating my first nitrifying bioreactor at the laboratory, while, on the other hand, an independent initiative for the recycling of university solid waste began with my friends from *SurSustenta* NGO, which was welcomed by the institution after a few years of persistence. It is not until my arrival at the *S.U.R.* political-organization I was able to broaden my perspective and give a more strategic approach to my scientific work, projecting it under a socio-environmental ethical framework and understanding that

world change responds more to political than scientific efforts, but that we can all be subjects of change if we organize ourselves.

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Abstract

Partial nitritation (PN)/Anammox process is an advance technology for the autotrophic nitrogen removal from wastewaters. Nitrogen removal by PN-Anammox allows to save costs associated with oxygen requirements, organic matter input and sludge disposal compared to the conventional nitrification-denitrification process. It has been mainly implemented in municipal wastewater treatment plants but also in industrial ammonia effluents, specifically used for anaerobic digester supernatants because of its high ammonia content and low organic carbon to nitrogen ratios. Its application has been limited to effluents with low chemical organic demand to nitrogen (COD/N) ratios since organic carbon promotes competition between heterotrophic and autotrophic bacteria for substrates (oxygen, ammonia and nitrite) and space. Otherwise, detection of denitrifying bacteria in PN-Anammox systems with organic carbon suggest that nitrogen removal efficiency can be improved by denitrifying the nitrate produced by Anammox, but operation conditions that promotes denitrification in PN-Ananmox systems are not already clear.

In this work, an experimental approach was used to find operational strategies to improve nitrogen removal efficiencies and to achieve a stable operation in granular PN-Anammox systems in presence of organic carbon. The research focused on the applied hydraulic residence time (HRT), aeration and feeding regime, studying the effect of these variables over the performance, efficiencies and microbial population dynamics.

Five independent experiments on PN-Anammox reactors at laboratory and bench scale were performed, evaluating the effluent quality to determine global and specific

nitrogen and COD removal rates and efficiencies in the reactor based on global balances. Contribution of aerobic and anaerobic ammonia oxidizing, nitrite oxidizing, heterotrophic and denitrifying bacterial groups were also determined based on biologic reactions stoichiometry. Microbial community analysis along the operation to detect composition, abundance and dynamics of the main bacterial groups complemented those results.

Stable operation range was found in granular PN-Anammox system with inlet organic carbon based on HRT (45 to 9.6 h) for the establishment of a hybrid biomass sludge composed by aggregated autotrophic bacteria (granules and flocs) and non-aggregated heterotrophic bacteria. High nitrogen removal efficiencies (NRE) from 74 to 86% and stable operation was achieved at least until a 3-COD/N inlet ratio operating at 30 h of HRT. A strategy to promote denitrification into an SBR PN-Anammox system was also developed, achieving Simultaneous Nitritation, Anammox and Denitrification (SNAD) into a PN-Anammox reactor with inlet COD, achieving NRE from 85 to 96% for inlet COD/N ratios of 2.3 and 0.57, respectively. This finding improves the nitrogen removal efficiency and decreases the aeration costs associated to aerobic COD oxidation. Furthermore, the developed strategies allow to maintain the PN-Anammox granules composition and the dominance of aerobic and anaerobic ammonium oxidizing bacteria in the granular sludge. The process was proved to be feasible for the nitrogen removal from digested poultry manure and fish canning effluents operating into the stable operation range experimentally found.

It was demonstrated that operation strategies based on HRT, feeding and aeration mode can improve the autotrophic nitrogen removal by PN-Anammox system with high COD/N ratio content. This will contribute to expand the full potential of this technology in industrial effluents treatment without losing efficiency and stability of the process due to the presence of organic carbon.

Resumen

El proceso de Nitritación parcial (NP)/Anammox es una tecnología avanzada que permite la remoción autotrófica de nitrógeno de aguas residuales. Esta tecnología comparada con el método tradicional de nitrificación-desnitrificación, permite ahorrar costos asociados a requerimientos de oxígeno, adición de materia orgánica y disposición de lodos. Ha sido implementada principalmente en plantas de tratamiento de aguas urbanas y en algunos efluentes industriales amoniacales, específicamente en el sobrenadante proveniente de digestores anaerobios por su alto contenido amoniacal y bajo contenido orgánico. Su aplicación se ha visto limitada a efluentes de baja razón de Demanda Química de Oxígeno a Nitrógeno (DQO/N) debido al desarrollo de bacterias heterotróficas promovido por la presencia de DQO, las cuales compiten con las bacterias autótrofas por sustratos (oxígeno, amonio y nitrito) y espacio disponible. Por otra parte, se ha detectado la presencia de bacterias desnitrificantes en algunos sistemas de NP-Anammox con carbono orgánico, lo cual ha llevado a sugerir que la eficiencia de remoción de nitrógeno puede ser mejorada mediante la desnitrificación del nitrato producido por la reacción Anammox. Sin embargo, aún se desconocen las condiciones que promueven la desnitrificación en sistemas de NP-Anammox.

Mediante un diseño experimental, este trabajo se enfocó en encontrar estrategias operacionales que permitieran mejorar las eficiencias de remoción de nitrógeno y obtener operaciones estables en sistemas de NP-Anammox en presencia de carbono orgánico. La investigación se enfocó en el estudio del tiempo de residencia hidráulico (TRH), el régimen de aireación y de alimentación aplicados al sistema,

evaluando el efecto de estas variables sobre el desempeño, la eficiencia y las dinámicas poblacionales de los microorganismos fundamentales.

Se realizaron cinco experimentos independientes en reactores de NP-Anammox a escala laboratorio y piloto, evaluando la calidad del efluente para la determinación de las velocidades y eficiencias de remoción de nitrógeno y COD mediante balances globales. Se determinó además la contribución de los grupos bacterianos principales (oxidantes de amonio aeróbicas y anaeróbicas, nitrito oxidantes, heterotróficas y desnitrificantes) en la remoción de nitrógeno global, basado en la estequiometría de las reacciones. Estos resultados fueron complementados con análisis de las comunidades microbianas a lo largo de la operación para detectar composición, abundancia y posibles dinámicas poblacionales.

Fue posible hallar un rango de operación estable para sistemas de NP-Anammox con carbono orgánico basado en el TRH (45 a 9.6 h) que permite el establecimiento de un sistema de biomasa híbrida compuesta por agregados bacterianos autótrofos (gránulos y flóculos) y bacterias heterótrofas no agregadas en suspensión. La mantención de un TRH de 30 h permitió obtener altas eficiencias de remoción de nitrógeno (74 a 86%) y una operación estable hasta al menos una razón DQO/N de 3.

Además, se desarrolló una estrategia que permite promover la desnitrificación en sistemas de NP-Anammox con afluente de carbono orgánico, permitiendo el desarrollo de un sistema SNAD (Nitrificación, Anammox y Desnitrificación Simultánea) de alta eficiencia (85 a 96% de remoción de nitrógeno para razones de DQO/N de 2.3 y 0.57 respectivamente). Este hallazgo permite mejorar la eficiencia de remoción de nitrógeno y a su vez, disminuir los costos de aireación asociados a la oxidación aeróbica del carbono orgánico.

Las estrategias desarrolladas permiten además mantener la composición y dominancia de las bacterias oxidantes de amonio aeróbicas y anaeróbicas en la biomasa granular de sistemas de NP-Anammox. La factibilidad del proceso de NP-Anammox fue probada con digestato de estiércol de gallina y efluentes de industria conservera de productos marinos, siendo exitosa en términos de estabilidad del proceso y eficiencias obtenidas en ambos casos.

En esta tesis ha sido posible demostrar que estrategias de operación basadas en el TRH, régimen de alimentación y de aireación permiten mejorar la remoción autotrófica de nitrógeno de efluentes con alta razón DQO/N mediante sistemas NP-Anammox de biomasa granular. Estas estrategias pretenden contribuir a la expansión de esta tecnología a nuevos efluentes industriales de alta carga amoniacal y contenido orgánico sin perder eficiencia y estabilidad debido a la presencia de carbono orgánico en el afluente.

Nomenclature

Abbreviations

AOB	Ammonia Oxidizing Bacteria
anAOB	Anaerobic Oxidizing Bacteria
AORx	Reactor specific removal rate by AOB
ARx	Reactor specific removal rate by anAOB
CANON	Completely Autotrophic Nitrogen removal Over Nitrite
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen concentration
DRx	Reactor specific removal rate by HDB
FA	Free Ammonia
FISH	Fluorescence In Situ Hybridization
FNA	Free Nitrous Acid
HB	Heterotrophic Bacteria
HDB	Heterotrophic Denitrifying Bacteria
HNa	Heterotrophic Nitrogen assimilation
HRT	Hydraulic Residence Time
IC	Inorganic Carbon
NLR	Nitrogen Loading Rate
NO_2^-–N	Nitrite-Nitrogen
NO_3^-–N	Nitrate-Nitrogen
NOB	Nitrite Oxidizing Bacteria
NORx	Reactor specific removal rate by NOB
NRE	Nitrogen Removal Efficiency
NRR	Nitrogen Removal Rate
OLAND	Oxygen-Limited Autotrophic Nitrification and Denitrification
OLR	Organic Loading Rate
OxLR	Effective Oxygen Loading Rate
SAA	Specific Anammox Activity
SBR	Sequencing Batch Reactor

SHARON	Single reactor for High activity Ammonium Removal Over Nitrite
SNA	Specific Nitrifying Activity
SNAD	Simultaneous Nitrification, Anammox and Denitrification
SRT	Sludge Retention Time
TAN	Total Ammonium Nitrogen
TAR	Total Ammonium nitrogen Removal efficiency
TN_{den}	Total Nitrogen denitrified
TOC	Total Organic Carbon
TS	Total Solids
VS	Volatile Solids
WWTP	Wastewater Treatment Plant
ΔN	total observed Nitrogen consumed in the reactor

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INTRODUCTION

Partial nitritation – Anammox

Since Anammox discovery on 1995 [1], a remarkably rapid growth of related articles has been observed, and bioprocesses based on Anammox has been identified as a novel and sustained technologies for wastewater treatment [2]. Anammox reaction coupled with partial nitritation (PN) allows to autotrophically remove nitrogen from wastewaters, since ammonia oxidizing bacteria (AOB) can partially oxidize ammonia to provide a suitable effluent for Anammox reaction (Eq. 1).

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13H^+ \rightarrow 1.02\ N_2 + 0.26NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad \text{(Eq. 1)}$$

The overall reaction of PN-Anammox (Eq. 2) compared to conventional nitrification-denitrification process decreases oxygen requirements around 60% [3], saves all the organic matter destined to denitrification for a potential biogas production [4] and reduces the sludge and N_2O production around 90% and 83%, respectively [5, 6].

$$NH_4^+ + 0.804\ O_2 + 0.071\ HCO_3^- \rightarrow 0.111\ NO_3^- + 0.436\ N_2 + 0.009\ C_5H_7O_2N + 0.028\ CH_2O_{0.5}N_{0.15} + 1.038\ H^+ + 1.46\ H_2O \qquad \text{(Eq. 2)}$$

Technologies based on Anammox has been extended, counting with more than 100 full scale installations, and at least 10 patented technologies which differ mainly in reactor configurations and operation procedures [7, 8]. Different solutions can be classified into one or two-stages arrangement, also into suspended, granular or biofilm biomass reactor, or by the feeding/aeration strategies used as continuous or intermittent mode, SBR operation, or others. A description of different classifications is presented in the following section.

Process arrangements

Autotrophic nitrogen removal needs the combination of both reactions of PN and Anammox, that could be achieved in separated reactors or by combining both reactions in a single-stage system. Those configurations are described as follows:

a. Two-stages systems: The first partial nitritation unit produces a suitable substrate with ammonia/nitrite ratio close to 1 to feed the second Anammox unit. The most used partial nitritation technology is called SHARON (Single reactor system for High Activity Ammonium Removal Over Nitrite) and owes it effectiveness to a strict control of temperature, hydraulic and solid retention time (HRT and SRT), dissolved oxygen concentration (DO) and pH [9, 10]. The two-stage system can be optimized in each unit individually, allows greater effectiveness in nitrite oxidizing bacteria (NOB) suppression in the first stage, eventual chemical organic demand (COD) loads are oxidized in the first stage avoiding COD inputs on the Anammox reactor and has lower risk of Anammox bacteria inhibition by DO [8].
b. Single-stage systems: The establishment of a cooperative relation between aerobic and anaerobic ammonium oxidizing bacteria (AOB and anAOB, respectively) in a single reactor is possible under oxygen-limited conditions [3]. It is the most applied configuration at industrial scale [7] since the use of only one reactor unit reduces the investment costs and simplify control and

operation systems [11]. Other advantages are a lower risk of nitrite inhibition and lower N_2O emissions compare to two-stage systems [11].

In both kind of arrangements, partial nitritation and Anammox reaction must be separated since the former needs oxygen and the latter reaction is anaerobic. In a two-stage system the separation is spatial, but in single-stage systems can be temporary, alternating aerobic and anoxic periods, or micro-spatial by the use of biofilms or granular biomass where AOB consumes the DO in the outer layers creating anoxic zones in the inner part of the granule/biofilm [12]. In any case, biomass retention is a prerequisite in the design process, since anAOB are slow growing bacteria with doubling time between 3.3 to 11 days [13, 14]. Retention strategies depends on the type of biomass used and are described below. Following the above reported advantages of single-stage systems, this configuration was chosen for all the experimental assays.

<u>Biomass retention strategies</u>

The wide variety of PN-Anammox technologies developed uses different strategies for biomass retention according to the aggregation state of the biomass:

a. Suspended biomass: Retention strategies are mainly based on SBR operation mode to retain the biomass by sedimentation and an hydrocyclone in some cases to separate and reintroduce granules, composed mainly of Anammox bacteria, from the flocculent biomass (mainly heterotrophs, AOB and NOB) which are purged [8, 15].

b. Granular or biofilm biomass: In all single-stage PN-Anammox systems that works with microbial aggregates as granules or biofilms, the biomass comprises a cooperative consortium of aerobic outer layer for AOB and an anoxic inner zone for anAOB [16]. Granular sludge is a kind of compact and dense bacterial aggregates commonly used in PN-Anammox systems because it has much better settling properties than flocculent sludge [17]. In biofilm systems, the microorganisms grow adhered to a carrier; thus, do not depend on settling properties to achieve high biomass retention [8].

Aeration and feeding strategies

It is well known that DO is one of the most important parameters in PN-Anammox systems [11]; the necessary oxygen to oxidize only half of the ammonia to nitrite has to be provided. Lower values produce not enough nitrite and lower nitrogen removal efficiencies, while higher values lead to nitrite oxidizing bacteria (NOB) competition with AOB and anAOB for oxygen and nitrite, respectively [18]. Therefore, an aeration regime to maintain micro-aerobic conditions must be provided. One strategy can be kept DO at a setpoint value or within a certain range, commonly around 0.5 mg O_2 L^{-1} [19-21], others report not optimal DO concentrations but optimal oxygen to nitrogen loading ratio to optimize nitrogen removal performance [22, 23]. Nevertheless, optimum concentration depends on the biofilm thickness and density, the boundary layer thickness, eventual inlet COD and temperature [12, 19], thus a DO control strategy adjusted to each particular system is needed. Aeration can be supplied by continuous or intermittent mode; some authors recommended continuous aeration to avoid nitrite accumulation [24, 25], but intermittent mode could lead to reduce aeration costs in some cases [26, 27]. A real-time DO control could be an effective strategy for highly fluctuating wastewaters as proposed by Wen et al. [18, 28]. Regarding to feeding regimes, continuous, semi-continuous or batch-fed reactors as SBR are used, but SBR technology is the most commonly applied [7]. Timing of feeding and exchanged volume per cycle can be optimized according to the sludge characteristics since these parameters determine the substrate concentrations experienced by the sludge [11].

In this work, continuous aeration and feeding regime was adopted in two experimental systems and SBR operation mode was proved in a third experiment. Also, an anoxic/oxic operation was assayed in the SBR system to detect possible performance improvements.

Applications

PN-Anammox has been widely applied on effluents rich in ammonia nitrogen but focused mainly on anaerobic digester supernatants because of its high ammonia content (500-1500 mg NH_4^+-N L^{-1}) and low content of organic carbon (COD/N < 1) [7]. The technology has been mainly implemented in municipal wastewater treatment plants but there are already more than 30 full scale installations treating industrial wastewater, most of them from the food processing industry but also from landfill leachate, fertilizer and semiconductor industry [8]. Wastewater composition is fundamental for a good performance of a PN-Anammox process, since several compounds that could be present in wastewaters as phosphate, sulphide, organic matter, salts, heavy metals, and others can inhibit AOB, anAOB or both [19]. In this sense, one of the main challenges to expand the applicability of PN-Anammox technologies has focused on wastewaters with high COD/N ratios, since industrial effluents contain in many cases organic matter concentrations that can negatively affect the process performance [12]. Several studies at laboratory scale has tested the response of the PN-Anammox process under different COD/N ratios [29-31] and also with more complex industrial effluents with high organic content as digested swine slurry [32-34] or landfill leachate [35]. Results vary depending on the kind of wastewater (synthetic or industrial effluent) and the operation parameters as temperature or hydraulic residence time (HRT). Generally, it is feasible to achieve high efficiencies working with low COD/N ratios (<1) [31, 35-37], but ratios around 2 or higher are associated with efficiency decrease due to microbial competition between heterotrophic bacteria (HB) with AOB for oxygen and space and with anAOB for nitrite and space [12, 38-41]. Zhang et al. [29] found a suppressing threshold of COD/N ratio on 1.7 in which denitrifiers out-compete Anammox bacteria. Find operational strategies to overcome the presence of organic carbon in PN-Anammox systems could expand the scope of this technology to a wider range of industrial wastewaters.

In addition to experimental assays to detect operational limits and strategies, this thesis deals with feasibility studies of PN-Anammox processes for the nitrogen removal from two industry effluents with COD content, being both not already treated using full scale Anammox technologies.

Organic carbon in PN-Anammox systems

The effect of organic carbon on PN-Annamox systems has been gaining attention in the last few years as we set before, but fundamental aspects of operational strategies to maintain a stable operation and good performance are not already clear. The out-competition of anAOB by HB growth in PN-Anammox systems with inlet COD is produced by different causes depending on the biomass. For suspended biomass systems, the higher sludge production due to HB growth often implies higher sludge loss, decreasing the sludge retention time (SRT) which affect the slowly growth of anAOB [42]. This problem can be solved providing a sufficiently high SRT and complementing with an hydrocyclone to separate Anammox bacteria from incoming solids [15]. In biofilm or granular systems, HB can growth over the autotrophic aggregates, limiting the oxygen availability for ammonia oxidation [43, 44]. Nevertheless, flocculent heterotrophic growth has been obtained in nitrifying granular systems by means of operate at HRT larger than reciprocal maximum specific growth rate of HB, since HRT acts as a selective pressure condition to promote microbial aggregation [43-45]. Thus, strategies based on HRT control could be effective to avoid HB growth over biofilm or granular PN-Anammox systems.

Modelling studies claim that N removal performance can be maintained at higher COD/N ratios as long as enough oxygen is provided to maintain partial nitritation despite oxygen uptake for the COD oxidation; but the contribution of Anammox reaction is gradually outcompeted by heterotrophic nitrogen removal with increasing COD load [46, 47]. Another problem derived from the oxygen load increase, besides increasing the aeration costs, is the promotion of NOB growth, which competes with anAOB for nitrite and the consequent nitrate concentration increase [48]. **Intermittent aeration strategy** has been shown to be effective for restraining NOB growth, most probably due to their elevated lag-phase experimented during the transition from anoxic to aerobic environment, compared with AOB [49]. During the aerobic phase under low DO (<0.5 mg O_2 L^{-1}), nitrite accumulates because the half saturation constant for DO is lower for AOB than NOB [19]. Then, the anoxic phase allows the nitrite consumption by anAOB, limiting nitrite oxidizing to nitrate by NOB and providing better conditions for anAOB to become a dominant group in the system [26, 50]. Besides, an intermittent aeration strategy has been proposed to **promote denitrification** in PN-Anammox systems since HB can denitrify the nitrate produced by anAOB during the anoxic phase [51, 52]. Denitrifying bacteria has been detected by molecular analysis (mainly fluorescence in-situ hybridization (FISH) and Real-time PCR) in several PN-Anammox reactors, either continuously [36, 53-55] or intermittently aerated [39, 56]. These kinds of systems have been called **SNAD process** (Simultaneous Nitrification, Anammox and Denitrification) [55], in which the nitrate generated by Anammox serves as an electron acceptor to oxidize organic carbon. Heterotrophic denitrification explains why small amounts of COD present in the influent positively affect the maximum nitrogen removal capacity [29, 30, 52]. However operational mechanisms that promotes the oxidation of COD by nitrate instead of oxygen are not already clear, neither which were the critical factors for a successful operation with elevated COD/N influent ratios in PN-Anammox systems.

This PhD project has been formulated aiming at finding operational strategies that allows a stable operation and the improvement of nitrogen removal efficiencies, based on the manipulation of HRT, aeration and feeding regime and studying the effect of those variables over the performance, efficiencies and microbial composition in granular PN-Anammox systems in presence of organic matter. The knowledge obtained from this work is expected to realize the full potential of this technology in industrial practice, including complex wastewaters with high COD/N inlet ratios as food processing industry effluents or animal manure wastewaters.

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process for removal of ammonia from effluent of a fertilizer industry, Bioresource Technology 130 (2013) 390-397.

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STRUCTURE OF PhD THESIS

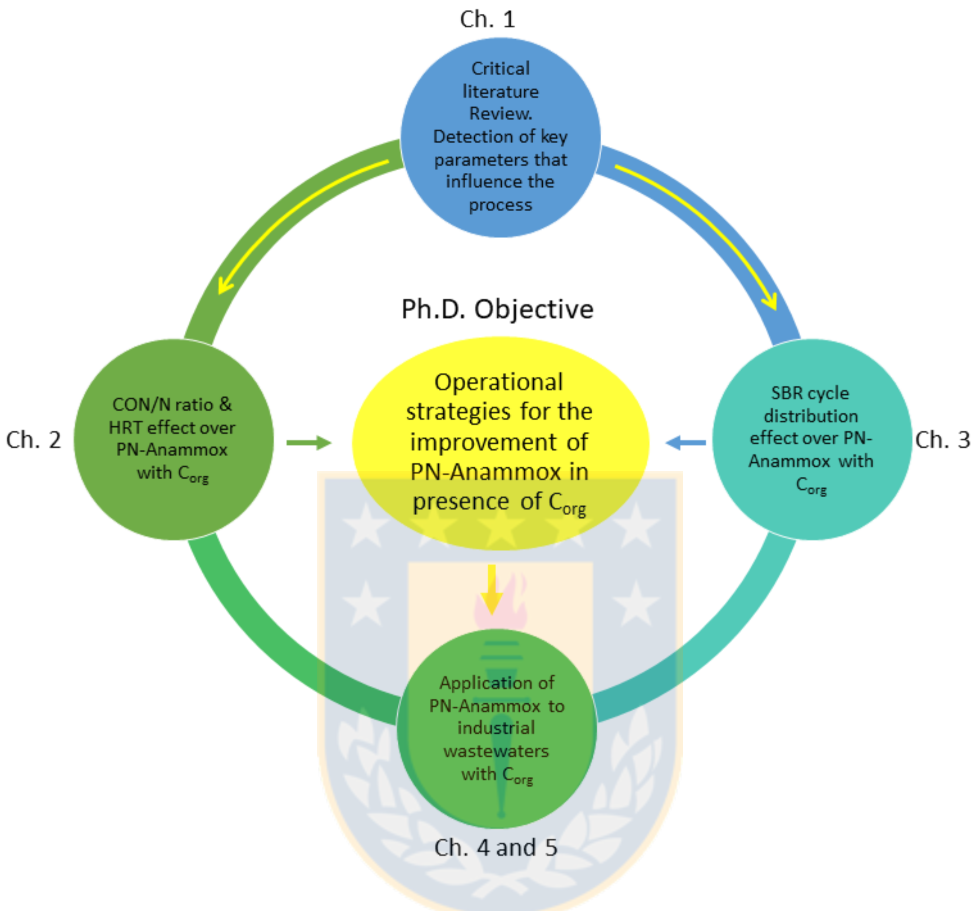

Figure 1: Graphical abstract of the Ph.D. project. The main objective is presented in the center and it is achieved through an experimental approach (Chapter (Ch.) 2 to 5) based on a critical review of main parameters affecting the process (Ch. 1).

This thesis is presented as a compendium of publications, composed by five articles or chapters (two already published and three in process of being sent). A graphical abstract that explain the organization of this work is presented on Figure 1. The first chapter was aimed in an extended literature critical review about SNAD process, the main operational aspects that influence the process focused on the different effects associated to the presence of organic matter in the PN-Anammox systems. The second chapter addresses the effect of COD/N ratio and the HRT with organic carbon presence over the PN-Anammox process, evaluated between 0.1 to 3 and 30 to 2 h, respectively. The third chapter proposed an aeration strategy to promote denitrification in a SBR for nitrogen removal by PN-Anammox with COD/N ratios from 0.3 to 2.3. The last two chapters address the application of the PN-Anammox process for the nitrogen removal from industrial wastewaters with COD content, digested poultry manure in one case and fish canning effluents in the last one. Each article/chapter include their own methodology, experimental data, discussion, conclusions and references. A final chapter of general conclusions is included. The format of all articles has been unified for the purposes of this thesis, thus, some format differences will be found between the first and the last chapters and the published articles.

- CHAPTER 1. E.A. Giustinianovich, J.-L. Campos, M.D. Roeckel, The presence of organic matter during autotrophic nitrogen removal: Problem or opportunity?, Separation and Purification Technology (2016); (166) 102-108.
- CHAPTER 2. E.A. Giustinianovich, J.-L. Campos, V. Guzmán-Fierro, L.A. Pereira, Y.B. Riquelme, G.A. Sobarzo, M.D. Roeckel, COD/N ratio and HRT influence on single stage partial nitritation-Anammox process.
- CHAPTER 3. E.A. Giustinianovich, C.B. Arriagada, J.-L. Campos, L.A. Pereira, Y.V. Weber, M.D. Roeckel, From CANON to SNAD: Aeration strategy to promote denitrification in a partial nitritation-Anammox process in presence of organic carbon.

- CHAPTER 4. E.A. Giustinianovich, C.B. Arriagada, J.-L. Campos, P.G. Golarte, V. Guzmán-Fierro, L.A. Pereira, M.D. Roeckel, Long-term operation of partial nitritation-Anammox process fed with digested poultry manure.
- CHAPTER 5. E.A. Giustinianovich, J.-L. Campos, M.D. Roeckel, A.J. Estrada, A. Mosquera-Corral, Á.V. del Río, Influence of biomass acclimation on the performance of a partial nitritation-Anammox reactor treating industrial saline effluents, Chemosphere. (2018 Mar); (194) 131-138. doi: 10.1016/j.chemosphere.2017.11.146.
- FINAL CONCLUSIONS

CHAPTER 1. *The presence of organic matter during autotrophic nitrogen removal: problem or opportunity?*

(Separation and Purification Technology 166 (2016) 102-108.)

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Keywords: Anammox, ammonia oxidation, heterotrophic denitrification, nitrogen removal, SNAD.

Highlights

- Literature-reported SNAD process performance is analyzed.
- Operational limits for maintaining high SNAD system N removal efficiency are reported.
- COD/N influent ratios and SRT operational limits are reported.
- Aeration-feeding regime strategies are reported.
- Different strategies for suspended biomass and granular/biofilm systems are presented.

Abstract

The simultaneous nitrification, Anammox and denitrification (SNAD) process discovered six years ago is an adaptation of the autotrophic denitrification process that allows for treating nitrogen-rich wastewater streams with moderate amounts of organic carbon. Several authors have noted that it is possible to utilize organic carbon to promote nitrogen removal via the action of denitrifying microorganisms, which can remove the remnant nitrate produced by Anammox bacteria. Thus, SNAD systems can achieve nitrogen removal efficiencies higher than 89%, which is what is expected under autotrophic conditions. Three bacterial groups are responsible for SNAD reactions: ammonium-oxidizing bacteria (AOB), anaerobic ammonium-oxidizing bacteria (AnAOB) and heterotrophic bacteria (HB). Because HB will compete with AOB and AnAOB for oxygen and nitrite, respectively, the system should be operated in such way that a balance among the different bacterial populations is achieved. Here, the results reported in the literature are analyzed to define suitable characteristics of effluents for treatment and operational conditions to allow the SNAD process to be carried out with different types of technologies.

1. Introduction

To fulfill disposal requirements, conventional nitrification-denitrification (ND) processes are generally used to remove both organic compounds and nitrogen from municipal and industrial wastewaters. These processes are well known, and their efficiency and reliability are beyond doubt. However, these processes have a major drawback: the availability of sufficient organic matter to carry out denitrification. Thus, ND processes can be applied only to effluents with chemical organic demand to nitrogen (COD/N) ratios higher than 5 [1]. The addition of an external organic carbon source required for COD/N ratios lower than 5 results in a consequent increase in operational costs [2]. Furthermore, application of the ND processes suggests that a portion of the organic matter present in the effluent is wasted by the aerobic route and cannot be used to produce biogas via anaerobic digestion [3]. To overcome this drawback, the application of partial nitrification-Anammox processes, instead of conventional ND processes, is being promoted because these processes occur under autotrophic conditions, and organic matter and nitrogen can therefore be removed in separate processes [4, 5].

Partial nitrification-Anammox processes involve two reactions in series. The first reaction (Eq. 1, [6]) consists of aerobic oxidation of ammonium to nitrite by ammonia-oxidizing bacteria (AOB), and the second (Eq. 2, [7]) is anoxic oxidation of ammonium to nitrogen gas using nitrite as an electron acceptor by the action of anaerobic ammonium-oxidizing bacteria (AnAOB).

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

$NH_4^+ + 1.3\ NO_2^- + 0.066\ HCO_3^+ + 0.03H^+ \rightarrow 1.02\ N_2 + 0.26NO_3^- +$
$0.066\ CH_2O_{0.5}N_{0.15} + 2H_2O$ (Eq. 2)

To couple these reactions, approximately half of the ammonium must be oxidized to nitrite by partial nitrification; thus, an appropriate substrate for anAOB is obtained, as shown in Eq. 3 [8].

$NH_4^+ + 0.85\ O_2 \rightarrow 0.11\ NO_3^- + 0.445\ N_2 + 0.14\ H^+ + 1.43\ H_2O$ (Eq. 3)

Autotrophic nitrogen removal can be carried out in two-reactor systems, one for partial nitrification (PN) and a second for Anammox, or by coupling the reactions in a single reactor operated under controlled dissolved oxygen (DO) levels. The latter configuration is the one applied most frequently at an industrial scale because it involves less complex control systems, lower investment costs, reduced risk of anAOB inhibition by nitrite and lower N_2O emissions [9]. In the past decade, a variety of technologies have been developed for performing PN-Anammox processes in a single-stage system, which can be divided into three types depending on the aggregation state of the biomass: suspended sludge, granular biomass and biofilm technologies.

Suspended sludge technologies utilize a mixed sludge containing flocculent bacteria (ammonia-oxidizing bacteria) and granular Anammox biomass. This technology includes a hydrocyclone to separate the granular Anammox bacteria from the flocculent biomass. The Anammox granules are returned to the reactor, whereas the flocs are separated and purged to avoid the proliferation of nitrite-oxidizing bacteria and thereby maintain a stable system operation [10]. When granular biomass or biofilms are used, AOB can grow in the outer part of the granule/biofilm and produce nitrite and consume oxygen, generating anoxic conditions in the inner part of the granule/biofilm. In this anoxic zone, ammonium and nitrite (produced during partial nitrification) must be present to allow the growth of Anammox bacteria [11, 12].

Most of the industrial-scale applications of partial nitrification–Anammox (PN-Anammox) processes are installed in Europe, though there is growing interest in both China and North America [13]. Currently, there are more than 100 plants in operation worldwide, and the most used technology is that based on suspended biomass (approximately 40% of the plants), followed by granular systems and biofilms [14]. Most of the industrial-scale facilities treat the supernatant of anaerobic sludge digesters with inlet ammonium concentrations of 500–1500 mg NH_4^+-N L^{-1} and COD/NH_4^+-N ratios of 0.5–1.5 [14]. The high hydraulic residence time (HRT) applied to anaerobic sludge digesters (20–30 d) guarantees almost no remaining biodegradable COD in the effluent, which could interfere with the autotrophic process.

Nevertheless, the HRTs applied in cases of anaerobic digester treatment of industrial effluents are shorter than those used for anaerobic sludge digesters; therefore, the presence of relatively high amounts of biodegradable COD in the effluents cannot be overlooked [8, 15-18]. A similar situation can occur during the application of PN-Anammox processes to the mainstream of municipal wastewater treatment plants (WWTPs) because organic matter is previously removed by an aerobic stage operated at a low solids retention time (SRT); thus, remaining biodegradable COD of approximately 20% of the inlet COD is commonly found due to the low SRT [19, 20]. For this reason, the effect of organic matter on the partial nitrification and Anammox process has been gaining attention over the past few years [21-23].

2. Effect of organic matter on the partial nitrification and Anammox processes

Because PN-Anammox systems were initially implemented in a two-stage configuration, the first works investigating the effect of organic matter focused on the Anammox process [24, 25]. Most research has shown that low organic levels do not significantly affect ammonia or nitrite removal but improve total nitrogen removal by denitrifiers [26]. In fact, the nitrate generated by Anammox bacteria can be reduced to nitrite in the presence of organic matter by heterotrophic denitrifying bacteria and can then be removed via the Anammox process [27]. However, if the inlet $COD_{biodegradable}/NO_2^-$-N ratio is greater than 1.9–3.1, Anammox bacteria are unable to compete with heterotrophic denitrifiers for both space and the electron acceptor (nitrite), with failed reactor performance [26, 28, 29]. Nevertheless, the coexistence of Anammox biomass with heterotrophic denitrifying biomass in the presence of organic matter has been reported [30]. This fact could be related to the capacity of Anammox bacteria to oxidize organic matter to CO_2 using nitrate and/or nitrite as the electron acceptor [31].

With regard to the effect of organic matter on partial nitrification, Mosquera-Corral et al. [15] found that an inlet $COD_{biodegradable}/NH_4^+$-N higher than 0.8 caused the washout of AOB from a chemostat operated at an SRT of 1 day and 30°C due to competition between the heterotrophs and AOB. This negative effect of organic matter on suspended nitrifying biomass can be overcome by increasing the SRT [32]. Such findings would indicate that inlet $COD_{biodegradable}/NH_4^+$-N should be controlled to avoid AOB loss when the reactor is operated at SRT values close to the minimum SRT for AOB (0.5 d at 30°C).

In the case of granular or biofilm nitrifying systems operated without devices to retain flocculent biomass, competition between heterotrophs and AOB can be avoided by HRT manipulation. If the HRT is larger than the reciprocal maximum specific growth rate of the heterotrophic biomass, the bacteria grow in suspension and do not form biofilms over the nitrifying biomass, which limits oxygen availability for ammonia oxidation [33]. If the HRT applied is shorter than the reciprocal maximum specific growth rate of the heterotrophic biomass, a heterotrophic layer will be formed on the nitrifying granular biomass or on the nitrifying biofilm, and its effect on the ammonia oxidation efficiency will depend not only on the $COD_{biodegradable}/NH_4^+$-N but also on the surface specific substrate load [33, 34].

When both PN and Anammox processes are carried out in one stage and in the presence of organic matter, destabilization of the nitrogen removal process could be expected due to the development of heterotrophic bacteria, which can displace AOB and anAOB by competing for oxygen and nitrite, respectively, due to higher growth rates [8, 35]. Nevertheless, if suitable operational conditions and inlet $COD_{biodegradable}/N$ ratios are provided, a balance among AOB, anAOB and HB activities can be achieved, thereby maintaining high nitrogen removal efficiency. According to Eq. 3, coupled reactions of partial nitrification and Anammox are capable of removing up to 89% of ammonium, leaving the remaining 11% of nitrogen in the form of nitrate. In the presence of organic matter, the remaining nitrate can be used by HB as an electron acceptor for organic carbon oxidation, which would allow the theoretical removal of 100% of the nitrogen by the combined action of these three bacterial groups (Eq. 4). This new process has been called the simultaneous nitrification, Anammox and denitrification (SNAD) process (Figure 1.1).

Since its emergence in 2009 [18], an abrupt increase in the number of published articles on the SNAD process has occurred compared with other N removal processes [36]. In the next sections, the fundamental aspects of operational strategies for maintaining a stable operation and reactor performance improvement are discussed.

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^- \quad \text{(Eq. 4)}$$

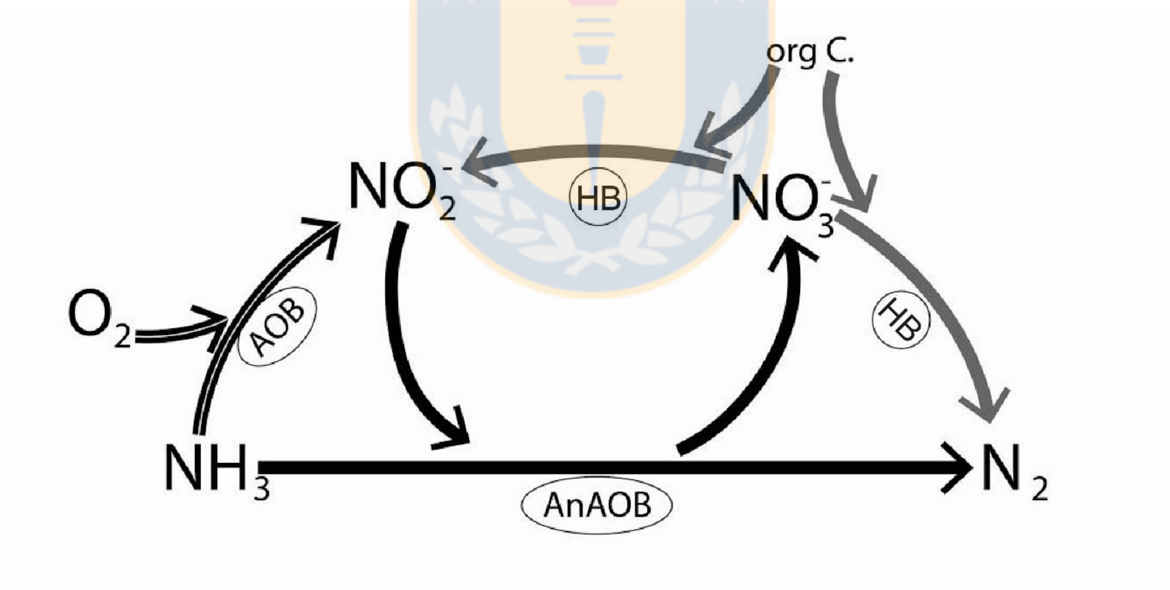

Figure 1.1. SNAD process reactions. Different arrows indicate the different reactions mediated by AOB, anAOB and HB, respectively.

3. Analysis of SNAD process performance

Much information about the performance of the SNAD process can be found in the literature (Table 1). Although this process has been applied to different types of effluents using different types of technologies and operational conditions, its operational limits for maintaining a high N removal efficiency have not yet been defined. For this reason, the main aspects that can affect competition between HB and AOB/anAOB, such as the COD/N influent ratio, solid retention time (SRT) and aeration regime, will be analyzed in the ensuing sections. The microbial characteristics of SNAD systems are also described.

3.1 COD/N influent ratio

To date, effluents from anaerobic digesters treating swine wastewater [35, 37] and sludge [38] from the fertilizer industry [39], opto-electronic wastewater [40] and landfill leachate [41, 42] have been tested in SNAD reactors.

In general, the inlet COD/N ratio reported in the literature takes into account the total COD; however, only the biodegradable fraction of organic matter should be considered because it is the available substrate for heterotrophic growth. For this reason, for the comparison of different works, the inlet COD/N ratio was calculated based on the organic matter degraded inside the reactor ($COD_{biodegradable}/N$). As shown in Figure 1.2, almost all SNAD studies have be conducted with effluents having a $COD_{biodegradable}/N$ ratio lower than 0.7; therefore, no negative effects on the Anammox biomass or the AOB are expected [15, 29]. These low applied

$COD_{biodegradable}/N$ ratios could explain the good performance of most of the SNAD experiments to date.

Table 1.1: Operational conditions of different SNAD systems reported in the literature.

#	Reference	Reactor Type / Operation mode	Biomass / T° (°C)	Substrate	Inlet COD/N ratio	TRH (d)	TN and COD loading rates (kg m^{-3} $\cdot d^{-1}$)	Aeration regime	DO (mg L^{-1})	Removal efficiency (%TN/%COD)	Inlet (COD_{biod}/N)* ratio
1	[39]	tubular up-flow reactor (1 L) / continuous	suspended / 30°C	effluent of a fertilizer industry. In mg L^{-1}: 700-800 TN, 46.6 COD	0.066	2.31	TN: 0.3-0.35 COD: 0.02	continuous	2.9-3.9 ox. zone 0.1-0.4 anox. zone	98.9/46.6	0.03
2	[41]	aeration tank (2.5 L) / SFBR	suspended /35°C	landfill leachate. In mg L^{-1}: 295-700 TN, 250-500 COD	0.85 0.85 0.55 0.71	2.5	TN/COD: 0.118/0.1 0.24/0.2 0.26/0.15 0.28/0.2	continuous	0.1	82/21 94/34 83/31 82/45	0.18 0.29 0.17 0.32
3	[43]	aeration tank (18 L) SFBR	suspended / 35°C	synthetic wastewater. In mg L^{-1}: 200 TN, 100 COD	0.5	9 4.5 3	TN: 0.02-0.067 COD: 0.01-0.03	continuous	0.5-1	93-96/72-87	0.36 - 0.44
4	[38, 44]	aeration tank (1400 m^3) / SFBR	suspended / 30°C	digested supernatant of a municipal WWTP. In mg L^{-1}: 650 TN, 300 COD	0.5 approx.	0.78	TN: 0.83 COD: 0.38	continuous	0.1-1	94.6/37	0,17
5	[37]	aeration tank (5 L) / SBR - SFBR	granules / 15-30°C (without control)	anaerobic digester liquor of swine wastewater. In mg L^{-1}: 662 ± 190 TN, 387 ± 145 COD	0.42 2.56	5 2.5	TN: 0.045-0.175 COD: 0.026-0.236	continuous	< 0.5	Batch: 52/57 Fed batch: 80/76	0.3 0.9
6	[40]	aeration tank (2.5 L) / SFBR	granules / 25°C	opto-electronic wastewater. In mg L^{-1}: 572 ± 6.6 TN, 100 ± 28 COD	0.17	2.5	TN: 0.23 COD: 0.04	continuous	0.1	83-93/79	0.13

7	[45]	bubble column (2.9 L) / SBR	granules / 18°C	synthetic wastewater. In mg L^{-1}: 200 TN, 100 COD	0.5	0.32	TN: 0.62 COD: 0.31	cycles ox/anox: 172 min/60 min	1.5 (oxic phase)	90/100	0.5
8	[42]	aeration tank (384 m^3) / continuous	granules / 30-33°C	landfill leachate. In mg L^{-1}: 634 TN, 554 COD	0.87	1.26	TN: 0.5 COD: 0.44	continuous	0.3	76/28	0.24
9	[46]	biofilm reactor (2.5 L) / SBR	biofilm / 25°C	synthetic wastewater. In mg L^{-1}: 600 TN, 300 COD	0.5	1.67	TN: 0.36 COD: 0.18	continuous	0.1	88/90	0.44
10	[47]	biofilm reactor (5 L) / SBR	biofilm / 32°C	synthetic wastewater. In mg L^{-1}: 428 TN, 150 COD	0.35	0.78	TN: 0.55 COD: 0.19	continuous	1-1.5	88.8/~50	0.175
11	[8]	packing up-flow biofilter (2.65 L) / continuous	biofilm / 25°C	synthetic wastewater. In mg L^{-1}: 200 TN, 40 COD	0.2	0.025	TN: 8 COD: 1.6	continuous (4.5 L air/min)	Not reported	76/81	0.162
12	[35]	packing biofilter (6.5 L) / SBR	biofilm / 30°C	swine digester liquor. In mg L^{-1}: 437 ± 4 COD, 472 ± 6 TN	0.81 0.65 1.24	1	TN/ COD: 0.20/0.16 0.25/0.16 0.20/0.25	cycles ox/anox: 3 h/1 h	1.5-2 ox. cycle 0.2-0.5 anox. cycle	47/39 62/39 29/56	0.32 0.25 0.69
13	[18]	NRBC[b] / continuous	biofilm / 35°C	synthetic wastewater. In mg L^{-1}: 200 TN, 150 and 100 COD	0.75 0.5	0.29	TN: 0.69 COD: 0.34	continuous	0.4-0.6	52/68 70/94	0.47 - 0.71

(a) non-woven rotating biological contactor

SBR: Sequencing Batch Reactor; SFBR: Sequencing Fed Batch Reactor; TN: Total Nitrogen.

*The COD_{biod}/N ratio was calculated based on the COD effectively degraded inside the reactor and the inlet N.

3.2 Applied solids retention time

Despite the importance of the SRT on the performance of biological systems, few works have provided the SRT values applied to SNAD reactors [42, 44]. Nonetheless, authors do provide the HRT values applied to their systems. For suspended biomass systems, the HRT ranges between 2–5 d, whereas these values for granular and biofilm systems range between 1–3 d and 0.025–1 d, respectively (Figure 1.2).

As mentioned previously, the SRT of the flocculent biomass in granular and biofilm systems is crucial to avoid heterotrophic growth over the nitrifying biomass which limits oxygen availability for ammonia oxidation. Thus, to separate autotrophic and heterotrophic biomasses spatially in granular and biofilm systems, the SRT imposed on the flocculent biomass should be larger than the reciprocal maximum specific growth rate of the heterotrophic biomass (approximately 0.1 days at 30°C [48]). Because SRT is equal to or larger than HRT, the development of a layer of heterotrophic biomass over biofilms could be expected only in the work of Liang et al. [8] (point 76[8] of Figure 1.2), in which a biofilm system was operated at an HRT of 0.025 d. Nevertheless, this system was able to operate with a nitrogen removal efficiency of 76%, most likely due to the low $COD_{biodegradable}/N$ ratio of the influent (0.16).

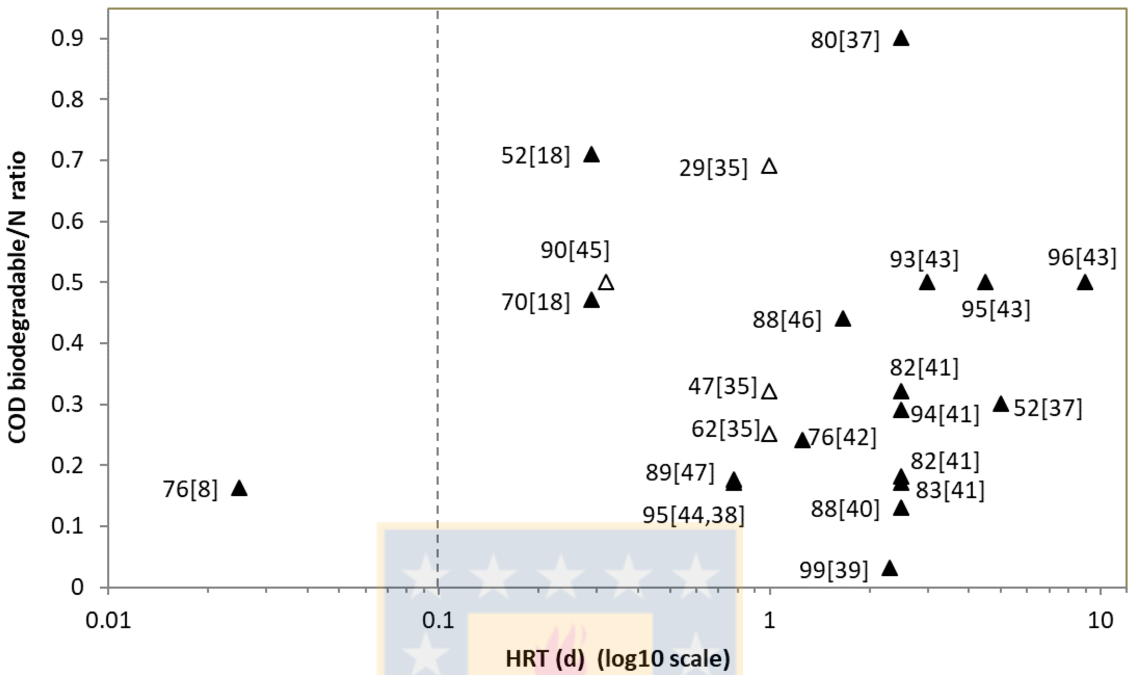

Figure 1.2: Plot of the solids retention time (SRT) and $COD_{biodegradable}/N$ ratios utilized by different authors for the SNAD process. Triangles are accompanied by N removal efficiency (%) obtained in each experiment, and the number in brackets indicates the reference. Filled triangles: continuous aeration mode; unfilled triangles: intermittent aeration mode.

3.3 Dissolved oxygen level and aeration strategy

Under autotrophic conditions, the theoretical nitrogen removal efficiency of the PN-Anammox process is 89%, though the efficiency obtained at an industrial scale is approximately 80–85% [14]. Therefore, if the SNAD process is carried out properly, efficiencies higher than the latter values are expected. Most SNAD processes have been performed under continuous aeration at DO levels between 0.1 O_2 L^{-1} and 0.5 mg O_2 L^{-1}, which are suitable for simultaneous nitrification and denitrification [49].

The nitrogen removal efficiency reported for these systems is 88±8% (calculated from the data shown in Table 1). Therefore, the promotion of simultaneous nitrification and denitrification under microaerobic conditions enhances the efficiency of the system. However, continuous aeration also causes a portion of the organic matter to be oxidized by oxygen instead of nitrate, which increases the operational costs. In this sense, the alternation of oxic and anoxic periods to promote the use of the organic matter present for denitrification would reduce aeration costs and increase the nitrogen removal efficiency [35, 45]. Thus, heterotrophic denitrification occurs during the anoxic phase, consuming most of the organic carbon and reducing the nitrate produced by Anammox; this in turn allows PN-Anammox processes to occur practically in the absence of organic carbon in the following oxic phase, during which ammonia is converted to N_2 by the combined action of AOB and anAOB (Figure 1.3). Depending on the availability of a carbon source during the anoxic period, nitrate can be completely reduced to N_2 or partially reduced to nitrite, which is consumed by the Anammox bacteria (Figure 1.3). During the oxic phase, denitrification is inhibited and organic carbon is preferentially consumed by the aerobic route, a time when oxygen levels are higher than 0.5 mg O_2 L^{-1} (Figure 1.3). Winkler et al. [45] used a granular SBR reactor initially fed with wastewater and operated under anoxic conditions to remove nitrate and organic matter via heterotrophic denitrification; air was then supplied (DO: 1.5 mg O_2 L^{-1}) to promote nitrogen removal by autotrophic bacteria. This system achieved a nitrogen removal efficiency of 90%, which was most likely limited by the volumetric exchange ratio applied (50%). Nevertheless, Zhang et al. [35] proposed a cycle in which wastewater was initially fed, and the system was then operated under aerobic conditions (1.5–2 mg O_2 L^{-1}), followed by an anoxic period. In this case, the nitrogen removal efficiency was 29–62%. This would indicate that better results are achieved when organic matter is mainly consumed under anoxic conditions.

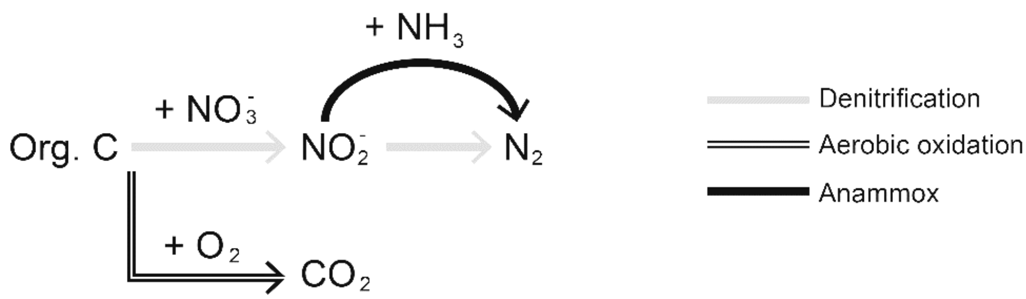

Figure 1.3: Organic carbon oxidation pathways.

If alternating oxic/anoxic conditions are applied in suspended biomass systems, the minimum SRT applied to the flocculent biomass should be increased with respect to those systems continuously aerated, as AOB can grow only during oxic periods. In the case of granular or biofilm systems, the minimum SRT applied to heterotrophic biomass will not be affected when compared to continuously aerated systems because this biomass can grow under both oxic and anoxic conditions.

3.4 Microbial community distribution

The coexistence of AOB, anAOB and HB communities has been proven in SNAD systems with high N removal efficiencies [18, 38, 50]. In addition, stratified distribution of different bacterial groups has been observed, with anAOB and denitrifiers being more abundant in anoxic zones [18] and AOB predominantly in oxic zones [18, 39]. However, the proportion of each type of bacterium has rarely been described. Liang et al. [8] detected a reduction in the relative abundance of AOB and anAOB from 35 and 40% to 30 and 33%, respectively, with COD addition in a CANON biofilm system, most likely due to the development of HB, such as denitrifiers. Keluskar et al. [39] detected different abundances of AOB and denitrifiers in a tubular up-flow reactor with suspended biomass, with AOB being more abundant in the oxic zone (61% versus 6% in the anoxic zone) and denitrifiers more abundant in the anoxic zone (22% versus 10% in the oxic zone). The dominance of anAOB in the anoxic zone was also suggested due to the detection of uncultured Planctomycetes in the biomass amplified. It appears that the functional proportion and distribution of different bacterial groups depends on the characteristics of the system, though further studies on microbial dynamics must be performed.

4. Conclusions

Most of the SNAD systems reported in the literature have achieved high nitrogen removal efficiencies. These systems have applied different technologies, were fed with different types of effluents and were operated under different operational conditions. However, the operational limits that guarantee the stability of the process are not well defined to date. In this sense, similar operational considerations to those used for maintaining the stability of the systems under autotrophic conditions must be taken into account [51], in addition to other considerations related to establishing a balance between heterotrophic and autotrophic populations:

- SNAD systems based on suspended biomass should be operated in such a way that the SRT applied to the flocculent biomass (heterotrophic and ammonia-oxidizing biomass) is sufficient to allow appropriate ammonia oxidation activity (Zone III, Figure 1.4A) and to avoid the washout of AOB (Zone I, Figure 1.4A). An effluent with a relatively high $COD_{biodegradable}/N$ ratio could cause the proliferation of heterotrophic biomass, which should be purged from the system. This would suggest a reduction in the SRT of the flocculent biomass and therefore a decrease in the ammonia oxidation capacity. Moreover, if the SRT applied to the flocculent biomass is close to the minimum SRT required by AOB, the development of heterotrophic biomass will have a more negative effect on the ammonia oxidation capacity (Zone II, Figure 1.4A).
- SNAD systems based on granular biomass or biofilms should be operated in such a way that heterotrophic biomass is allowed to grow in suspension by applying an SRT higher than 0.1 d for systems operated at 30°C (Zone III, Figure 1.4B). In this way, the risk of process stability loss due to oxygen limitations during ammonia oxidation by a heterotrophic biofilm is minimized. This risk will also depend on the $COD_{biodegradable}/N$ ratio of the influent and the applied specific surface-loading rate. Therefore, operation at an HRT smaller than 0.1 d and a relatively high $COD_{biodegradable}/N$ ratio is not advisable for this type of system (Zone I, Figure 1.4B), whereas an HRT smaller than 0.1 d and a relatively low $COD_{biodegradable}/N$ ratio will promote the growth of heterotrophic biomass on the granular biomass or biofilm, with oxygen still being available for AOB (Zone II, Figure 1.4B).

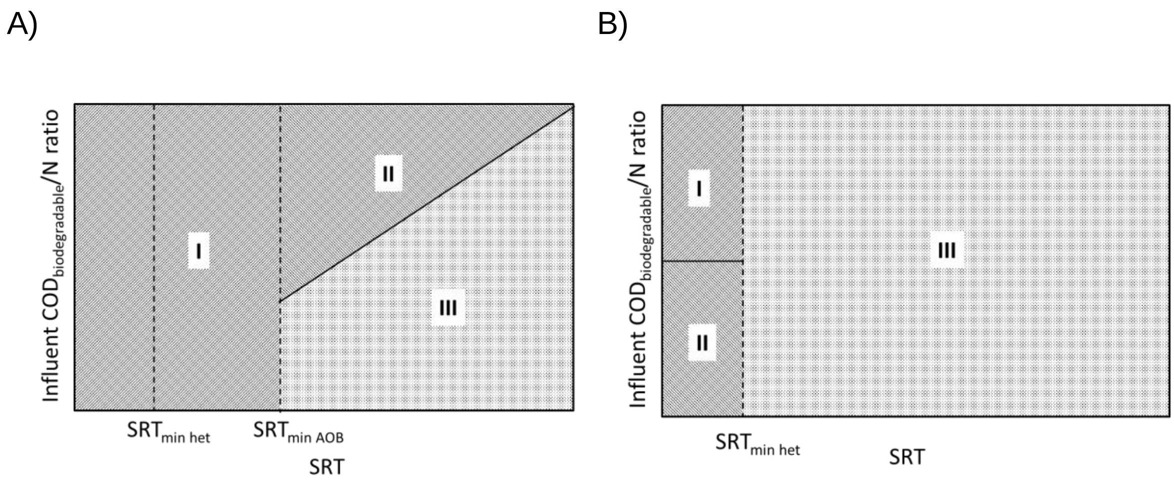

Figure 1.4. Different operational zones depending on the influent COD$_{biodegradable}$/N ratio and on the solid retention time (SRT) applied to flocculent biomass (heterotrophic biomass in the case of granular and biofilm systems and heterotrophic and ammonia-oxidizing biomass in the case of suspended biomass systems). A) Suspended biomass systems: zone I - washout of AOB; zone II - proliferation of heterotrophic biomass; and zone III - stable operation. B) Granular biomass and biofilm systems: zone I - formation of heterotrophic biofilm causing oxygen limitation; zone II - formation of heterotrophic biofilm not causing oxygen limitation; and zone III - stable operation.

To improve the nitrogen removal efficiency of the system, alternating oxic/anoxic periods could be implemented to promote organic carbon consumption via denitrification. To achieve this, the feeding strategy must promote the presence of organic carbon during anoxic periods.

5. Prospects

As mentioned above, most of the applications of the SNAD process have been focused on treating effluents from industrial anaerobic digesters containing high ammonia concentrations at a temperature of approximately 30 °C. However, due to the recent interest in improving the energy efficiency of WWTPs by means of the application of Anammox-based processes in the mainstream, future efforts will be addressed at studying the stability of the SNAD process operated at ammonia concentrations of approximately 50 mg N/L and ambient temperatures [45, 52, 53]. COD can be mainly removed from mainstream by an aerobic reactor operated at a low SRT to save oxygen and to maximize methane production via sludge digesters; the main effluent with an expected COD/N ratio of approximately 2 [20, 52, 54] would then be treated via the SNAD process. Because Anammox bacteria have the slowest growth rate among the microorganisms involved in the SNAD process, they could be expected to be the bottleneck of this process. Nevertheless, several works have demonstrated that Anammox bacteria can maintain a stable activity even at temperatures between 10-20 °C [55-58] and in the presence of organic matter [20, 54]. Moreover, the ability of anAOB to reduce nitrate to nitrite using short-chain fatty acids [31, 59] could contribute to improving the effluent quality in terms of nitrogen compounds [60]. Recent studies report that the actual limitation of SNAD application to the mainstream of WWTPs appears to be the proliferation of nitrite-oxidizing bacteria, which contribute to an excessive presence of nitrate in the effluent. New strategies have been proposed for partial nitrification to suppress nitrite oxidizing bacteria, as alternating aerobic/anoxic conditions. However, the full-scale application of this strategy implies a precise design of operation conditions as oxic/anoxic cycles, different solids retention times for each types of biomasses and a strict control of the dissolved oxygen and ammonia concentrations [51].

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CHAPTER 2. COD/N ratio and HRT influence on single stage partial nitritation-Anammox process

(Article to being sent for publication)

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Keywords: partial nitritation; Anammox; organic carbon, COD/N ratio, hydraulic residence time.

Highlights:

- Partial nitritation/Anammox operated with variable COD/N ratio and HRT.
- High efficiency obtained until COD/N ratio of 3.0.
- Decrease HRT produces efficiency loose.
- Low HRT favored competition between nitrite oxidizers and Anammox bacteria.

Abstract

The performance of the partial nitritation/Anammox process was evaluated under COD/N ratios between 0.1 to 3 and HRT between 30 to 2 h in two parallel reactors. The same NLR of 240 mg TAN L^{-1} d^{-1} was used in both reactors and an OLR of 120 mg COD L^{-1} d^{-1} was applied to the second one. Progressive increase of the COD/N ratio had no negative effects over the nitrogen removal performance operating at 30 h of HRT, obtaining a maximum nitrogen removal efficiency of 85% with COD/N ratio of 2 and 82% with COD/N ratio of 3. Progressive decrease of HRT produced a strong decrease of nitrogen removal efficiency from 82% to 40%. The increase in the nitrite oxidation activity and a direct relationship between Anammox removal rate and nitrite concentration confirm that competition between nitrite oxidizing bacteria and Anammox bacteria for substrate was the cause of the reactor performance decrease. These results demonstrate that granular PN/Anammox systems are highly sensitive to HRT, but keeping HRT at high values as 30 h, effluents with high COD/N ratios until 3.0 can be treated without losing removal efficiency.

1. Introduction

Single stage partial nitritation/Anammox (PN-Anammox) process is one of the novel developed technologies for the complete removal of nitrogen from wastewaters based on Anammox process, and it has been preferred over other configurations for its technical and economic advantages [1] being the most applied at full scale [2]. Full scale applications have been focused on anaerobic digester supernatants because of its high ammonium content (500–1500 mg NH_4^+-N L^{-1}) and low COD/N ratios below 1 [3], make it the most suitable effluent for this process. However, several industrial effluents have COD/N ratios >1, specially livestock manure wastewaters with high ratios but with great variability [4], hindering the nitrogen removal via nitrification-denitrification for lack of organic carbon to achieve complete denitrification [5]. Those effluents can also be treated by partial nitritation/Anammox, but the drawback of having higher COD/N ratios allows the development of heterotrophic biomass which can interfere with the autotrophic nitrogen removal process [6]. Heterotrophic bacteria (HB) can compete for both oxygen and nitrite with aerobic and anaerobic ammonium oxidizing bacteria (AOB and anAOB), respectively ([7-9]), displacing autotrophic bacteria due to their higher growth rates [10, 11].

Zhang et al. [12] studied the effect of COD/N ratio from 0.5 to 4 in PN-Anammox membrane bioreactors with synthetic wastewater and glucose as organic source, obtaining an improvement of nitrogen removal with ratios from 0.5 to 1 but a decrease was observed with ratios greater than 2, finding a suppressing threshold of COD/N ratio at 1.7. Chen et al [13] observed a decrease from 70 to 52% with an increase of COD/N ratio from 0.5 to 0.75, working with a rotating biological contactor and synthetic substrate also. Nevertheless, other authors obtained higher efficiencies with similar COD/N ratios between 0.8 and 1.2 and more complex substrates as landfill leachate [14] or anaerobic digester liquor from swine and poultry manure [15, 16]. Enhancement of nitrogen removal by PN-Anammox in presence of organic carbon has been attributed to denitrification contribution over the process, since the remnant nitrate produced by Anammox reaction can be denitrified to nitrite and then removed by Anammox [17]. But operating conditions that determine enhancement or inhibition effect by organic carbon over PN-Anammox process are not already defined.

Jenni et al. [18] identified sludge retention time (SRT) as a relevant factor that affects PN-Anammox performance in presence of organic matter. Since organic carbon leads to heterotrophic growth and higher sludge production, often entails higher sludge loss, decreasing the SRT which mainly affects the slowly growth of anAOB. In this sense, biofilm and granular systems have the advantage to allow operation control at higher SRT and the possibility to develop a hybrid biomass system composed by autotrophic aggregates and heterotrophic suspended biomass [19, 20]. The growth of heterotrophic bacteria (HB) on biofilms or suspended on the bulk is strongly determined by the HRT applied to the system [21], since HRT shorter than reciprocal maximum specific growth rate of HB acts as a selective pressure condition to promote microbial aggregation [19-21].

Thus, the goal of this study was to test the effect of COD/N ratio from 0.1 to 3.0 over the removal performance and microbial composition in a continuous PN-Anammox reactor with hybrid biomass composed by granules, flocs and non-aggregated biomass. Additionally, progressive HRT decrease from 30 to 2 hours was tested in another identical reactor with a COD/N ratio of 0.5 and constant nitrogen loading rate (NLR) in order to avoid substrate inhibition by overloads. Results obtained in both reactors were compared in order to determine operational limits to ensure a good performance of PN-Anammox process in the presence of organic matter.

2. Materials & Methods

2.1 Reactor set-up

Two identical bubble column reactors with an effective volume of 2.0 L and built with completely transparent glass were used. This design was chosen due the good performance presented by Varas et al. [22] and Arriagada et al. [16]. Both reactors had a total height of 63 cm and were structured by three sections. The bottom section had a height and inner diameter of 33 cm and 7.0 cm, respectively. The middle section had a height and inner diameter of 20 cm and 9.5 cm, the higher diameter allowed reducing upflow velocity of the granular biomass. Moreover, in this section a three-phase separator such as described by Arriagada et al. [16] was installed. Finally, the top section had a height and inner diameter of 10 and 9.5 cm; in this section a gas chamber is located that allows the recirculation and the agitation by gas.

The air and the influent were supplied to the bottom section of the reactors, while the gas (recirculation and overpressure) and the effluent were discharged from the top section. All the stream of liquid and gas, except overpressure, circulate through peristaltic pumps (LongerPump, BT100-2J, China). Oxygen control system was performed with optical dissolved oxygen electrode (WTW, FDO 925, Germany) according to the strategy proposed by Varas et al. [22]. Temperature was maintained at 35° C using a thermostatic bath (TIC Controller, Thermo Haake, DC30, Germany).

2.2 Substrate and operational conditions

Both reactors were operated with continuous feed and aeration regime, fed with synthetic media. Five different stages were evaluated in each reactor to test the effect of COD/N ratio and the HRT effect over the PN-Anammox performance (Table 1). The COD and nitrogen sources were added as sodium acetate and ammonium sulfate, respectively. Inlet NLR was maintained constant at 240 mg TAN L^{-1} d^{-1} in both reactors and COD concentration was supplied according to the COD/N ratio (Table 1). Inorganic carbon (IC) was supplied as sodium bicarbonate, maintaining a molar IC/N ratio of 2.5 [23]. Synthetic substrate composition was: KH_2PO_4 25 mg L^{-1}; $MgSO_4{\cdot}7H_2O$ 200 mg L^{-1}; $CaCl_2{\cdot}2H_2O$ 300 mg L^{-1}; $FeSO_4{\cdot}7H_2O$ 11 mg L^{-1}; EDTA 6 mg L^{-1} and trace elements added as concentrate solutions in order to obtain: EDTA 22,5 mg L^{-1}; $ZnSO_4{\cdot}7H_2O$ 0,645 mg L^{-1}; $CoCl_2{\cdot}6H_2O$ 0,360 mg L^{-1}; $MnSO_4{\cdot}4H_2O$ 6,3 mg L^{-1}; $CuSO_4{\cdot}5H_2O$ 0,375 mg L^{-1}; $Na_2MoO_4{\cdot}2H_2O$ 1,4 mg L^{-1}; $NiCl_2{\cdot}6H_2O$ 0,285 mg L^{-1}, Na_2SeO_3 anhydrous 0,675 mg L^{-1} y H_3BO_3 0,021 mg L^{-1}.

Table 2.1: Operational conditions applied to both reactors.

Parameter		Reactor 1					Reactor 2				
NLR	(mg TAN/L·d)	240					240				
OLR	(mg COD/L·d)	24	120	240	480	720	120				
C/N ratio	-	0.1	0.5	1	2	3	0.5				
HRT	(h)	30					30	20	10	4	2

NLR: Nitrogen loading rate; OLR: Organic loading rate; HRT: Hydraulic residence time.

2.3 Characteristics of the partial nitritation-Anammox inoculum

The inoculum reactor was identically as the experimental ones, and biomass was obtained from the reactor described by Varas et al. [22]. The inoculum reactor was operated 138 days until reach steady state with COD/N ratio of 0.1 with 430 mg TAN L^{-1} and HRT of 1.27 d. The final biomass concentration in the inoculum reactor was 2.80 g VSS L^{-1}, the granule diameter and sedimentation rate were 1.35 ± 0.45 mm and 62.4 ± 9.25 m h^{-1}, respectively, and specific nitrification and Anammox activities were 0.064 and 0.197 g N g VSS^{-1} d^{-1}, respectively. The experimental reactors were inoculated with 1 g VSS L^{-1} each one.

2.4 Analytical methods

Periodically, influent and effluent of both reactors were sampled. Nitrogen compounds such as nitrate, nitrite and total ammonia nitrogen (TAN) were measured by spectrophotometry using Double Beam Spectrophotometer (Techcomp, UV-2300, China) according to standard methods [24]. Total organic and inorganic carbon concentrations (TOC and IC) were measured via combustion analysis followed by a nondispersive infrared gas analyzer in the TOC (Shimadzu, TOC-

5000[a], Japan). Measurement of pH, total solids (TS), volatile solids (VS) and chemical organic demand (COD) were performed according to standard methods [24].

2.5 FISH analysis

The identification of the different bacterial groups, the analysis of the structure and relative abundance of the different populations of microorganisms, as well as the monitoring of the spatial and temporal dynamics of microbial populations that integrates the SNAD granular biomass samples, was carried out by the technique of fluorescent in situ hybridization (FISH) according to the technique described by Pernthaler et al. [25]. The granular biomass was prepared as follows: fixation in 4% paraformaldehyde / PBS solution for 12 hours at 4°C, then washed and stored in 1:1 ethanol / PBS, until hybridization with the probes solution. 100 µL of each sample were hybridized in the presence of each probe solution for 2 hours at 46°C. The probes solution for each hybridization was prepared mixing 1 µl of probe with 9 µl of hybridization buffer. The solutions and buffers as well as the procedures for each step were carried out as set forth. Probes used for in situ hybridization were EUB-338, NEU653, Ntspa-1026, DEN124 and Amx-0820; complementary to a region of the 16S specific domain of DNAr for Eubacteria, AOB, NOB, heterotrophic denitrifying bacteria (HDB) and anAOB, respectively (Table 2). The analysis was performed on MOTIC-BA310 fluorescence microscope using the specific filters for each probe, using Eq. 1 to determine number of cells per gram of biomass. DAPI and EUB-338 were used as controls. From the analysis, the relative percentages of abundance of each bacterial group were determined with respect to the total biomass of eubacteria, combining each specific probe with the probe EUB338; targeted to the total population of eubacteria. The difference between eubacteria and the sum of anAOB, AOB, HDB and NOB was termed unidentified eubacteria (UI).

$$N\left(\frac{cells}{biomass\ (g)}\right) = Number\ of\ cells\ on\ the\ visual\ field\ \times 3.62 \times 10^{8} \qquad \text{(Eq. 1)}$$

Table 2.2: FISH oligonucleotides used in this study.

Probe	Sequence (5´–3´)	rRNA target sites[a]	Specificity	Formamide[b] (%)	Reference
EUB338	GCTGCCTCCCGTAGG-AGT	16S, 338–355	Most Eubacteria	20	[26]
NEU653	CCCCTCTGCTGCACT-CTA	16S, 653–670	(AOB) Halophilic and halotolerant members of the genus of Nitrosomonas	40	[27]
Ntspa1026	AGCACGCTGGTATTG-CTA	16S, 1026–104	(NOB) Nitrospira moscoviensis, activated sludge clones A-4 and A-11	20	[27]
Amx-0820	AAACCCCTCTACTTA-GTGCCC	16S, 820–841	(AnAOB) Genera "*Ca.* Brocadia" and "*Ca.* Kuenenia"	40	[28]
DEN124	CGACATGGGCGCGT-TCCGAT	16S, 124–143	(HDB) Acetate-denitrifying cluster	40	[26]
DAPI	Regions enriched in adenine and thymine in DNA sequences	Unspecific	DNA of all microorganism		

(a) 16S rRNA position according to Escherichia coli numbering; (b) Formamide concentration in the hybridization buffer; DAPI: 4′,6-Diamidine-2′-phenylindole dihydrochloride.

2.6 Calculations

Nitrogen removal rate (NRR) and nitrogen removal efficiency (NRE) were calculated based on nitrogen global balances. Removal rates in the reactor by AOB (AOR_x), NOB (NOR_x) and Anammox (AR_x) were estimated based on the Anammox stoichiometry (Eq. 1) and nitrogen balances, according to the equations proposed by Vázquez-Padín et al. [29] but modified to include heterotrophic nitrogen assimilation (HNa) and finally dividing by the reactor biomass concentration to obtain the specific removal rates, expressed as g N g VSS^{-1} d^{-1}. Negative values for NOR_x were attributed to specific denitrifying removal rate (DR_x). Used equations and modifications were:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \text{Eq. (1)}$$

$$\Delta N = TAN_{inf} - \left(TAN_{ef} + NNO_2{}^-{}_{ef} + NNO_3{}^-{}_{ef}\right) \quad \text{Eq. (2)}$$

$$AR_x = \frac{(\Delta N - HNa)}{HRT \cdot X} \quad \text{Eq. (3)}$$

$$AOR_x = \frac{(\Delta TAN - HNa)}{HRT \cdot X} - \frac{AR_x}{2.04} \quad \text{Eq. (4)}$$

$$NOR_x = \frac{-\Delta NNO_3{}^-}{HRT \cdot X} - \frac{0.26 \cdot AR_x}{2.04} \quad \text{Eq. (5)}$$

Being TAN_{inf} and the TAN_{ef} the total ammonium nitrogen concentration in the influent and effluent (g N/L), respectively, and the $NNO_2{}^-{}_{ef}$ and $NNO_3{}^-{}_{ef}$ the nitrogen as nitrite and nitrogen as nitrate concentrations in the effluent (g N L^{-1}), respectively. ΔN correspond to total observed nitrogen consumed in the reactor.

Heterotrophic nitrogen assimilation (HNa) was calculated based on observed biomass yield coefficient of heterotrophic biomass [30] (Eq. 7), COD consumption and nitrogen biomass content, according to equation (6):

$$HNa = Y_{obs}\left[\frac{gVSS}{gCOD}\right] \times \frac{14}{113^*}\left[\frac{gN}{gVSS}\right] \times \left(COD_{in} - COD_{ef}\right)\left[\frac{gCOD}{L}\right] \times \frac{1}{TRH}\left[d^{-1}\right] \quad \text{Eq. (6)}$$

And,

$$Y_{obs} = \frac{Y}{1+K_d \times SRT} \quad \text{Eq. (7)}$$

Where:

Y = Maximal growth yield coefficient for heterotrophs =0.43 g VSS g COD^{-1}

K_d = Decay coefficient for heterotrophs = 0.96 d^{-1}

SRT = Solids retention time = HRT (for non-aggregated biomass only)

COD_{in} = influent COD

COD_{ef} = effluent COD

113* = molar weight considering $C_5H_7O_2N$ as biomass molecule.

The potential maximum heterotrophic denitrification contribution over the nitrogen removal (considering absence of aerobic oxidation of organic carbon) was calculated based on COD balances considering 6.6 g of acetate COD per g of NO_3^--N denitrified, according to denitrification stoichiometry [31] (see Eq. 8).

$$1.0NO_3^- + 1.0H^+ + 0.33NH_4^+ + 1.45CH_3COO^- \rightarrow 0.5N_2 + 0.33C_5H_7O_2N + 0.13CO_2 + 1.12HCO_3^- + 1.62H_2O \quad \text{Eq. (8)}$$

3. Results

3.1 Partial nitritation-Anammox process performance with progressive COD/N ratio increase

Nitrogen compounds concentrations at the effluent for progressive COD/N ratio are shown on Figure 2.1(a). Since the inoculum biomass had moderate nitrifying and

Anammox specific activities (0.073 ± 0.012 and 0.197 ± 0.009 g N g VSS^{-1} d^{-1}, respectively), and the initial biomass concentration was 1.64 g VSS L^{-1}, a NLR of 0.134 g N L^{-1} d^{-1} was used for the start up. Subsequently, an acclimation to higher TAN concentrations was achieved during the first stage, with a stepwise increase from 170 to 300 mg TAN L^{-1}. A nitrogen removal rate (NRR) of 0.179 ± 0.005 g N L^{-1} d^{-1} was achieved during the first stage and a nitrogen removal efficiency (NRE) of 75.91 ± 2.02 % with a COD/N ratio of 0.1 (Table 3). At the end of the first stage, clogging problems at the effluent tube produced a destabilization on day 32; once the tube was unclogged, the stability was restored on day 43, obtaining a NRR of 0.176 ± 0.002 g N L^{-1} d^{-1} with a COD/N ratio of 0.5. During stages III to V, slightly differences of NRR and NRE were observed, with a maximum NRR of 0.202 ± 0.004 g N L^{-1} d^{-1} and 85.54 ± 1.65 % of NRE, obtained with a COD/N ratio of 2.0.

The analysis of the reactor specific removal rates for each stage, were obtained dividing the removal rates by the biomass concentration inside the reactor at each steady state. The increasing of NRE and NRR observed in the last three stages (Table 3) can be explained by the increase observed in specific Anammox removal rate (AR_X) (Figure 2.2.a), which augmented from 0.109 ± 0.003 at COD/N ratio of 0.1 to 0.123 ± 0.002 g N g VSS^{-1} d^{-1} at COD/N ratio of 2, obtaining an average of 0.120 ± 0.002 g N g VSS^{-1} d^{-1} during the last three stages corresponding to COD/N ratios from 1 to 3. The AOR_X had a slight increase from 0.070 ± 0.001 to 0.076 ± 0.003 g N g VSS^{-1} d^{-1} at COD/N ratios of 1.0 and 2.0, but at the last stage a similar value to the first stage was obtained (0.066 ± 0.002 g N g VSS^{-1} d^{-1}). NOR_X remained inactive during all the operation. Also, denitrification contribution over the total nitrogen removal could explain the observed increasing tendency, since larger amounts of COD were applied capable to remove theoretically until 2, 10, 18, 35 and 55% of the removed nitrogen as nitrate by denitrification in each stage, respectively. Nevertheless, the continuous aeration used during the operation hinders the oxidation of organic matter by nitrite or nitrate route, promoting the organic oxidation by the aerobic route. The difference between total produced nitrate and produced nitrate by Anammox was attributed to NOB removal rate (NOR_x), but negative results was obtained at the last two stages. Those values indicate specific denitrifying rate contribution (DR_x) over the nitrogen removal process, achieving a maximum of 7% of the total nitrogen removed in the last stage (Figure 2.2.a), a much lower value than the maximum theoretic 55% calculated with COD removed, supporting that COD was mainly aerobically oxidized in spite that low DO values were measured along the operation (0.09-0.14 mg O_2 L^{-1}). In fact, heterotrophic nitrogen assimilation (HNa_x) represents a major contribution than denitrification (Figure 2.2.a), achieving an 8.6% of nitrogen assimilation with a COD/N ratio of 3.

3.1 Partial nitritation-Anammox process performance with progressive HRT decrease

The second partial nitritation-Anammox reactor, identical to the first one was operated in parallel, modifying the HRT applied but maintaining a constant NLR during all the operation. A rapid acclimation from 200 to 300 mg TAN L^{-1} was achieved in the first seven days; once stabilized, decreasing HRT values of 30, 20, 10, 4 and 2 h were applied. Exit nitrogen compounds profiles maintained stable with low values during all the operation (Figure 2.1(b)), but the low inlet TAN concentrations applied in the last three stages in order to maintain the constant NLR, negatively affecting the nitrogen removal rate as well as the removal efficiency of the process (Table 3). NRR gradually diminished from 0.193 ± 0.002 to 0.093 ± 0.013 g L^{-1} d^{-1} with the decreasing of HRT from 30 to 2 h, respectively. In addition, NRE decayed progressively from 81.79 ± 0.96 to 39.62 ± 5.74 % from the first to the last stage.

Analysis of the specific removal rates obtained in the reactor revealed that AR_X was negatively affected with the HRT decrease, diminishing from 0.118 to 0.057 g N g VSS^{-1} d^{-1} at the first and last stage, respectively (Figure 2.2.b). On the other hand, AOR_X was improved with HRT decrease, promoting a rate increasing from 0.076 to 0.103 g N g VSS^{-1} d^{-1} with 30 and 2 h of HRT, respectively. DR_x and HNa_x were despicable during all the operation as seen on Figure 2.2.b due to the low COD/N ratio applied, but a strong increase of NOR_X was observed with the decrease of HRT, suggesting that anAOB were outcompeted by NOB for nitrite.

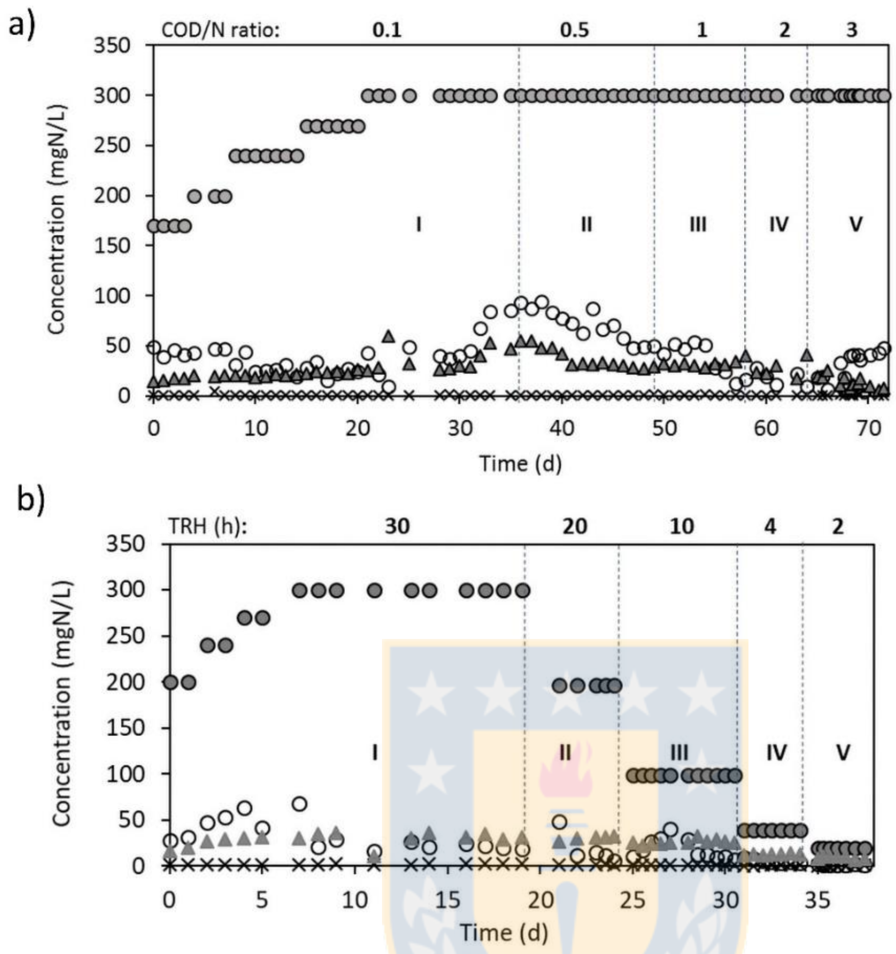

Figure 2.1: Nitrogen compounds profile in the different operational stages of PN-Anammox reactors at constant NLR of 240 mg N L^{-1} d^{-1}, with: (a) variable COD/N ratios at 30 h of HRT; and (b) variable HRT at COD/N ratio of 0.5. TAN in the influent (○) and TAN (○), NO_2^--N (×) and NO_3^--N (▲) in the effluent.

Table 2.3: Nitrogen removal rates and efficiencies (NRR and NRE, respectively) obtained in the different operational stages with variable COD/N ratios at 30 h of HRT and variable HRT at COD/N ratio of 0.5.

Stage	COD/N (p/p)	NRR (g/L·d)	NRE (%)
I	0.1	0.179 ± 0.005	75.91 ± 2.02
II	0.5	0.176 ± 0.002	74.38 ± 0.75
III	1	0.193 ± 0.004	81.76 ± 1.56
IV	2	0.202 ± 0.004	85.54 ± 1.65
V	3	0.195 ± 0.002	82.41 ± 0.92
Stage	**TRH (h)**	**NRR (g/L·d)**	**NRE (%)**
I	30	0.193 ± 0.002	81.79 ± 0.96
II	20	0.184 ± 0.004	77.48 ± 1.65
III	10	0.136 ± 0.016	58.03 ± 6.81
IV	4	0.123 ± 0.003	53.24 ± 1.42
V	2	0.093 ± 0.013	39.62 ± 5.74

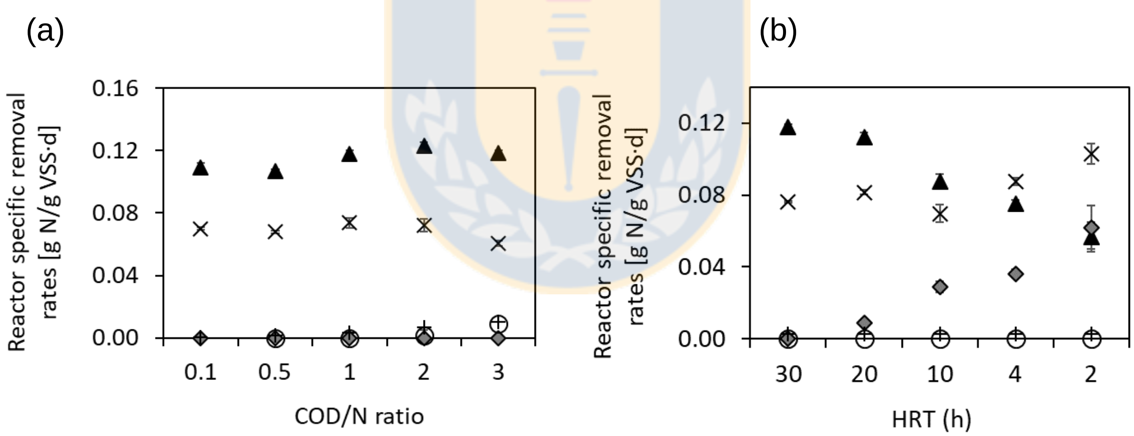

Figure 2.2: Reactor specific nitrogen removal rates by Anammox (AR_X) (▲), ammonia oxidizing (AOR_X) (×) nitrite oxidizing (NOR_X) (◆), denitrifying (DR_x) (○) bacteria, and heterotrophic nitrogen assimilation (HNa_x) (+) obtained at constant NLR of 240 mg N L^{-1} d^{-1}, with: (a) different COD/N ratios at 30 h of HRT; and (b) variable HRT at COD/N ratio of 0.5.

3.2 COD/N ratio and HRT influence over the main microbial groups

The relative abundance of main microbial groups was analyzed by FISH in granules and flocs separately, along the operation of both experimental reactors (Figure 2.3). The low concentration of non-aggregated biomass made impossible to analyze it by FISH but was measured as volatile solids at the effluent.

In the first reactor of variable COD/N ratio, a stable composition of granules and flocs was observed along all the operation, being anAOB the dominant microbial group in granules (48.9 ± 1.2 %) and the AOB in case of flocs (46.4 ± 1.3 %). Inversely, the second main abundance group in granules are the AOB (42.0 ± 0.9 %), and the anAOB in flocs (37.1 ± 1.2 %). This is consistent considering that granules are more compact, dense and has greater size than flocs, allowing the existence of an anoxic zone in the inner structure due to diffusional limitations that allows the development of stable anAOB communities. Regarding the NOB group, it remained with low abundance in granules during all the operation (2.2 ± 0.6 %) and a slightly greater presence in flocs (7.0 ± 1.3 %), although there were probably inactive given that no specific nitrite oxidizing rate was detected in the reactor along all the operation. Finally, an abundance of 4.4 ± 2.1 % of HDB was detected in granular biomass and 6.1 ± 1.3 % in flocs, suggesting possible denitrifying activity in the reactor.

The same behavior was observed in the second reactor with variable HRT. Greater abundance of AOB in flocs than in granules (51.0 ± 3.1% and 37.9 ± 2.4%, respectively), and greater abundance of anAOB in granules than in flocs (49.63 ± 1.9% and 33.6 ± 1.6%, respectively), was detected. Also, the NOB group growth was limited along all the operation, maintaining low abundance in granules (3.6 ± 0.8%)

and higher abundance in flocs (7.5 ± 1.1%). HDB was also detected, with similar abundances in granules and in flocs (5.4 ± 0.9% and 4.8 ± 0.6%, respectively).

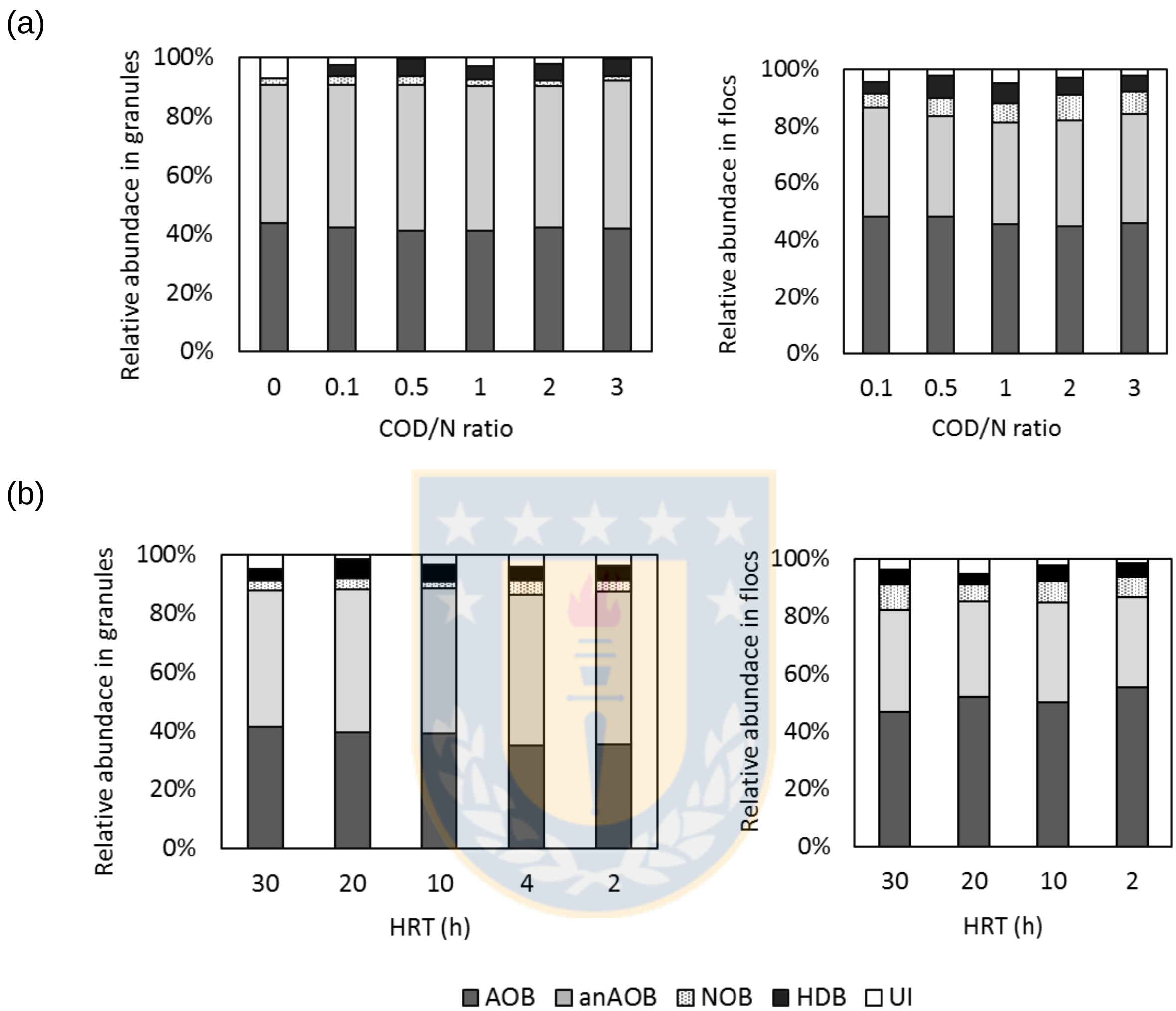

Figure 2.3: Relative abundance of main bacterial groups of the partial nitritation-Anammox process obtained in the different operational stages with: a) COD/N ratios of 0, 0.1, 0.5, 1, 2 and 3 at 30 h of HRT; b) HRT of 30, 20, 10, 4 and 2 at COD/N ratio of 0.5. AOB: ammonia oxidizing bacteria; anAOB: anaerobic ammonia oxidizing bacteria; NOB: nitrite oxidizing bacteria; HDB: heterotrophic denitrifying bacteria; UI: unidentified eubacteria.

3.3 COD/N ratio and HRT influence over the biomass properties

Specific nitrifying and Anammox activities (SNA and SAA, respectively) performed to granules at the first and the last stages of the first reactor with variable COD/N ratio, shows no significant differences between the beginning and the end of the operation, confirming no inhibition effect of organic carbon over the main bacterial groups along the operation.

The second reactor with variable HRT showed a 28% increase in SNA and a 25% decrease of SAA. Those tendencies are consistent with the specific removal rates calculated for the reactor, sustaining that performance decrease was due to Anammox activity decrease.

Granules size for both reactors, were maintained stable along the operation showing no significant differences with average values of 1.35 ± 0.02 mm and 1.38 ± 0.10 mm in the first and second reactor, respectively. Volatile solids at the effluent corresponded mainly to non-aggregated biomass since no granules neither flocs were observed at the discharge. An increase of 53% of VS at the end of the operation of the first reactor was detected. Instead, the second reactor show no variations of solids concentration at the effluent but an increase of nine times of VS load at the discharge due to the HRT increase. Nevertheless, biomass concentration inside the reactors remains almost constant, with average values of 1.62 ± 0.02 and 1.69 ± 0.08 g VS L^{-1} in the first and second reactor, respectively.

4. Discussion

4.1 Influence of COD/N ratio and HRT on NP-Anammox performance

COD/N ratio increase evaluated on the first reactor had no negative effects over the nitrogen removal performance, observing a maximum NRE of 85% with COD/N ratio of 2 followed by a NRE of 82% obtained with the greatest ratio assayed equal to 3. Nitrogen removal enhancement with the COD addition to NP-Anammox systems has been already observed by other authors [12, 18, 32] but lower threshold ratios were detected in all cases. Zhang et al. [32] determined a threshold of COD/N ratio of 2.2, when HB start to outcompete anAOB for nitrite, displacing Anammox by denitritation reaction. Even though a COD/N ratio of 2.2 is enough for partially denitrify NO_2-N to N_2, since 4.5 g of acetate COD are needed per gram of NO_2-N according to stoichiometry of denitritation reaction [31], in continuous aeration systems as the preceding case of Zhang et al., COD would be mainly oxidized by the aerobic route since enzymes responsible for denitrification are immediately inactivated in presence of O_2 [33]. Explanation for the good performance observed in our experiments are on the reactor type and HRT applied to the system. Granular or biofilm systems can be operated without retention of non-aggregated biomass since HRT is equal to SRT for non-aggregated microorganisms, and if HRT is larger than the reciprocal maximum specific growth rate of the heterotrophs, they grow in suspension without forming biofilms over the granules [34]. The use of HRT near or below 3 h stimulates the cell aggregation and heterotrophic layer will be form in the outer granules or biofilms, as reported by Van Benthum et al. [19]. In the case of Zhang et al. [12, 32], the use of a membrane bioreactor and suspended biomass favored the retention of all microbial groups, allowing HB to outcompete AOB and anAOB. Working with moderate HRT higher than 3 h on granular or biofilm systems it is possible to achieve higher tolerance to organic carbon, maintaining high nitrogen removal efficiencies in the system [8, 18]. Otherwise, the sensitivity of granular PN-Anammox systems at low HRT has been demonstrated with the second experiment, since identical conditions were applied to both reactors, and, maintaining a moderate COD/N ratio of 0.5, a strong decrease of nitrogen removal performance was observed with progressive decrease of HRT. Even though the nitrogen load applied to the system was constant for all the operation (NLR of 240 mg N L^{-1} d^{-1}), the way of application decreasing TAN concentration proportional to HRT decrease in order to maintain a constant NLR, produced an unexpected effect on TAN and nitrite concentrations in the bulk, obtaining lower values at each stage that clearly affect the removal rates due to the kinetic dependence on substrate concentration.

In Fig. 2.4, the specific Anammox removal rate versus the square root of nitrite concentration is represented, showing a linear tendency with apparent ½ order kinetic. Although the nitrite half saturation constant of Anammox bacteria is very low (0.05 mg NO_2-N L^{-1}) [35], ensuring maximal anammox activity for higher concentration values (0 order kinetic), the half order kinetic obtained represents the transition between 1 and 0 order caused by the substrate penetration into the granules, that means a kinetic limited by nitrite diffusion [36]. Thus, a condition for better performance in this kind of systems operated at low HRT below 20 h, should be to work with higher nitrogen loads than the assayed in this experiment.

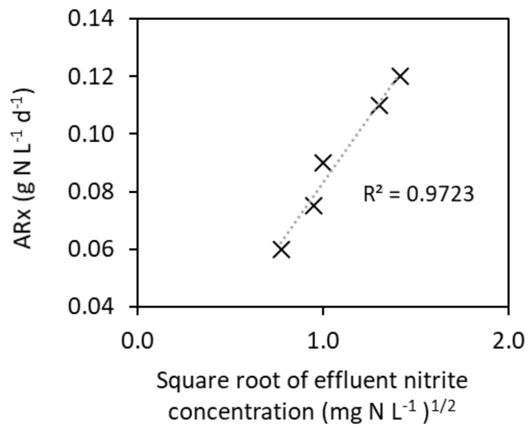

Figure 2.4: Correlation between specific nitrogen removal rate by Anammox (AR_X) and the square root of the nitrite concentration (×) measured at the effluent of reactor 2 with variable HRT.

4.2 Nitrogen removal by AOB, anAOB, NOB and HDB

The stable operation achieved on the first reactor when the COD/N ratio was increased is consistent with the calculated specific Anammox and AOB reactor activities that maintained similar values in all stages with a slightly increase of AR_x with COD/N ratios 1, 2 and 3 that can explain the removal improvement observed with COD/N ratio of 2. This stability was reflected on specific Anammox and AOB batch activities, which maintained the initial measured value at the end of the operation. Even communities maintained a high stability along the operation in granules and flocs, being anAOB and AOB the most abundant microbial groups in the cell aggregates. The high stability of the communities added to the low HDB fraction detected in aggregated biomass by FISH during the COD/N ratio increase, confirm that COD was mainly oxidized by non-aggregated heterotrophic biomass by the aerobic route. Some authors have reported for aerobic sludge systems that long SRT (10-20 d) has a positive effect on flocculation [37, 38] by changing the surface properties of the sludge as hydrophobicity and surface charge [39]. This means that working with SRT lower than 10 d it is possible to avoid the flocculation of heterotrophic sludge, allowing the manipulation of heterotrophic SRT by HRT control. Thus, the HRT of 1.25 d used in this experiment was high enough (higher than 3 h) to avoid the HB aggregation over the autotrophic granules and low enough (lower than 10 d) to avoid HB flocculation. This condition allowed the easy separation between granules/flocs and the non-aggregated heterotrophic biomass, the latter reflected in the effluent volatile solids, which increase in 53% with the increment of COD/N ratio from 0.1 to 2.

On the other hand, the performance decline observed with the HRT decrease has a correlation with a loss of specific Anammox activity measured in batch and Anammox rate calculated for the reactor also. Nevertheless, no rate decrease was observed over the AOB, in fact, an improvement of specific AOB activity was detected at the end of the operation. This fact confirms the effectiveness of maintaining the heterotrophic biomass in a non-aggregated state, since ammonia oxidation can occur simultaneously with the organic carbon oxidation without affect the ammonia oxidation rate of the reactor.

When HRT was decreased at fixed NLR, Anammox reaction was limited by substrate as mentioned previously, since the nitrite concentration in the bulk decreased gradually within each stage, being 2.3 ± 0.1 mg NO_2-N L^{-1} at the first stage and 0.6 ± 0.1 mg NO_2-N L^{-1} at the last one. TAN concentration decreased also, from 20.1 ± 1.1 to 1.7 ± 0.2 mg TAN L^{-1}, but no limitation on the ammonia oxidation rate neither on the specific AOB activity was observed. These results suggest that Anammox bacteria were affected by diffusional limitations of the substrates. Given the characteristics of the microbial groups distribution in the granules, AOB are less conditioned than anAOB to diffusion of substrates since they growth at the granule surface where dissolved oxygen is available. AnAOB instead are in the inner part of the granules but depends on the diffusion of TAN and nitrite which in turn depends on the concentration gradient driving force. As nitrite and TAN concentration in the bulk decreased gradually within each stage, the lower gradients determine lower diffusional rates. On the other hand, the increase of NOR_x observed during the operation revealed that nitrite began to be oxidized by NOB, outcompeting anAOB for substrate. Competition between NOB and anAOB can be solved working in a two-reactor system of partial nitrification and Anammox separately, by nitratation suppression in the first stage [1], considering that NOB cannot growth in the second anoxic stage. That could be useful to treat low loaded wastewaters, otherwise, Anammox inhibition by nitrite could cause process failure [1, 40].

In spite of nitrogen removal decline due to the HRT decrease, biomass aggregates maintained stable along the operation, showing a constant granular size and microbial composition, thus, a population balance can be maintained at low HRT under proven conditions, but higher NLR is recommended to avoid substrate limitation.

5. Conclusions

Stable performance was achieved in partial nitritation/Anammox reactor with increasing COD/N ratio from 0.1 to 3.0, with a maximum nitrogen removal of 86 % obtained with a COD/N ratio of 2. No effects over the granules and flocs composition were observed with the increase of COD/N ratio until the value of 3.0, denitrifying communities were detected but nitrogen was removed mainly by PN-Anammox and organic carbon through the aerobic route by non-aggregated heterotrophic bacteria. Also, no variations of the specific Anammox and nitrifying activities were observed, showing high stability of the biomass under the studied conditions.

Instead, decreasing HRT applied to the same operation conditions with COD/N ratio of 0.5 and keeping constant the nitrogen loading rate, produced a progressive performance decline. Competition between NOB and anAOB for nitrite was identified as the most probable cause of nitrogen rate decrease. Nevertheless, no negative effect over the biomass composition was observed, staying stable the main bacterial groups in granules and flocs.

These results demonstrated the high sensitivity of the PN-Anammox process to HRT below 20 h at moderate NLR of 240 mg N L^{-1} d^{-1} but the feasibility to operate with high COD/N ratios until 3.0 at 30 h of HRT in continuous granular reactor systems. These findings contribute to expand the potential of PN-Anammox technology, including wastewaters with high COD/N inlet ratios as food processing industry effluents or animal manure wastewaters.

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CHAPTER 3. From CANON to SNAD: Aeration strategy to promote denitrification in a partial nitritation-Anammox process in presence of organic carbon

(Article to being sent for publication)

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Keywords: partial-nitritation; Anammox; denitrification; SNAD.

Highlights:

- Anoxic/oxic phases promote denitrification in CANON reactor with COD.
- Continuous CANON with COD can be enhanced operating in SBR mode.
- Complete anoxic oxidation of COD during the anoxic phase was achieved.
- Anoxic/oxic phases promotes denitrifying growth in flocs, not in granules.
- Nitrogen removal of 96% and 85% with COD/N ratios of 0.57 and 2.3, respectively.

Abstract

An aeration strategy aimed to develop denitrification and to remove COD anoxically into a CANON reactor at different COD/N ratios was evaluated. An initial anoxic phase before the aerobic reaction was included into the sequencing batch reactor (SBR) cycle and was compared with a completely aerobic cycle and a continuous CANON reactor. Nitrogen load was maintained at 0.24 kg N m^{-3} d^{-1} and COD/N ratios of 0.3, 0.57 and 2.3 were assayed. High nitrogen removal efficiencies of 96% and 85% with COD/N ratios of 0.57 and 2.3 were observed, respectively. Complete anoxic oxidation of COD was achieved, and denitritation reaction was detected during the anoxic phase. An increase from 6.8 to 16.4% of denitrifying organisms was detected in flocculent biomass by FISH with the inclusion of the anoxic phase into the cycle, while Anammox and ammonia oxidizing bacteria dominated in granules with abundances of 43.6% and 38.2%, respectively. In sum, the proposed operation enhanced SNAD performance for the treatment of ammonia wastewaters with high organic content reducing aeration costs associated to COD oxidation.

1. Introduction

One-stage Partial nitritation-Anammox reaction, commonly known as CANON process (Completely Autotrophic Nitrogen Over Nitrite), has been widely implemented along the last decade for the ammonia removal from wastewaters [1]. The application of CANON systems has been limited to effluents with low ratios of organic carbon to nitrogen content, since COD/N ratios around 2 or higher are associated with efficiency loss due to microbial competition between heterotrophic bacteria (HB) with ammonia oxidizing bacteria (AOB) for oxygen and space and with Anammox bacteria (anAOB) for nitrite and space [2-4]. Nevertheless, several authors have detected heterotrophic denitrifying bacteria (HDB) in CANON systems treating ammonia effluents with low COD content [5-8], suggesting that nitrogen removal efficiency can be improved by denitrifying the nitrate produced by Anammox reaction. The development of simultaneous nitritation, Anammox and denitrification process (SNAD) depends on the cooperative relation between AOB, anAOB and HDB to oxidize ammonia avoiding that the latter outcompete AOB for oxygen uptake and anAOB for nitrite consumption.

In aerobic systems, denitrification of nitrate is difficult to achieve since organic carbon would be oxidized mainly by the aerobic route [9]. For low COD/N ratio effluents, the organic carbon does not mean a problem for the CANON reaction as long as enough oxygen is provided to maintain partial nitritation despite oxygen uptake for the COD oxidation, but no significant denitrification will occur because of the aerobic abatement of COD [10]. Several authors have proved the feasibility to achieve N removal by partial nitritation-Anammox process in presence of low COD/N ratios (<1), [11-14], obtaining N removal efficiencies greater than 80% but low denitrification contribution is expected in all cases due to the continuous aeration. Modelling studies have proved that N removal can be maintained at higher COD/N ratios but contribution of Anammox is gradually displaced by heterotrophic nitrogen removal with increasing COD load [3, 9], and as increasing optimal bulk oxygen concentration is also required, NOB growth is also promoted resulting in competition with anAOB for nitrite and nitrate accumulation [15]. In any case, the aerobic oxidation of COD demands more energy to the system, raising operation costs.

To overcome this drawback, intermittent aerobic systems have presented as an operational alterative to reduce aeration costs and even increase the nitrogen removal efficiency by promoting denitrification during anoxic phases [16, 17], but aerobic oxidation of COD occur during aerobic phases anyway [18].

In this work, we propose an operation strategy that allows the totally anoxic oxidation of COD and the development of denitrification in a CANON system for the treatment of ammonia effluents with COD/N ratio content from 0.3 to 2.3, allowing to save aeration costs associated with aerobic COD oxidation. The proposed strategy consists in a SBR system with an initial anoxic phase that promotes the anoxic COD consumption at the beginning of the batch cycle, using the remaining nitrate from the previous cycle as electron acceptor. Data collected was compared with continuous operation mode and its eventual conversion into a SNAD system was evaluated by physicochemical analysis and microbial community analysis by FISH.

2. Materials & Methods

2.1 Reactor set-up

A bubble column reactor made of glass with an effective volume of 2.0 L was used. The air and the influent were supplied from the bottom section of the reactor, while the gas (recirculation and overpressure) and the effluent were discharged from the top section. All the stream of liquid and gas, except overpressure, circulate through peristaltic pumps (LongerPump, BT100-2J, China). Oxygen supply was initially fixed at 135 mL h^{-1} to obtain a loading ratio of 1.9 g O_2 g N^{-1} according to the optimal ratio determined by Vansgaard et al. [19] and increased according to the increasing COD load applied and the concentration of the nitrogen compounds at the effluent. Dissolved oxygen (DO) concentration was measured daily with an optical dissolved oxygen electrode (WTW, FDO 925, Germany) and fluctuated between 0.05-0.1 mg L^{-1} along the operation. Temperature was maintained at 35° C using a thermostatic bath (TIC Controller, Thermo Haake, DC30, Germany).

2.2 Substrate and operational conditions

The sequential batch reactor was operated in 6-hours cycles comprising: 4.7 min of feeding, 349 min of reaction, 1.5 min of sedimentation and 4.8 min of withdrawal. Volume exchange was 20%, obtaining a mean HRT of 30 h. The reactor was initially started with continuous aeration regime until stabilized the nitrogen compounds profile at the effluent. Subsequently, an initial anoxic phase of 30 min was included in the reaction phase of the cycle (30 min of anoxic reaction followed by 319 min of aerobic reaction), in order to promote the organic carbon oxidation in anoxic conditions with nitrate as electron donor by denitrification. Three COD/N ratios were assayed under anoxic/oxic operation mode (A/O) of 0.3, 0.57 and 2.3. COD and nitrogen sources was added as sodium acetate and ammonium sulfate, respectively. Inlet total ammonia nitrogen (TAN) was maintained constant at 300 mg TAN/L and COD concentration was supplied according to the desired COD/N ratio.

2.3 Characteristics of the partial nitritation-Anammox inoculum

Experimental reactor was inoculated with partial nitritation-Anammox granules, obtaining a final concentration of 2 g L^{-1} of volatile suspended solids (VSS). Biomass was obtained from the reactor described at Varas et al. [20] but previously acclimated to organic carbon at COD/N ratio of 0.1 and 430 mg TAN/L. Characteristics of the inoculum biomass were: granule diameter of 1.35 ± 0.45 mm, sedimentation rate of 62.4 ± 9.25 m h^{-1}, specific nitrification and Anammox activities of 0.064 and 0.197 g N g VSS^{-1} d^{-1}, respectively.

2.4 Analytical methods

Influent and effluent samples were taken periodically to measure TAN (NH_3 + NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), chemical organic demand (COD), pH, total and volatile suspended solids (TSS and VSS, respectively), according to the standard methods [21]. Specific Anammox and nitrification activities (SAA and SNA, respectively) were determined by batch assays according to the methodology described by Dapena-Mora et al. [22] and López-Fiuza et al. [23], respectively.

2.5 FISH analysis

The identification of the different bacterial groups, the analysis of the structure and relative abundance of the different populations of microorganisms, as well as the monitoring of the spatial and temporal dynamics of microbial populations that integrates the SNAD granular biomass samples, was carried out by the technique of fluorescent in situ hybridization (FISH) according to the technique described by Pernthaler et al. [24]. The granular biomass was prepared as follows: fixation in 4% paraformaldehyde /phosphate buffered saline (PBS) solution for 12 hours at 4°C, then washed and stored in 1:1 ethanol / PBS, until hybridization with the probes solution. 100 µL of each sample were hybridized in the presence of each probe solution for 2 hours at 46°C. The probes solution for each hybridization was prepared mixing 1 µl of probe with 9 µl of hybridization buffer. The solutions and buffers as well as the procedures for each step were carried out as set forth. Probes used for in situ hybridization were EUB-338, NEU653, Ntspa-1026, DEN124 and Amx-0820; complementary to a region of the 16S specific domain of DNAr for Eubacteria, AOB, NOB, heterotrophic denitrifying bacteria (HDB) and anAOB, respectively (Table 1). The analysis was performed with a MOTIC-BA310 fluorescence microscope using the specific filters for each probe, using Eq. (1) to determine number of cells per gram of biomass. DAPI and EUB-338 were used as controls. From the analysis, the relative percentages of abundance of each bacterial group were determined with respect to the total biomass of eubacteria, combining each specific probe with the probe EUB338; targeted to the total population of eubacteria. The difference between eubacteria and the sum of anAOB, aerAOB, HDB and NOB was termed unidentified eubacteria (UI).

$$N\left(\frac{cells}{biomass\ (g)}\right) = Number\ of\ cells\ on\ the\ visual\ field\ \times 3.62 \times 10^{8} \qquad \text{Eq. (1)}$$

2.6 Calculations

Nitrogen removal rate (NRR) and nitrogen removal efficiency (NRE) were calculated based on nitrogen global balances. Removal rates in the reactor by AOB (AOR_x), NOB (NOR_x) and Anammox (AR_x) were estimated based on the Anammox stoichiometry (Eq. 1) and nitrogen balances, according to the equations proposed by Vázquez-Padín et al. [25] but dividing by the reactor biomass concentration to obtain the specific removal rates, expressed as g N g VSS^{-1} d^{-1}. Used equations were:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad \text{Eq. (2)}$$

$$\Delta N = TAN_{inf} - \left(TAN_{ef} + NNO_2{}^-{}_{ef} + NNO_3{}^-{}_{ef}\right) \qquad \text{Eq. (3)}$$

$$AR_x = \frac{(\Delta N)}{HRT \cdot X} \qquad \text{Eq. (3)}$$

$$AOR_x = \frac{(\Delta TAN)}{HRT \cdot X} - \frac{AR_x}{2.04} \qquad \text{Eq. (4)}$$

$$NOR_x = \frac{-\Delta NNO_3{}^-}{HRT \cdot X} - \frac{0.26 \cdot AR_x}{2.04} \qquad \text{Eq. (5)}$$

Being TAN_{inf} and the TAN_{ef} the total ammonium nitrogen concentration in the influent and effluent (g N/L), respectively, and the $NNO_2{}^-{}_{ef}$ and $NNO_3{}^-{}_{ef}$ the nitrogen as nitrite and nitrogen as nitrate concentrations in the effluent (g N/L), respectively.

COD and nitrate mass balances in the anoxic phase were done to compare with stoichiometric acetate-COD requirements of denitrification reactions (Eq. 6 and 7) described by Capadoglio et al. [26].

Denitratation: $1.0NO_3^- + 1.0H^+ + 0.33NH_4^+ + 1.45CH_3COO^- \rightarrow 0.5N_2 + 0.33C_5H_7O_2N + 0.13CO_2 + 1.12HCO_3^- + 1.62H_2O$ Eq. (6)

Denitritation: $1.0NO_2^- + 1.0H^+ + 0.24NH_4^+ + 0.975CH_3COO^- \rightarrow 0.5N_2 + 0.24C_5H_7O_2N + 0.015CO_2 + 0.735HCO_3^- + 1.235H_2O$ Eq. (7)

Therefore, 6.6 g of acetate COD are needed per g of NO_3-N denitrified (Eq. 6), 4.5 g acetate COD per g of NO_2-N (Eq. 7) and, by subtraction, 2.2 g of acetate COD are needed per g of NO_3-N denitrified up to nitrite (partial denitrification).

Table 3.1: FISH oligonucleotides used in this study.

Probe	Sequence (5´–3´)	rRNA target sites[(a)]	Specificity	Formamide[(b)] (%)	Reference
EUB338	GCTGCCTCCCGT AGG-AGT	16S, 338–355	Most Eubacteria	20	[27]
NEU653	CCCCTCTGCTGC ACT-CTA	16S, 653–670	(AOB) Halophilic and halotolerant members of the genus of Nitrosomonas	40	[28]
Ntspa1026	AGCACGCTGGTA TTG-CTA	16S, 1026–104	(NOB) Nitrospira moscoviensis, activated sludge clones A-4 and A-11	20	[28]
Amx-0820	AAACCCCTCTACT TA-GTGCCC	16S, 820–841	(anAOB) Genera "*Ca.* Brocadia" and "*Ca.* Kuenenia"	40	[29]
DEN124	CGACATGGGCGC GT-TCCGAT	16S, 124–143	(HDB) Acetate-denitrifying cluster	40	[27]
DAPI	Regions enriched in adenine and thymine in DNA sequences	Unspecific	DNA of all microorganism		

(a) 16S rRNA position according to Escherichia coli numbering; (b) Formamide concentration in the hybridization buffer; DAPI: 4′,6-Diamidine-2′-phenylindole dihydrochloride.

3. Results and discussion

3.1 Reactor performance under different aeration regimes

The reactor was initially started with continuous aeration (CA) and a COD/N ratio of 0.3 and was quickly stabilized as shown in Figure 3.1. Consequently, the initial anoxic phase was implemented (A/O operation mode), maintaining low and stable concentrations of nitrogen compounds at the effluent. Afterward, inlet COD/N ratios of 0.57 and 2.3 were assayed in stages III and IV, respectively. During the third stage, an initial destabilization was observed but on day 61, the biomass started to acclimate to the new condition and achieved the steady state on day 75. During the fourth stage, higher values of TAN were obtained but almost no nitrite neither nitrate were detected in the effluent. COD was totally consumed during stages I to IV.

Analysis of the nitrogen removal rates and efficiencies (NRR and NRE, respectively), showed the highest values during the operation with initial anoxic phase (A/O) with COD/N ratio of 0.57, obtaining a NRR of 0.231 mg N L^{-1} d^{-1} and a NRE of 96.1% (Figure 3.2). No significative differences were obtained in terms of NRR and NRE during the stages I, II and IV, showing an average NRR of 0.208 mg N L^{-1} d^{-1} and a NRE of 86.8% at steady state.

Another interesting comparison included on Figure 3.2 was the average removal rates and efficiencies obtained with another 2 identical reactors operated in parallel but operated at continuous feeding and aeration modes [30]. Same inoculum and operation conditions as HRT, temperature and substrate was used except for the continuous mode, revealing a greater effectivity of the SBR over the continuous operation for CANON systems with inlet COD. The main drawback detected in continuous reactors was the accumulation of nitrate, not observed in the SBR. Fast feeding of the SBR can contribute to inhibit NOB, since initial dilution of the fresh inlet feeding at the cycle beginning provides at least 60 mg TAN L^{-1} in the bulk, corresponding to a 3.5 mg L^{-1} of free ammonia (FA), and inhibition of NOB began at 0.1-1.0 mg FA L^{-1}, being critical at 4.0 mg FA L^{-1} [31, 32]. Care must be taken with high ammonia wastewaters, since AOB inhibition begins at 10 mg FA L^{-1} [31], thus lower exchange volumes or slow feeding must be applied in SBR systems to avoid shock loading.

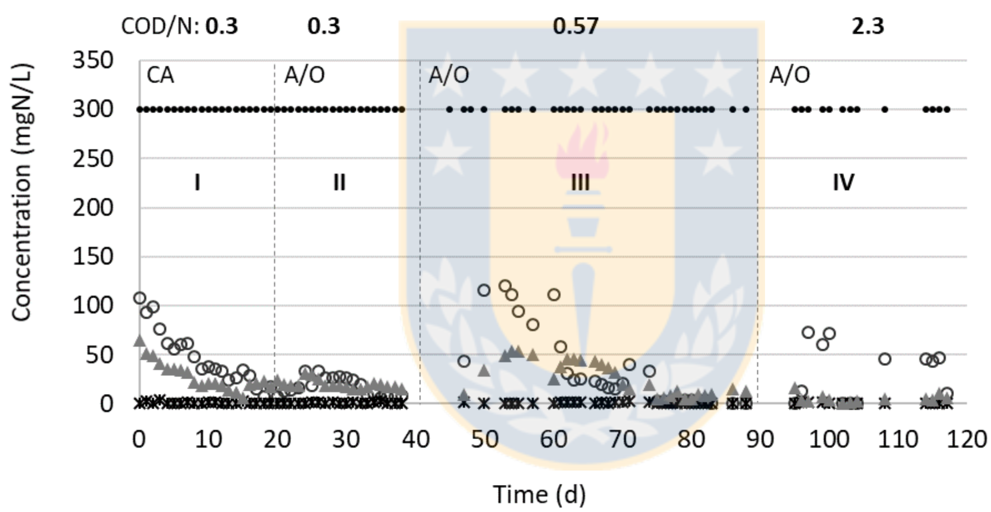

Figure 3.1: Nitrogen compounds profile in the different operational stages: TAN in the influent (●) and TAN (○), NO_2^--N (×) and NO_3^--N (▲) in the effluent. COD/N: inlet COD/N ratio; CA: Continuous aeration; A/O: Anoxic/Oxic operation mode.

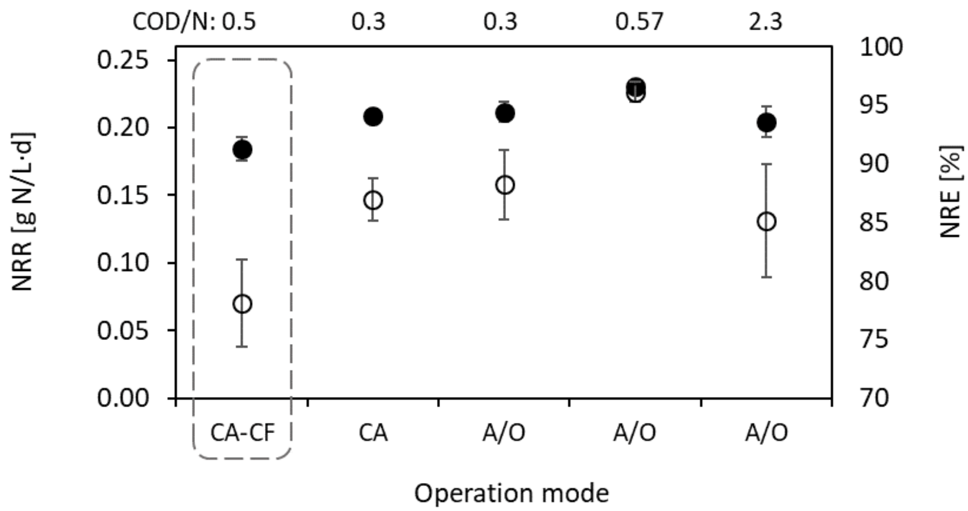

Figure 3.2: Nitrogen removal rates and efficiencies (NRR and NRE, respectively) obtained in the different operational stages: NRR (●) and NRE (○). COD/N: inlet COD/N ratio; CA: Continuous aeration; CF: Continuous feeding; A/O: Anoxic/Oxic operation mode; CA-CF: Experimental average data obtained with two identical reactors operated with continuous regime [30].

The highest NRE of 96.13 ± 0.8% obtained on stage III, could be explained by denitrifying reaction, since theoretical maximum NRE of PN-Anammox process is 89% and the other 11% remains as nitrate due to Anammox stoichiometry [33]. The total consumption of COD detected during the anoxic phase of stage III supports this idea (Figure 3.3.b), since the remainder nitrate at the end of the cycles was more than enough to oxidize all the inlet COD, considering a ratio of 6.6 g of acetate COD per g of NO_3-N according to the stoichiometry of denitrification reaction [26]. Nitrate and COD mass balances done to the anoxic phase of a representative cycle of stage III showed an observed ratio of 4.1 g of oxidized COD per g of reduced NO_3-N. As complete denitrification requires 6.6 g of COD per g of NO_3-N according to eq. (6) and partial denitrification, has a COD/ NO_3-N ratio of 2.1 (from eq. 6 and 7), the observed ratio found in the anoxic stage suggest that part of the nitrate was reduced only up to nitrite. Actually, nitrogen compounds profile presented in Figure 3.3.b showed a transient nitrite accumulation during anoxic phase that together with ammonia consumption revealed Anammox activity during this phase. The comparison of concentration profiles obtained with continuous aeration (Stage I) and the anoxic/oxic operation mode (Stage III) presented on Figures 3.3.a and 3.3.b, respectively, showing total COD consumption during the first 30 min of operation but higher nitrate values achieving with continuous aeration since non-consumption was observed.

The main difference of the anoxic/oxic operation mode assayed with intermittent aerobic systems proved in other works [17, 18, 34, 35] was the totally anoxic consumption of COD observed in our study. While it is possible to denitrify in intermittent aerobic systems during the anoxic phases, aerobic oxidation of COD will inevitably happen as clearly shown in cycle compounds profiles presented by Zhang et al. [18]. The initial anoxic phase ensures the anoxic oxidation of inlet COD by denitrifying the nitrate produced by Anammox reaction, being an easy way to turn from CANON to SNAD process, reducing aeration costs associated to the aerobic oxidation of COD.

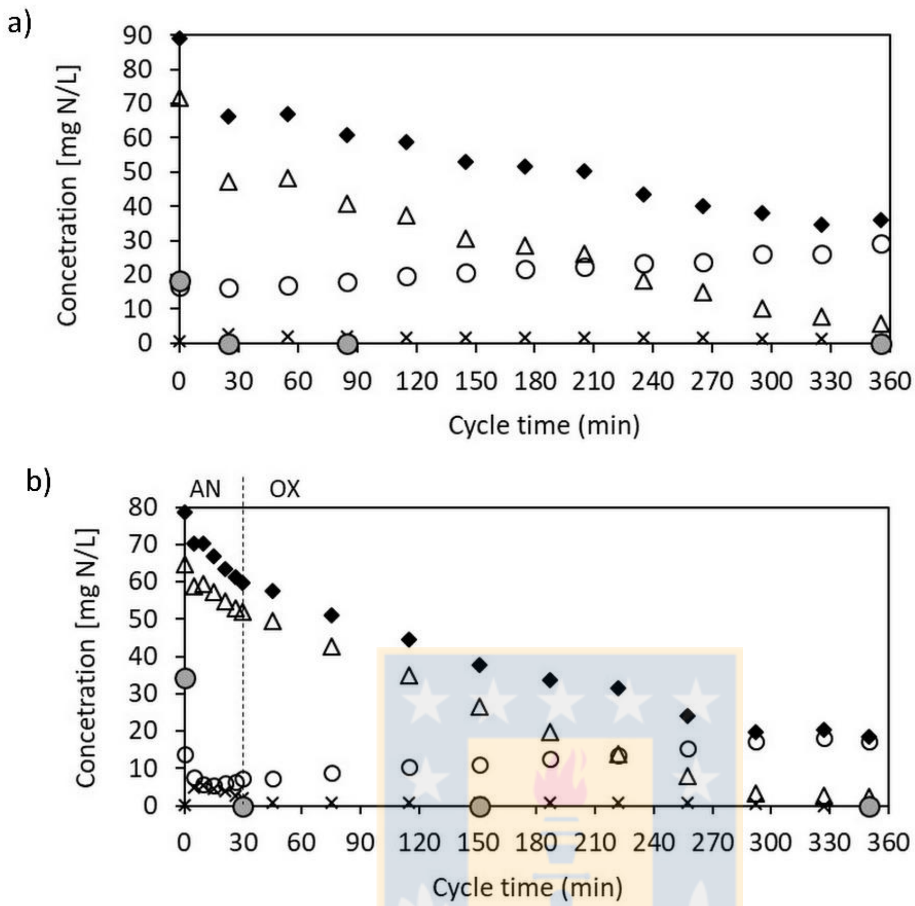

Figure 3.3: Evolution of COD and nitrogen compounds concentrations inside the reactor: COD (○) TAN (△), NO_2^--N (×), NO_3^--N (○) and TN (◆), for an operational cycle: (a) Continuous aeration operation mode, COD/N ratio = 0.3 and (b) Anoxic/Oxic operation mode, COD/N ratio = 0.57.

The NRE of 85.1% obtained with COD/N ratio of 2.3 was lower than the 96.1% obtained with ratio of 0.57. No nitrite neither nitrate but TAN was detected in the effluent at this last stage, suggesting a lack of oxygen to oxidize the remnant ammonia.

A comparison of different operation stages in terms of the reactor specific removal rates of main bacterial groups showed small variations of Anammox specific rates (AR_x) along all the operation but ammonia oxidizing specific rate (AOR_x) showed a decrease of 43% at the IV stage with COD/N ratio of 2.3 (Figure 3.4). This analysis confirms that the efficiency decay observed during the last stage was due to AOR_x decay but anAOB maintained their activity. Anyway, the high NRE obtained with the higher COD/N ratio of 2.3 demonstrated the effectiveness of the proposed anoxic/oxic SBR operation mode, since reaction suppression has been reported with lower COD/N ratios [6, 36]. In fact, no significate difference of removal rates was obtained between the lowest and the greatest COD/N ratios assays during the operation of the SBR, confirming that the proposed operation mode is highly tolerant to organic carbon at least until a COD/N ratio of 2.3.

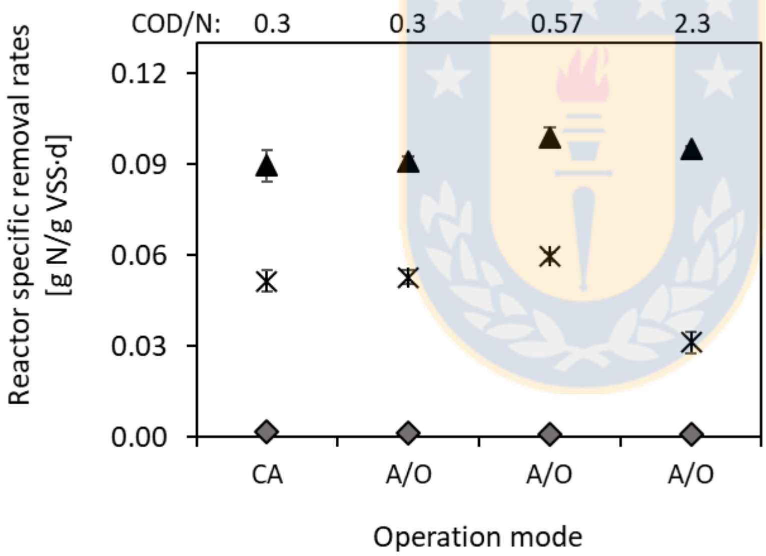

Figure 3.4: Reactor specific removal rates for Anammox (AR_X) (▲), ammonia oxidizing (AOR_X) (×) and nitrite oxidizing (NOR_X) (◆) bacteria, obtained at different operation modes. CA: Continuous aeration; A/O: Anoxic/Oxic operation mode; COD/N: inlet COD/N ratio.

3.2 Operation mode influence over the biomass composition

The relative abundance of main microbial groups was analyzed by FISH in granules and flocs separately, along the SBR operation (Figure 3.5). A stable composition of granules was observed during stages I to III, being AOB and anAOB the dominant groups with relative abundances of 38.2 ± 0.8 and 43.6 ± 0.6%, respectively. Nevertheless, notorious increase from 6.8 to 16.4% of HDB was detected in flocs with the change from continuous aeration (CA) to anoxic/oxic operation mode (A/O) after 3 weeks of implementing the initial anoxic phase (Figure 3.4.b).

Some authors have detected denitrifying organisms in SNAD systems [6-8, 34], but no specific conditions for denitrification stimulation has been reported. Indeed, the presence of HDB not necessarily means denitrifying activity in the reactor, since the enzymes responsible for reducing nitrate are immediately inactivated by O_2 due to competition for electrons [10]. In this work, the increase of HDB clearly indicates that the initial anoxic stage implemented in the SBR cycle promotes the denitrifying activity and their consequent growth. Contrary to the growth over PN-Anammox biofilms and granules predicted by Mozumbder et al. [15] and Hao and Van Loosdrecht [9] in CANON models with inlet COD, this study shows a denitrifying-heterotrophic growth not over granules but in flocs, conditioned by the SBR operation mode with the initial anoxic stage. Sedimentation time could be a key parameter to achieve the flocculent heterotrophic growth, since selective pressure parameters as liquid upflow velocity, solids in the effluent, or removal of excess sludge from reactor are crucial to obtain anaerobic granulation [37], otherwise growth take place as dispersed biomass. In this work a settling time of 1.5 min was used that seems to be a low selective pressure that avoid the heterotrophic granule formation into the SNAD reactor. Further research must be done in this field to determine the SBR conditions that triggers the aggregation of heterotrophs in SNAD reactors.

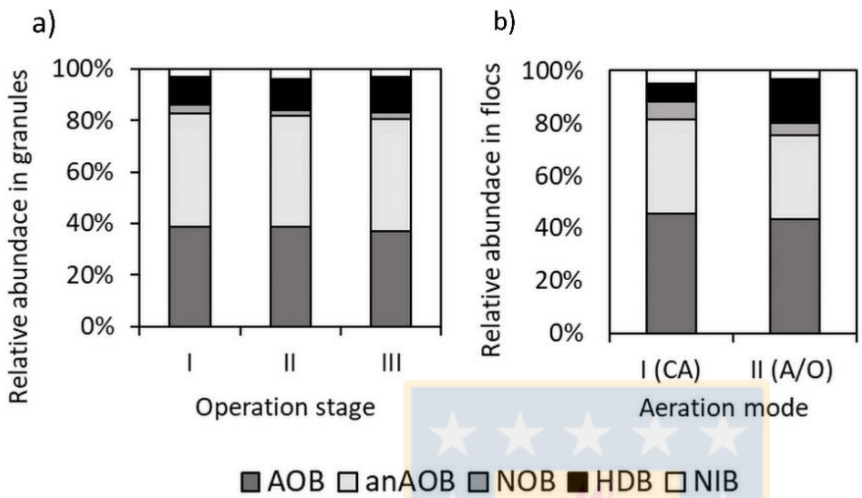

Figure 3.5: Relative abundance of main bacterial groups of the partial nitritation-Anammox process obtained in granules (a) and flocs (b), at different operational stages and modes. CA: continuous aeration; A/O: anoxic/oxic operation mode. AOB: ammonia oxidizing bacteria; anAOB: anaerobic ammonia oxidizing bacteria; NOB: nitrite oxidizing bacteria; HDB: heterotrophic denitrifying bacteria; NIB: non-identified bacteria.

4. Conclusions

Successful conversion from CANON reactor into SNAD was done in a SBR by implementing an initial anoxic step into the aerobic cycle. The main goals achieved with the proposed operation mode are:

- To maintain high nitrogen removal efficiencies with high COD/N ratios at least until 2.3;
- To allows the anoxic COD oxidation at the initial anoxic phase, reducing the aeration costs;

- To promote the development and growth of flocculent denitrifying bacteria in the system, contributing with denitritation reaction on the nitrogen removal process.

Also, comparison of nitrogen removal rates and efficiencies demonstrated the better performance of SBR than continuous reactor. The proposed operation strategy seems to be a good alternative to work with ammonia effluents with fluctuating organic content as for example livestock manure or mainstream wastewaters.

Acknowledgements

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CHAPTER 4. Long-term operation of partial nitritation-Anammox process fed with digested poultry manure

(Article to being sent for publication)

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Keywords: partial-nitritation; Anammox; poultry manure.

Highlights:

- 1-year operation of PN-Anammox reactor for N removal from digested poultry manure
- N removal rate of 0.88 g N L^{-1} d^{-1} (83% of efficiency) obtained at a HRT of 9.6 h
- Overload caused granules floatation but fast recovery was observed
- A HRT of 3.6 h promotes filamentous growth over granules
- Stable anAOB abundance of 44% and decrease from 43 to 39% of AOB was observed

Abstract

Partial nitritation-Anammox processes have been applied to many industrial effluents mainly from anaerobic digesters, but few experiences with digested animal manures have been reported. This study proves the feasibility of N removal from digested poultry manure in a PN-Anammox reactor under different values of HRT between 9.6 to 45 h. Stable performance was achieved along the operation, obtaining a maximum N removal rate and efficiency of 0.88 g N L^{-1} d^{-1} and 83%, respectively, when the system was operated at a HRT of 9.6 h. One event of granules floatation was provoked by a hydraulic overload, but fast performance recovery was observed in three days. Granular biomass maintained anAOB dominance with a relative abundance of 44.2 ± 1.5% and the presence of AOB decreased from 43 to 39% at the end of the operation, possibly caused by heterotrophic growth over granules.

1. Introduction

Animal manure management has been gained a growing concern due to the increasing animal husbandry for productive purposes during the last two decades [1]. Problems associated with the on-land application of manures as water pollution, food contamination, nutrient surplus and greenhouse gases emissions [1-3], urgently demands a change towards responsible management. Many efforts have been done to develop nutrients recovery techniques as air or steam stripping, reverse osmosis, struvite precipitation and vacuum evaporation, but the energy demand added to the product transport in scenarios of nutrient excess, limits their application [3].

Anaerobic digestion (AD) of animal manures is considered the best alternative for manures treatment since produces energy (biogas) and reduces greenhouse gases emissions [4]. Nevertheless, AD does not affect the nitrogen content, indeed, it is negatively affected by ammonia content due to methanogenesis inhibition by free ammonia, limiting biogas production [5]. In this sense, autotrophic nitrogen removal based on the combination of partial nitritation (PN) and Anammox reactions (Equation 1) have demonstrated its effectiveness on anaerobic digester supernatants [6] and it has been referred over other processes for its economic and technical advantages [7].

$$NH_4^+ + 0.804\ O_2 + 0.071\ HCO_3^- \rightarrow 0.111\ NO_3^- + 0.436\ N_2 + 0.009\ C_5H_7O_2N + 0.028\ CH_2O_{0.5}N_{0.15} + 1.038\ H^+ + 1.46\ H_2O \quad \text{(Eq. 1)}$$

Preliminary studies at laboratory scale have proven the feasibility of nitrogen removal by PN-Anammox processes from digested swine slurry and poultry manure [8-10], but much efforts must be done to find the operational limits that ensures a good performance and to improve the efficiency, in order to scale the process. Regarding to reactor design, a low HRT allows decreasing the reactor size required, reducing investment and operation costs. Thus, the feasibility of a long-term operation of a PN-Anammox reactor fed with digested poultry manure under different and decreasing values of HRT was studied, focused on the effect of the HRT decrease and the consequent increment of substrate load over the global performance of the process and also on the microbial composition of the biomass.

2. Materials & Methods

2.1 Reactor set-up

A bubble column reactor made of glass with an effective volume of 3.0 L and granular PN-Anammox biomass obtained from the reactor described at [11] was used. This reactor was previously operated and enriched with synthetic substrate for 9 months and short experiences with digested poultry manure were assayed to evaluate the response of the system to this substrate (not reported data). The air flow was supplied from the bottom and the liquid influent from the middle section of the reactor, while the liquid effluent and gas overpressure were discharged from the top section. The agitation was done by recirculating the gas from the top to the bottom and a three-phase separator allows the retention of biomass. Liquid and gas streams circulate through peristaltic pumps (LongerPump, BT100-2J, China). Temperature was maintained at 35°C through a heating jacket and a thermostatic bath (Fried electric, TEPS-1, China). Dissolved oxygen was measured on-line by an optical dissolved oxygen sensor (WQ-FDO 925, Global Water, USA).

2.2 Substrate and operational conditions

The reactor was operated with continuous feed and aeration regime. Eleven stages were defined; the first one corresponds to synthetic substrate feeding and all the following ones to digested poultry manure feeding. In these stages, the HRT was stepwisely decreased. There were also two last stages corresponding to a recovery period due to overload experimented in stage IX (Table 1). Synthetic substrate composition was: KH_2PO_4 25 mg L^{-1}; $MgSO_4 \cdot 7H_2O$ 200 mg L^{-1}; $CaCl_2 \cdot 2H_2O$ 300 mg

L^{-1}; $FeSO_4 \cdot 7H_2O$ 11 mg L^{-1}; EDTA 6 mg $L^{-1}$and trace elements added as concentrate solutions in order to obtain: EDTA 22,5 mg L^{-1}; $ZnSO_4 \cdot 7H_2O$ 0,645 mg L^{-1}; $CoCl_2 \cdot 6H_2O$ 0,360 mg L^{-1}; $MnSO_4 \cdot 4H_2O$ 6,3 mg L^{-1}; $CuSO_4 \cdot 5H_2O$ 0,375 mg L^{-1}; $Na_2MoO_4 \cdot 2H_2O$ 1,4 mg L^{-1}; $NiCl_2 \cdot 6H_2O$ 0,285 mg L^{-1}, Na_2SeO_3 anhydrous 0,675 mg L^{-1} and H_3BO_3 0,021 mg L^{-1}. COD and nitrogen sources was added as sodium acetate and ammonium sulfate, respectively, while inorganic carbon (IC) was supplied as sodium bicarbonate, maintaining a molar IC/N ratio of 2.5 [12]. Digested poultry manure was diluted in tap water to obtain a COD concentration of 280 mg L^{-1} and supplemented with ammonium sulfate in order to set the COD/N ratio in 0.7, minimizing the fluctuation of inlet load and isolating the effect of HRT into the system. Final inlet concentrations and COD/N ratios obtained for each stage are presented in Table 1.

Table 4.1: Hydraulic residence time (HRT) and characterization of inlet substrate in terms of TAN and COD, applied for each operational stage of the partial nitritation-Anammox reactor.

Steps	HRT (h)	NAT_{in} (mg N L^{-1})	COD_{in} (mg COD L^{-1})	NLR (g N L^{-1} d^{-1})	OLR (g COD L^{-1} d^{-1})	COD/N ratio
I	45	360 ± 0	0	0.19 ± 0.0	0.00	0.0
II	45	397 ± 28	257 ± 33	0.21 ± 0.02	0.14 ± 0.02	0.7
III	36	399 ± 27	235 ± 21	0.27 ± 0.02	0.16 ± 0.01	0.6
IV	28.8	406 ± 31	235 ± 39	0.34 ± 0.03	0.20 ± 0.03	0.6
V	23	394 ± 43	241 ± 30	0.41 ± 0.04	0.25 ± 0.03	0.6
VI	17.5	406 ± 32	205 ± 48	0.56 ± 0.04	0.28 ± 0.07	0.5
VII	14.4	401 ± 14	267 ± 74	0.67 ± 0.03	0.45 ± 0.12	0.7
VIII	9.6	425 ± 0	175 ± 2	1.06 ± 0.00	0.44 ± 0.00	0.4
IX	3.6	362 ± 20	131 ± 71	2.41 ± 0.13	0.87 ± 0.00	0.4
Recovery	32.6	378 ± 14	210 ± 45	0.28 ± 0.01	0.15 ± 0.03	0.5
Recovery	9.8	408 ± 17	264 ± 8	1.00 ± 0.04	0.65 ± 0.02	0.7

2.3 Analytical methods

Influent and effluent samples were taken periodically to measure nitrogen compounds (TAN, nitrite and nitrate), COD and pH according to the standard methods [13]. Biomass samples were taken at the end of each stage to measure average granule diameter according to Varas et al. [11] and microbial characterization by FISH was done to obtain the relative abundance of main bacterial groups.

2.4 FISH analysis

The relative abundance of main microbial populations present in granular biomass was characterized by FISH technique according to methods described by Pernthaler et al. [14]. Biomass preparation includes fixation and permeabilization, probe hybridization, washing for non-hybridized probes removal and detection of marked cells by microscopy (MOTIC-BA310 fluorescence microscope). Probes used for in situ hybridization for detection of Eubacteria, ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), and anaerobic ammonia oxidizing bacteria (anAOB) were presented on Table 2, DAPI and EUB-338 were used as controls. Relative percentages of abundance of each bacterial group were determined with respect to the total biomass of eubacteria, combining each specific probe with the EUB338 probe; targeted to the total population of eubacteria. The difference between eubacteria and the sum of anAOB, AOB and NOB was termed unidentified eubacteria (UI).

Table 4.2: FISH oligonucleotides used in this study.

Probe	Sequence (5´–3´)	rRNA target sites[(a)]	Specificity	Formamide[(b)] (%)	Reference
EUB338	GCTGCCTCCCGTAGG-AGT	16S, 338–355	Most Eubacteria	20	[15]
NEU653	CCCCTCTGCTGCACT-CTA	16S, 653–670	(AOB) Halophilic and halotolerant members of the genus of Nitrosomonas	40	[16]
Ntspa1026	AGCACGCTGGTATTG-CTA	16S, 1026–104	(NOB) Nitrospira moscoviensis, activated sludge clones A-4 and A-11	20	[16]
Amx-0820	AAACCCCTCTACTTA-GTGCCC	16S, 820–841	(anAOB) Genera "*Ca.* Brocadia" and "*Ca.* Kuenenia"	40	[17]
DAPI	Regions enriched in adenine and thymine in DNA sequences	Unspecific	DNA of all microorganism	-	-

(a) 16S rRNA position according to Escherichia coli numbering; (b) Formamide concentration in the hybridization buffer; DAPI: 4′,6-Diamidine-2′-phenylindole dihydrochloride.

2.5 Calculations

Nitrogen removal rate and efficiency (NRR and NRE, respectively), as organic removal rate and efficiency measured as COD (ORR and ORE, respectively), were calculated based on nitrogen and COD global balances [18, 19]. Maximum NRR was calculated based on Specific Anammox Activity (SAA) and the reactor biomass concentration according to equation 2. HB production was calculated based on COD consumption and the observed growth yield coefficient for heterotrophs (Y_{obs}) [20], according to equations 3 and 4. The effective oxygen loading rate (OxLR) was calculated by subtracting the consumed COD load to the total oxygen load applied to the system, which corresponds to the effectively OxLR available to carry out the partial nitritation reaction.

$$NRR_{max} = SAA\left[\frac{gN_2-N}{gVSS \cdot d}\right] \times X\left[\frac{gVSS}{L}\right] \qquad \text{Eq. (2)}$$

$$HB_{prod} = Y_{obs}\left[\frac{gVSS}{gCOD}\right] \times \left(COD_{in} - COD_{ef}\right)\left[\frac{gCOD}{L}\right] \times \frac{1}{TRH}\left[d^{-1}\right] \qquad \text{Eq. (3)}$$

And,

$$Y_{obs} = \frac{Y}{1+K_d \times SRT} \qquad \text{Eq. (4)}$$

Where:

Y = Yield coefficient for heterotrophs =0.43 g VSS g COD^{-1}

K_d = Decay coefficient for heterotrophs = 0.96 d^{-1}

SRT = Solids retention time = HRT (for non-aggregated biomass only)

COD_{in} = influent COD

COD_{ef} = effluent COD

3. Results and discussion

3.1 Reactor performance

The reactor performance in terms of nitrogen compounds can be observed on Figure 4.1. A good response of the system was observed after changing the synthetic substrate by digested poultry manure on day 16. Few days of TAN accumulation was observed at the begging, but stable nitrogen removal rate (NRR) and efficiency (NRE) of 0.14 ± 0.02 g L^{-1} d^{-1} and 66.7 ± 3.4%, respectively, were obtained after adjusting the inlet air flow. After 72 days of stable operation, HRT was changed and other seven lower values of HRT were tested in the reactor. An improve of NRE was observed with HRT of 28.8 h and was maintained above 74% until 9.6 h of HRT as seen on Figure 4.2. Since nitrogen loading rate (NLR) increased with the HRT decrease, the NRR got higher in each stage, obtaining a maximum NRR of 0.88 g L^{-1} d^{-1} with 9.6 h of HRT. When a HRT of 3.6 h was assayed, the stability of the system was lost and an abrupt drop in NRR and NRE was observed. The process failure was accompanied with granules floatation and the consequent wash out of the biomass.

Although high nitrite concentrations were obtained with the minimum HRT of 3.6 h (121.4 ± 12.4 mg N-NO_2 L^{-1}), concentrations below 280 mg L^{-1} are expected to be suitable to maintain Anammox activity [21]. Nitrite accumulation was produced because the last NLR increase from 1.06 to 2.41 g N L^{-1} d^{-1} due to HRT reduction from 9.6 to 3.6 h exceeded the maximum NRR of 1.33 g N L^{-1} d^{-1} calculated with the SAA of the preceding stage (0.34 g N g VSS^{-1} d^{-1}). Campos et al. [22] related granule floatation with the accumulation of substrates in the bulk, as they determine N_2 concentration inside the granule. They determined by Anammox kinetics a threshold value of 16 mg N-NO_2 L^{-1} for granules with average radius size of 0.2 cm and 30°C, when N_2 concentration exceed the gas solubility and started to accumulate, leading to floatation episodes. Safety values are below 16 mg N-NO_2 L^{-1} and for granule radius size below 0.038 cm according to these authors. The high nitrite concentrations achieved with a HRT of 3.6 h added to the measured average radius of 0.08 cm determined in this case the floatation of granular biomass and the wash out.

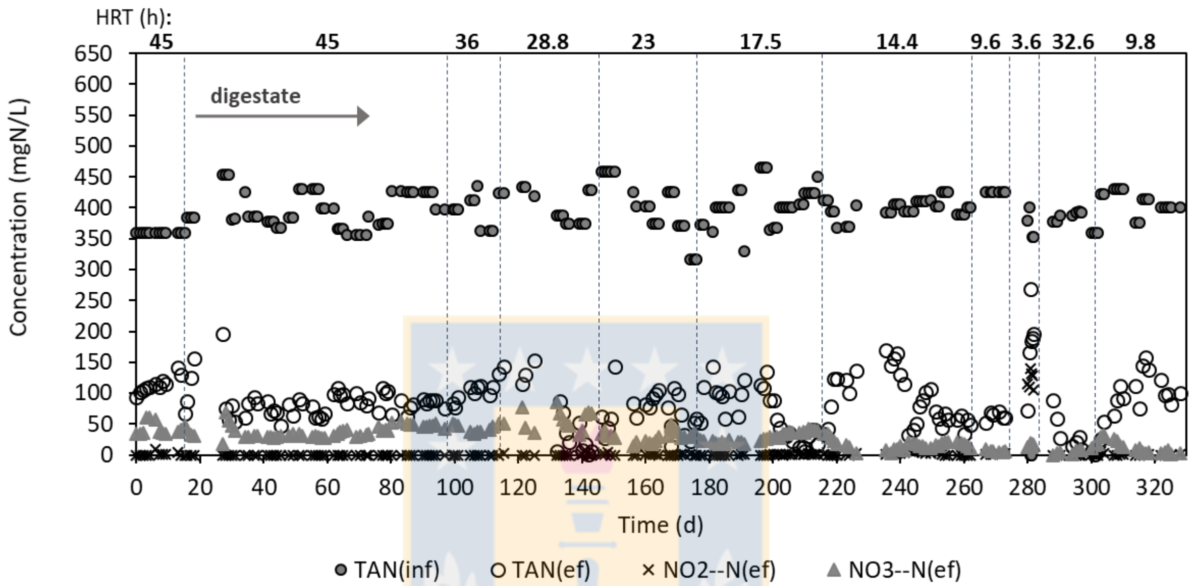

Figure 4.1: Nitrogen compounds profile in the different operational stages with HRT from 45 to 3.6 h. TAN in the influent (●) and TAN (○), NO_2^--N (×) and NO_3^--N (▲) in the effluent. Grey arrow indicates the change of synthetic substrate to digested poultry manure.

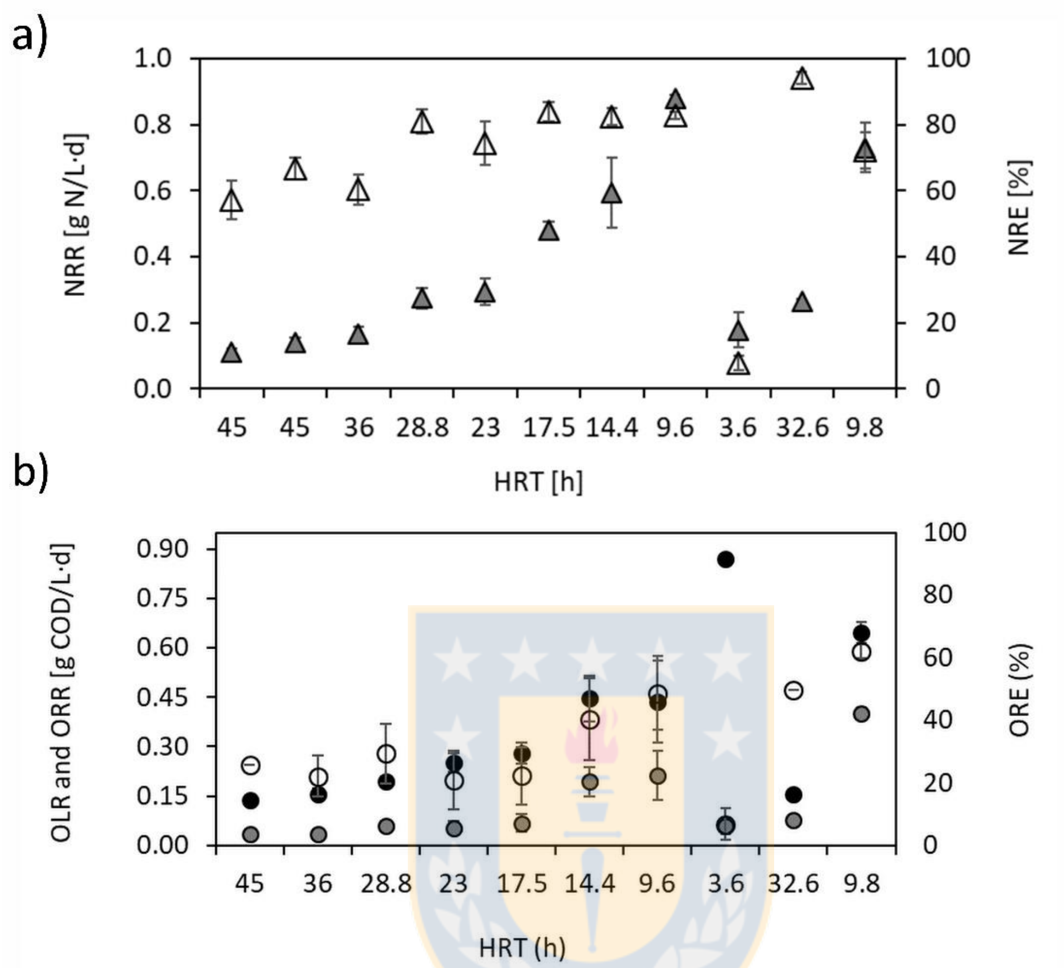

Figure 4.2: Nitrogen and organic removal rates and efficiencies obtained along the operation with variable HRT. The first and last stages correspond to synthetic substrate feeding and recovery periods, respectively. a) Nitrogen removal rates and efficiencies (NRR (▲) and NRE (△), respectively); b) Organic loading rates (OLR (●)) and organic removal rates and efficiencies (ORR (○) and ORE (○), respectively).

Different photographs taken along the operation showed the notorious development of filamentous biomass over the outer layer of the granules in the last HRT assayed (Figure 4.3). As lower the HRT greater the OLR, and the load increment was accompanied with a greater COD consumption (Figure 4.2.b) which implied a higher production of heterotrophic biomass. The maximum COD consumption obtained with

9.6 h of HRT implies a heterotrophic biomass production of 0.07 g VSS L^{-1} d^{-1}, that could be left the reactor with the effluent or stay in the reactor attached to granular biomass. Van Benthum et al. [23] related the HRT (equal to SRT for suspended biomass) with the heterotrophic bacteria (HB) attachment in autotrophic granular or biofilm systems, since operation with HRT lower than the reciprocal maximum specific growth rate (μ_{max}^{-1}) of HB acts as a selective pressure over the suspended growing bacteria, and biofilm layers will start to growth to avoid the wash out. Thus, the low HRT applied to the reactor stimulate the attachment of suspended growing bacteria, probably HB but could be AOB also, since AOB can growth not also in the granule but suspended in the bulk while the applied HRT not wash them. Since μ_{max} of AOB is 3.28 d^{-1} at 35°C [24], an HRT below 7.3 h would promote the wash out of suspended AOB and could be a cause of filamentous growth over the granules.

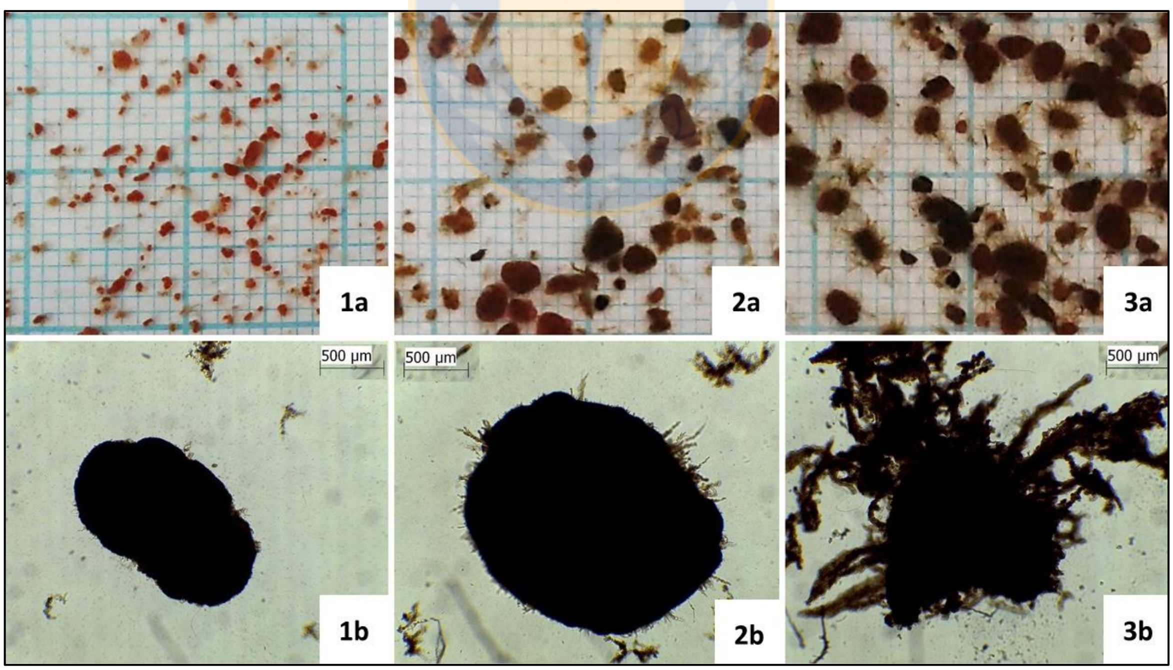

Figure 4.3: Granules images over millimeter paper (1a, 2a, 3a) and under optical microscope (1b, 2b, 3b). 1a,b: Inoculum granular biomass; 2a,b: Granules after 140 days of operation with digestate at 28.8 h of HRT; 3a,b: Granules after last HRT proved of 3.6 h.

Nevertheless, in spite of the development of filamentous biomass in the outer layer of granules, a quick recovery was observed when returning into safety operation conditions with 32.6 h of HRT and 0.28 g N L^{-1} d^{-1}. In three days was possible to return into a steady state condition with a NRE of 94%. In spite of filamentous bacteria did not disappear from the outer granule layer, no floatation events were observed during recovery nor after it, and low HRT of 9.8 h was again implemented after 2 weeks of recovery.

The most affected parameter was the effective OxLR to NLR ratio needed to maintain the efficiency of N removal. Vansgaard et al. [25] found an optimal loading ratio of 1.90 (g O_2 m_3^{-1} d^{-1})/(g TAN-N m_3^{-1} d^{-1}), slightly higher than the theoretical stoichiometry ratio of 1.84 g O_2 per g N from Eq. (1). Nevertheless, we observed not a fixed ratio but a potential relation between the OxLR and the NLR applied as seen on Figure 4.4. The increase of NLR by the HRT decrease (instead of NLR increase by TAN concentration increase at constant HRT) should had an effect over the oxygen requirements, since HRT has an effect on the volumetric oxygen transfer coefficient (K_La) needed to achieve partial nitritation reaction [26]. Shorter HRTs require higher K_La values, and for constant operation conditions as temperature, stirring, reactor design and gas-liquid properties, K_La must be controlled trough aeration flow [24].

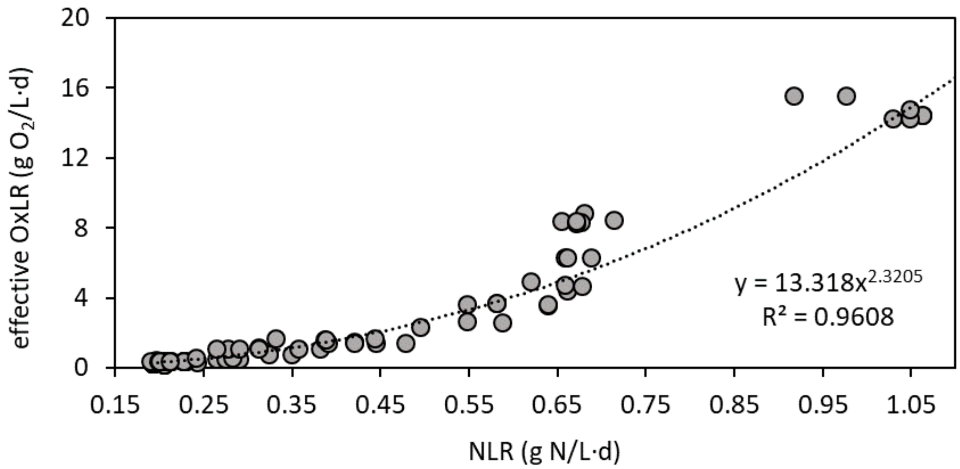

Figure 4.4: Effective Oxygen Loading Rate (OxLR) applied to maintain the nitrogen removal efficiency at the different Nitrogen Loading Rates (NLR) assayed in the system.

As the authors knowledge, there are not full-scale CANON experiences with digested animal manures, but some studies with digested swine and poultry manure have shown the feasibility at lab-scale. Table 3 summarizes those experiences in order to compare with this study, showing a high efficiency obtained with the lowest HRT and highest NLR but moderate COD/N ratio. A high NLR of 1.06 kg N m^{-3} d^{-1} with a low HRT of 0.4 d demonstrated to be suitable for nitrogen removal by partial nitritation Anammox process, but care must be taken to apply high NLR, considering always not to exceed the maximum specific removal rate capacity of the microorganisms.

Table 4.3: Comparison between lab-scale partial nitritation-amnammox reactors fed with digested animal manures reported in literature.

Reactor Type	Substrate	TRH (d)	NLR (kg N m^3 d^{-1})	OLR (kg N m^3 d^{-1})	COD/N ratio	NRE (%)	Reference
Biofilm SBR	Digested swine liquor	1	0.25	0.16	0.64	62	[8]
Granular SBR	Digested swine slurry	0.5	0.6	0.84	1.4	77	[9]
Granular continuous BCR	Digested poultry manure	1.24	0.34	0.89	2.63	91.7	[10]
Granular continuous BCR	Digested poultry manure	0.4	1.06	0.44	0.41	83.2	This study

BCR: Bubble column reactor; SBR: Sequencing batch reactor; HRT: Hydraulic residence time; NLR: Nitrogen loading rate; OLR: Organic loading rate; COD: Chemical oxygen demand; NRE: Nitrogen removal efficiency.

3.2 HRT influence over the main microbial groups

FISH analysis made to granular biomass shown a high stability of the bacterial group composition. However, a slight decrease of AOB from 42.9 to 33.9% with an increase of unidentified (UI) bacteria from 9.3 to 18.7% as the HRT was decreased from 45 to 3.6 h (Figure 4.5). This can be explained as a promotion of heterotrophic growth over the granule at lower HRTs due to higher COD load and the consequent decrease of AOB proportion. anAOB population maintain their dominance in granular biomass with an average fraction of 44.2 ± 1.5% along the operation. Also, the granules distribution size maintained a constant average of 1.51 ± 0.1 mm. The low COD/N ratio applied in this work could be the reason why no negative effects on population balances were observed [28]. Also, despite that the lowest HRT applied provoked a filamentous growth over granules surface, no complications were detected operating at higher HRT with this granular biomass with filamentous growth at the surface. As predicted by Hao and Van Loosdrecht [29], good performance of CANON systems with COD presence can be achieved while enough oxygen was supplied to cope with COD oxidation and partial nitritation reaction.

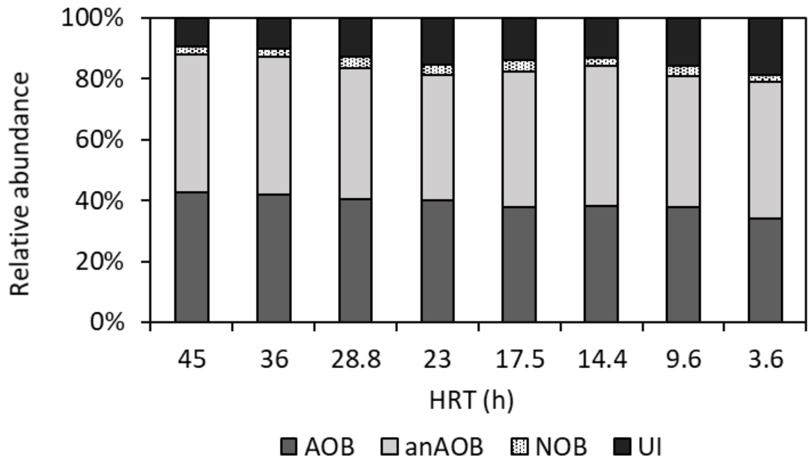

Figure 4.5: Relative abundance of main bacterial groups of the partial nitritation-Anammox process obtained in the different operational stages with the different HRT assayed. AOB: ammonia oxidizing bacteria; anAOB: anaerobic ammonia oxidizing bacteria; NOB: nitrite oxidizing bacteria; HDB: heterotrophic denitrifying bacteria; UI: unidentified eubacteria.

4. Conclusions

A partial nitritation-Anammox reactor fed with digested poultry manure with variable HRT was operated stably with efficiencies over 74% for 11 months. A maximum NRR of 0.88 g N L^{-1} d^{-1} was obtained at a HRT of 9.6 h. The greatest NLR assayed of 2.4 g N L^{-1} d^{-1} at 3.6 h of HRT caused nitrite accumulation and granular biomass floatation, but a quick recovery was observed returning into previous conditions of good performance.

The lowest HRT assayed (3.6 h) promotes the development of filamentous growth over granules, but this shape modification did not affect the nitrogen removal rate when the HRT was increased.

A potential relation of OxLR requirements for partial nitritation-Anammox and the NLR applied was found; shorter HRTs require higher loading ratios of O_2 to TAN, fluctuating between 1.8 and 13.6 (g O_2 m^3 d^{-1})/(g TAN-N m^3 d^{-1}) for HRT from 45 to 9.6 h, respectively.

HRT fluctuations did not affect microbial population balance neither the anAOB dominance in granular biomass, but a slight AOB decrease was observed at the lowest HRT tested probably caused by HB growth over granules.

The feasibility of nitrogen removal from digested poultry manure by partial nitritation-Anammox processes has been demonstrated for a wide range of HRT values (9.6-45 h), but for scaling the process, low HRT values should be avoided since fluctuations of digestate composition could lead to process failure caused by overloads.

Acknowledgements

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CHAPTER 5. Influence of biomass acclimation on the performance of a partial nitritation-Anammox reactor treating industrial saline effluents

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Keywords: Anammox, canning industry effluents, nitrogen removal, partial nitritation, saline effluents

Highlights:

- Partial nitritation/Anammox SBR operated to treat saline canning effluents
- Intermittent aeration avoids NOB activity at low NaCl and nitrite accumulation
- Anammox activity loss of 94% after 160 days of salt rise from 2 to 18 g-NaCl L^{-1}
- Direct exposure to high salt (18 g-NaCl L^{-1}) avoids progressive Anammox weakening
- Partial nitritation/Anammox start-up is shortened by direct exposure to high salt

Abstract

The performance of the partial nitritation/Anammox processes was evaluated for the treatment of fish canning effluents. A sequencing batch reactor (SBR) was fed with industrial wastewater, with variable salt and total ammonium nitrogen (TAN) concentrations in the range of 1.75 - 18.00 g-NaCl L^{-1} and 112 - 267 mg-TAN L^{-1}. The SBR operation was divided into two experiments: (A) progressive increase of salt concentrations from 1.75 to 18.33 g-NaCl L^{-1}; (B) direct application of high salt concentration (18 g-NaCl L^{-1}). The progressive increase of NaCl concentration provoked the inhibition of the Anammox biomass by up to 94% when 18 g-NaCl L^{-1} were added. The stable operation of the processes was achieved after 154 days when the nitrogen removal rate was 0.021±0.007 g N/L·d (corresponding to 30% of removal efficiency). To avoid the development of NOB activity at low salt concentrations and to stabilize the performance of the processes dissolved oxygen was supplied by intermittent aeration. A greater removal rate of 0.029±0.017 g-N L^{-1} d^{-1} was obtained with direct exposure of the inoculum to 18 g-NaCl L^{-1} in less than 40 days. Also, higher specific activities than those from the inoculum were achieved for salt concentrations of 15 and 20 g-NaCl L^{-1} after 39 days of operation. This first study of the performance of the partial nitritation/Anammox processes, to treat saline wastewaters, indicates that the acclimation period can be avoided to shorten the start-up period for industrial application purposes. Nevertheless, further experiments are needed in order to improve the efficiency of the processes.

1. Introduction

The amount of saline wastewater that needs to be treated worldwide is rising due to the increase in industrial activities (petroleum, food, tannery, pharmaceuticals, etc.), the use of seawater for flushing toilets, the infiltrations of seawater in wastewater treatment plants (WWTPs) located in coastal regions, the separation of grey and black wastewaters with decentralized treatment in residential areas, etc.

One of the sectors that generates high salinity wastewaters is the fish canning industry [1]. This sector is very important in the region of Galicia (northwest Spain), which dominates the national market that places Spain as the leading fish and seafood canning county in the European Union [2]. The sector comprises approximately 65 companies distributed mainly along the coast. Similarly, Chile is an important fishing-aquaculture nation in Latin America, generating 48.5% of its aquaculture production and is the fifth country in the world in fishery production [3]. At the present time, the new stringent regulations and the need for preserving the quality of the marine environment in coastal areas make the development of efficient technologies to tackle fish canning-wastewater treatment necessary.

According to the concept of “circular economy”, wastewater can be considered as a “source of materials” instead of as “waste”, to produce for example energy. It is known that the energy contained in the wastewater is enough to compensate for the energy required for its treatment [4]. One of the alternatives to achieve the energy self-sufficiency of the WWTPs is the application of combined partial nitritation/Anammox processes for autotrophic nitrogen removal, which enables the maximization of organic matter valorization via anaerobic digestion for biogas production [5].

In particular, fish canning wastewater treatment normally consists of a physico-chemical process for the removal of solids and fats by dissolved air floatation. In some cases, an additional anaerobic digester is placed subsequently for organic matter valorization as biogas. More rarely, a coupled nitrification-denitrification system is added to remove nitrogen and fulfill the discharge requirements in terms of organic matter and nitrogen removal. However, the nitrification-denitrification processes present two main disadvantages that make the nitrogen removal inefficient: (1) the nitrification step consumes large amounts of oxygen, which involves high energy consumption and (2) the organic matter consumed for the denitrification process cannot be valorized as biogas in the anaerobic digestion step. Therefore, the application of the combined autotrophic partial nitritation/Anammox processes is of great interest [6]. This option allows a 50% saving in the energy needed for aeration, as only half of the ammonium contained in the wastewater is oxidized to nitrite (partial nitritation). Furthermore, all the organic matter previously used in the denitrification process can be used now to produce biogas in the anaerobic digester. This advantage relies on the fact that in the Anammox process the ammonium is oxidized to nitrogen gas using the nitrite as an electron acceptor, without the need for an organic source. However, the Anammox bacteria are very sensitive to different parameters and compounds, such as salinity [7]. There are a significant number of studies that report on the inhibition of Anammox bacteria by salinity and also their possible adaptation [8]. However, there are no studies available that refer to the performance of the combined partial nitritation/Anammox processes for saline industrial wastewater conditions. Furthermore, the research published to date used synthetic saline wastewater [9, 10]. Therefore, specific studies with industrial wastewater are necessary to verify if a specific company can shift from an existing nitrification-denitrification treatment system to a partial nitritation/anammmox one.

Thus, the present study focuses on testing the feasibility of implementing a combined partial nitritation/Anammox system to treat the effluents produced from an anaerobic digester in operation in a fish canning facility.

2. Materials & Methods

2.1 Reactor set-up

A pilot scale sequencing batch reactor (SBR) with a useful volume of 25 L was operated in 6 h cycles comprising: 5 min of feeding, 340 min of reaction with stirring and variable aeration, 5 min of sedimentation and 10 min of withdrawal. The exchange volume was 4.5 ± 0.3 L. The aeration was supplied from the bottom of the reactor by applying a controlled on/off strategy (Table 1) to guarantee an adequate balance between the activities of the partial nitritation and Anammox processes. The oxygen concentration ranged between 0.1 and 1.5 mg-O_2 L^{-1} during the whole operational period. The temperature was maintained at 29 ± 1 °C and the hydraulic retention time (HRT) was varied between 1.35 and 2.57 d. A mechanical stirrer (50 rpm) was installed to ensure the mixing of the reactor bulk when the aeration was too low and to favor the mass transfer.

2.2 Industrial fish canning wastewater and SBR operational periods

The SBR was fed with the effluent from an anaerobic digester treating fish canning industrial wastewater. This wastewater was periodically collected at the industry and stored at 4 °C. During the SBR operation, the influent composition varied according to the different collected batches. In this period, different products were processed in the industrial facility (tuna, sardines, mussels, etc.). The main inlet difference in composition corresponded to the NaCl concentration, which increased from 1.75 to approximately 18 g-NaCl L^{-1} in the 6 months of operation. Then, the NaCl concentration of the industrial wastewater was stabilized at 18 g-NaCl L^{-1} when the company processed mussels. Therefore, the SBR was operated in two different experiments: (A) to evaluate the effects of increasing salt concentrations from 1.75 to 18.33 g-NaCl L^{-1}; and (B) to evaluate the effects when a high salt concentration of 18 g-NaCl L^{-1} was directly applied. The main characteristics of the industrial wastewater fed to the SBR in each operational period are summarized in Table 1.

The first experiment (A) lasted 175 days and was divided into four stages according to the aeration strategy applied (Table 1). In Stage A-I the aeration was continuously supplied; in Stage A-II the aeration was intermittent; in Stage A-III the aeration was supplied in continuous mode but an initial anoxic phase of 60 min was included in the operational cycle; finally, in Stage A-IV the previous anoxic phase was maintained and the aeration was intermittent. The second experiment (B) lasted 40 days, after re-inoculation, without modifications in the SBR operational conditions and using the same batch of industrial wastewater.

Table 5.1: Characteristics of the fish canning industrial wastewater fed to the partial nitritation/Anammox SBR and operating conditions applied during each operational stage.

Stage (range of days)	TAN ($mg\text{-}N\ L^{-1}$)	TOC ($mg\text{-}C\ L^{-1}$)	NaCl ($g\ L^{-1}$)	HRT (d)	Anoxic/aerobic (min/min)	Air on/off (min/min)
A-I (0 - 42)	136 ± 14	48.3 ± 9.4	1.75 ± 0.26	1.41 ± 0.04	0/340	340/0
A-II (43 - 94)	161 ± 25	52.6 ± 9.2	2.38 - 6.62	1.37 ± 0.04	0/340	2/3 4/2 9/1
A-III (95 - 139)	196 ± 31	29.8 ± 5.8	6.08 - 10.30	1.35 ± 0.01	60/280	280/0
A-IV (140 - 175)	215 ± 22	36.9 ± 7.1	10.33 - 18.33	2.57 ± 0.07	60/280	15/45
B (0 - 40)	157 ± 20	35.5 ± 7.4	17.44 ± 1.08	2.01 ± 0.06	0/340	40/20

TAN: total ammonium nitrogen; TOC: total organic carbon.

2.3 Partial nitritation-Anammox inoculum

The SBR was inoculated with granular biomass from an ELAN® pilot plant (200 L) performing the combined partial nitritation-Anammox processes operated at 30 °C [11]. This pilot plant was fed with the effluent from an anaerobic sludge digester containing ammonium and salt concentrations of 500 - 1,000 mg-TAN L^{-1} and 1 - 2 g-NaCl L^{-1}, respectively. The maximum specific Anammox activity (SAA) of the inoculated biomass, determined by batch activity tests performed at 30 °C, was 0.562 ± 0.078 and 0.421 ± 0.056 g-N g-VSS^{-1} d^{-1} for experiments (A) and (B), respectively.

2.4 Analytical methods

Analytical determinations of total ammonium nitrogen (TAN = NH_3 + NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), pH, total suspended solids (TSS) and volatile suspended solids (VSS) were carried out according to the standard methods (APHA-AWWA-WPCF, 2005). Total Organic Carbon (TOC) concentration was determined by a Shimadzu analyser (TOC-L, automatic sample injector Shimazdu ASI-L) as the difference between the Total Carbon (TC) and the Inorganic Carbon (IC) concentrations. Cation and anion concentrations were determined by ion chromatography with an Advanced Compact IC system (861, Metrohm). The SAA values were determined by batch assays in vials with a total volume of 38 mL and a liquid volume of 25 mL according to the methodology described by Dapena-Mora et al. [12].

2.5 Calculations

Free nitrous acid (FNA) and free ammonia (FA) concentrations were calculated according to Anthonisen et al. [13] from the nitrite and ammonium concentrations, respectively. The operational temperature and the pH values measured in the bulk liquid were considered.

Ammonia and nitrite oxidation rates (AOR and NOR, respectively) as well as nitrogen removal rate by the Anammox process (AR) were estimated based on nitrogen balances and the Anammox process stoichiometry as g-N L^{-1} d^{-1}, according to Morales et al. [14]. The reactor specific removal rates (AOR_x, NOR_x and AR_x) were determined dividing the oxidation rates by the biomass concentration inside the reactor (X, g-VSS L^{-1}).

The maximum total nitrogen removal percentage by a possible heterotrophic denitrification process ($\%TN_{den}$) was determined based on organic matter balances and according to equation (1).

$$\%TN_{den} = \frac{(TOC_{\inf} - TOC_{eff}) \cdot 0.933}{TN_{\inf}} \cdot 100 \qquad \text{(Eq. 1)}$$

Where TOC_{inf} and TOC_{eff} are the concentrations of total organic carbon (as mg-C L^{-1}) in the influent and in the effluent, respectively; TN_{inf} is the total nitrogen concentration present in the feeding (as mg-N L^{-1}); and 0.933 is the stoichiometric coefficient (as g-NO_3^--N g-C^{-1}) that relates nitrate and organic carbon consumption in the denitrification process, considering acetic acid as a source of carbon. The acetic acid was selected as source of carbon because the inlet measured soluble chemical oxygen demand and TOC ratio (COD_S/TOC) in the feeding was close to it value (average of 1.9 ± 0.5 with maximum value of 2.6 g·g^{-1}). Note that part of the organic matter could be consumed aerobically due to the presence of oxygen and/or used for biomass growth, which will decrease the maximum denitrification capacity. Therefore, the equation (1) considers only the “maximum potential” value for the heterotrophic denitrification, not the actual value.

The inhibitory effect of NaCl on the Anammox activity was expressed as a percentage of activity maintained and calculated according to equation (2):

$$\%SAA = \frac{SAA}{SAA_0} \cdot 100 \qquad \text{(Eq. 2)}$$

Where SAA_0 and SAA are the specific Anammox activities measured for the inoculum (without salt addition) and with the presence of variable NaCl concentrations (0, 5, 10, 15, 20, 25 and 30 g-NaCl L^{-1}), respectively.

3. Results and discussion

3.1 Partial nitritation-Anammox processes performance with progressive NaCl concentration increase

In the first experiment (A) the wastewater collected in the fish cannery was characterized by a progressive and significant increase in the salt concentration, from 1.75 ± 0.26 to 17.40 ± 0.64 g-NaCl L^{-1}, while the nitrogen concentration as ammonium slightly increased from 136 ± 14 to 215 ± 22 mg-TAN L^{-1}. Thus, the fluctuations of the output parameters should be attributable mainly to the NaCl effect on the combined processes (Figure 5.1).

In Stage A-I the high specific Anammox activity of the inoculated biomass, of 0.562 ± 0.078 g-N g VSS^{-1} d^{-1}, allowed a quick response of the system to the new conditions. During the first 20 d of operation the achieved total ammonium nitrogen removal efficiency (TAR) and total nitrogen removal efficiency (NRE) were approximately 100% and 80%, respectively. In this stage, the aeration was continuously supplied and the dissolved oxygen (DO) concentration was regulated between 0.5 and 1.5 mg-O_2 L^{-1}. However, due to biofouling problems with the oxygen probe which caused underestimated measurements, higher levels of DO concentration may in fact have been achieved during this first stage. Consequently, the nitrate concentration in the effluent was higher than the expected value according to the Anammox stoichiometry. In this way, the ratio of (NO_3^--N)-produced to TAN-consumed was 0.30 ± 0.10 g-N g-N^{-1}, which revealed the presence of nitrite oxidizing bacteria (NOB) activity. The TAR was maintained approximately at 100%, but the NRE decreased from 83% on day 11 to 49% on day 37 (Table 2).

From day 43 onwards, an intermittent aeration strategy was used in order to limit the NOB activity (Stage A-II). Different rates of air pulses (on/off) were applied between days 43 and 94 (Table 1). As a consequence, the TAR decreased to an average value of 77% in this stage (Table 2). The total ammonium concentration in the effluent increased from negligible values to concentrations as high as 75 mg-TAN L^{-1} (Figure 5.1). However, the NRE was improved from 45% on day 44 to 70% on day 70. This improvement was due to the NOB activity limitation observed by the decrease of the nitrate concentration in the effluent. From the analysis of the results obtained from the monitoring of the operational cycle of day 74 (Figure 5.2.a) the nitrite concentration inside the reactor was not significant and low nitrate accumulation was measured. These results indicated the good performance of the partial nitritation/Anammox processes. However, at the end of Stage A-II the nitrite and TAN started to accumulate, reaching values as high as 50 mg-NO_2^--N L^{-1} and 86.9 mg-TAN L^{-1}, respectively, in the effluent. The nitrite accumulation inside the SBR was probably due to the combined effect of a decrease in NOB and Anammox activities provoked by the increase in NaCl to values of 6 - 7 g-NaCl L^{-1}. Although the estimated FA concentration also increased to values as high as 16.8 mg-N L^{-1} (Table 2), this concentration was not expected to be inhibitory for granular Anammox biomass [7], since no effects have been previously detected for FA concentrations up to 20 mg-N L^{-1} [15]. The FNA concentration in this stage was lower than 10^{-4} mg-N L^{-1}, thus neither the FNA inhibition effect on Anammox [7] nor that on the NOB population [16] was considered.

In order to stop the increasing inhibition of Anammox bacteria activity by salt and substrates (NH_4^+ and NO_2^-) concentrations, in Stage A-III an anoxic phase of 60 min was implemented at the beginning of the reaction phase. In some cases, sequential oxic/anoxic phases have been implemented in partial nitritation-Anammox reactors to separate the two reactions in time. Then, the nitritation occurred during the oxic phase while the Anammox reaction took place during the anoxic one [17, 18]. Taking this into consideration, in the present study the aeration was stopped for 1/6 of the cycle length. The implemented anoxic phase aimed at shortening the reaction time available for aerobic ammonium oxidation and decreasing the nitrite accumulation, while promoting the Anammox reaction. Following this strategy, the nitrite concentration inside the reactor diminished. Simultaneously, the decreasing tendency of the NRE stopped and was maintained at approximately 20% during this stage. However, the Anammox bacteria were not able to consume the nitrite left from the previous cycle, as indicated by the nitrogen compounds profiles corresponding to the operational cycle measured on day 130 (Figure 5.2.b). This observation correlates with the low value of the SAA, 0.033 ± 0.025 g-N g-VSS^{-1} d^{-1}, of the biomass inside the reactor on this date, which confirms the 94% decrease in activity in comparison with the inoculum.

As FA concentration started to reach inhibitory values at the end of Stage A-III (up to 23.55 mg L^{-1}), the HRT was doubled to 2.57 d for the next stage, in order to avoid substrate inhibition. Also, intermittent aeration was applied for the next step to promote the nitrite consumption during anoxic steps by Anammox reaction.

In Stage A-IV, the combined strategies applied promoted a decrease in the nitrite but an increase in ammonium concentrations in the effluent (Figure 5.1). In this case the ammonium oxidizing bacteria (AOB) activity limited the process. As the nitrite concentration was negligible, the NRE increased from values of 20% up to 30%. However, the high NaCl (up to 18.33 g-NaCl L^{-1}) and FA (up to 38 mg-N L^{-1}) concentrations reached in this stage were inhibitory for Anammox bacteria. Since a higher HRT could decrease FA concentration but not the NaCl concentration, the decision to stabilize the reactor under the applied operational parameters was taken. FNA concentration was lower than 0.01 mg-N L^{-1} at this stage (Table 2), with maximum values of 0.009 mg-N L^{-1}. Although no inhibition has been reported for Anammox biomass at higher FNA values [7], Fernandez et al. [15] suggest avoiding concentrations higher than 0.0005 mg-N L^{-1} to maintain a stable Anammox operation since inhibitory effects are stronger in long-term experiments. For this reason, the SAA measured at the end of the operation was again 0.035 ± 0.004 g-N g-VSS^{-1} d^{-1}. On the other hand, NOB activity inhibition has been reported for FNA concentrations higher than 0.02 mg-N L^{-1} [16]. Thus, in the present study, no inhibitory effect was expected on NOB populations at any time during the operation, the NOB decay being attributable mainly to the salt effect.

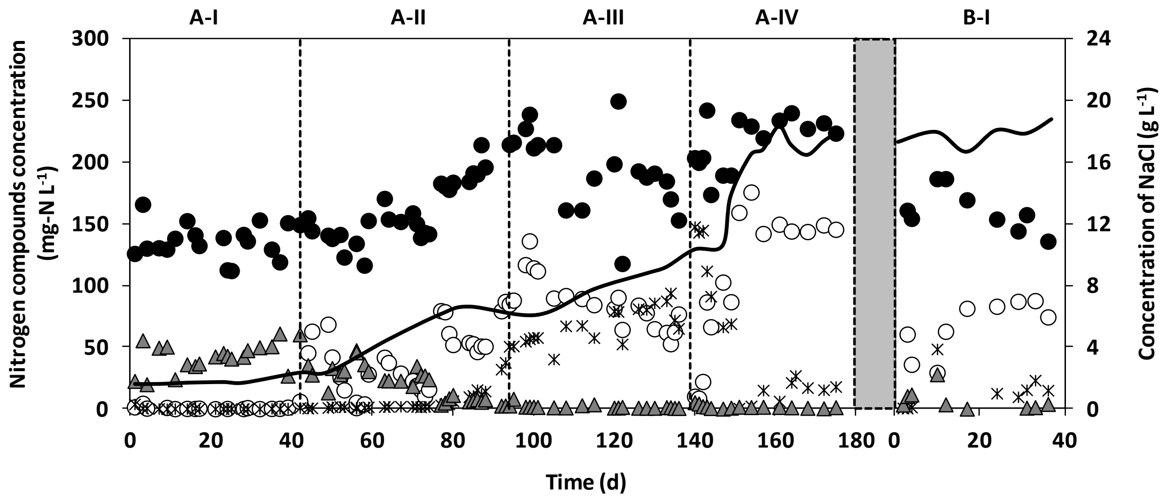

Figure 5.1: Profiles of salt and nitrogen compounds concentrations in the different operational stages: NaCl (–), TAN in the influent (●) and TAN (○), NO_2^--N (∗) and NO_3^--N (▲) in the effluent.

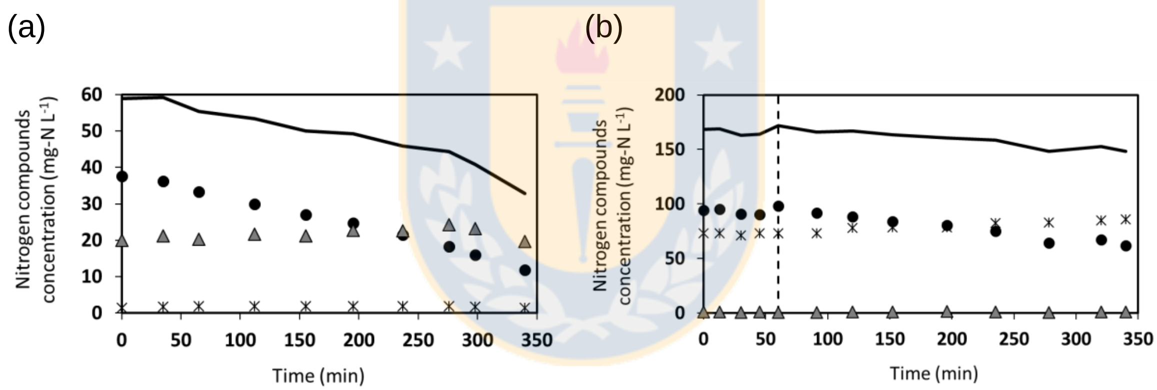

Figure 5.2. Evolution of nitrogen compounds concentrations inside the reactor: TAN (●), NO_2^--N (∗), NO_3^--N (▲) and TN (–), for an operational cycle: (a) Stage A-II, day 74 and (b) Stage A-III, day 130.

Table 5.2: Operational conditions in the different operational stages with increasing salt concentration.

Operation periods (d)	NaCl ($g\ L^{-1}$)	NRR ($g\text{-}N\ L^{-1}\ d^{-1}$)	TAR (%)	NRE (%)	TN_{den} (%)	FA ($mg\text{-}N\ L^{-1}$)	FNA ($mg\text{-}N\ L^{-1}$)
A-I (0-42)	1.75 ± 0.26	0.067 ± 0.013	99.4 ± 1.0	68.3 ± 9.6	16.7 ± 5.0	$<10^{-4}$	$<10^{-4}$
A-II (43-94)	2.38 - 6.62	0.197 ± 0.022	76.9 ± 12.3	61.6 ± 7.2	13.0 ± 4.7	0.83 - 16.80	$<10^{-3}$
A-III (95-139)	6.08 - 10.30	0.031 ± 0.011	55.0 ± 8.7	19.7 ± 11.0	4.8 ± 1.1	2.66 - 23.55	<0.01
A-IV (140-175)	10.33 - 18.33	0.021 ± 0.007	37.7 ± 8.6	26.3 ± 7.4	4.6 ± 2.0	27.86 ± 9.08	<0.01
B (0-40)	17.44 ± 1.08	0.029 ± 0.017	64.1 ± 16.4	46.3 ± 17.1	8.1 ± 2.4	4.86 - 36.11	$<10^{-3}$

NRR: Nitrogen removal rate; TAR: total ammonium nitrogen removal efficiency; NRE: nitrogen removal efficiency; TN_{den}: total nitrogen denitrified; FA: free ammonia; FNA: free nitrous acid.

In fact, the analysis of reactor specific removal rates obtained for each bacterial population at the different salt concentrations tested revealed that the most affected parameter was the NOR_x, associated with NOB activity, suffering a total inhibition at concentrations higher than 4 g-NaCl L^{-1} (Figure 5.3). On the other hand, the AOR_x remained stable throughout the operational period up to concentrations of 9.02 g-NaCl L^{-1}. Meanwhile, the decrement of the nitrogen removal in the system was finally due to the decay of the specific nitrogen removal rate (AR_x) associated with the Anammox activity decrease, which took place at concentrations over 7.15 g-NaCl L^{-1}.

To the knowledge of the authors the present research seems to be the only experience in which partial nitritation/Anammox processes are applied to the treatment of industrial saline wastewater. Although, Dapena-Mora et al. [19] and Vázquez-Padín et al. [20] worked with similar substrates, they contained lower salt concentrations than in the present case and operated with a 2-steps SHARON-

Anammox process instead of a single partial nitritation-Anammox reactor (Table 3). High nitrogen removal rates (NRR) were obtained in both cases due to the high nitrogen loading rates (NLRs) applied to the system, and they also achieved high NRE, probably due to the lower salt concentrations present. The difficulty with obtaining a SHARON effluent with the needed stoichiometric ratio of nitrite/ammonia for the Anammox reactor was the main cause of instability and the failure of that process where nitrite accumulation at ratios greater than 1.0 - 1.3 took place [12, 20]. The other reported studies performed appropriately [9, 10, 21] and allowed the achievement of larger NRRs due to the greater NLRs applied, even the highest NRR of 609 mg-N L^{-1} d^{-1} (84% of NRE) was reached at 30 g-NaCl L^{-1} after 172 days of progressive salt increase/acclimation [21]. These high removal percentages are possible mainly due to the utilization of synthetic substrates, which make it possible to work without the inlet fluctuations of the industrial effluents. Furthermore, the industrial effluents also contain other compounds such as sulfates, phosphates, remains of COD that can interfere with the nitrogen removal process.

Different causes for process instability have been proposed in these studies. Some of them reported ammonia accumulation and others nitrite accumulation, indicating AOR or AR decrease, respectively. Both phenomena have an explanation based on the degree of acclimation to salt of the biomass in the reactor. In this respect, the community composition (since there are species more tolerant to high salt levels [9, 22]) and the biomass story (since an adapted biomass increases its resistance to high salt concentrations) are relevant factors [23-25].

Several authors have reported salt inhibition on nitrification [12, 26, 27] accompanied by nitrite accumulation since the NOB population is more sensitive to NaCl than AOB

[28, 29]. This strategy for NOB inhibition could be useful in partial nitritation/Anammox systems, especially when they operate at low nitrogen concentration and temperature, since NOB suppression by salt can improve NRR in these systems [30]. On the other hand, salt acclimation has been reported for total nitrifying (AOB and NOB) biomass [26, 31], showing higher specific activities for biomass acclimated to high salt levels (13.7-18 g-NaCl L^{-1}) than the non-exposed one. She et al. [32], also achieved highly efficient nitritation reaction in a nitritation-denitritation reactor after a long-term acclimation period with increasing salt concentration, obtaining high nitrite accumulation percentages (>92%) at concentrations up to 37.7 g-NaCl L^{-1}.

Also, many studies have been focused on determining the Anammox tolerance and adaptability to high NaCl concentrations. Dapena Mora et al. [12] reported an IC_{50} value of 13.4 g-NaCl L^{-1} for Anammox cultures and no inhibitory effect for concentrations below 8.77 g-NaCl L^{-1}, measured in batch activity tests. Nevertheless, good performances of Anammox reactors with high salt concentrations using synthetic substrates have been obtained with previous acclimation strategies. Dapena-Mora et al. [24], measured high SAA of adapted biomass with salt concentrations up to 15 g-NaCl L^{-1} compared to experiments without salt addition. These authors achieved a NRE of 99% and an NRR of 0.32 g-NO_2^--N L^{-1} d^{-1} with 15 g-NaCl L^{-1}. Also, Gonzalez-Silva et al. [22], Jin et al. [33], Wei et al. [34] and Yang et al. [35] obtained stable Anammox reactor performances at 30 g-NaCl L^{-1} by applying long previous acclimation periods, which remained even up to 50 g-NaCl L^{-1} with 85% of NRE, working with marine Anammox cultures [34]. On the other hand, Ma et al. [36], observed decreasing NRE testing NaCl shocks in an

Anammox reactor for a range of concentrations between 5 and 60 g-NaCl L^{-1}, diminishing with increasing saline concentrations.

Therefore, from these studies it can be concluded that long acclimation periods seem to be the key factor for a stable nitrogen removal process based on Anammox and nitrifying microorganisms, in order to select the microbes with high tolerance to salt [9, 22]. In the present research work, the first experiment regarding the performance of partial nitritation/Anammox processes for the treatment of fish canning effluents with fluctuating salt concentrations is reported. Stable conditions were established, which made it possible to achieve a NRE of 30% at 18 g-NaCl L^{-1} and strongly inhibited the Anammox biomass, which was only 6% of the initial value measured for the Anammox biomass used as inoculum.

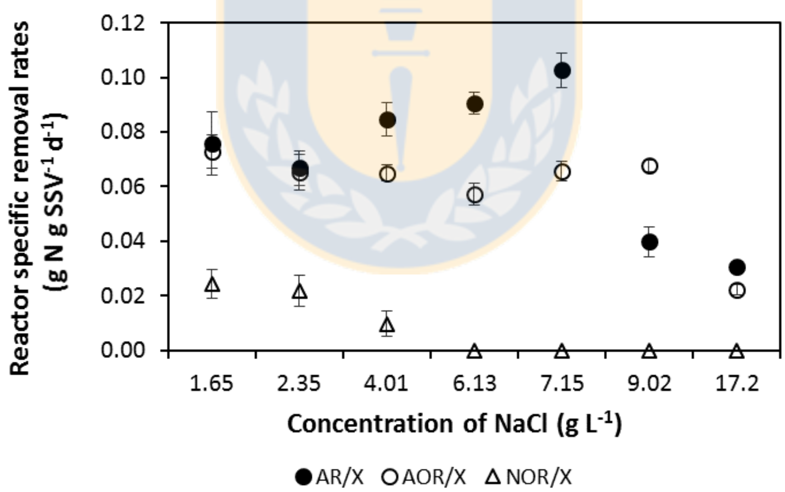

Figure 5.3: Reactor specific removal rates for Anammox (AR_x) (●), ammonia oxidizing (AOR_x) (○) and nitrite oxidizing (NOR_x) (△) bacteria obtained at different NaCl concentrations during the SBR operation. Specific rates were calculated by dividing removal rates by the biomass concentration inside the reactor (X, g-VSS L^{-1}).

Table 5.3: Different autotrophic nitrogen removal processes with high salt content reported in the literature

Process	Substrate	Operation conditions	NLR (mg-N L^{-1} d^{-1})	OLR (mg-TOC L^{-1} d^{-1})	NaCl (g L^{-1})	NRE (%)	$NRR_{máx}$ (mg-N L^{-1} d^{-1})	Cause of instability	Reference
SHARON-Anammox	Anaerobic digested fish canning effluents	SBR (Anammox reactor), 3 L, TRH=1.8 d, 35 °C	300-700	28-67	10	90	460	Nitrite/ammonia ratios larger than 1.0 g N/g N	[12]
SHARON-Anammox	Anaerobic digested fish canning effluents	SBR (Anammox reactor), 3 L, TRH=1.8 d, 35 °C	340-670	0-555	5.9	80	300 (average)	High OLR produced nitrite accumulation	[20]
Partial nitritation/ Anammox	Anaerobic digestion reject water supplemented with NaCl	MBBR reactor, 10 L, TRH=2.27 - 4.67 d, 26-27 °C	120-300	na	10	59	78	Ammonia and nitrite accumulation	[10]
CANON	Synthetic	SBR, 2 L, TRH=6 h, 33 °C	800	0	10 15 20	29 24 -	230 190 -	Ammonia accumulation (AOR decreased)	[9]
OLAND	Synthetic	RBC reactor, 50 L, TRH=0.77 - 0.91 d	525-1100	0	30	84	609	Ammonia accumulation	[21]
Partial nitritation/ Anammox	Anaerobic digested seafood canning effluents	SBR, 25 L, TRH = 1.35 - 2.57	78-145	21.5-38.4	18	46	29	Ammonia and nitrite accumulation	This study

na: not available; OLR: organic loading rate; NLR: nitrogen loading rate.

Besides the salt, the wastewater also contains soluble organic matter at very low concentrations (between 36-53 mg-C L^{-1} as TOC, Table 1). Furthermore, the balance between the TOC in the influent and the effluent indicated that the removal efficiency was lower than 50% for the whole operational period, showing a low content of biodegradable organic matter in the influent. Considering a conversion factor of 2.7 g g^{-1} between TOC and COD, the $COD_{biodegradable}$/N ratio was <0.5 during the whole operational period. Therefore, no significant negative effects on the Anammox biomass or the AOB are expected [37, 38]. The consumption of small fractions of organic matter in a partial nitritation/Anammox one-stage process can take place in aerobic or anaerobic conditions. In the latter case, the organic matter can be used by heterotrophic bacteria during denitrification to consume the produced nitrite or nitrate. Therefore, its removal might indicate the presence of active denitrifying bacteria in the system [39, 40]. Although DO levels lower than 0.5 mg-O_2 L^{-1} allow for the simultaneous nitrification and denitrification processes to take place [41], continuous aeration causes a portion of the organic matter to be oxidized by oxygen instead of nitrate. This extra oxygen consumption increases the operational costs of the process. In the present study, the maximum TNR by a possible denitrification process based on the TOC consumption was determined to be around 15% in Stages A-I and A-II and 5% in Stages A-III and A-IV (Table 2). Furthermore, to determine the contribution of denitrification to the process, a completely anoxic cycle was performed on day 35 (data not shown). In this case only 4 mg-NO_3^-–N L^{-1} were reduced at the end of the cycle, while the TOC concentration varied slightly. This experiment confirmed that the denitrification contribution to the total nitrogen removal was negligible. Thus, an organic removal rate of between 4 - 30 mg-TOC L^{-1} d^{-1} was obtained, oxidized mainly by the aerobic route.

3.2 A partial nitritation-Anammox process at high NaCl concentration without acclimation

The second experiment (B) lasted 40 days and the industrial wastewater fed had a salt concentration of 17.44 ± 1.08 g-NaCl L^{-1}. The partial nitration/Anammox biomass inoculated was directly exposed to this salt concentration without previous acclimation. The NRR was 0.029 ± 0.017 g-N L^{-1} d^{-1} (Table 2). This value is comparable to the NRR in the previous experiment in Stage A-III at moderate salt concentration (6 - 10 g-NaCl L^{-1}), but with progressive acclimation of the biomass for more than 100 days. These preliminary results indicate that, although the Anammox bacteria are very sensitive to salt, the acclimation period is not essential and it is therefore feasible to speed up the start-up process for industrial application purposes. In order to clarify this observation, the SAA values obtained for the biomass of this experiment B were compared with those from the previous one and are discussed in the following section.

3.3 Effects of NaCl concentration and acclimation on the specific Anammox activity (SAA)

The SAA, using a medium supplied with different NaCl concentrations, was evaluated for the biomass used as an inoculum and for the biomass of the first experiment (A) on day 142 and of the second experiment (B) on days 11, 32 and 39 (Figure 5.4). The Anammox activity of the inoculum was strongly inhibited at concentrations of 15 g-NaCl L^{-1}; between 15 and 30 g-NaCl L^{-1} the percentage of SAA remaining being approximately 17% with respect to the initial values. After 142 days of continuous operation with increasing salt concentrations (experiment A) the percentage of SAA remaining was only 7% at all the NaCl concentrations tested. However, this low SAA cannot be attributed only to the effect of high NaCl concentrations, as the use of industrial wastewater contains different compounds at different concentrations which might also affect the correct performance of the process, as mentioned before.

The start-up periods of a partial nitritation-Anammox reactor located in an industrial facility are prolonged due to the acclimation procedure needed by the biomass to reach maximum capacity, which in many cases requires the use of dilution water to adjust the applied loads. Furthermore, the possible loss of Anammox activity in the acclimation period must be considered too. Thus, in practice the acclimation period in full scale systems should be shortened to attain stable conditions as soon as possible. For this reason, in the case of the fish canning industry, which produces high saline effluents, the possibility of applying the partial nitritation/Anammox process without previous acclimation is of interest. Considering this aspect, experience B was performed to understand the direct effect of high saline industrial wastewater (18 g-NaCl L^{-1}) on the SAA of the inoculated biomass. The results showed that a start-up of the partial nitritation/Anammox process directly fed with high saline industrial wastewater is possible. After 11 days of continuous operation at 18 g-NaCl L^{-1} the SAA values worsened in comparison with those of the inoculum at almost all the NaCl concentrations tested (Figure 5.4). However, on day 31 the SAA values were similar to those on day 11, and a slight improvement between 15-25 g-NaCl L^{-1} was even observed. The results obtained on day 39 for the SAA values showed a clear recovery of Anammox activity in comparison with day 11. Furthermore, the activity of the biomass at 15 and 20 g-NaCl L^{-1} improved in relation to that of the inoculum. To date, long acclimation periods with small salt concentration increments have been successfully tested in partial nitritation-Anammox reactors [9, 10, 21], but no comparison with direct biomass exposure to salt has been made. In this research better results were obtained with direct exposure with regard to nitrogen removal efficiencies obtained, lower Anammox inhibition and faster recovery from the initial salt shock.

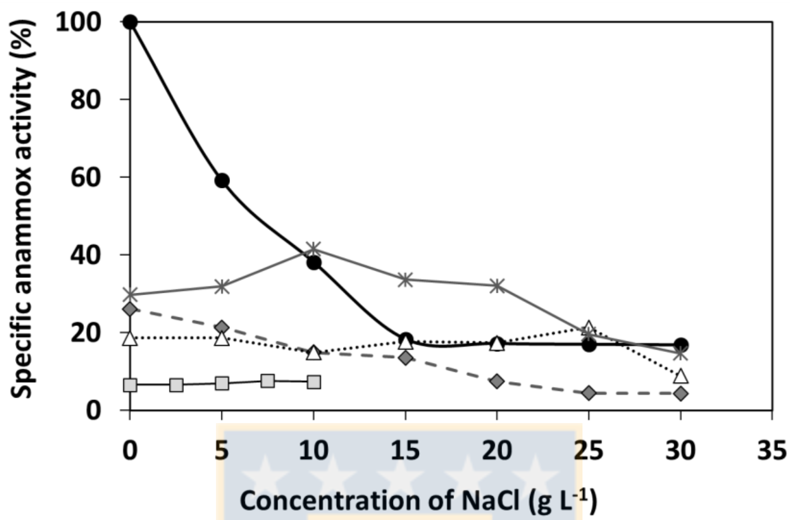

Figure 5.4: Percentage of remaining SAA in the assays with the salt concentration for the SBR biomass on different operational days: inoculum (●), Stage A-IV on day 142 (■), Stage-B on days 11 (◆), 31 (△) and 39 (✱). The values correspond to average values of the experiments performed in triplicate (standard deviation lower than 10%).

4. Conclusions

The removal of nitrogen from fish canning effluents with high salt content was accomplished in a partial nitritation/Anammox reactor.

Percentages of 61.6 ± 7.2% of nitrogen removal efficiencies were obtained with effluents containing concentrations of up to 6.6 g-NaCl L^{-1}; nitrite accumulation and the instability of the process were observed at higher salt levels.

Stable conditions were reached in the partial nitritation-Anammox SBR after 154 days of progressive increase in salt concentration up to 18 g-NaCl L^{-1}. In these conditions, the nitrogen removal efficiency was 30% (0.021 ± 0.007 g-N L^{-1} d^{-1}) which corresponded to a remaining SAA of 6% compared to that of the inoculum.

Intermittent aeration was more effective than continuous mode, allowing decreasing NOB activity when low salt concentrations were present (approximately 2 - 6 g-NaCl L^{-1}), and it stabilized the process performance in the other assayed conditions.

Better results were obtained without progressive acclimation to salt than with acclimated biomass. The nitrogen removal rate obtained after 40 days of inoculation with non-adapted biomass was 0.029 ± 0.017 g-N L^{-1} d^{-1} (46.3% of efficiency) at 17.44 ± 1.08 g-NaCl L^{-1}. Furthermore, the specific Anammox activities of the biomass were higher after 39 days of operation with salt concentrations of 15 and 20 g L^{-1}.

These results confirm that the acclimation of Anammox biomass to high salt concentrations can be done without applying long start-up periods where the salt concentration is progressively increased. In this way, the time required for the start-up of industrial reactors might be significantly shortened, although this strategy is only possible if there is enough inoculum available to couple with the initial strong decrease in Anammox activity. Further experiments with industrial saline wastewaters are needed to achieve stable operation under fluctuating salt concentrations, which are very common in these kinds of effluents.

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FINAL CONCLUSIONS

This thesis has been focused on finding operational limits and strategies that guarantee the stability of the PN-Anammox process in presence of organic carbon. In the first chapter, the heterotrophic SRT, the COD/N ratio, the aeration and feeding regimes were identified as critical parameters to maintain a balance between autotrophic and heterotrophic populations; in the following articles were experimentally proven.

Stable operation and performance was achieved in granular PN-Anammox system with a wide range of COD/N ratios from 0.1 to 3, maintaining the HRT in 30 h (SRT applied to heterotrophic biomass). This finding supports the proposed *stable operation zone* based on the SRT applied to granular or biofilm systems discussed in chapter 1 (Fig. 1.4.b, chapter 1).

The HRT operational limits of PN-Anammox with organic carbon showed to be dependent to the nitrogen load of the wastewater. The treatment of low strength wastewaters (e.g. 0.24 g N L^{-1} d^{-1}) suffer a progressive efficiency loss with HRT below 20 h, while high efficiency and stable operation can be achieved with higher nitrogen loads (e.g. 0.88 g N L^{-1} d^{-1}) operating at low HRT at least until 9.6 h.

Shorter HRTs require higher loading ratios of O_2 to TAN to maintain the nitrogen removal efficiency, fluctuations between 1.8 and 13.6 (g O_2 m^{-3} d^{-1})/(g TAN-N m^{-3} d^{-1}) for HRT from 45 to 9.6 h were measured.

SBR operation mode has demonstrated to be more efficient than continuous operation for nitrogen removal by PN-Anammox with organic carbon. Besides, PN-

Anammox system can be easily convert into a SNAD process by means of including an initial anoxic step into the aerobic SBR cycles. The initial anoxic step allows the anoxic COD oxidation by denitrification and promotes the denitrifying growth, decreasing the aeration costs.

The presence of denitrifying bacteria in PN-Anammox systems with organic carbon does not imply denitrifying activity in the system, as well as OLR increase does not imply an increase in denitrifying population abundance. Denitrifying population increase and activity in-situ was only detected applying the strategy of initial anoxic phase in an SBR PN-Anammox system (reactor described in chapter 3). In continuous aerated reactors the organic carbon was removed through the aerobic route.

Stable composition of partial nitritation/Anammox granules and dominance of AOB and anAOB groups can be achieved operating in the *stable operation zone*. Low HRT (e.g. 3.6 h) promotes the development of filamentous heterotrophic growth over granules, displacing AOB population.

Regarding to industrial wastewaters applications, the feasibility of autotrophic nitrogen removal from digested poultry manure was proved, obtaining high efficiencies and long-term stability operating into the *stable operation zone* (Fig. 1.4.b, chapter 1). Stable operation was also achieved with fish canning effluents, but the high salt content determines lower efficiencies for salt concentration higher than 6.6 g NaCl L^{-1}.

Other collaborative works

During the development of the thesis project, other collaborative works related to the Anammox subject was done. Some of them were collaborations to research projects from the Group of Environmental Biotechnology, Department of Chemical Engineering of the University of Santiago de Compostela, Spain, and the others correspond to research projects from the Bioengineering laboratory, Department of Chemical Engineering of the Universidad de Concepción. References and Abstracts of each work are listed below.

[1] *R. Varas, V. Guzmán-Fierro, E. Giustinianovich, J. Behar, K. Fernández, M. Roeckel, Startup and oxygen concentration effects in a continuous granular mixed flow autotrophic nitrogen removal reactor, Bioresource Technology 190 (2015) 345-351.*

Abstract: The startup and performance of the completely autotrophic nitrogen removal over nitrite (CANON) process was tested in a continuously fed granular bubble column reactor (BCR) with two different aeration strategies: controlling the oxygen volumetric flow and oxygen concentration. During the startup with the control of oxygen volumetric flow, the air volume was adjusted to 60 mL h^{-1} and the CANON reactor had volumetric N loadings ranging from 7.35 to 100.90 mg N L^{-1} d^{-1} with 36–71% total nitrogen removal and high instability. In the second stage, the reactor was operated at oxygen concentrations of 0.6, 0.4 and 0.2 mg L^{-1}. The best condition was 0.2 mg O_2 L^{-1} with a total nitrogen removal of 75.36% with a CANON reactor activity of 0.1149 g N g VSS^{-1} d^{-1} and high stability. The feasibility and effectiveness of CANON processes with oxygen control was demonstrated, showing an alternative design tool for efficiently removing nitrogen species.

[2] *C. Arriagada, V. Guzman-Fierro, E. Giustinianovich, L. Alejo-Alvarez, J. Behar, L. Pereira, V. Campos, K. Fernandez, M. Roeckel, NOB suppression and adaptation strategies in the partial nitrification-Anammox process for a poultry manure anaerobic digester, Process Biochemistry 58 (2017) 258-265.*

Abstract: Poultry manure contains high levels of ammonia, which result in a suboptimal bioconversion to methane in anaerobic digesters (AD). A simultaneous process of nitrification, Anammox and denitrification (SNAD) in a continuous granular bubble column reactor to treat the anaerobically digested poultry manure was implemented. Thus, two strategies to achieve high efficiencies were proposed in this study: (1) ammonia overload to suppress nitrite oxidizing bacteria (NOB) and (2) gradual adaptation of the partial nitrification–Anammox (PN–A) biomass to organic matter. During the NOB-suppression stage, microbial and physical biomass characterizations were performed and the NOB abundance decreased from 31.3% to 3.3%. During the adaptation stage, with a nitrogen loading rate of 0.34 g L^{-1} d^{-1}, a hydraulic retention time of 1.24 d and an influent COD/N ratio of 2.63 ± 0.02, a maximum ammonia and total nitrogen removal of 100% and 91.68% were achieved, respectively. The relative abundances of the aerobic and the anaerobic ammonia-oxidizing bacteria were greater than 35% and 40% respectively, during the study. These strategies provided useful design tools for the efficient removal of nitrogen species in the presence of organic matter.

[3] *J. Campos, A.V. del Río, A. Pedrouso, P. Raux, E. Giustinianovichc, A. Mosquera-Corral, Granular biomass floatation: A simple kinetic/stoichiometric explanation, Chemical engineering journal 311 (2017) 63-71.*

Abstract: Floatation events are commonly observed in Anammox, denitrifying and anaerobic granular systems mostly subjected to overloading conditions. Although several operational strategies have been proposed to avoid floatation of granular biomass, until now, there is no consensus about the conditions responsible for this phenomenon. In the present study, a simple explanation based on kinetic and stoichiometric principles defining the aforementioned processes is provided. The operational zones corresponding to evaluated parameters where risk of floatation exists are defined as a function of substrate concentration in the bulk liquid and the radius of the granule. Moreover, the possible control of biomass floatation by changing the operating temperature was analyzed. Defined operational zones and profiles fit data reported in literature for granular biomass floatation events. From the study the most influencing parameter on floatation occurrence has been identified as the substrate concentration in the bulk media.

[4] *Á.V. del Río, A.P. Fuentes, E.A. Giustinianovich, J.L.C. Gomez, A. Mosquera-Corral, Anammox Process: Technologies and Application to Industrial Effluents, Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater, IGI Global2017, pp. 264-289.*

Abstract: Application of Anammox based processes is nowadays an efficient way to remove nitrogen from wastewaters, being good alternative to the conventional nitrification-denitrification process. This chapter reviews the possible configurations to apply the Anammox process, being special attention to the previous partial nitritation, necessary to obtain the adequate substrates for Anammox bacteria. Furthermore a description of the main technologies developed and patented by different companies was performed, with focus on the advantages and bottlenecks of them. These technologies are classified in the chapter based on the type of biomass: suspended, granular and biofilm. Also a review is presented for the industrial applications (food industry, agricultural wastes, landfill leachates, electronic industry, etc.), taking into account full scale experiences and laboratory results, as well as microbiology aspects respect to the Anammox bacteria genera involved. Finally the possibility to couple nitrogen removal, by Anammox, with phosphorus recovery, by struvite precipitation, is also evaluated.