POLITECNICO DI MILANO

Scuola di Ingegneria Civile e Ambientale

Corso di Laurea in Ingegneria per l'Ambiente e il territorio

Autotrophic Process for Nitrogen Removal from Biowaste Digestate

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Anno Accademico 2014 – 2015

Ringraziamenti

Ringrazio in primo luogo la Prof.ssa Ficara, che mi ha aiutato da remoto e da vicino, con la sua competenza e la sua pazienza.

Debo agradecer el profesor Juliàn Carrera y el Doctor Carlos Ramos por el ayudo y todas las cosas que me ensenaron. “Sin miedo”.

Ringrazio mia mamma e mio papà, i miei modelli di lavoratori. Sono tutto il sostegno e la cieca fiducia che mi hanno sempre dato che mi hanno permesso di arrivare fin qui.

Ringrazio mia zia Silvia e mio zio Walter, le loro risposte e i loro consigli sono sempre arrivati nel momento giusto.

Ringrazio Tommaso e Davide, correlatori e amici di anammox. Delirio.

E poi vorrei ringraziare i miei amici.
I poliamici, Federica, Anna, Giulia, Marco, Roberto, Davide, Simone.
Quelli di sempre, Paolo, Enrico, Cecilia, Silvia, Giulia e Andrea Giulia.
Quelle dell’Olanda, Adele, Isabella e Veronica.
Quelle del martedì, Gaia, Carol e Marta.
Quelli di Civitanova, Luca, Carlo, Fede e Bea.
Mis amigos de Barcelona, Alba, Pedro, Jose y Victor.
My innovative friends from the Asp, Eleonora, Silvia, Nicoletta e Francesco.

Se questi anni sono stati bellissimi, è grazie a voi.

Infine, ringrazio la mia persona preferita. Sarà la cosa che mi mancherà di più. Anche perché so che oggi sarebbe stata la più felice di tutti.

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1 Abstract

The organic fraction of municipal wastes represent the 35% of total wastes production in Europe. Composting and anaerobic digestion of those wastes are the most sustainable alternatives for dealing with those wastes. Anaerobic digestion allows both the recovery of energy and material through biogas production and digestate generation (that can be used as fertilizer once stabilized) respectively.

However, the treatment of the liquid fraction of the digestate is highly expensive thus limiting the exploitation of the whole process.

The main objective of this study is to evaluate the feasibility of the application of an innovative treatment such as the biological autotrophic nitrogen removal to the liquid fraction of the digestate with the final purpose of promoting the energetic valorization of the organic fraction of urban wastes through its anaerobic digestion.

The biologic process, completely autotrophic, is made up of two stages: in the firs one (Partial Nitration PN) anout half the amount of ammonium is oxidated to nitrate in an aerated tank by ammonium oxidinzing bacteria (AOB); the effluent from the process is then fed to second stage (anammox process): here, forced anaerobic bacteria use the left ammonium and nitrite from the first stage for their growth thus producing molecular nitrogen that is discharged in the atmosphere, thus completing the removal of the nitrogen part of the waste.

The work is split in two work packages (WP): the first one was carried out at the Universitat Autònoma de Barcelona and the second one at the Politecnico di Milano.

Working package I: Partial Nitritation

The activity consisted in the operation and monitoring of an air-lift reactor with granular biomass where the partial nitration process takes place. The PN reactor was fed on the liquid fraction of the digestate from anaerobic digestion of organic wastes.

The monitoring activity included the determination of the main input and output parameters (nitrogen compounds concentration, organic matter and suspended solids): Moreover, specific tests were performed to determine the main kinetics of the process in/ex reactor. The obtained data provided information on the stability of the process and on the possibility of the suppression of the NOB, catalizing the (unwanted) nitratation process. Finally, the inhibiting effect on the bacteria that catalyze the partial nitration process in the medium and long term was evaluated.

At the end of the 101 days of operation, the effluent from the partial nitritation reactor with an applied nitrogen loading rate of 0.61 mg N $L^{-1}d^{-1}$ was showing a stoichiometric ratio of 1.49, with an average TOC removal efficiency of the 44% and TOC/ammonium ratio in the effluent of 0.57 $g_{TOC}\, g_N^{-1}$, achieving a suitable influent for an anammox reacotr.

Moreover, the mentioned effluent was tested in a specific anammox activity test achieving an inhibition value of the 20%.

Working package II: Politecnico di Milano

Similarly to what was done in the first phase, the activity in Milano, concerned the management and monitoring of an anoxic SBR reactor with anammox granular biomass, fed on the supernatant from a fill scale anaerobic digestion treating biowastes to which nitrite and ammonium were supplemented in order to achieve a desired concentration and nitrite to ammonium ratio so to mimic the chemical composition that would be obtained by PN process. The aim was to evaluate the process kinetics and the potential inhibition on the anammox biomass in the medium and long term.

The treatment of the real wastewater was performed at different dilution factors, with decreased removal efficiencies with higher concentrated influent. Particularly, in the most successful phase (1:2 dilution) the average nitrogen removal rate was 370± 95 mg N – $NO_2^- L^{-1}d^{-1}$ with a removal efficiency of the 98%.

Adaptation phenomena of the biomass were observed and a further confirmation of the feasibility of the treatment was achieved through specific anammox activity tests.

2 Sommario

Obiettivi generali

La frazione organica dei rifiuti urbani (FORSU) è costituita dalla frazione umida (scarti di cucina domestici, ristoranti, mense etc.) e verde (sfalci d'erba, potature etc.). Questa frazione rappresenta circa il 35% della produzione complessiva di rifiuti urbani in Europa. Tra le tecniche di smaltimento della FORSU a basso impatto ambientale si annoverano la digestione anaerobica e il compostaggio. Tra le due la digestione anaerobica é considerata più efficiente dal punto di vista ambientale per le sue caratteristiche di processo: le reazioni sono di tipo anaerobico e producono sia compost, che può essere riutilizzato come fertilizzante previa stabilizzazione aerobica, sia biogas composto prevalentemente da metano, che è utilizzato per produrre elettricità e calore nei motori a cogenerazione (CHP). La digestione anaerobica consente quindi di produrre un gas ad alto potere calorifico a partire dall'energia chimica associata ai composti organici presenti nella FORSU, al contrario del processo di compostaggio che richiede energia per essere completato, ed è il motivo per il quale la sua applicazione sulla frazione organica dei rifiuti risulta interessante. Il trattamento della componente azotata della frazione liquida del digestato, principale prodotto di scarto della digestione anaerobica, risulta tuttavia molto oneroso limitando di fatto lo sviluppo di tutta la filiera di recupero energetico dalla FORSU. L'obiettivo generale di questo studio é pertanto quello di valutare l'applicabilitá di un processo innovativo altamente efficiente come il processo biologico completamente autotrofo di nitritazione parziale/anammox al trattamento della frazione liquida del digestato da FORSU con lo scopo ultimo di promuovere la valorizzazione energetica della FORSU tramite sua digestione anaerobica.

Obiettivi specifici

Il processo biologico completamente autotrofo si compone di due stadi distinti posti in cascata: nel primo stadio (nitritazione parziale, PN), circa la metá della componente ammoniacale del refluo viene ossidata a nitrito in ambiente aerato ad opera di batteri ammonio ossidanti (AOB); l'effluente del processo di nitritazione parziale viene quindi alimentato al secondo stadio (processo anammox) dove batteri anaerobici obbligati usano l'ammoniaca restante ed il nitrito prodotto nello stadio precedente per il proprio sostentamento risultando nella produzione di azoto molecolare che viene liberato in atmosfera, chiudendo cosi il processo di rimozione della componente azotata dal refluo trattato. Lo studio in oggetto si divide in due work package (WP) svolti rispettivamente nei laboratori dell'Università Autonoma di Barcellona e del Politecnico di Milano, i cui rispettivi obbiettivi specifici sono qui di seguito riportati:

WP1. Processo PN: analizzare le cinetiche batteriche e le potenzialitá di processo, la qualitá dell'effluente prodotto da un reattore di PN alla scala laboratorio in termini del rapporto finale di concentrazione nitrito/ammonio in vista del suo accoppiamento col processo anammox, monitorare la presenza/attività di batteri nitrito ossidanti (NOB) catalizzanti il processo (indesiderato) di nitratazione; analizzare e quantificare eventuali fenomeni di inibizione dovuti a sostanze presenti nel refluo e/o a suoi ossidati prodotti di ossidazione;

WP2. Processo anammox: analizzare le cinetiche di processo e la sua stabilità, la qualità dell'effluente prodotto da un reattore anammox alla scala laboratorio in termini di concentrazione di azoto; analizzare e identificare eventuali fenomeni di inibizione dovuti a sostanze presenti nel refluo e/o a suoi prodotti di degradazione prodotti nel precedente processo di PN.

Contenuto Scientifico

Valutando la tipologia di refluo oggetto dello studio (con alto contenuto salino e di solidi colloidali) e sulla base di quanto pubblicato nella letteratura tecnico-scientifica (Fernández et al., 2008; Vyrides and Stuckey, 2009; Lotti et al., 2012; Hu et al., 2013; Lackner et al., 2014), si ritiene che delle alternative tecnologiche ad oggi disponibili (impianti a biomassa anammox granulare, sospesa o adesa) la soluzione granulare possa assicurare maggiore efficacia e garanzie di continuità di funzionamento. Ciò per i seguenti motivi: a) l'aggregazione della biomassa in granuli ha un effetto protettivo nei confronti dei batteri presenti al suo interno, che, in caso di reflui con elevate concentrazioni di Sali o di potenziali sostanze inibenti, costituisce un vantaggio non trascurabile rispetto alle altre forme di aggregazione; b) i granuli hanno una conformazione compatta e presentano una elevatissima velocità di sedimentazione (da 15 fino a 90 m/h) e ciò consente un facile disaccoppiamento del tempo di ritenzione idraulico dal tempo di ritenzione dei granuli e comporta la possibilità di operare con una concentrazione di microrganismi elevata e quindi con reattori molto compatti.

Inoltre il processo biologico completamente autotrofo che sfrutta la conformazione granulare della biomassa viene per la prima volta applicato a digestati derivanti dal trattamento anaerobico della FORSU.

Obiettivi della sperimentazione

Obiettivo 1: Verificare la fattibilità dell'applicazione del processo innovativo di rimozione autotrofa dell'azoto al trattamento della frazione liquida del digestato da FORSU, investigando distintimente i due processi in cascata che lo compongono: nitritazione parziale e anammox.

Obiettivo 2: Ottenere dati di interesse pratico/operativo quali la valutazione dei carichi di azoto applicabili ai reattori granulari studiati e le corrispondenti rese di rimozione dell'azoto.

Descrittivo delle attività svolte

Attività svolte presso l'Università Autonoma di Barcelona

L'attività ha riguardato principalmente la gestione e il monitoraggio di un reattore air-lift a biomassa granulare nel quale si è realizzato il processo di nitritazione parziale alimentato da digestato derivante dalla digestione anaerobica della FORSU. A questo scopo si è sfruttata la disponibilità del reattore di nitritazione parziale a biomassa granulare già presenti ed operativi presso il laboratorio di GENCOV di cui il Prof. Juliàn Carrera è a capo nonché l'esperienza del suo gruppo di ricerca nella gestione di questi reattori.

Il monitoraggio ha riguardato la determinazione dei principali parametri di interesse (concentrazione delle specie azotate, della sostanza organica e dei solidi sospesi) in ingresso ed in uscita dal reattore. I dati raccolti consentono di ottenere informazioni sulla stabilità del processo di nitritazione parziale ed in particolare sulla possibilità di garantire una efficiente soppressione dei batteri nitrito ossidanti (NOB) catalizzanti il processo di nitratazione nonché di valutare i potenziali effetti inibitori sui batteri che catalizzano il processo di nitritazione parziale a medio e lungo termine.

Al termine dei 101 giorni di operazione, l'effluente del reattore di nitritazione parziale a cui era applicato un carico di 0.61 mg N $L^{-1}d^{-1}$ mostrava un rapporto ammonio/nitrito pari a 1.49, con una rimozione del TOC del 44% e un rapporto di TOC/ammonio effluente di 0.57 $g_{TOC}\ g_N^{-1}$, raggiungendo un influente idoneo per un processo anammox.

Inoltre, si è testato il suddetto effluente in una prova specifica di attiità anammox ottenendo valori di inibizione pari al 20%.

Attività svolte presso il Politecnico di Milano

Similmente a quanto svolto nella prima fase, l'attività presso il Politecnico di Milano, a completamento del progetto ha riguardato la gestione e monitoraggio di un reattore anossico SBR a biomassa granulare alimentato da un refluo reale con aggiunta di nitrito (refluo semisintetico) per la valutazione delle cinetiche di processo e degli effetti inibitori sulla biomassa anammox a medio e lungo termine.

Anche in questo caso si sono utilizzati reattori già operativi presso il laboratorio di ingegneria sanitaria del DICA sfruttando l'esperienza pregressa del gruppo di ricerca nella gestione di reattori anammox granulari.

Il trattamento del refluo reale è stato effettuato a diversi fattori di diluizione, con efficienze di rimozione tanto più basse quanto più il refluo era concentrato. In particolare nella fase più performante (influente diluito 1:2) il carico di azoto rimosso medio era 370± 95 mg N – NO_2^- $L^{-1}d^{-1}$ con un'efficienza pari al 98%.

Fenomeni di adattamento della biomassa sono stati osservati e una conferma ulteriore della trattabilità si è ottenuta attraverso prove di attività specifica anammox.

3 Nitrogen

3.1 The nitrogen cycle and its alteration

Nitrogen forms the 78% of Earth's atmosphere and is the most abundant uncombined element. However, due to the extremely strong triple bond in the elemental nitrogen, atmospheric nitrogen cannot be used as such and has to be converted to reactive Nitrogen (N_r).

Currently, mankind has almost doubled the amount of N_r (reactive nitrogen) converted from N_2. The superfluous nitrogen accumulates in soils, water, atmosphere and biomass. Once lost to the environment, this nitrogen migrates through the Earth's atmosphere, forests, grasslands and waters, causing a cascade of environmental changes that negatively impact both people and ecosystems. These changes include smog, acid rain, forest dieback, coastal 'dead zones', biodiversity loss, stratospheric ozone depletion, and an enhanced greenhouse effect (Galloway et al. 2003). According to Sutton et al. (2011) the excess nitrogen in the environment can cost between 70 and 320 billion euro per year for the European Union.

The global population growth trend will increase this issue in the following years.

Nitrogen is present in different oxidation states and the inter-conversion from one form to the other is performed through biologic reactions carried on by specific microorganisms that need nitrogen as election accepter or donor.

Those processes can be engineerized and implemented in wastewater treatment in order to promote the removal of ammonium or organic nitrogen, that are the most common form of nitrogen in municipal wastewaters.

The most consolidate process for nitrogen removal are:

- Nitrification/Denitrification. Ammonium is oxidized to nitrate and nitrate is consequentially reduced to nitrogen gas
- Nitritation/Denitritation. Ammonium is oxidized to nitrite and nitrite is consequentially reduced to nitrogen gas
- Partial Nitritation /Anammox. Partial oxidation of ammonium to nitrite and utilization of both nitrite and ammonium in the anammox metabolism to produce denitrogen gas.

3.2 Traditional nitrogen removal processes: Nitrification/ Denitrification

This is a mature technology and it is also the most widespread process in municipal wastewater treatment. In the first step ammonium is oxidized to nitrate (nitrification) that is then reduced to nitrogen gas with the need for an organic substrate as electron donor.

Results of this process are the mineralization of organic carbon to CO_2 as well as the conversion of nitrogen in a no-reactive N_2 form.

3.2.1 Nitrification

In the nitrification process ammonium is firstly converted to nitrite (nitritation) by AOB (ammonium oxidizing bacteria) and then to nitrate (nitratation) by NOB (nitrite oxidizing bacteria). Those bacteria are autotrophic – thus meaning that there is the need of an external inorganic carbon source.

Those reactions are respectively:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$$

$$2NO_2^- + O_2 \rightarrow 4NO_3^- + 2H^+ + 2H_2O$$

The overall reaction, comprehensive of the required alkalinity is:

$$NH_4^+ + 2O_2 + 2HCO_3^- \rightarrow NO_3^- + 3H_2O + 2CO_2$$

It can be noticed that there is alkalinity, oxygen and inorganic carbon consumption.

3.2.2 Denitrification

In this process nitrite and nitrate are converted to gaseous N_2 by facultative anoxic heterotrophic bacteria. The anoxic environment allows nitrates to be the electron acceptor of the process with the contemporary biologic degradation of the organic substrates.
Hence the availability of ready degradable organic carbon is an important parameter to implement the process. One of the main operational costs of those processes is indeed the addition of an external carbon source (c-source), mainly methanol. The metabolic reaction is strictly dependent on the c-source, for example in the case of methanol is:

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 6H_2O + 3N_2 + 6OH^-$$

Main advantages of the process are the long term and consolidated knowledge that results in a reliable and stable process management and easy process control (Tchobanoglous and Burton, 2003).

3.3 Alternative processes

3.3.1 Nitritation/Denitritation

Usually municipal wastewaters have an ammonium concentration lower than 100 mg N – NH_4^+ L^{-1} and nitrogen is eliminated through traditional nitrification/denitrification. However, there are wastewater streams that show high ammonium concentration such as anaerobic

digestion effluents, landfill leachate and wastewaters from the food industry or particular industrial wastewater.
For a specific application, alternatives should be evaluated on the basis of economic, operational experiences, energy and chemicals requirements and environmental impact. Actually, the main driver in the choice of an alternative above another is the cost-effectiveness. For this reason, a nitritation/denitritation process is a valid alternative to conventional nitrification/denitrification because of its less oxygen and carbon demand (Mulder et al., 2011).

Another name for the process is DENO2 (Scaglione, 2012) or short-cut denitrification. The ratio behind this process is the role of nitrite as intermediate of both nitrification and denitrification. Hence, there is a first phase of nitritation in which ammonium is oxidized to nitrite and a second reaction of denitration with the reduction of nitrite to nitrogen gas. The name of this process comes from the fact that both nitratation and denitrification are avoided, allowing a “short-cut” in the biologic reaction.

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \rightarrow NO_2^- + 2CO_2 + 3H_2O$$

$$4NO_2^- + CO_2 + 2H_2O + 3C \rightarrow 4HCO_3^- + 2N_2$$

The above reactions immediately suggest that lower amounts of oxygen and carbon are required to remove ammonium nitrogen if compared to those presented in 1.2.2 and 10. From the economic point of view, this results in savings in the amount of air blown in the reactor and in the external carbon source, respectively of the 25% and of the 40% (Turk and Mavinic, 1986).

3.3.1.1 Achievement of nitritation process

The bottleneck for the implementation of nitritation/denitritation is the out-competition of NOB bacteria whose kinetics are favored by the usual operating wastewater plant conditions process temperature, pH, and concentrations of the nitrogen forms.

In order to achieve this goal, the main parameters to be controlled are:

- Temperature. It is complex to state the exact influence of temperature because it affects mass transfers, growth rates and chemical equilibria. However, the SRT in a reactor is function of the temperature as well. It was demonstrated that above 25°C the AOB bacteria maximum growth rate is higher than that of NOB (Hellinga et al., 1998), as it can be seen from Figure 1 where the minimum residence time (inversely proportional to the SRT) is represented against temperature. Therefore, above this temperature, the minimum SRT value for keeping NOB in the system is higher than the one for AOB that are thus the only population maintained in the reactor with NOB selectively washed out.

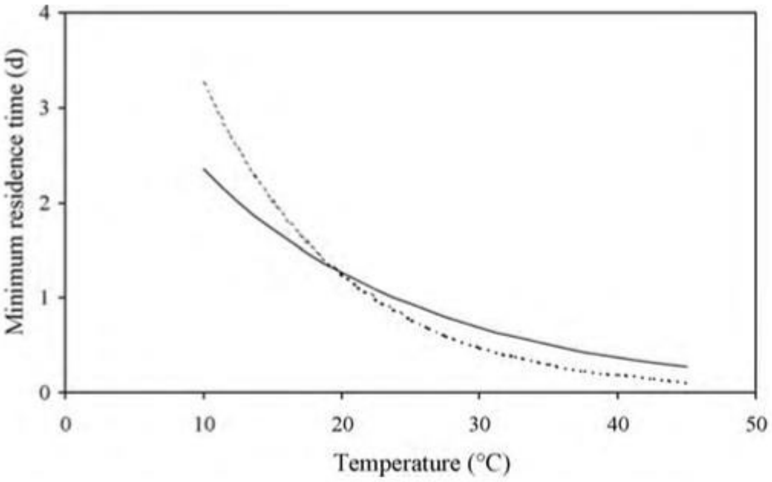

Figure 1 Effect of temperature on the minimal required residence time for AOB and NOB. Above 25°C it is possible to wash out NOB (-) while manteining AOB (--). The picture was adapted from Hellinga et al. (1998)

- Dissolved Oxygen (DO). There is accordance on the fact that NOB have a higher k_s (semi-saturation constant) than AOB thus meaning a lower affinity for DO. However, in literature different values have been calculated for this constant, ranging from 0.16-0.74 mg O_2 L^{-1} to 0.54-2.5 mg O_2 L^{-1} for AOB and NOB respectively (Guisasola 2005, Hunik 1994, Barnes 1983, Ciudad 2005). Such a variety of values can be explained with the differences in oxygen mass transfer efficiency for the different experimental setup. Indeed, the apparent O_2 affinity is strictly dependent on the experimental setup and therefore on biomass density, floc size, mixing intensity and the rate of oxygen in the floc. (Manser et al. 2005). As a general consequence it can be concluded that nitritation is favored at low DO (Pollice et al 2002, Ruiz et al 2003)
- Free ammonia (NH_3) and free nitrous acid (HNO_2) concentration. These unionized forms for a pH>8 and <7.5 are the actual substrates/inhibitor for ammonium and nitrite oxidation (Van Hulle et al.,). Indeed ammonium concentrations between 1-5 mg NH_3-N L-1 inhibit nitratation but not nitritation (Abeling and Seyfried, 1992). Knowing total ammonium nitrogen (TAN) and total nitrite (TNO_2), free form concentration can be calculated taking into account pH and temperature (acid-base equilibrium).

  NH_3 increases with increasing pH while HNO shows the opposite tendency, which promotes AOB and suppress NOB. Hence a weak alkaline environment allows the outcompetion of nitrite oxidizers. In literature different threshold for inhibition have been calculated (Anthonisen et al. 1976), however they are once again function of the experimental setup that determines bacteria acclimation and response.

  Relying on this strategy alone might not lead to a sufficient stability as NOB bacteria were found to be able to adapt to FA inhibition (Turk and Mavinic 1989), however it can still be coupled with others strategies (Peng and Zhu, 2006; Bartrolì et al., 2010).
- pH. Despite the effect of pH being controversial, it is universally recognized that the optimum pH for both NOB and AOB lies between 7 and 8. In the first place, pH has

an effect on the equilibria between ionized and no ionized forms (NH_4^+-NH_3 and NO_2^-—HNO_2). AOB growth rate is higher in a slight alkaline environment, probably because they use NH_3 as substrate. Moreover, at pH 7 it was observed a eight fold decrease in NOB bacteria growth rate, while AOB were not effected (Hellinga et al., 1998).

Those parameters are usually exploited to give stability to the process and they are usually combined. There are examples in literature of implementations where the process is successfully stopped at the nitritation stage (Canziani et al., 2006, Castro Daniel et al., 2008) even with complex influents (Peng et al., 2007).

3.3.2 The PN / anammox process

Anaerobic ammonium oxidation (anammox) is the biological oxidation of ammonium with NO_2^- as electron acceptor producing N_2 and NO_3^-. A more correct name for the process would be "anoxic ammonium oxidation" (Van Hulle et al., 2010) since the electron acceptor of the process is nitrite. NO_2^- is not just oxidizing NH_4^+ but it is also oxidized to NO_3 thus providing the necessary reducing capacity to fix carbon to new biomass.

Anammox microorganisms are autotrophic, accordingly there is in no need for an organic C– source.

The simplified (and not balanced) reaction is as follows (Van Dongen, 2001):

$$NH_4^+ + NO_2^- \rightarrow H_2O + 2N_2$$

A partial nitritation stage should be performed before the anammox process in order to achieve a suitable ratio between the two substrates.

The challenge of this step is the oxidation of just half the ammonium to nitrite and the prevention of its further oxidation to nitrate.

In order to prevent NO_2^- conversion to NO_3 growth the strategies presented before in 1.3.1.1. The counter-ion of ammonium in the wastewater is one of the main factors to determine the conversion of just half of the ammonium present to nitrite. Particularly, nitritation is limited by pH when bicarbonate is present. Indeed, only half of the produced protons can be buffered by bicarbonate. Therefore, the ratio bicarbonate/ammonium in the wastewater determines the equilibrium pH that allows a conversion of ammonium between the 50 – 60% (Van Dongen et al., 2001)

The anammox process is particularly convenient for the treatment of high strength ammonium wastewaters with a ratio COD/N lower than 0.5 g COD g N^{-1} (Josse et al., 2009; van der Star et al., 2007). Examples are anaerobic digestion effluents, landfill leachate or digestate from food industry. However, Jenni et al. (2014) achieved the treatment of a wastewater with a C/N ratio of 1.4 gCOD gN^{-1} in a mixed culture (very close to typical stored urine ratio of 1.5 gCOD gN^{-1}).

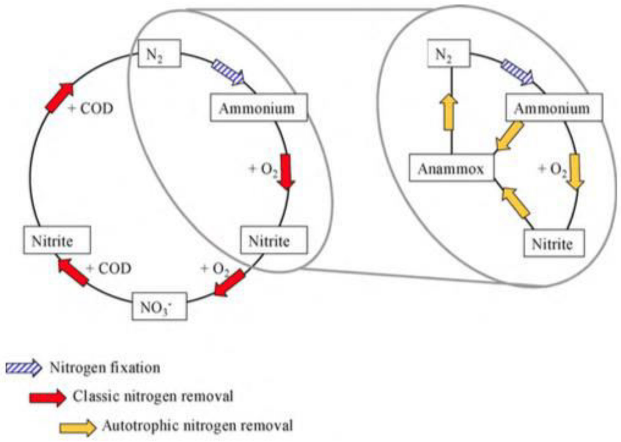

Figure 2 Representation of nitrogen cycle with the contribution of anammox bacteria (Van Hulle 2010)

3.3.3 Achievement of a stable partial nitritation process

In the following Table 1 some control strategies implemented at lab scale are presented, along with the setup and the NLR treated.

Table 1 Examples of PN lab scale studies

Reference	NLR [g N L^{-1} d^{-1}]	Experimental Set Up	Control Method
Liang et al. 2010	0.26	Fixed bed biofilm reactor	HRT, pH, low DO
Jin et al., 2013	1.19	Airlift	RSM[1] to find the optimal combination of DO, Alk and influent ammonium
Shinohara 2009	3	Gel Carriers	NOB inhibition by FA
Garrido et al, 1997	5	Airlift	DO concentration
van Dongen et al., 2001	0.36	CSTR	alkalinity, temperature, DO
Fux et al., 2002	0.35[2]	CSTR	pH, DO
Wyffels et al., 2004	0.55	MBR	Microfiltration (SRT), DO
Padin et al.,	0.08[3]	SBR	ratio fresh/air recirculation (DO)
Ganigue et al (2010)	1.2	MBR	alkalinity, step feed in order to reduce total N

A stable PN process is particularly important for the following anammox biomass because of its vulnerability from substrates' high concentration as explained in 1.3.5.
The main configurations that have been used to achieve PN are: CSTR (completely stirred tank, also implemented by applying an air-lift mixing), MBR (membrane bioreactor) and SBR (sequencing batch reactor).

- SBR. It was successfully implemented for the treatment of anaerobic sludge reject water and leachate (Galì et al. 2007, Vàzquez-Padin et al. 2009, Ganiguè et al.

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[1] Response surface metholodogy
[2] experiments were carried out at temperature as low as 24°
[3] at temperature as low as 24°

2006). They all controlled the process with the available alkalinity and oxygen limitation, Galì set manually the proper SRT as well. There have been also experiences of controlling the process with the aeration pattern alone (Pollice et al., 2002) thus resulting in energy savings along with simpler operating conditions.

- MBR. Due to the high difficulties in setting the SRT, it is difficult to suppress NOB growth (Xue et al. 2009).
- SHARON (Single high-activity removal over nitrite). Is the most famous implementation (van Dongen et al. 2001) and it consists in a CSTR operated as a chemostat with a HRT (equal to the SRT) lower than 1 d at 35°C. Nowadays the SHARON is implemented at full-scale in the WWTP of Sluisjesdijk (Rotterdam, The Netherlands). In this application, the alkalinity present in the reject water (dewatered anaerobic sludge) is already adequate to oxidize just half of the ammonium present.

  Main drawbacks of this system are the impossibility of biomass retention as well as the difficulties in coping with fluctuating influents or loads since pH and DO must be strictly controlled to obtain the oxidation of just half of the ammonium present to nitrite. Therefore the maximum volumetric loading rate of the reactor is the bottleneck to the increase of the treatment capacity in the WWTP of Sluisjesdijk.

A recent strategy to control the process explained above can be the implementation of advanced control loops on the inflow flow rate (Torà et al. 2014, Jemaat et al., 2013) at a fixed DO. With this configuration, ammonium is measured on-line and actions to limit the full conversion of NO_2^- to NO_3 are taken on the basis of the difference between the measured value and the fixed set-point thus resulting in an high process flexibility

3.3.4 Stoichiometry and comparison with conventional nitrification/denitrification

The stoichiometry of the anammox process was obtained by Strous et al. (1998) by means of mass balances over different microbial culture:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H_2^+$$
$$= 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

A simplified scheme of anammox catabolism can be the following:

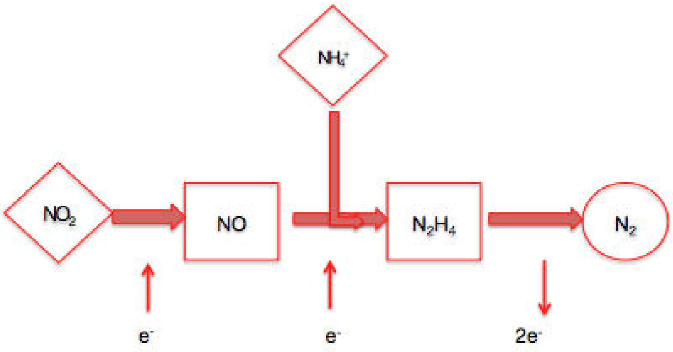

Figure 3 A simplified scheme for Anammox catabolism. Diamond represent substrates, squares are the intermediates and circles the products. The scheme is taken from Lotti et al. (2015)

Nitrate production is the result of the CO_2 fixation process (Van de Graaf et al., 1996). Being nitrate a byproduct of the anabolism, it can thus be used as an indicator of anammox growth.

According to the anammox reaction, the ratio between the stoichiometric conversion rates of nitrite to ammonium and of nitrate to ammonium should be 1.32 and 0.26, although they can vary in the range from 1.1-1.3 and 0.1-0.3. Observations of lower and higher ratios respectively are an index of the contemporary presence of other processes such as the heterotrophic bacteria performing denitrification.

In literature, different maximum specific growth rate are reported: 0.02 d^{-1} (Fux et al., 2004), 0.05 d^{-1} (Strous et al., 1999) up to 0.21 d^{-1} (Lotti et al., 2014). They correspond to a doubling time of 30, 14 and 3.4 d respectively, the lower value ever reported in literature. Such differences may due to different analytical methods or differences at microbial level in the bacteria population. Indeed, different Anammox species seldom occur in the same WWTP and they occupy their own niche in the environment.

Because of the slow growth rate of the anammox biomass, the start up of the process and the recovery from inhibition episodes are particularly difficult and time-consuming. Start up processes may require up to two and a half years (Wett, 2007). Particular care should be taken in not washing out the active biomass, therefore systems should guarantee an high biomass retention. Particularly suitable for this purpose are systems that promote the aggregation of the biomass in flocks, granules or biofilms.
Actually, for the precedent reason, the start up of anammox systems is often performed through the inoculation of biomass already acclimated from plants or processes in operation.

Table 2 Comparison between different nitrogen removal processes (Adapted from Van Hulle et al., 2010)

Process	Oxygen needed [gO2 gN^{-1}]	COD needed without assimilation [gCOD gN^{-1}]	COD needed with assimilation [gCOD gN^{-1}]
Nitrification-denitrification	4.57	2.86	4
Nitritation-denitritation	3.43	1.72	2.4
PN - Anammox	1.72	-	-

From the stoichiometry and from Table 2 it can immediately be concluded that the PN/A process requires 60% less oxygen and lower (or not at all) carbon consumption if compared to nitrifying/denitrifying systems.

Moreover, Mulder calculated that due to the autotroph metabolism of anammox biomass, the overall sludge production in PN/A and DENO2 is 90 % less if compared with traditional processes (0.1 $g_{\text{dry weight}}\, g_N^{-1}$ against 1 $g_{\text{dry weight}}\, g_N^{-1}$) and this results in substantial savings in sludge treatment and disposal.

A WWTP that implements nitrification/denitrification has investment costs that are comparable with PN/anammox but it shows an overall 85% savings with comparison to traditional nitrification/denitrification (Vlaemink et al., 2009). Moreover, nitrogen removal efficiency is higher (Nielsen et al., 2005).

Finally, overall N_2O emissions are globally lower (Jetten et al, 1997), although this last point will need further investigations.

Nitrate produced from the anammox process requires organic carbon as well, however the amount of COD that is usually found in traditional wastewater is enough to oxidize the small amount of NO_3^- in the effluent.

Main inhibitors to the anammox process

Due to the low growth rate of anammox biomass, inhibition phenomena are particularly critical because of the long recovering time of the biomass. The main inhibitors to the process are:

- Nitrite. The inhibiting concentration is once again difficult to determine and it is function of the type of process. In literature there are experiences of irreversible loss of the activity with NO_2^- concentrations of 100 (Strous et al., 1999) and 40 mg N - NO_2^- L^{-1} (Fux et al., 2012). From recent studies, the IC_{50}[4] was found to be 350 mg N - NO_2^- L^{-1} - 400 mg L^{-1} (Dapena-Mora et al. 2010, Lotti et al., 2012) but inhibition was also found to be reversible up to 1000 mg N - NO_2^- L^{-1} (Lotti et al., 2012).

  Ammonium. It was found an IC_{50} for ammonium of 700 mg N – NH_4^+ with the actual inhibitor being the FA (Dapena-Mora et al., 2010). However, in the short term a FA concentration of 38 mg N – NH_4^+ L-1 (Dapena-mora et al. 2007) determines a 50% reduction in the anammox activity, while in the long term a concentration of 25 mg N – NH_4^+ L^{-1} was detrimental to the biomass (Fernandez et al., 2012). Once again, differences between the obtained values can be due to different setup along with different microbial characteristics.

  Hence, those values are strictly dependent on the type of process. Since FA concentration depends on total nitrogen and pH, the latter is the parameter that is usually controlled

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[4] The concentration that corresponds to the 50% inhibition

- Oxygen. A concentration of oxygen 0.25 - 2% of air saturation was found to be already reversibly inhibiting (Egli et al., 2011). This reversibility allows the implementation of systems performing simultaneously PN/A in one reactor (Strous et al., 1997). The topic will be deepened in the following paragraph 1.3.6.
- Organic carbon. High ammonium strength wastewaters usually contain elevated amount of organic matter as well. As anammox bacteria have a lower growth rate than the one of denitrifiers, they are outcompeted if a certain amount of organic carbon is present. Although the overall effect is a decrease in the nitrogen removal, this is not a proper inhibition phenomenon.
  However, organic matter can also by toxic for the anammox. For example, methanol and ethanol irreversibly inhibit the process.
- Salts. The general effect of an increase in the conductivity medium is an increase in the osmotic pressure across the membrane thus impacting on transport phenomenon and leading to a decrease in the microbial activity. However, annammox bacteria were found active in marine ecosystems (Arrigo et al., 2005; Dalsgaard et al., 2005) and they showed capacity of adaption to salinity in several literature studies. A deeper insight into the topic will be given in chapter 1.3.8.

3.3.5 Different process configurations: one stage and two stages

Conversion of ammonium to nitrogen gas through the anammox process requires a first partial nitritation of the influent. The two stages of the process can be implemented in the same reactor (single stage) or in two separated reactors (two stages).

3.3.5.1 Single stage configuration

In this configuration PN and anammox processes take place in the same reactor. The contemporary presence of both the organisms make the management of the process difficult since their optimal and inhibiting characteristics are different. For example, oxygen is inhibitory for the anammox while it is a substrate for the AOB. Moreover, AOB minimum doubling time is 7-8 hours (Bock et al., 1986), certainly smaller than that of the anammox, thus resulting in the need for a high SRT for the whole system.
In order to guarantee aerobic and anoxic conditions for the growth of both microbial populations different system configuration have been implemented at a full scale:

- Sequencing batch reactor (SBR). It is the most applied (50% of the installations). The DEMON (DEaMONification) configuration is the most popular. It implements a pH-based feed control. Another SBR system is the NH_4^+ controlled PA/A implemented in Zurich. Also Degremont (Cleargreen) and Aqualia (ELAN) are developing their own patented SBR technologies.
- Moving bed biofilms reactors (MBBR). Aerated MBBRs are equipped with 50% carriers in the DeAmmon (Rosenwinkel and Cornelius 2005) process. Another

implementation is the one from Veolia, named ANITAMox (Christensson et al., 2011)

- Rotating biological contactor (RBC). In the OLAND process (Vlaeminck et al., 2009) the control parameter are rotating velocity (and thus the DO) and the pH
- Granular sludge process. The CANON (complete autotrophic nitrogen removal over nitrite) process foresees the elimination of nitrogen in one single oxygen limited step (Third et al., 2010). There is also an experience at full scale at Olburgen (Albma et al., 2010)

  In this system, AOB bacteria oxidize ammonium taking up all the available O_2 in the outer layer of the granule thus limiting its diffusion in the biofilm and allowing an anoxic environment in the core of the granule where the anammox process takes place.

In the single stage reactor there will be competition for NO_2^- between anammox/NOB, for O_2 between AOB/NOB and for NH_4^+ between anammox and AOB. The challenge of this configuration is to put NOB bacteria in the condition of not competing for the substrates and being completely inhibited.

In order to do that, biomass retention and mixing are key factors in order to allow the growing of biomass and making all the substrates available for the metabolism.

3.3.5.2 Two stages configuration

In a two stages configuration, two different tanks are implemented and independently controlled thus allowing their separate optimization. The first tank is aerated in continuous while the second is anoxic with no aeration.

As for the first stage, there are different strategies to guarantee a partial nitrification and to fix the effluent nitrite to ammonium ratio to the optimal value For the following anammox reactor while preventing nitrate formation, as previously explained in paragraph 1.3.1.1.

In the second tank the anammox process takes place. The key parameters are the mixing of the reactor and biomass retention. The importance of the mixing relies in the dilution of the influent thus preventing the annamox to be locally inhibited by nitrite. Biomass retention is also of fundamental importance because of the slow growth rate of microorganisms. Typical SRT of the system should be about 30d.

The most common full-scale implementation of the two stage configurations are:

- Traditional flocculent activated sludge. They can be used to retrofit an existing plant. Examples are PANDA (partial augmented nitritation/denitritation with alkalinity recovery) and NAS (new activated sludge), respectively a two stage suspended sludge process (aerated reactor, mixing reactor and settlers) and a combination of PN/A and N/DN (Desloover et al., 2011).

  Suspended growth system usually implements ditch aeration and operates at very low O_2 concentration.

- Granular. The most widespread implementation is the SHARON/ANAMMOX from Paques (The Netherlands) whose configuration will be further explained in the following paragraph. The challenge of granular systems is the capacity to retain biomass that is able to form granules. The main criterion to select for this kind of biomass is the settling velocity (de Kreuk et al., 2005).
  Here the SHARON reactor is loaded with 750 kg_N d^{-1} in a 40 m^3 reactor, thus making a NLR of 18.8 kg_N d^{-1} m^3. Mixing is provided by the influent, the downcomer flow and gas recirculation. The following anammox reactor has a bigger volume of 70 m^3, coherently with the longer doubling time of the Anammox bacteria compared with the AOB, and the NLR is 10.7 kg_N d^{-1} m^3. The reactor was scaled up directly from laboratory to full scale (van der Star et al., 2007), this experience clearly asses the possibility of implementation of such a process to a full scale.
- Hybrid systems. TERRANA foresees the injection of bentonitic material in an activated sludge plant in order to let the biomass grown attached and retained in the system (John et al., 2010)
- SBR. There are no full scale implementations yet of PN/SBR but there are some interesting studies at lab scale (Ganiguè et al., 2009)

A two stages system allows coping with variable influents in terms of suspended solids and fluctuating organic matter loading rate. Therefore, if there are peaks of COD – that are likely in a process that is not constant in time as anaerobic digestion, its removal can be separately optimized in the first tank in order not to favor heterotrophic denitrification in the sequent anammox tank: this result in a higher stability of the process.
The kinetics in the anoxic phase is quicker: up to ten folds if compared with a single tank (van der Star et al. 2007) – thus allowing for treating higher ammonium loads in this stage.

Moreover, a two stages system is particularly interesting when toxic compounds are to be treated: the anammox process is more sensitive than nitritation and inhibition can be avoided with the preventive degradation of potentially inhibiting compounds (Jemaat et al., 2013 and Ramos et al., 2015).

On the other hand, a two stages system also requires more space and higher capital costs. Indeed single stage systems are often preferred because of the simplified setup and the lower occurrency of nitrite accumulation. Of all the known full-scale applications of the process, about 100 installations worldwide, the 88% are operated as a single-stage system (Lackner et al., 2014).

3.3.6 Main advantages of granular systems

Granules are to be understood as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which subsequently settle significantly faster than activated sludge flocks. (de Kreuk et al., First Aerobic Granule Workshop 2004, Munich, Germany).

Granular systems have a high biomass retention capacity that results in an high biomass concentration and therefore a capacity to treat relatively high volumetric loading rates. The main features of granular systems are (de Kreuk et al., 2004):

- Good settling properties that determine high biomass settling velocity but no coagulation if the shear is reduced so that they settle as separate units
- The possibility of high SRT in the reactor that is of extreme importance with slow growing bacteria as anammox and AOB
- Granular sludge can cope with various wastewater and loadings, thus guaranteeing flexibility in the process
- Less surfaces needed, a more straightforward process that results in lower costs and energy consumption

3.3.7 Experiences of high salinity wastewaters with PN and anammox, a review

Influents with high ammonium strength usually show high salinity as well, and there are many literature studies upon the feasibility of their treatment with PN/A.
Therefore, literature comparison can only be partial and should focus on some characteristic of the wastewater treated, that is to say, the salinity. Similar influents are leachate, synthetic high conductivity wastewater and wastewaters from the food industry.
Moreover, because of differences in the experimental setup and in the influents nature, results might differ significantly also for similar salinity.

From literature studies, it emerges that there is a value of salinity that actually enhance the anammox activity. This value was not univocally determined, for example it is 15 mg NaCl L-1 for Dapena-Mora. High concentrations of NaCl could enhance biomass aggregation and consequentially its retention in the system. Moreover, salinity can also be used as a strategy to enhance granulation. In fact, saline precipitate can act as aggregators for granules thus helping biomass retention in an SBR reactor (Fernandez et al., 2008).

However, it is common opinion that a concentration of 45 mg NaCl L^{-1} irreversibly ceases annamox activity (Liu et al., 2009, Kartal et al., 2006, Dapena-Mora et al., 2006). However, Windey et al (2005) could assess anammox activity at 45 mg NaCl L^{-1}.

Adaption phenomena were also assessed in literature in order to understand whether there could be activity recovery after an acclimation phase (Kartal et al. 2006, Liu et al., 2009). In those studies the biomass was tested both with no adaption and after a stepwise increase of salts concentration, showing that anammox biomass is not intrinsically resistant to salinity, but it can be adapted.

It has also been assessed in literature the feasibility of the treatment of real wastewaters, such as leachate (Ruscalleda et al., 2010), manure (Karakashew et al., 2008) or swine slurry (Zhang et al, 2012) that due to their high conductivity can be assimilated to the influent used in this study.

Anammox biomass appears to be highly resistant to salinity. This is not surprising since it might be responsible to the 70% of N_2 production form sediments in marine environment (Dalsgaard et al., 2002). However, anammox behavior under salinity stress is also strictly dependent on the microbial active population, on the reactor setup and on to the biomass story.

Also PN was tested with saline influents, although there are less experiences. Adaption phenomena were observed in this case as well and they will be presented in 5.3.2.

3.3.8 Full scale implementations

The cumulative development of PN/A installation has been exponentially growing worldwide in the last ten year, above all in Europe. In the following Table 3 some of the most famous implementation are presented.

Despite some technical problems that are deeply site-specific, PN/A is now a well-established technology for nitrogen removal, allowing energy and chemical savings

Table 3 Full-scale applications of the anammox process, both in single and two stage. The table was adacted from Lackner et al. (2014)

Plant surveyed	**Amersfort**	**Apeldoorn**	**Balingen**	**Heidelberg**	**Ingolstadt**	**Nieuwegein**	**Zurich**	**Malmo**	**Olburgen**	**Lichtenvoorde**	**Landshut**	**Rotterdam**	**Bergen op Zoom**
Type of reactor	DEMON	DEMON	DEMON	DEMON	SBR	DEMON	SBR	ANITAMox	ANAMMOX	CIRCOX / ANAMMOX	Terrana	(SHARON) ANAMMOX	NAS
Number of stages	Single	Single	Single	Single	Single	Single	Single	Single	Two	Two	Two	Two	Two
Volume [m^3]	780	2400	705	2 x 570	2 x 560	450	2 x 14400	4 x 50	600	150 + 75	288 + 495	1800 + 70	7920 (2370, 1650, 1600, 2300)
TSS [g L^{-1}]	4.5	3.5 - 4	1.2	1 - 2.5	2.0 - 4.0	-	3.5 - 4.5	16	25	25	10 - 12, 5-6	0.27, 7-10	0.3, 5, 5, 5 (2-7.6)
HRT [h]	26	58	94	114	75	42	45	24	5	8	25, 42	36, 6	80 (total)
Vol. loading operation [kgN m^3 d^{-1}]	0.65	0.54	0.04 - 0.11	0.2	0.18 - 0.2	0.61	0.4	1.0 - 1.2	1.0 - 2.33	0.89 - 1, 0.78	1.11, 0.65	0.27, 7.03	0.1
Sludge loading design/operation [gN kgTSS-^{1} d-^{1}]	145	155	35 - 95	119	71	-	134	64	80	62	110, 118	260	26 (average)
Energy demand [kW kgN^{-1}]	-	1.1.	0.92	1.67	1.92	0.8	1.11	64	93	71	101, 108	238	18 (average)

4 Literature Review

4.1 OFMSW: production collection and treatment

Generation of wastes from household activities, according to the last Eurostate update (2012), was 242 ton/year. It traduces in a pro capita production of about 481 kg/pro capite /year. Italy and Spain place themselves respectively below and above the Europe average: 449 and 491 kg/pro capite /year. Although the trend for waste production is slightly decreasing (basically because of the economic crisis), those numbers are still impressive.

The lack of a unique method to compute biodegradable municipal wastes (BMW) makes it difficult to give an average value of their production in Europe, however they are estimated to account for the 35% of total wastes production in Europe. The Italian production of the organic fraction of municipal solid wastes (OFMSW) was 11.3 megatonnes per year (Mt/y) in 2010.

According to Eurostat there are four possible treatment possibilities: landfill, incineration, recycling and composting. Statistics provided by the European Union do not differentiate between composting and anaerobic digestion, merging everything in the general category "compost". Composting allows the recovery of organic material and its annual rate shows a growth on average of the 5.3% on average from 1995 to 2013. (Eurostat Data). Indeed, in the famous inverted pyramid of wastes introduced in the Directive 2008/98/CE, which lists all the possibilities for solid waste management, material recovery is to be preferred to incineration (that is to say, energy recovery).

Member States stipulated the Directive31/ 1999 that forced them to reduce the amount of organic (BMW) wastes headed to landfills to 75% by 2006, to 50% by 2009 and to 35% by 2016 (compared with 1995 values). It was up to the different states to decide how to reach this goal– either with composting (including fermentation and anaerobic digestion), incineration or pre-treatment.

4.2 Italian situation: organic waste generation

The percentage of organic waste over the total recycled urban waste is estimated to be the 38% in 2013 by ISPRA (Isituto Superiore per la Protezione e la Ricerca Ambientale). Actually, this value is derived from the input to the treatment facilities for composting and anaerobic digestion. Hence all the losses in weight during the collection or the treatment steps are not taken into account and the real percentage must be higher that the reported value.

Moreover, it is estimated that the overall organic waste that is now recovered is the 40-45% of the amount produced (with a value of 30-35 % in the Centre and 20-25% in the South).

When considering data about waste production, one should be aware that the computation and processing of this type of data is challenging because there are consistent shift of

wastes from one region to the other (taking the specific Italian name of “Rifiuti Speciali”) making it difficult to follow properly waste streams.
The amount of organic wastes that is being differentiated and treated pro capita is on average 71 kg / inhabitant /year, with very different values for the North, the Centre and the South of Italy: respectively 105, 60 and 33 kg / inhabitant / year.
Those data indeed do not match the real situation of the collection of organic wastes since the lack of proper facilities for the treatment in the South and Center involves the transportation of all those materials towards the North.

4.3 Anaerobic digestion to treat OFMSW

Anaerobic digestion is the name given to the set of processes by which microorganisms in the absence of oxygen (anaerobic) break down biodegradable matter in order to produce methane, hydrogen and carbon dioxide – that is to say, energy in the from of biogas. The digestate is rich in nutrients and can be used as fertilizer. Consequentially, anaerobic digestion can be considered both as energy and material recover. There have been many improvements in the last twenty years and the technology can be considered as mature.

Due to a continuous growth in the efficiency of the collection process, the amount of organic waste treated in composting and anaerobic digestion facilities has been increasing constantly. The number of operative plants in Europe is now 244 with a treatment capacity of almost 8 million tons of organics per year, thus accounting for the 25% of the biological treatment in Europe (De Baere, 2014). Spain has one of the largest installed capacity, 1.6 million tons; it is second only to Germany and Italy that pass the 2 million tons. Of course data should be corrected for the inhabitants, thus making the Netherlands and Switzerland the leading countries as for the capacity per million people (respectively 52400 and 49000 tons/million people).

4.3.1 Anaerobic digestion in Italy

The growing interest in the anaerobic digestion process is demonstrtaed by the constant increase of the number of existing operating plant. In 2013 the classified plants were 50 (of which 43 in operation), 45 in 2012 and 35 in 2011. They are located for the 86% in the North, 2.3% in the Centre and 11.3 in the South. This classification does not include the plants that are a preliminary phase for the production of compost. About 1Mt/y of OFMSW is treated in those plants.
Both the amount of total wastes in input and its urban waste (UW) fraction are increasing: in 2013 more than a billion tons were treated and of those about 527 (50.5% of the total) come from UW; with the rest being sludge, green fraction and agro industrial wastes. It is important to notice that the amount of organic wastes collected separately are slightly decreasing (7,9%), thus meaning that relative importance of anaerobic digestion has increased. The amount of treated waste was almost the same in 2012 that instead shows a great increase if compared to the previous year (27.7% more).

Another important thing that can be noticed is that the installed (and authorized) capacity is far higher than the actual amount treated: in fact the potential treatment is almost two billion tons: plants work on average at the 52 % of their capacity (ISPRA data).

4.3.2 Treatment schemes

Anaerobic digestion can be “dry” or “wet”. The former is designed to process solid wastes without the addition of water, while the latter is performed with the addition of water thus improving the mixing of material and making substrates more available for bacteria and increasing the global production of biogas but requiring on the other hand higher volumes.

40% of the digesters currently operated are “wet”. The effluent from the digester needs to be further separated into a solid and liquid fraction (many alternatives are currently in use for this step).

The solid fraction is aerobically composted after a maturation stage or it is used as such as organic amendment achieving material recovery. As far as the liquid fraction is concern, it is usually treated in dedicated wastewater treatment plants before being headed to the sewage or to receiving water bodies.

The criticality of the disposal of the liquid fraction is currently one of the major limiting factors to the widespread of anaerobic digestion.

4.4 The Italian case study

The wastewater employed for the WP II was provided by an anaerobic digestion plant located in the north of Italy that performs wet AD in a CSTR with no digestate recirculation The general scheme of the plant is shown in figure 1. The first step of the process is a pretreatment in order to remove extraneous fractions such as metals, plastic; then the waste is headed to the digester where electricity and heat are produced in cogeneration. The residue from the digestion has to be dewatered thus generating a solid and a liquid fraction that are separated by a centrifuge. The solid fraction is aerobically composted in order to produce a high grade fertilizer; through this step material recovery is accomplished as prescribed by the WasteFramework (2008/98/EC).

The liquid fraction is currently treated on site in a dedicated wastewater treatment plant (WWTP). Phosphorus removal is performed together with recalcitrant COD removal by adsorption with PAC, while nitrogen is removed by conventional nitrification/denitrification including both pre-denitrification and post-denitrification with external COD dosage. The average daily discharge is about 60 m^3/d, corresponding to an average nitrogen load of 2500 kg-N d^{-1}. The effluent of the WWTP is discharged into the sewer (sewer discharge limits: 50 mgN-NH_4^+ L^{-1}, 50 mgN-NO_3 L^{-1}, 2 mgN- $NO_2^-$$L^{-1}$, 500 mgCOD L^{-1}, 2500 mgCl L^{-1}). Currently, in order to meet standard effluent limits, operational costs are very high (approximately 1 Meuro/y).

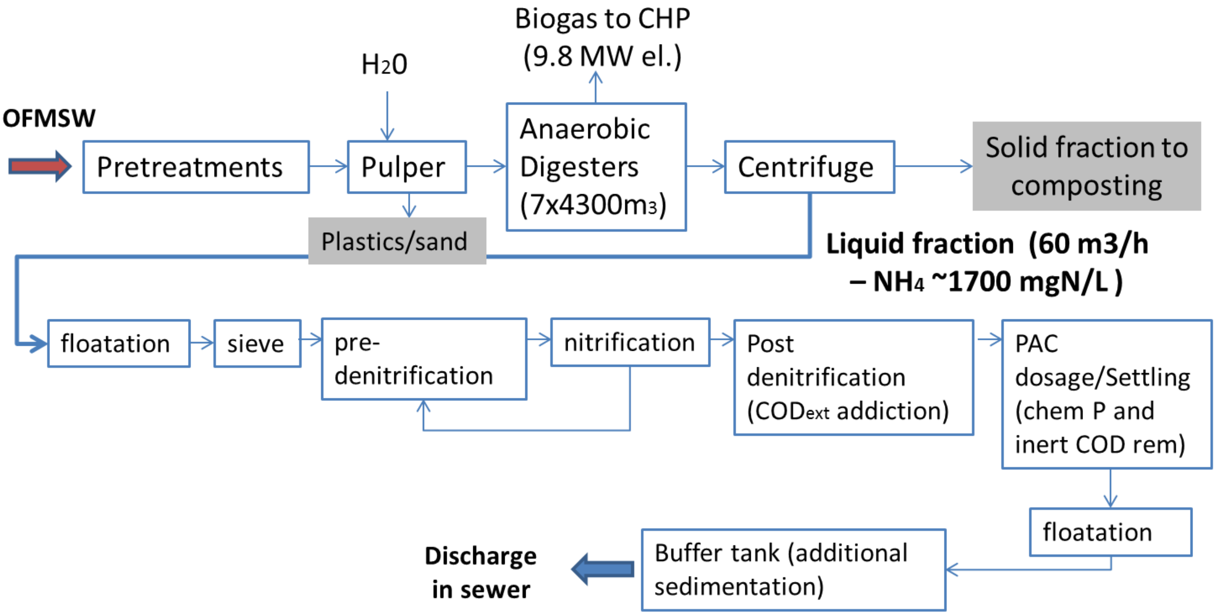


Figure 4 General layout of the AD plant used in the WP II

5 Materials and Methods - Working Package I

5.1 2.1 Experimental setup and reactor

An airlift reactor made of glass (2.6 L of working volume) was used. The reactor configuration was as follows: the internal diameter of down-comer was 62.5 mm; the riser had a height of 750 mm and an internal diameter of 42.5 mm and it was at 8 mm from the bottom of the down-comer. Compressed air was supplied through an air diffuser placed at the bottom of the reactor at an upflow velocity of 0.2-0.3 cm s^{-1}. Airflow rate in the reactor was regulated manually between 150 to 330 mL min^{-1} (230 on average) by a rotameter (Aalborg, USA) and it was enough to ensure an appropriate flow in the airlift reactor. The reactor was equipped with DO (Crison DO 6050), temperature (Crison Pt1000) and pH probes (Crison pH 5333) that were connected to a data monitoring system (Crison Multimeter 44). DO was not automatically controlled and varied between 0.5 and 9.0 mg O_2 L^{-1} according to the applied airflow. A Programmable Logic Controller (PLC) coupled to a Supervisory Control And Data Acquisition (SCADA) system regulated temperature, pH and feeding (element 10 in Figure 5). pH was maintained at 8.0 ± 0.2 by a regular addition of $NaHCO_3$ whereas temperature in the reactor was maintained at 30 ± 0.5 ºC using a temperature controller coupled with a belt-type heating device (Horst, Germany). Feeding to the reactor was made with a membrane pump (ProMinent Gamma/L).

The reactor was fed in continuous and biomass retention was maintained through a three-phases separator (element 3 in the following Figure 5).

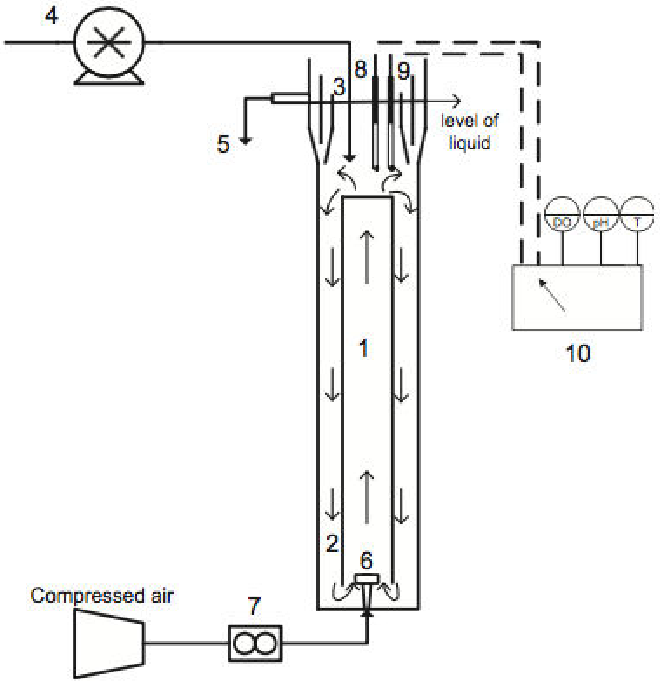


Figure 5 Experimental set-up of the continuous granular airlift reactor: (1) Riser; (2) down comer; (3) separator; (4) feed pump; (5) effluents port; (6) air sparger; (7) rotameter; (8) pH probe; (9) DO probe; (10) monitoring panel. Scheme adopted from Jamaat et al. (2014)

5.2 The tested influents

5.2.1 First phase: the industrial wastewater

In the first phase the influent was an industrial wastewater from the a plant in the area of Barcelona. The influent showed the following composition: 3.6 g L^{-1} NH_4Cl (950 ± 25 mg $N–NH_4^+$ L^{-1}) and the following compounds and micronutrients (concentrations are expressed in mg L^{-1}): CH_3COONa, 48.0; glucose, 12.5; sucrose, 11.9; $CaCl_2•2H_2O$, 88.0; KH_2PO_4, 41.0; NaCl, 176.0; $MgCl_2•7H_2O$, 198.0; $FeSO_4•7H_2O$, 4.0; $MnSO_4•H_2O$, 3.0; $ZnSO_4•7H_2O$, 4.0; $CuSO_4•5H_2O$, 2.0; and H_3BO_3, 0.02; $CO(NH_2)_2$, 12.0 and yeast extract, 2.0.

5.2.2 Second phase: origin of the wastewater

The influent to feed the reactor was taken from the Catalunya-Eco Park 2, Montcada i Reixach (Àrea Metropolitana de Barcelona-AMB).

This is a facility where the organic wastes separately collected in Barcelona and in the surrondings are converted into compost and biogas through “dry” anaerobic digestion and post composting. The nominal capacity of the plant is 100 000 t of organic wastes per year.

The biogas is used as fuel to drive a cogeneration plant, thus producing both electrical and thermal energy. The former is split between internal consumption and sale to the electrical grid, the latter completely used to heat the digesters. The other product of the process is an effluent that is called “digested sludge” as shown in Figure 6: this is firstly dewatered by a screwpress and a centrifuge. The solids are headed to composting (with an addition of shredded vegetable fraction) and the liquid phase is partly recirculated and partly sent to a wastewater treatment plant. The aim of this study was to treat this wastewater in the airlift reactor described in the paragraph 2.1 to achieve effluent with characteristics suitable for a hypothetic anammox reactor: nitrite/ammonium concentration ratio in the order of 1-1.3 gN gN

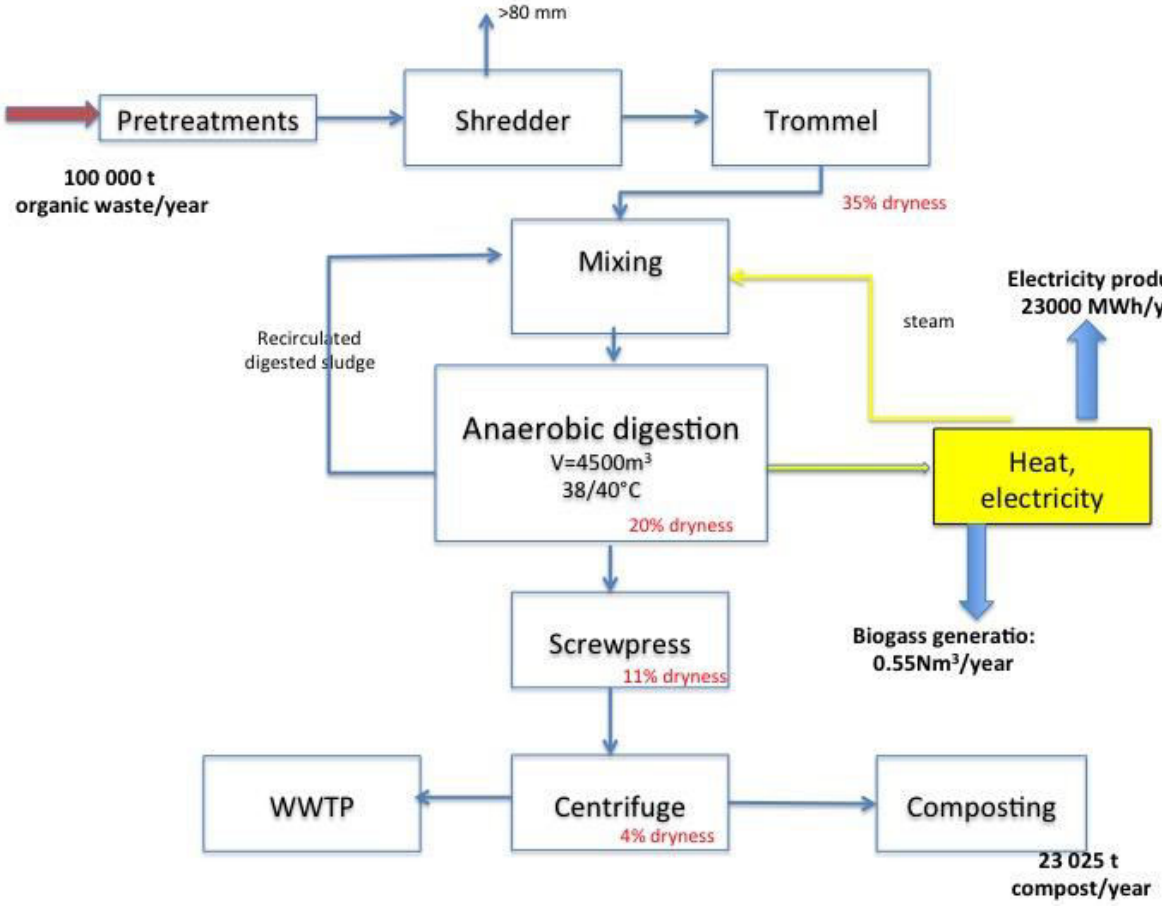


Figure 6 Scheme of the treatment of the sludge from the digester. Flux of material and energy on a year basis are also provided.

5.2.3 Sampling, characterization

The wastewater was sampled two times directly in the Ecopark facility.
Results of physical /chemical characterization of the wastewater is presented in the following Table 4**:**

Table 4 Characterization of the effluent of the anaerobic digester of the Ecopark used as wastewater in these study

Sampling Time	$N\text{-}NH_4^+$ concentration $[g_N\ L^{-1}]$	Conductivity @20°C [mS/cm]	TS [g/L]	VS [g/L]	TOC [g/L]
1st: 24/04/2015	4.00	30.2	33.8	19.0	9.7
2nd: 15/06/2015	4.48	28.0	39.0	13.2	6.9

The criticality of the wastewater relied in high strength ammonium concentration and salinity, along with the residual presence of a consistent amount of solids, both volatile and total. No nitrite nor nitrate were detected out of the anaerobic digester. Once collected from the plant, it was stored at 4°C to avoid possible modification of the influent.

5.3 Operational plan

5.3.1 Inoculum and operation before experimentation

Aerobic granular sludge from a continuous airlift reactor performing simultaneous nitritation and biodegradation of an industrial wastewater from the chemical sector in the boundaries of Barcelona was used as inoculum. This reactor exhibited successful partial nitritation and complete biodegradation of the organic compounds at a nitrogen and organic loading rates of 0.67 g N L^{-1} d^{-1} and 0.65 g COD L^{-1} d^{-1} (0.97 gCOD gN^{-1}) respectively (data not shown). Stable operation and granulation were obtained throughout long term-operation (more than 150 days). Some of the granular biomass characteristics were as follows: average granule size of 0.95 ± 0.03 mm, sludge volumetric index (SVI) at 5 min of 15 ± 5 5 mL g^{-1} of total suspended solids (TSS) and SVI30/SVI5 ratio of 1.0 (Ramos et al., 2015).

5.3.2 Experimental Phases

The first experimental phase was conducted with the industrial wastewater described in 3.2.1.

This phase lasted 19 days. In phase 2, from day 20 to day 101, the real wastewater was fed. In both phase 2 and phase 2-bis (starting from day 75) the influent was the wastewater sampled from the Ecopark 2, it was just collected in different moments as explained before. The Table 5 sums up all the operational conditions adopted.

Table 5 A summary of the experimental phases

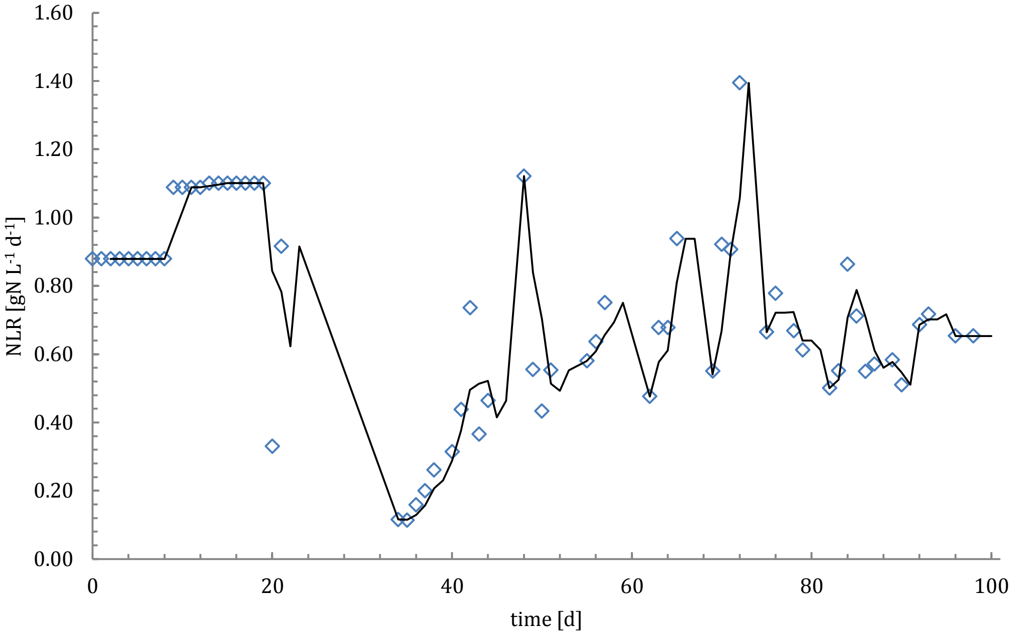
Phase	Day Started	Day Ended	Average NLR [gN d^{-1} L^{-1}]	Type of influent	Dilution Factor
1	0	19	1.02 ± 0.11	Industrial wastewater	-
2	20	74	0.59 ± 0.31	Diluted wastewater from the 1st sampling	6
2-bis	75	101	0.64 ± 0.21	Diluted wastewater from the 2nd sampling	5,3

Since the high load of ammonium and salinity was very likely to stress bacteria, it was decided to dilute the wastewater. The initial concentration of ammonium was 4gN L^{-1} and conductivity was 30.2 mS cm^{-1}, so it was decided to dilute the effluent in order to reach a concentration of ammonium lower than the one of the industrial wastewater that was about 1000 mgN – NH_4^+ L^{-1}. Therefore, a dilution of 6 was adopted, resulting in a concentration of was 770 ± 70 mgN – NH_4^+ L^{-1} on average and a conductivity of 7.2 mS cm^{-1} throughout phase 2.

5.3.3 Operating conditions

The feeding was done with a peristaltic pump that suctioned from a 50 L tank stored at ambient temperature.

The Hydraulic Retention Time (HRT) in the reactor depends on the inflow rate. In the first experimental phase, it was between 1 and 1.2 day. When the feeding was switched to real wastewater in phase 2, the high amount of solids (more than 30g L^{-1}in the raw water) prevented the pump from working properly: consequentially the inflow could not be kept constant and the HRT cannot be determined. The same can be argued for the nitrogen loading rate (NLR) that was 0,9 and 1,1 $g_N L^{-1} d^{-1}$ in phase 1 and was highly fluctuant in phase 2. Data for the NLR are shown in Graph 1.



Graph 1 The operational NLR in time with the moving average over three days

5.3.4 Control strategies

In order to do reach an adequate influent for the Anammox reactor, half the amount of ammonium has to be converted to nitrite. Therefore, a partial nitritation reactor should be operated in order to achieve this goal. To this aim, two were the operational parameters to be controlled: the load of ammonium and the air provided – and consequentially the operational dissolved oxygen.

The implemented strategy to obtain the partial oxidation of ammonium to nitrite was the control of the DO/N–NH_4^+ ratio to a target value. Indeed, this ratio was previously proven to be a successful strategy to limit NOB growth in a full nitritation reactor (Bartrolí et al., 2010) where this value was set between 0.17 – 0.35 mg O_2 mg^{-1} N. This strategy was applied to the granular PN reactor, however the set-point value was reduced to 0.02 mg

O_2 mg^{-1} N in order to achieve a partial nitritation process thus limiting ammonium oxidation to only half of the influent load. This value was identified empirically in a previous experimentation on a similar granular partial nitritation (parnit) reactor.

5.3.5 Monitoring plan

In order to assess the reactor performance the following parameters were measured: conductivity, pH, N species (Ammonium, Nitrate and Nitrite) and TOC were determined daily in the effluent. TS and VS determination in the reactor mixed liquor was performed four times. Ammonium concentration, pH and conductivity in the influent were determined every time a new influent was prepared (globally, ten times).

5.4 Analytical methods

Samples were regularly withdrawn (3 to 6 samples per week) from the influent and effluent and filtered through 0.20 µm syringe filter driven unit from Milipore® provided with a high-density polyethylene housing and membrane of hydrophilic Durapore® (PVDF) prior to analysis. Ammonium, nitrite, nitrate, TOC and conductivity were determined.

5.4.1 VS and TS

The procedure was adapted from Standard Methods (APHA et al, 1998).
Total and volatile solids from the reactor were measured taking a known volume of sample (25mL). Since there were no significant differences between the determination of VSS and VS, the latter were determined. Moreover, VS's determination includes a lower error, because filtration is not performed. Samples were put directly in the crucible and in the stove at 105°C for 24 hours. After all the water has been evaporated the crucible are then weighted and TS are determined. VS are finally determined after putting the crucible at 550°C for two hours and weighing it, they are the difference between the TS and what has not volatized (the so called ash).

All the determinations were done in triplicate.

5.4.2 Ammonium

Ammonium as TAN (total ammonium nitrogen) was measured daily by a continuous flow ammonia analyser (Hach Lange AMTAX sc), based on potentiometric determination. The instruments converts all the NH_4^+ into NH_3 gas that is then measured by the Gas Sensitive Electrode (GSE). Since the range of the instrument was from 1 to 100 mg L^{-1} a ten dilution factor was used.

5.4.3 Nitrite and nitrate

Nitrate and nitrite concentrations were analysed daily with ionic chromatography using an ion cromatographer ICS-2000 Integrated Reagent-Free IC system (DIONEX Corporation), which performs ion analyses using suppressed conductivity detection. Ion-exchange chromatography (or ion chromatography) is a chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger.

5.4.4 TOC

The soluble TOC (Total Organic Carbon) was measured with the OI Analytical Model 1020A Total Organic Carbon Analyzer, an automated system for the selective analysis of total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC).

Fundamentally, all TOC analyzers perform two tasks: the oxidation of organic carbon to form carbon dioxide and the subsequent detection of carbon dioxide. However, those tasks can be performed in different ways. The 1020A involves a combustion system in which an aqueous sample is heated to 680°C in an oxygen- rich environment. Water is evaporated and removed from the system, and the total carbon content of the aliquot is oxidized to form CO_2. The carbon dioxide is then swept quantitatively to the infrared cell. The time-integrated absorbance of the carbon dioxide flowing through the nondispersive infrared (NDIR) detector is directly proportional to the amount of carbon in the sample (TC).

Indeed, inorganic carbon is present as carbonate, bicarbonate and carbon dioxide. Through acidification the equilibrium is shifted to CO_2, that is released from the aqueous phase and measured another time from the NDIR, this time giving the value of the Total Inorganic Carbon (TIC). The TOC value is then given from the difference between TC and TOC.

5.4.5 FISH

The FISH-CLSM technique (Fluorescence In Situ Hybridization and confocal laser scanning microscope) was used to identify specific microorganisms: betaproteobacterial ammonia-oxidising bacteria (*βAOB*), nitrite-oxidizing bacteria (NOB) and general heterotrophic population.

FISH is a cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. This is used to detect and localize the presence of specific DNA sequences on chromosomes. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes.

Hybridization was carried out employing probes targeting specific microorganisms, as reported in Table 6. FISH protocol was adopted from Suarez – Ojeda et al. (2011).

A Leica (Leica Microsystem Heidelberg GmbH; Mannheim, Germany) TCS-SP5 confocal laser scanning microscope (CLSM) was used. The confocal microscope was equipped with a Plan-Apochromatic 63x objective (NA 1.4, oil).

Table 6 Probes used for targeting the specific microorganisms

Probe name	Specificity	Reference
Nso 190	*βAOB*	Mobarry et al. (1996)
NIT3	*Nitrobacter sp.*	Wagner et al. (1996)
UNIV1390	All organisms	Zheng et al. (1996)

5.4.6 Granules diameter distribution

The diameter of the granules was measured about once a week in order to be sure that there was no loss of granulation. Measurements were made with Mastersizer 2000 with Hydro 2000sm dispersion unit (Malvern). This is a laser diffraction particle sizing: during the measurement, particles are passed through a focused laser beam. These particles scatter light at an angle that is inversely proportional to their size. The angular intensity of the scattered light is then measured by a series of photosensitive detectors.

All particle-sizing techniques have problems with an adequate sample dispersion thus giving a great amount of error in the measure: that is why sample dispersion is an integral part of the measurement process. The samples were put in the dispersion unit and were analyzed in triplicate.

What the machine gives back is the distribution of particles' diameter.

5.4.7 Sludge Volumetric Index (SVI) and settling velocity

Procedure for SVI's determination was done according to APHA et al. (1998).

It consists in the measurement of the volume occupied by the biomass after 5 and 30 minutes settling.

$$SVI = \frac{settled\ sludge\ volume\ (\frac{mL}{L})}{biomass\ concentration\ (\frac{gVSS}{L})}$$

SVI was determined in duplicate and solids were determined as described above.

Settling velocity was determined by putting single granules in a transparent column (Ø 5cm) and then measuring the time spent to drop an eight of 40 cm. 30 granules were used and the result is the average of the single velocities.

SVI and settling velocity were performed twice, on day 6 and 91, in order to assess whether there had been changes in the biomass because of the switching from synthetic to real wastewater.

5.4.8 Specific Anammox Activities (SAA): manometric test

The following procedure is adapted from Dapena-Mora 2007.

Bottles with a working volume of 50 mL (59.4 mL total volume) were used. Anammox biomass was added to the bottle after washing it three times with a phosphate buffer (1.4 g L^{-1} KH_2PO_4, 7.5 g L^{-1} K_2HPO_4, pH 7.8). The volume of biomass taken was as such to achieve a final concentration of about 1 g_{vs} L^{-1}.

The tested liquids were a synthetic wastewater[5] and the effluent from the partial nitritation reactor and also a blank was included containing just the phosphate buffer, in order to show there were no external causes of pressure's changes. The amounts of substrate added were as such to achieve a final concentration between 40 and 90 mg N L-1 in each bottle.

Bottles were closed and air was eliminated by flushing gaseous N_2 in the headspace. Then bottles were incubated at 30°C in a thermostatic shaker for 30 minutes at 150 rpm before injecting synthetic wastewater in the control and the effluent from the reactor in the other bottles.

Before starting the test, bottles were depressurized and from now on, pressure was manually measured about every 30 minutes with a manometer Centrepoint Electronics (o-5 psi). The experiment was stopped when no further pressure increase was observed. To compute the specific activity, biomass concentration was determined as TS and VS as described above.

Pressure's increase is a measure of Nitrogen gas production by Anammox bacteria - that is to say nitrite and ammonium consumption, according to the anammox stoichiometry presented in paragraph 1.3.4. The conversion between nitrogen production and pressure measurement, obtained from the ideal gas law, is as follows:

$$N_{production\ rate} = \frac{\Delta p * V_{gas}}{0{,}08206 \frac{L\ atm}{mol\ K} * (T_{gas} + 273{,}15K)} * \frac{28\ g_N}{mol} * \frac{1}{V_l} * \frac{1000 mg_N}{g_N}$$

Where V_{gas} is the headspace volume, V_l is the volume of the liquid in the bottle and T_{gas} is the temperature of the gas in the headspace (considered equal to the temperature in the shaker).

The measured pressure values are reported in a graph against time as shown in Graph 2

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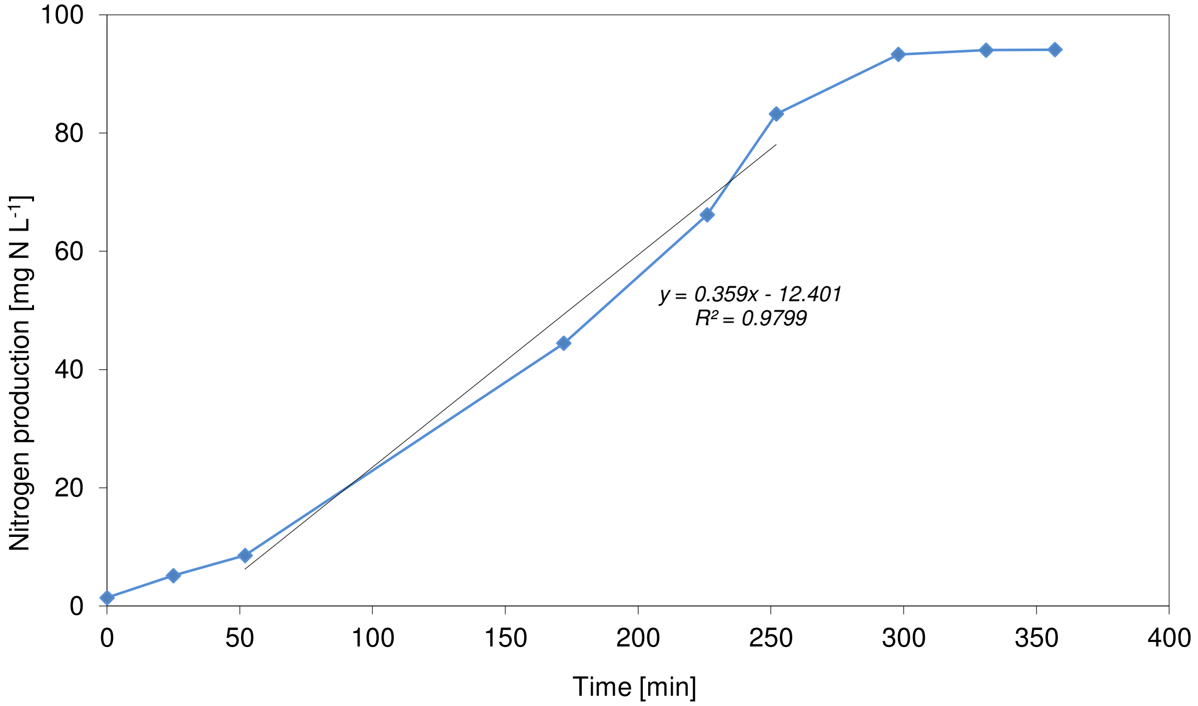
[5] The composition is shown in appendix



Graph 2 Nitrogen production in time, it is also reported the line of the maximum slope of the graph

The maximum slope of the graph, called R, is the maximum consumption rate observed in during the experiment and it expressed as mg N $L^{-1}min^{-1}$.

Finally, after the determination of biomass concentration, the specific Anammox activity is given by the following equation:

$$SAA = \left[\frac{gN}{g_{VS} * d}\right] = \frac{R * 24 * 60}{VS}$$

Where VS is the biomass concentration in the bottle and 24*60 comes from the conversion of minutes into days.

Activities were determined in triplicate.

5.4.9 2.4.11 N_2O measurement

Off gas was collected continuously (at 0.5 L/min) from the reactor headspace which was covered with a plastic container (a nitrite glove) and consequentially transferred to a clean plastic bag that was connected via a gas tube to a gas conditioning unit (series CSS, M&C Tech group). The gas outlet from the conditioning unit was connected to the multicomponent online gas analyser (VA-3000, Horiba, Japan) that provided an online measurement of N_2O concentrations from the gas flow.

N_2O determination was done in triplicate. The measurement of N_2O was done in the operational day 98.

6 Material and Methods – Working Package II

6.1 Experimental setup and reactor

6.1.1 The Sequencing batch reactor

The pilot plant employed in this study was a sequencing batch reactor (SBR) produced by Pharming (Italy). The reactor volume is 5.5L although the working volume used in this study was 2L.

The temperature was controlled to 35 ± 0.5 by mean of a heating jacket and a cooling water system. The reactor was sealed in order to guarantee the anoxic environment required by the anammox process. Mixing was provided with a rotating shelf with blades placed at different heights. The reactor was equipped with temperature, redox (Crison DO 6050) and pH probes (Crison pH 5333) that were monitored in continuous along with a manometer that measures the pressure in the headspace. There are two solenoid valves connected to nitrogen gas (95%N_2 and 5%CO_2) to flush the headspace.

Three peristaltic pump are connected to the reactor: feeding, discharge and security discharge.

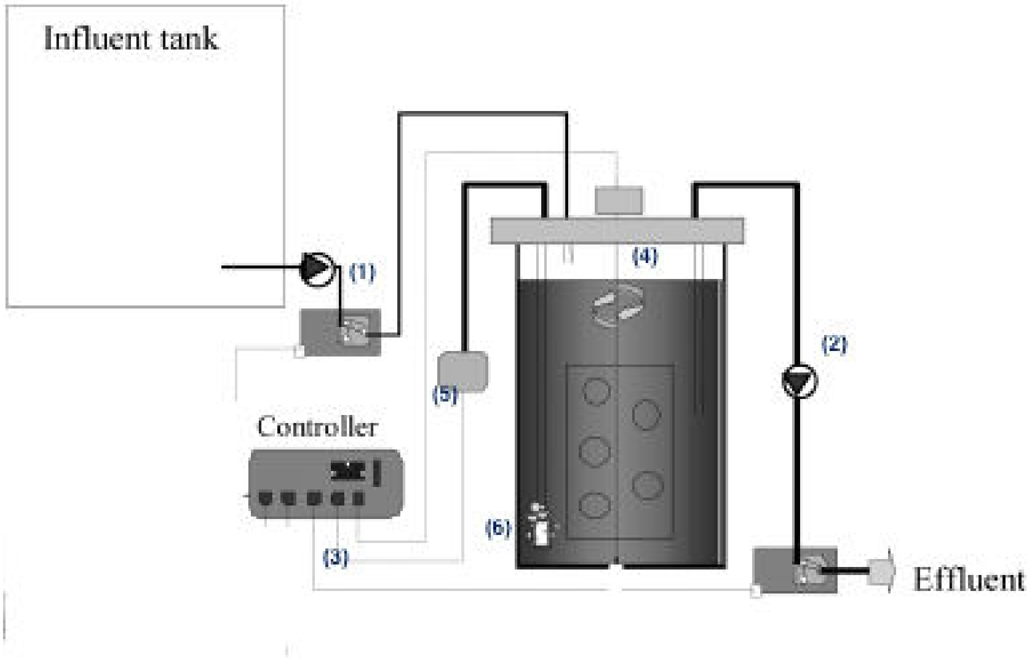


Figure 7 Experimental set-up of the SBR: (1) Influent peristaltic pump; (2) Effluent peristaltic pump; (3) controlling system; (4) mixer; (5) solenoid valves; (6) pH and DO probes.

The reactor can be managed through a control panel that allows to set the inflow rate, the control parameters and the duration of every phase.

Alike every SBR system, the reactor had five operational phases:

- Feeding. The wastewater is provide to the reactor and mixing is turned on in order to assure that the anammox bacteria have access to the substrate.
- Reaction. Only mixing is provided and the reaction continues.
- Settling. The mixing is turned off and the biomass settles in the reactor.
- Discharge. The discharge pump is activated and the treated water discharged until the minimum working volume is achieved (2L). The solenoid valve is turned on in order to maintain the overpressure and avoid air infiltration
- Idle. The mixing is turned on and the biomass is ready for starting another cycle

The duration of the different phases is provided in the following Figure 8, along with a scheme of the operation of the reactor.

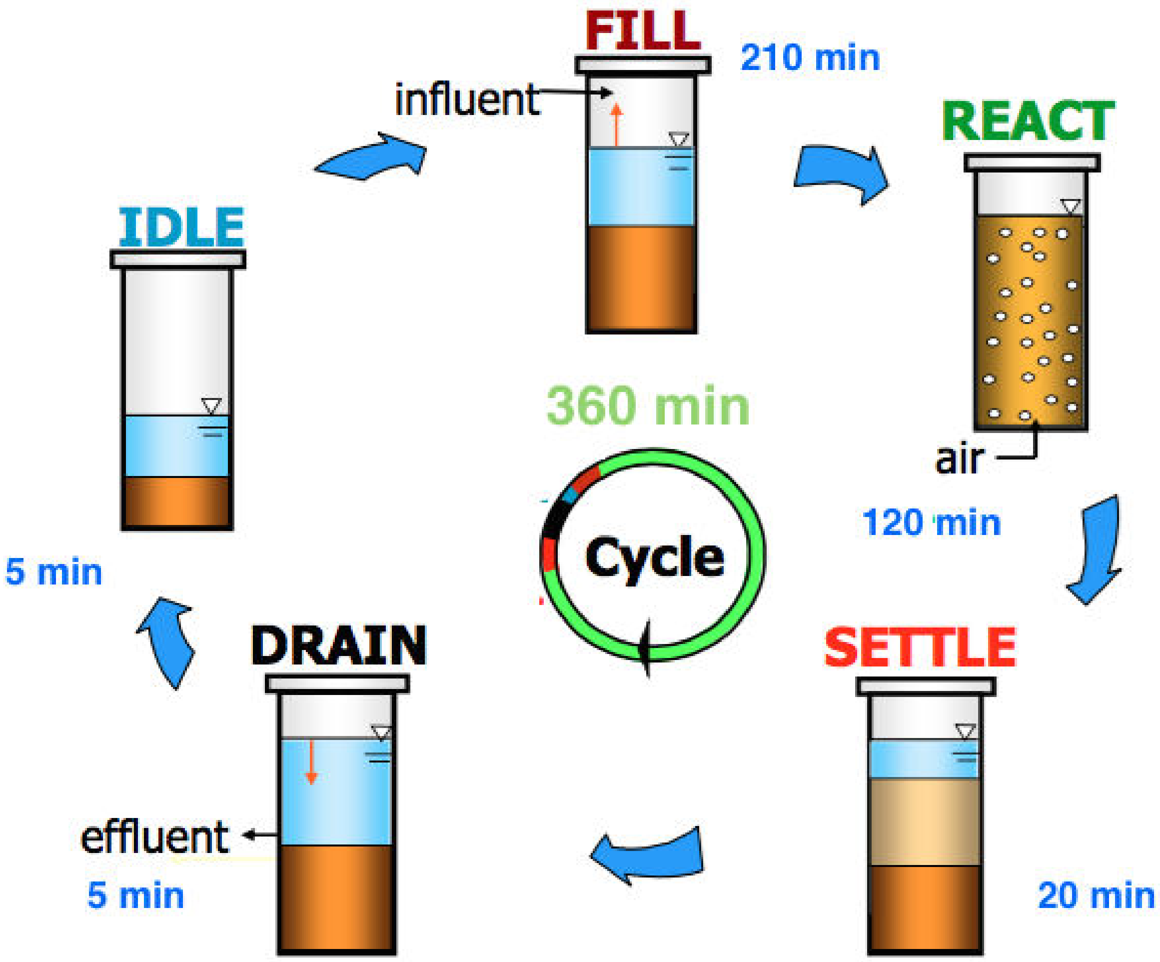


Figure 8 A scheme for the functioning of the SBR and duration of the different phases of the cycle

6.2 The tested influent

6.2.1 Origin

In the first and third experimental phases the influent was a synthetic wastewater *with the following compounds and micronutrients (concentrations are expressed in g* L^{-1}*): KCl, 1.088; NaCl, 3.232;* $NaHCO_3$*, 1.932;* $KHCO_3$*, 1.069;* K_2HPO_4*, 0.042. As for the micronutrients (concentrations are expressed as mL* L^{-1}*):* Ca^{2+}*, 0.625;* Mg^{2+} *, 3.5;* Fe^{2+}*, 2; trace elements (solution 2), 1.25. The conductivity was 12 mS* cm^{-1} *before adding the nitrogen forms.*

The effluent was paper filtered before feeding it into the reactor to remove gross matter that could bring technical problems to the feeding pump. Because of the upstream treatment, residual nitrite and ammonium concentrations were close to zero. Therefore, salts of ammonium, nitrite and nitrate were dissolved in the raw wastewater to treat the desired loading rate thus obtaining a semisynthetic influent. Those salts were respectively NH_4HCO_3, $NaNO_2$ and KNO_3 and the final nitrite and nitrogen concentration in the influent varied through the different phases.

6.2.2 Sampling, characterization

Sampling from the treatment plant was frequent because continuous storage could have modified the characteristic of the influent.
In Table 1 average values are provided for the four sampling time (19/06/2015, 9/07/2015, 2/09/2015 and 8/10/2015):

Table 7 Characterization of the average effluent from the AD digestion plant in the north of Italy

Parameter	Min/Max value	Average
Alkalinity [mg L^{-1}]	1984/3296	2541 ± 548
Conductivity [mS cm^{-1}]	11.6/15.8	12.8 ± 2.0
TS [mg L^{-1}]	7.27/8.52	7.90 ± 0.55
VS [mg L^{-1}]	1.05/1.31	1.19 ± 0.11
SST [mg L^{-1}]	377/622	467 ± 107
SSV [mg L^{-1}]	358/526	414 ± 76
TKN [mg L^{-1}]	109/124	114 ± 7
NH_4^+ [mg L^{-1}]	12-42/15.4	14.0 ± 1.4
COD [mg L^{-1}]	2303/2486	2417 ± 88
BOD5 [mg L^{-1}]	144/251	193 ± 52
BOD20 [mg L^{-1}]	327/561	406 ± 108
BOD5/COD[-]	6.2/10	8.0 ± 2.2
pH	8.02/8.59	8.39 ± 0.32

The effluent showed high conductivity and suspended solids. It can also be noted that the activated sludge treatment did not guarantee a complete removal of nitrogen and COD. Indeed, residual nitrogen forms could be found, especially nitrite and nitrate, as well as residual COD.

Moreover, the addition of salts to the influent induced a further increase in its conductivity.

6.3 Operational Plan

6.3.1 Inoculum

The SBR reactor was inoculated with 181.3 g_{WW} Anammox biomass from the SHARON/Anammox plant full-scale of Sluisjesdijk wastewater treatment plant on the 9/06/2015. It corresponded to a final biomass concentration in the reactor of 7.7 ± 0.2 $g_{VSS}L^{-1}$.

At day 66, more granular biomass was added to the reactor to improve the nitrogen removal capacity. Specifically, 272.5 g_{WW} was added. This second biomass addition is later on called “bioaugmentation”.

6.3.2 Experimental phases

The experimentation lasted 129 days. In the very first experimental phase a synthetic wastewater was fed in order to acclimate the biomass. Then the influent was switched to the synthetic *high* conductivity wastewater described in 4.2.1. From day 15 onward, the real wastewater was fed without any dilution. However, a serious activity loss was observed and therefore wastewater feeding was temporarily stopped and synthetic wastewater was used instead in order to try to restore the activity. Since no relevant activity increase was observed, bioaugmentation was implemented. Finally, the wastewater was treated with two different dilutions.

Table 8 A summary of the experimental phases

Phase	Day Started	Day Ended	Average NLR[6] [g N d^{-1} L^{-1}]	Type of influent	Dilution Factor	HRT [d]
1	0	6	0.77	Control	-	1.0
1-bis	7	14	0.77	Synthetic high conductivity wastewater	-	1.0
2	15	33	0.52	Raw wastewater	1	2.5
3	34	65	0.24	Synthetic high conductivity wastewater	-	1.7
4	66	93	0.84	Diluted wastewater	2	1.0
5	94	?	0.60	Diluted wastewater	0.43[7]	2.5

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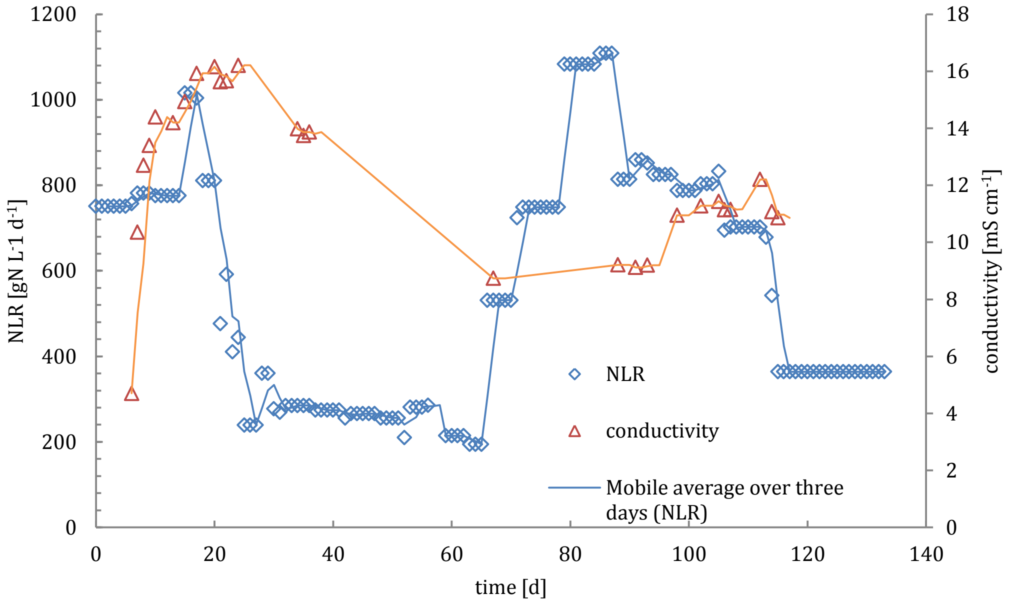
[6] In the computation of the NLR nitrogen is computed considering both ammonium and nitrite

[7] this is equivalent to 70% raw watewater and 30% tap water

6.3.3 Operating conditions

T, pH, feeding strategies, NLR, HRT, SRT

The temperature was set to 35°C and pH was controlled by flushing the reactor head space with a N2/CO2 (95%/5%) gas mixture which stabilized the pH of the suspension at 7 ± 0.2.



Graph 3 The value of the NLR and of the conductivity in time with the moving average over three days

The HRT changed through the time because the average inflow rate was varied. In fact, when switching from the synthetic to the real wastewater, the inflow flow rate was lowered to reduce the NLR in view of an expected reduction of the anammox activity. The values for the different phases are provided in Table 8.

6.3.4 Monitoring plan

Performances of the reactor were determined by monitoring the effluent concentrations of nitrite, ammonium and nitrate three times a week and of COD once a week. Also batch activity tests were carried out once a week according to the procedure described in the following paragraph. VSS and TSS in the reactor were determined at the time of the inoculum and bio-augmentation.

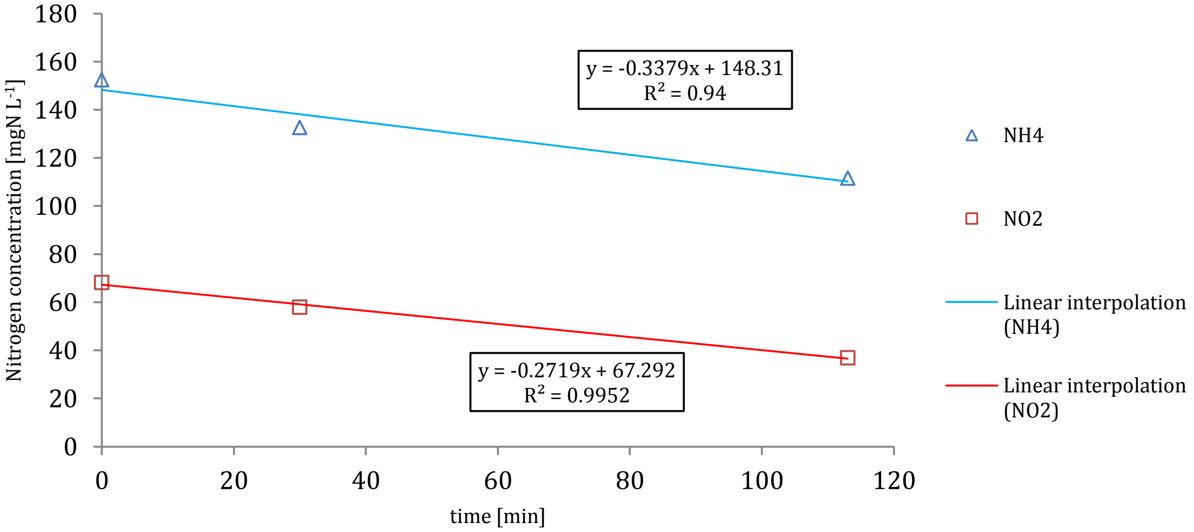
Ammonium, nitrite and nitrate, and COD were also assessed in the influent although the nitrate and nitrite concentrations were almost negligible.

6.3.5 Activity determination – batch test in the reactor

The anammox activity in the reactor was constantly monitored because it is the most precise measure of the actual capacity of the biomass to degrade nitrogen-forms.

To this purpose, a spike of a known nitrite and ammonium amount was done in the reactor after switching it in batch mode. After few minutes required to achieve homogeneous conditions in the bulk liquid, the first sample is taken. The following samples are taken at different time intervals, adjusted according to the activity observed in previous activity tests and on the type of influent in the reactor. Hence, the slower the activity that is expected the longer the interval between two sampling points.
Samples are taken directly from the liquid phase of the reactor and nitrite, ammonium and nitrate concentrations are measured.

The objective is to understand the maximum consumption rate in the reactor.
In order to do that, concentrations data are plotted against time and the average consumption rate is computed as the slope of the fitting line, as shown in Graph 4. Since consumption rates of both nitrite and ammonium are determined, the stoichiometry of the reaction can be also assessed.



Graph 4 The determination of the maximum nitrogen consumption in the reactor

6.4 Anammox inhibition tests

Inhibition tests were performed by assessing the specific Anammox activity (SAA) of anammox granules suspended into the wastewater to be tested and by comparing it to the SAA that the Anammox bacteria expresses when suspended in a reference mineral medium.

6.4.1 Inoculum

The tested biomass is the same as that used to inoculate in the SBR reactor from the Anammox reactor of Sluisjesdijk. Anammox bacteria were stored in a tank at ambient temperature in a control solution.[8]

6.4.2 Tested influents

A synthesis of the tested influent is provided in Table 9. Batch tests were performed in order to assess influent toxicity in the reactor. Due to the high consistency of such tests, they were preliminary performed to obtain trustworthy indications upon influents to be test in the reactor.
Therefore, potential inhibition was determined for all the influents prior to their use for feeding preparation, with the exception of the raw wastewater that was first optimistically fed to the reactor.

6.4.3 Method

The SAA was determined through a manometric method that was previously applied and validated (Scaglione et al., 2009). Specifically, the Anammox granular suspension is poured into a batch reactor. Micronutrients solutions and nitrate are added. The addition of nitrate is performed in order to avoid sulphate reduction. The reactor consists in a closed bottle (335 mL) provided with two lateral necks closed with a rubber septum for substrate injection, depressurization and flushing of the headspace for deoxygenating. The bottle is provided with a manometric sensor and a data logger registering up to 360 measuring points (OxiTop Control system) achieving a curve as the one in Graph 5.

A control was always added to every set of tests in order to relate the measured activity with an ideal influent for the anammox reactor. Inhibition is calculated as the observed reduction in the activity. An overview of the different activities carried out is presented in Table 9.

The amount of wet biomass in the bottle was weighted and added with the tested liquid in the bottles in order to reach an overall liquid volume of about 200 mL. Bottles were flushed to reach anoxic conditions with a gas containing 5% CO_2 and 95% N_2.
Bottles were consequentially put in a shaker and temperature was controlled at 35°C. After biomass acclimatization, substrates were added with a spike of 1mL concentrated solution of 50 $mg_N\ L^{-1}$ of ammonium and nitrite. The ratio between nitrite and ammonium was not the stoichiometric one because it is preferable to work in nitrite-limiting condition.
The test can last between 18 and 24 hours, depending on the nitrogen removal rate. Once the substrate has been consumed the overpressure is eliminated and another spike is

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[8] recipe in the appendix

done. Up to five subsequent spikes were carried out .The final value of nitrite consumption is the average of the observed values.

Once the test is terminated, the amount of TS and VS were determined in order to calculate the specific activity of the biomass. Also pH and conductivity were monitored at the beginning and at the end of the test in order to verify that optimal conditions for microorganisms' metabolism were kept throughout the test.

Table 9 A synthesis of the manometeric tests carried out. Tests performed using the same biomass sampled from the storage tank are merged together

Tested Influent	Conductivity [mS cm^{-1} @ 20°C]
Control	3.96
High conductivity synthetic wastewater[9]	12.3
50 % raw wastewater(sampling #3)	7.5
Raw wastewater (sampling #3)	11.6
Control	3.71
Very high conductivity synthetic wastewater[10]	16.0
70 % raw wastewater (sampling #4)	9.3
Raw wastewater (sampling #4)	11.6
Control	3.78
Raw wastewater – may (sampling #0[11])	12
High conductivity synthetic wastewater	11.4

6.4.4 Data processing

Data processing is done with a calculation sheet where the values of pressure are converted into nitrogen removal rates, under the assumption that all the overpressure is only due to nitrogen gas release and that no solubilisation of the gas takes place.

The bacteria responsible for N_2 production are anammox as well as heterotrophic denitrifiers. Indeed, a fraction of denitrifiers is always present in the inoculum and they take the necessary carbon to be used as electron donor for their metabolism from the organic matter in the influent while NO_x^- are the electron acceptors.

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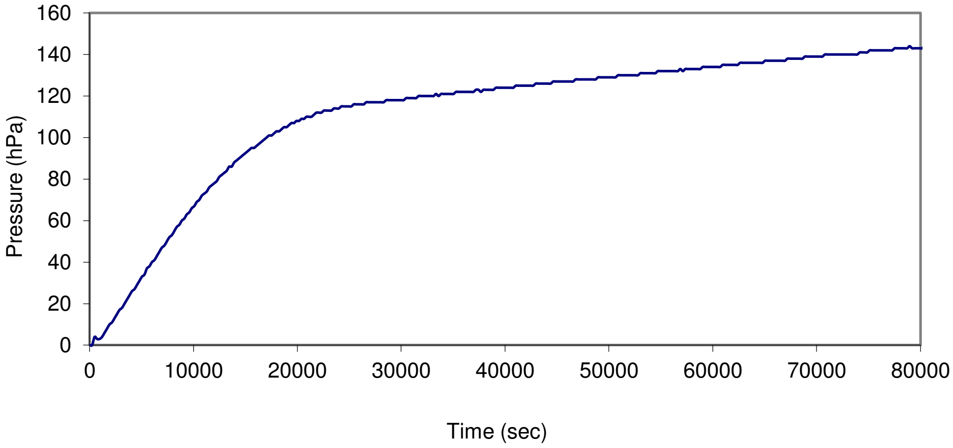
[9] The mentioned wastewater had the same composition as the one fed to the reactor and described in 2.2.1

[10] The composition is included in appendix 9.1

[11] Those data are taken from a previous experimentation in which the influent had the same origin as in this study. The same biomass of this study was used, only stored in different conditions, reported in the Table 20



Graph 5 The typical trend for overpressure measurement from the OxiTop system data in time. The residual pressure increase after all the substrate has been consumed is due to heterotrophic activity. In order to determine the specific anammox activity, this contribute is to be subtracted to the total pressure increase measure.

Pressure increase should consequentially be converted to nitrogen removal rate using the perfect gas law, the operative conditions and the anammox stoichiometry given in paragraph 1.3.4:

$$\Delta P_i * V_g = \Delta n_i * RT$$

$$\Delta n_i = \frac{\Delta P_i * V_g}{RT} \quad [mol]$$

$$\Delta NO_2 = \frac{\Delta n_i * 14\frac{gN}{mol\ N} * 1.32\frac{molNO_2}{mol\ NH_4}}{1.02\frac{molN_2}{mol\ NH_4}} \quad [gNO_2 - N]$$

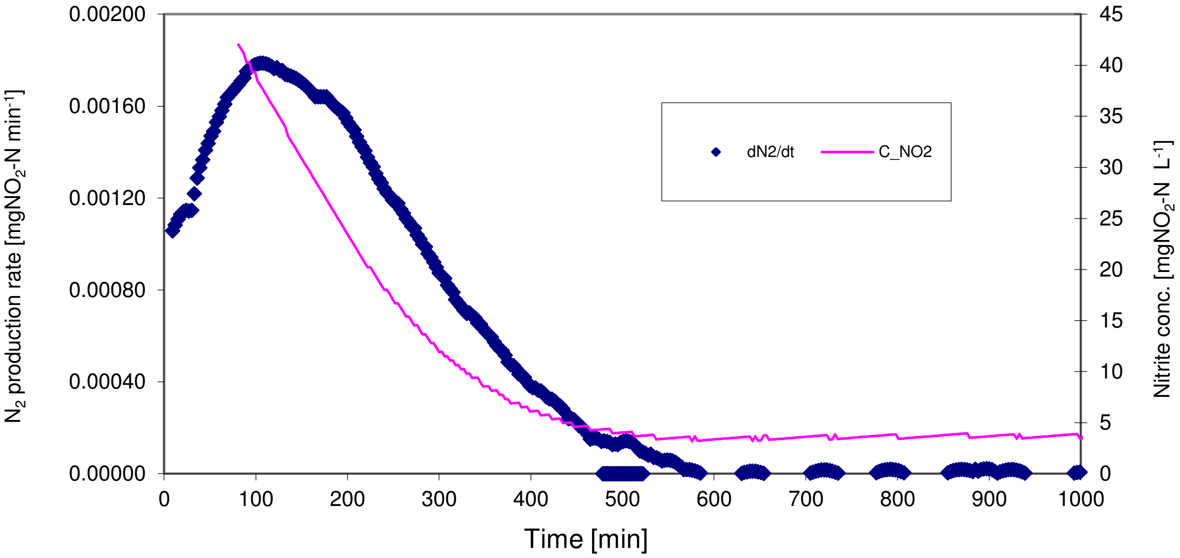
Knowing the time interval in which the production has been measured, it is possible calculate the production rate in mg NO_2^- – N min $^{-1}$.

The conversion is the same as used in 3.4.8.

The curves all share the same shape:

- there is a first phase where no activity is shown. At the time of the spike, a pressure jump is observed. Later, the biological nitrogen production begins, leading to a linear pressure increase over time. During this phase, the SAA is assessed.
- After all the nitrite has been consumed, the only increase in pressure is due to heterotrophic activity.

An example is shown below in Graph 6. To determine the contribution of anammox bacteria alone, data should be corrected for the heterotrophic activity. Therefore, with a linear regression on the last part of the curve denitrifiers contribution is assessed and subtract to the net pressure production measured.



Graph 6 Example of processed data: the rate of nitrogen gas production and of nitrite consumption during the test. After the injection of the substrates there is a small interval in which the biomass adaptation, consequentially the plateau of the curve represents the maximum activity of the anammox biomass (SAA).

6.5 Analytical methods

6.5.1 Nitrite, nitrate, ammonium and COD

Samples were regularly withdrawn (2 to 4 samples per week) from the effluent and filtered through 0.45 µm acetate filter. Determination of COD, total ammonium nitrate and nitrite nitrogen is performed through commercial KIT (Hach Lange Gmbh, Düsseldorf, Germania) with subsequent spectrophotometric measurement (Lange Xion500) after the necessary dilution to match the range of measurement of the KIT.

6.5.2 VSS and TSS

VSS and TSS were determined as in 3.4.1, according to APHA methodology, with the addition of the initial filtration step.

7 Results and Discussion - Working Package I

7.1.1 Comparison between the characteristics of the Italian and Spanish digestate

From the comparison of the two wastewaters reported in Table 10 and Table 11, it is immediate to conclude that the Spanish digestate shows higher concentration of ammonium and total solids. Moreover, it also presents higher metals concentration. For example zinc, iron, nickel and lead show a concentration that is 25, 13, 6 and 15 fold the one of the Italian digestate. All those data are coherent with the different process configuration.

It can be concluded that the effluent from a “dry” digester has a higher complexity and it is therefore less compatible with an autotrophic nitrogen removal process. Such complexity justifies the higher dilution of the influent of the PN reactor in Barcelona.

Table 10 Comparison between the raw wastewaters used in the two WP

Raw Influent	$N\text{-}NH_4^+$ concentration $[g_N\ L^{-1}]$	Conductivity $[mS\ cm^{-1}$ @ 20°C]	TS $[g\ L^{-1}]$	VS $[g\ L^{-1}]$
WP I	4.2	29	36.4	16.1
WP II	2.2	12	7.9	1.2

Table 11 A comparison between the Italian and Spanish digestate in terms of metals

Metal	Units of measure	Plant in the north of Italy (4th sampling)	Plant in the north of Italy (3rd sampling)	Ecopark (1st sampling)	Ecopark (2nd sampling)	Analytical Method
Alluminium	$mg\ L^{-1}$	7	7.1	21	13	UNI EN ISO 17294-1 2007
Chloride	$mg\ L^{-1}$	2200				ISO 10304-1, 1999
Arsenic	$ug\ L^{-1}$	<10	<10	213	685	UNI EN ISO 17294-1 2007
Sulphates	$mg\ L^{-1}$	54				APAT IRSA CNR 4020 Man 29/2003
Phosphates	$mg\ L^{-1}$	11				UNI EN ISO 17294-1 2007
Silver	$\mu g\ L^{-1}$	<5	<5	<5	<5	UNI EN ISO 17294-1 2007
Calcium	$mg\ L^{-1}$	655	655	740	680	UNI EN ISO 17294-1 2007
Cadmium	$mg\ L^{-1}$	<2.5	<2.5	<2.5	<2.5	UNI EN ISO 17294-1 2007
Cobalt	$ug\ L^{-1}$	38	41	117	348	UNI EN ISO 17294-1 2007
Total Chrome	$mg\ L^{-1}$	<5	<5	<5	555	UNI EN ISO 17294-1 2007
Copper	$mg\ L^{-1}$	<0.0005	<0.005	0.71	1.7	UNI EN ISO 17294-1 2007
Iron	$mg\ L^{-1}$	0.983	1.1	17	11	UNI EN ISO 17294-1 2007
Mercury	$\mu g\ L^{-1}$	<5	<5	<5	<5	UNI EN ISO 17294-1 2007
Potassium	$mg\ L^{-1}$	1390	1440	3700	3400	UNI EN ISO 17294-1 2007
Magnesium	$mg\ L^{-1}$	57	59	30	37	UNI EN ISO 17294-1 2007
Molybdenu	$\mu g\ L^{-1}$	<25	<25	<25	<25	UNI EN ISO 17294-1 2007

m						
Manganese	ug L^{-1}	65	65	473	1150	UNI EN ISO 17294-1 2007
Sodium	mg L^{-1}	1420	1340	2900	2400	UNI EN ISO 17294-1 2007
Nickel	µg L^{-1}	171	226	655	1900	UNI EN ISO 17294-1 2007
Lead	µg L^{-1}	<10	<10	231	57	UNI EN ISO 17294-1 2007
Selenium	µg L^{-1}	<25	<25	<25	<25	UNI EN ISO 17294-1 2007
Strontium	µg L^{-1}	2900	2800	3500	3500	UNI EN ISO 17294-1 2007
Vanadium	µg L^{-1}	<10	<10	<10	100	UNI EN ISO 17294-1 2007
Zincs	mg L^{-1}	0.18	0.23	3.1	7.1	UNI EN ISO 17294-1 2007

7.2 The partial nitritation process

7.2.1 Control strategies and issues

As already explained, pH and temperature were automatically controlled while oxygen concentration was manually set by providing an amount of air proportional to the NLR.

The amount of air provided during the whole experimental period was on average 280 mL /min. Air was given in large excess with respect to the stoichiometric needs because in a gaslift system it plays multiple roles: it is used both for mixing and keeping granules in suspension. Accordingly, air supply in the reactor provides both the necessary oxygen for AOB metabolism and mixing, thus making the system easy to operate. Moreover, the O_2 mass transfer from the gas-liquid phase interface and liquid phase bulk transport to cells is more efficient if compared with other systems because the interfacial area is higher. Finally, the great advantage at full scale is the absence of mechanical rotating parts, which are onerous in term of investment and maintenance costs.

Therefore, it was decided to control the process by adjusting the NLR instead and by regulating to the level that allowed the oxidation of just the half of the TAN load. According to previous experiences of partial nitritation on this same gas lift reactor, this value was expected to be around 1 $g\ N\ L^{-1}d^{-1}$.

However, in the first 60 experimental days of phase 2 there were technical issue in the control of the rate of the feeding pump and thus of the NLR because of the amount of solids in the influent that caused clogging issues, thus not allowing the control of the inflow rate to the desired value, as it can be observed in Graph 1.

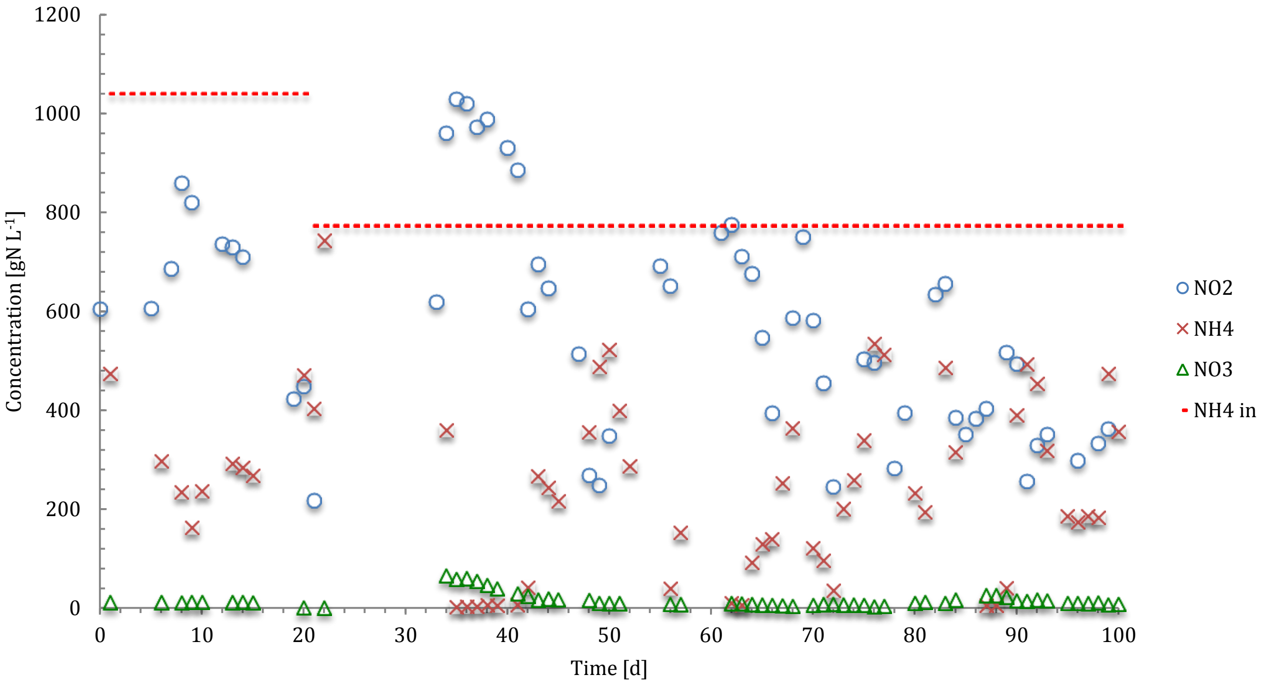
Indeed, the reactor was undercharged most of the time and nitritation was thus very efficient, with TAN conversions beyond the typical desired value of 50% for PN systems. The average conversion of ammonium to nitrite during the whole period was 72 ± 22 %. An anammox reactor would be inhibited by such ratios and the treatment would not be successful. However, when the inflow was kept around the optimal value corresponding to a NLR of about 1 g N $L^{-1}d^{-1}$ an effluent NO_2^-/NH_4^+ ratio around 1 was achieved (see results from day 80 to 100 in Graph 6). This evidence suggests that, in a full-scale implementation where inflow rate can be reliably controlled and pretreatment are available to control the *amount of solids, the applied control strategy would be successfully implemented and an appropriate effluent for a downstream anammox reactor could be achieved.*

7.2.2 Results of the operation of the gas-lift reactor

In Graph 7 the concentrations of the main N forms in the effluent are shown. It is clearly enlighten that nitrate was never present in the effluent.
The average conversion of NH_4^+ to NO_3^- during the whole experimentation was 1.8±1.5%. This suggests that NOBs activity was suppressed as confirmed by FISH and pyrosequencing analysis (reported below in 5.2.6 and 9.3.1).

As shown in Graph 1, during the first 20 days of operation the NLR could be maintained constant to its design value. *Unfortunately, the airflow was higher than the design value and the ammonium conversion efficiency was higher than 50%.* Around day 20, i.e. at the end of phase 1, a proper control of both the NLR and the air flow rate allowed achieving the desired nitrite to ammonium ratio in the effluent. The DO/TAN ratio was 0.0024 mg O_2 mg^{-1} NH_4^+-N in this phase 1.
In phase 2, the influent to the PN reactor was changed and the influent synthetic wastewater was substituted by the reject water from the Ecopark 2. This wastewater was diluted 6 times in order to achieve a total ammonium concentration in the inflow of *770* ± 70 mgN L^{-1} corresponding to a NLR of 0.59 ± 0.31 g N –NH_4^+ L-^{1} d^{-1}.
With the changing to the real wastewater, technical issues became relevant. The feeding pump could not be operated at a constant flow rate because the high amount of solids and impurity in the influent wastewater caused serious clogging issues. Thus the operational NLR was varying in time and lower than the desired design value.
For this reason, in the first experimental days (from day 21 to about day 80) the inflow and consequentially the TAN concentration could not be set to meet the target DO/TAN ratio empirically determined to be 0.0025 mg O_2 mg^{-1} NH_4^+-N from previous experiences on that air-lift reactor.
Average NH_4^+ and NO_2^- concentrations were respectively 241± 181 mg N - NH_4^+ L^{-1} and 552 ± 221 mg N – NO_2^- L^{-1}.

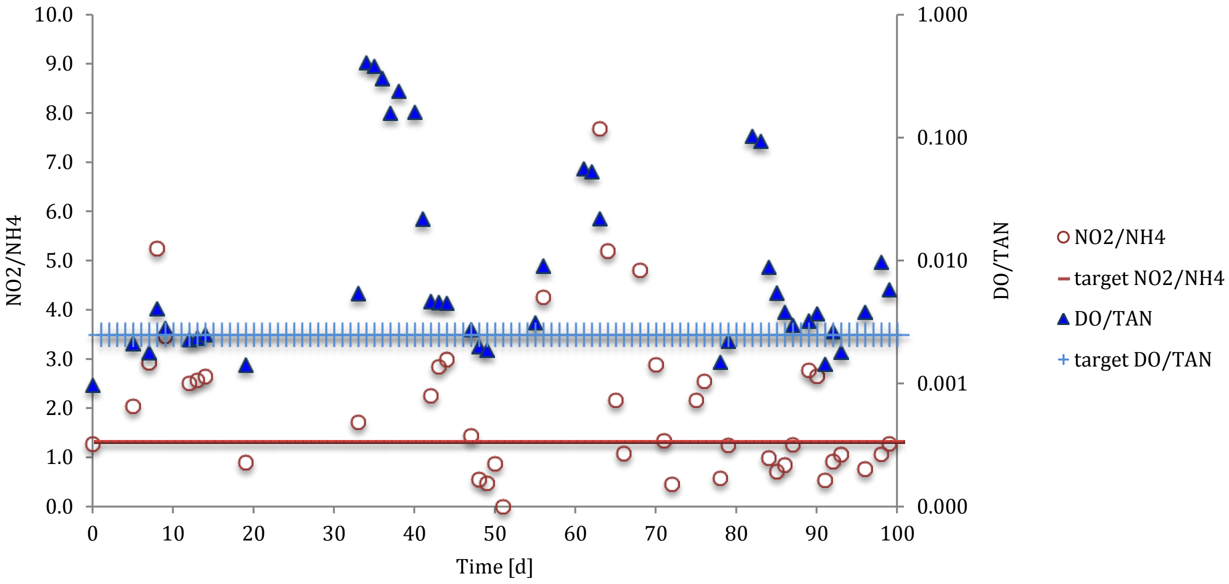
Nonetheless, as shown in Graph 7, from day 80 onward nitrite and ammonium have almost the same concentration in the effluent. The DO/TAN ratio could be finally regulated to an average value of 0.0043 ± 0.0039 mg O_2 mgNH_4^+-N^{-1} and this allowed the nitritation of just half of the ammonium load. In this phase the average ammonium, nitrite and nitrate concentrations were 324 ± 111 mgN L^-1, 390 ± 82 mgN L^{-1} and 10 ± 3.9 mgN L^{-1} respectively. As for the NO_2^-/NH_4^+ ratio it was 1.46 ± 0.48. The correspondent applied NLR was 0.65 ± 0.1 mg N – NH_4^+ d^{-1} L^{-1}.



Graph 7 Ammonium concentration in the influent and the trend of N forms' concentrations in the effluent throughout the whole experimental period are reported. The observed nitrite concentration higher than the ammonium load will be explained in 5.2.2.1

Moreover, the following Graph 5 compares the DO/TAN ratio in the bulk liquid with the NO_2^-/NH_4^+, along with the lines corresponding to their target values.

Although in the first phase there were high fluctuations in the NLR that caused fluctuations in the nitrite to ammonium ratio, in the final phase the reactor was oxidizing almost half of the ammonium fed and the ratio between NO_2^-/NH_4^+ was close to the unit, which is the optimal value for an anammox reactor. Nevertheless, the ratio NO_2^-/NH_4^+ in the effluent was slightly higher than the stoichiometric optimum. The reactor was indeed undercharged and could oxidize more ammonium to nitrite than what observed, about 1 g N $L^{-1}d^{-1}$ as seen in the first experimental phase.



Graph 8 The ratio between nitrite and ammonium and the DO/TAN against time

TOC was analyzed almost as frequently as the nitrogen forms: indeed, the removal of the biodegradable organic matter before an anammox reactor is very important in order to limit the growth of conventional heterotrophs that may outcompete the anammox bacteria as explained in paragraph 1.3.5).

The applied TOC loading rate through the three phases was 0.80 ± 0.9, 0.85 ± 0.44 and 0.61 ± 0.09 g TOC $L^{-1}d^{-1}$ or phase 1, 2 and 2-bis respectively. Besides, the inflow TOC/N ratio was 0.86, 1.46 and 0.97 respectively for phase 1, 2 and 2-bis.

The average value for TOC removal efficiency was 47 %. As shown in the average TOC removal efficiency was higher in phase 1 and remained around the 62%. When the real wastewater was fed, higher effluent concentrations were measured, and the TOC removal efficiency dropped to 42% and 44% respectively for phase 2 and 2 bis.

The average TOC/N ratio in the outflow for the three phases is 0.32, 0.75 and 0.57 g_{TOC} g_N^{-1} respectively for phase 1, 2 and 2-bis. There are experimental evidence that at a COD/N ratio higher than 1 heterotrophic denitrifiers area able to outcompete the annammox (Guven et al., 2005). However, it is difficult to convert the measured TOC to a COD concentration. At any rate, during anaerobic digestion, fast biodegradable organic carbon is converted to biogas. Therefore, the remaining organic fraction in the effluent is slowly degradable. Actually, a more suitable indicator for the removal efficiency of organic matter would have been the BOD_5 since this is the fraction that is taken up more easily.

Additionally, as in the raw wastewater all the organic matter was highly refractory, it would be resistant to degradation and it was supposed not to be inhibiting for the following anammox reactor, as demonstrated in the batch tests (see below 5.2.5).

It can be enlighten that a higher air (and thus oxygen) availability[12], as from day 49 onward, led to a lower TOC concentration in the outflow since the higher amount of

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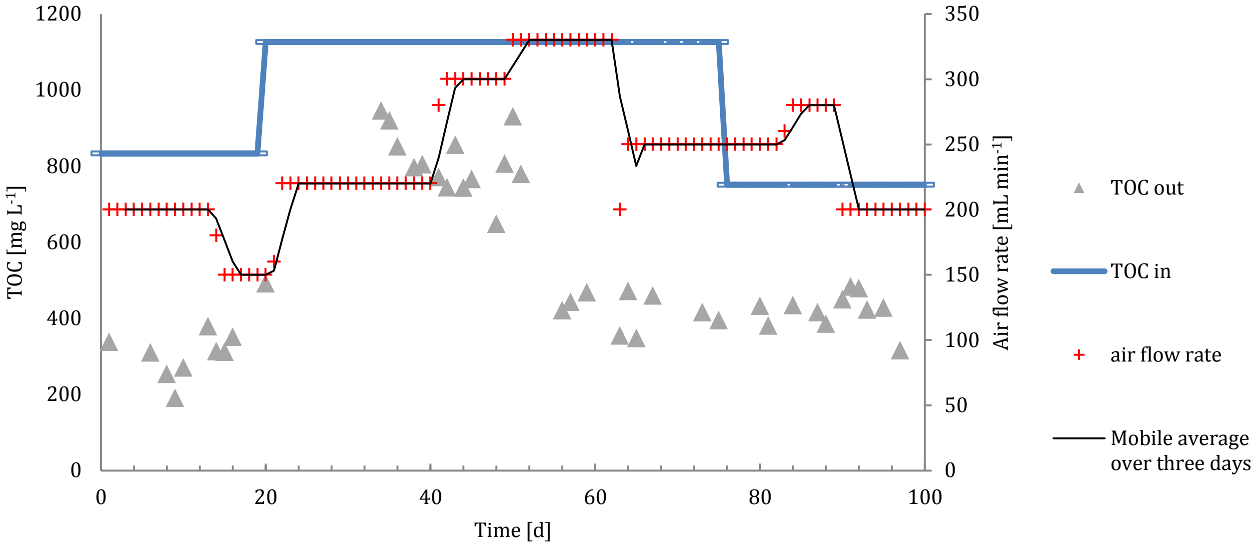
[12] In the graph is provided the amount of air and not of oxygen because this datum was available throughout the whole experimental period, while the DO measurement was interrupted for a period because of technical issues.

oxygen supported the organic matter oxidation. Moreover, in phase 2-bis the overall TOC in the influent was lower and this also contributed to an average lower concentration in the effluent, despite the air-flow rate being decreased.



Graph 5Graph 5 TOC concentration in the influent and in the effluent

7.2.2.1 *Protein hydrolysis*

From day 33 to day 47, it was noticed that in the effluent that the total N (the sum of nitrite and ammonium) in the effluent was higher than the ammonium present in the feed (up to the 29% more).

It was observed that the exceeded ammonium was strictly dependent on the HRT as shown in. The higher the time the wastewater was kept in the reactor (i.e. higher HRT), the higher the ammonium production. It was concluded that hydrolysis of the proteinaceous organic matter was taking place. The ΔN reported in Graph 6 in the ordinate axe is the observed difference between nitrogen in the influent and in the effluent.

The observed data are well fitted with a hyperbolic curve. The model is in the form:

$$\Delta N_{observed} = \Delta N_{max} * (1 - \frac{1}{1 + k * HRT})$$

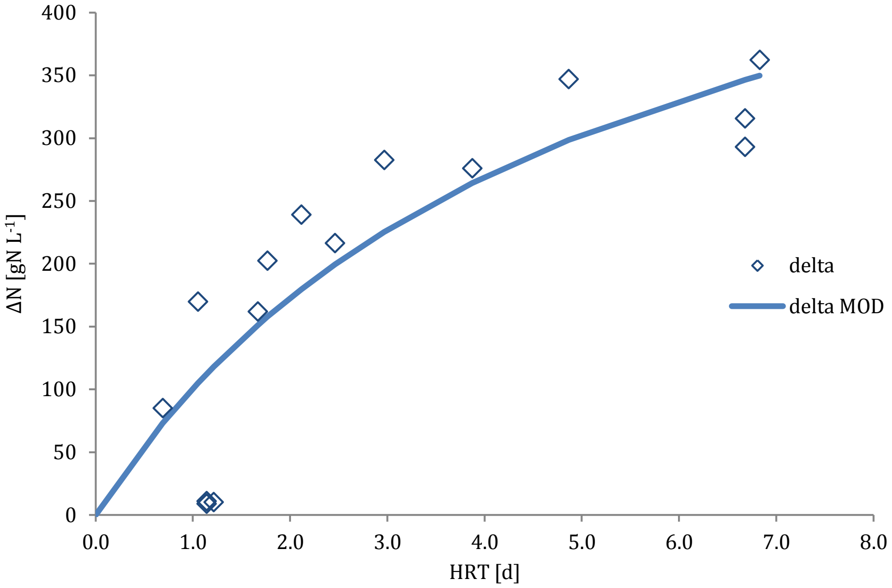
Where:

k is a first order kinetic constant

ΔN_{max} is the maxium theoretically hidrolizable nitrogen

This equation comes from the hypothesis that the hydrolysis rate of hydrolysable proteins can be described as a first order kinetics, as suggested by the kinetic model adopted for hydrolysis in the Anaerobic Digestion Model (Batstone et al., 2002).

Where k and ΔN_{max} of the model were obtained through a fit to the data to the kinetic model. If the limit of the HRT goes to infinite the model foresees a theoretical limit to the amount of ΔN that can be measured in the effluent that is the ΔN_{max}, when all the amino acids in the influent have hydrolyzed and are found in the effluent. Parameters of the model are shown in Table 6.



Graph 6 Empirical correlations between the HRT and the Delta Nitrogen

Table 12 Parameters of the regression model

ΔN_{max} [mgN L^{-1}]	608
K [d^{-1}]	0.20
R2[-]	0.75

7.2.3 Analysis of the stability of the reactor: maintenance of granulation

7.2.3.1 Biomass concentration in the reactor

The amount of VS and TS were determined as explained in the paragraph 2.1. Results are showed in Table 12

VS concentration remained between 2.2 and 4.2 g/L. Those oscillations may be caused by occasional variations in the air-flow rate which resulted in variations in the amount of granules that are lifted and dragged out of the reactor in the effluent stream. The small increase in the VS/TS ratio prove the inorganic part of the granules slightly decreased.

Table 13 Biomass concentration in the reactor during the whole operational period

Operational Day	**TS [g/L]**	**VS [g/L]**	**% VS/TS [-]**
6	10.5 ± 0.1	2.7 ± 0.2	25.4
14	11.8 ± 0.1	4.2 ± 0.2	35.6
78	5.0 ± 0.2	2.5 ± 0.2	50.3
91	9.4 ± 0.02	4.2 ± 0.1	44.9

7.2.3.2 SVI and settling velocity

SVI and settling velocity were monitored in order to assess the state of granulation in the reactor. Although their determination is easy, they are important parameters that permit to discriminate between granular or flocculent systems. The following Table 13 shows the results.

Table 14 Biomass characterization in terms of SVI and settling velocity

Operational Day	**SVI5 [mL g_{TS}^{-1}]**	**SVI30 [mL g_{TS}^{-1}]**	**SVI5/SVI30**	**Settling Velocity [m/h]**
6	11.9	11.9	**1**	88.7
91	6.6	6.6	**1**	99.5

The ratio between the SVI_5 and the SVI_{30} is a measure of the settling ability of the biomass: granules settle much faster than flocculent biomass. Therefore, in a granular system the ratio between the two values should be close to one.

A macroscopic difference between granules and flocks is the faster sedimentation of the formers. Settling capacity is then an important parameter because it is related to biomass retention and solid liquid separation in the reactor.

In Table 14 some references are given as comparison of the values obtained in this study.

SVI for flocculent systems are usually above 50 mL g_{TS}^{-1}. The low values achieved are indexes of a good settling of the biomass.

Moreover, the high settling velocity (compared to literature values) and the fact that all the biomass had already settled after five minutes are evidence that granulation was never lost.

Both the slight decrease in the SVI and increase in the settling velocity should suggest a small enhancement of granulation, maybe due to the higher conductivity of the wastewater in phase 2.

Table 15 Literature values for SVI and settling velocity

SVI5 [mL gTS^{-1}]	Settling velocity [m/h]	Kind of biomass	Reference
23	18 – 90	PN Granules	Zheng et al, 2005
-	55	CANON system	Vlaeminck et al., 2009
70-416	6.6	Anammox Granules	Dulekgurgen et al., 2008
11 - 15	40 - 60	PN Granules	Jemaat et al, 2013
-	70 - 150	Anammox Granules	Padin et al., 2009
8 -26	45 -91	PN Granules	Ramos et al., 2016
100	-	PN Floccular	Desloover et al, 2011
40	-	PN activated sludge	Ciudad et al, 2004

7.2.3.3 Diameter of the granules inside and in the outflow

Biomass could be characterized not only with solids concentration: throughout the whole experimental period diameters of the granules were determined with Mastersizer 2000.

As the following Table 15 shows, there never was any significant loss in the granulation in the reactor with the exception of the value found on day 61 that was due to a technical issue.

Conventionally, granules are considered to have a diameter higher than 0.200 mm: under this value we are talking about flocks (de Kreuk et al., 2007).

It is interesting to notice that despite the granules becoming a little bit smaller (20% reduction between the beginning and the end of the experimentation) the amount of VS in the reactor did not change significantly.

From the overall presented results on granules stability, it can be concluded that granulation was maintained stable in the reactor throughout the whole experimental period.

Table 16 Biomass diameter determination

Operational Day	Reactor	Effluent	Average diameter [μm]	D > 0.2 mm [%]
7	x		860 ± 453	96,4
34	x		587 ± 548	67,1
41	x		774 ± 124	84,4
		x	95 ± 544	20,1
61	x		703 ± 382	79,0

		x	306 ± 552	48,6
71	x		795 ± 209	86,1
		x	160 ± 512	31,1
79	x		767 ± 277	84,5
		x	255 ± 542	49,8
96	x		686 ± 63	80,5
		x	45±53	4,9

7.2.4 N_2O determination

Recently, high attention has been drowned to N_2O emissions. The GWP (global warming potential) is a measure of how much a gas is capable of trapping heat in a certain time horizon, compared with the heat trapped by CO_2: it is then an indicator of the impact of the gas on the greenhouse effect and global climate change. For N_2O, GWP_{20} and GWP_{100} are respectively 289 and 298 (for a comparison, those of methane are 72 and 25). Nitrous oxide emissions from wastewater treatment plant accounts for the 2.8% of anthropogenic emissions, mostly from biological nitrogen removal (IPCC, 2007).

DO is the main parameter affecting N_2O emissions: the lower the DO the higher the emissions (Kampschreueur et al., 2009). However different emissions factor were found in literature and it is hence difficult whether it can be assessed if there is a threshold value for minimizing emissions.
N_2O concentration was determined in the off-gas on day 98: the average of the three measured values in the off-gas was $7.5 \pm 0.2 * 10^{-5}$ ppm_v. However, N_2O emission rate depends from the air blown in the reactor.

The N_2O specific emission rate was 14.6 mg N_2O – N $L^{-1}d^{-1}$.[13]

That referred to the NLR that was applied makes:

$$N_2\,O_{rate} = 100 * {}^{14.6\frac{mg\,N}{L*d}}\Big/_{650\frac{mg\,N}{L*d}} = 2.2\%$$

Moreover, it can be interesting to also calculate the rate of N_2O conversion referred to the oxidized nitrogen as ammonium because it is a better indicator of the metabolic activity of the AOB:

$$N_2\,O_{rate} = 100 * {}^{14.6\frac{mg\,N}{L*d}}\Big/_{282\frac{mg\,N}{L*d}} = 5.2\%$$

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[13] In appendix 8.3 calculations and assumption made are shown

Where 282 mg N L^{-1} d^{-1} is the ammonium that was removed and converted to nitrite on day 98. This is a more accurate index of N_2O emissions. The DO concentration on day 98 was 3.0 mg O_2 L^{-1}.

It can be concluded that N_2O emissions were the 5.2 % of the N oxidized to NO_2^-, this value can be compared to other literature studies. Pijuan et al. (2014) with the same experimental setup (granular airlift reactor) assessed that the average N_2O emissions ranged from 6.1 - 2.2% with a DO 1.1 - 4.5 mg O_2 L^{-1}. De Graaf (2010) measured a 3.2% with a DO of 4.1 mg O_2 L^{-1} in a lab-scale PN reactor with flocculent sludge while Kampschreur (2009) a 2.5% at a DO of 5.0 in a CANON reactor at full scale. Those values are highly coherent with each other and with what found in this study.

Comparable N_2O emissions for conventional WWTP ranged from of 0.12% (von Schluthess 1994) –15% (Wicht 1995) of the N-load were found. Nevertheless, for a full understanding of the net CO_2 emissions from the two processes also higher aeration costs and external c-source supply should enter the balance. Further investigations are needed, but this is very likely to result in a global lower impact, in terms of GHG emissions, of autotrophic nitrogen removal over conventional nitrification/denitrification.

7.2.5 Specific Anammox Activity

When the feasibility of the PN process on the diluted influent was clear, also the Specific anammox Activity (SAA) test was performed on experimental days 93 and 100: this was done to show that the effluent from the partial nitritation stage would not be toxic for a following anammox reactor.

SAA were performed twice with very different biomass concentration with no significant difference between the two values obtained.
The specific inhibition of the real wastewater was then calculated as the ratio between its activity and the one of the synthetic wastewater, used as a control. Results are shown in Table 16.
Indeed, according to the SAA obtained, the effluent from the reactor would not be toxic for a subsequent anammox reactor.

Table 17 Summary of the obtained Specific Anammox Activities

Tested Influent	Biomass concentration [g_{VSS} L^{-1}]	SAA [gN g_{VSS}^{-1} d^{-1}]	Inhibition [%]
Control	0.66	0.21	-
Effluent from the PN reactor (day 93)	1.08	0.14	33
Control	2.76	0.18	-
Effluent from the PN	2.34	0.14	21

reactor (day 100)

7.2.6 Identification of microbial species in the granules

On day 98 FISH was performed to confirm that the main active population were the AOB. In the following Figure 9 are reported some of the obtained photograph: in the first one general microorganisms are identified, in green. Then there are the photos of the specific microorganisms: in the second only *βAOB* are shown (blue) and finally – barely visible - in red *Nitrobacter sp.*

Indeed, the low occurrence of *Nitrobacter sp.* and relevant presence of *βAOB* demonstrate, once again, that the latter was the only active nitrifying population and it can be concluded that NOB activity was successfully suppressed.

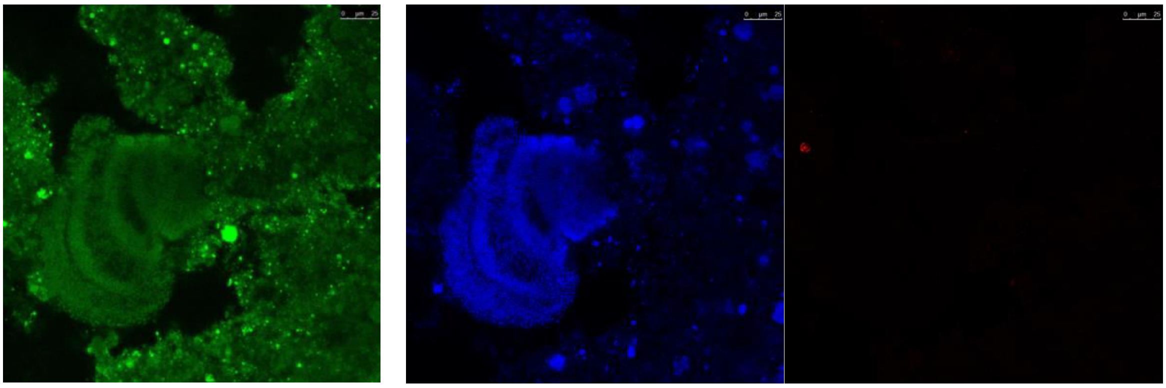

Figure 9 Different microorganisms populating the granules

7.3 Comparison with literature values

7.3.1 Successful PN through DO/TAN ratio control systems

As explained before, different strategies are possible to achieve PN.

In this experimentation, the theoretical strategy was the setting of a proper DO/TAN ratio in the bulk liquid and there are successful experiences of it in literature. With the same principle, full nitritation can be achieved as well, only by increasing the ratio and thus the amount of oxygen available to oxidize the TAN. Bartrolì et al. (2010) implemented two control loops for TAN and the DO thus achieving full nitritation of a synthetic wastewater with a NLR of 6.1 gN L^{-1} d^{-1}.

Torà (2013) added one more control loop for pH and achieved both full and partial nitritation of 9.3 ± 0.5 gN L^{-1} d^{-1} and 5.1± 1.0 gN L^{-1} d^{-1} respectively for synthetic and real wastewater in a 25L reactor. Another similar experience is the one of Jubany (2009) that coupled pH and DO set-points for NOB inhibition together with an inflow control loop based on OUR measurement to achieve an influent for an anammox reactor.

All the mentioned control loops were automatically implemented and therefore stability and reliability was intrinsic to those systems. The main advantage of the systems mentioned above is the possibility of working at the reactor maximum volumetric loading rate as ammonium is always limiting. This explains the higher volumetric loading rates achieved.
The system could be optimized to work in ammonium limiting conditions at higher DO concentrations. This might be a choice to mitigate N_2O emissions as discussed above in 5.2.4.
Besides, there is the possibility of switching from full to partial nitritation only by changing TAN set-point value.

The practical implementation of this strategy requires the availability of a reject water storage capacity to allow for a flexible regulation of the inflow rate to the PN reactor in order to achieve the desired TAN conversion efficiency. However, being sludge dewatering hardly ever a continuous process, reject water storage is typically available. In addition, such systems have the possibility of coping with fluctuations of ammonium concentration in the influent, that are typical of variable processes as anaerobic digestion.
Another aspect that could be taken into account is the economic cost of implementation of such a system at full-scale.

7.3.2 Experiences of similar influents

To our best knowledge, there are no experiences of implementation of a two stages PN/anammox process to treat the liquid fraction from the anaerobic digestion of OFMSW.

However, still comparison can be made with similar influents. In the following Table 18 some literature results are presented, along with the experimental setup and conductivity of the influent.
The experiences in literature are successful in achieving a suitable effluent to feed an anammox reactor with even higher loading rate than the one applied in this study (0.8 – 3 gN $L^{-1}d^{-1}$). The wastewaters employed are similar to the one of this study and conductivities even higher (up to 60.1 mS cm^{-1}) thus suggesting that adaption to high salinity is possible for the AOB population.

However, the level of criticality of the wastewater is very different from previous experiences because of the different origin. It would be interesting to assess, through a longer experimentation, whether it is feasible to treat this influent with a lower or not at all dilution.

Indeed, such ammonium load, conductivity and amount and variety of metal were difficultly experienced in other waste stream and could thus be toxic. On the other hand, the biomass might show some kind of adaption to the influent and a partial oxidation of the ammonium load might be achieved for the effluent employed in this study.

Table 18 Literature examples of application of PN process to similar influents

Author	**Influent for the reactor**	**Experimental setup**	**NaCl concentration [g $_{NaCl}$ L^{-1}]**	**Conductivity [mS cm^{-1}][14]**	**NLR treated [gN $L^{-1}d^{-1}$]**	**NO_2^-/NH_4^+ [-]**	**Biomass**
Liang and Liu 2006	Leachate	Fixed bed biofilm reactor	-	-	1	1-1.3	no adapted
Shinoahora et al., 2009	Brine waste	Biomass carrier	18	29.3	0.8	1.14	no adapted
Furukawa, 2009	Digester liquor	PN in an airlift with a gel carrier	-		3	1 -1.4	no adapted
Ganigue et al., 2009	Urban landiffil leachates	SBR	-	60.1	1.5	1.0 - 1.5	adapted in the study
Mosquera-Corral, 2005	Fish canning effluent with salt addition	SHARON	4,93	-	1	1	no adapted
Mosquera-Corral, 2005	Fish canning effluent with salt addition	SHARON	30	48.2	0.9	1	no adapted
Mosquera-Corral, 2005	Fish canning effluent with salt addition	Batch	5,8	10.0	fluctuating	-	no adapted
Yamamoto, 2009	Swine wastewater	Up-flow fixed bed	-	-	1	1.4	no adapted
Ganigue et al., 2009	Urban landiffil leachates	PN - SBR	-	60.1	1.5	1 - 1.3	adapted in the study
This study	Liquid fraction from AD of OFMW	Air Lift	-	8.1	0.6	1.46 ± 0.49	no adapted

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[14] The conversion between conductivity and NaCl concentration is shown in the appendix 9.2

7.3.3 Future perspective for the stability of the process

The presence of solids in the influent was not topic of investigation. The difficulties met in the experiment would have been dramatically reduced if the influent would have been filtered in order to remove colloidal and extraneous compounds, even grossly. Indeed the high amount of solids disturbed the operational setup and gave some technical issues, as already discussed.

Moreover, the control loop implemented was “manual”: adjustments of the inflow and of air-flow rate were done according to empirical considerations and not with on-line measurement. A more refined strategy to optimize the process would be an automatic control loop that, once the concentration of ammonium in the influent is measured, can set a suitable value for the inflow in order to load the reactor with the amount of Nitrogen that we know can be treated at the fixed DO. There are experiences in literature of such control systems. (Jemaat et al. 2013).

8 Results and discussion - Working Package II

8.1 Phases and reactor: influent and effluent concentrations of main parameters

The results presented in this study correspond to the operation of the SBR reactor throughout 129 Days.
In Graph 3, data of the influent and effluent nitrogen forms-concentration throughout the whole experimental period are shown. When evaluating concentration in the outlet a better indicator of reactor performances is given by nitrite concentration since ammonium was given in excess in order to work in nitrite-limiting conditions. This was done to prevent nitrite accumulation that is a strongly inhibitor of anammox process as explained before in paragraph 1.3.5.

As shown in Graph 9, during the first experimental phase (until day 14) there were low NO_2^- and NH_4^+ concentration in the effluent, thus suggesting high efficiency[15] of the anammox process (91% ± 12%). When switching to the raw wastewater on day 15 (phase 2), the worst reactor performances were observed. Nitrite was found in the effluent and there was a dramatic drop of both maximum activity and average removal rates, as shown

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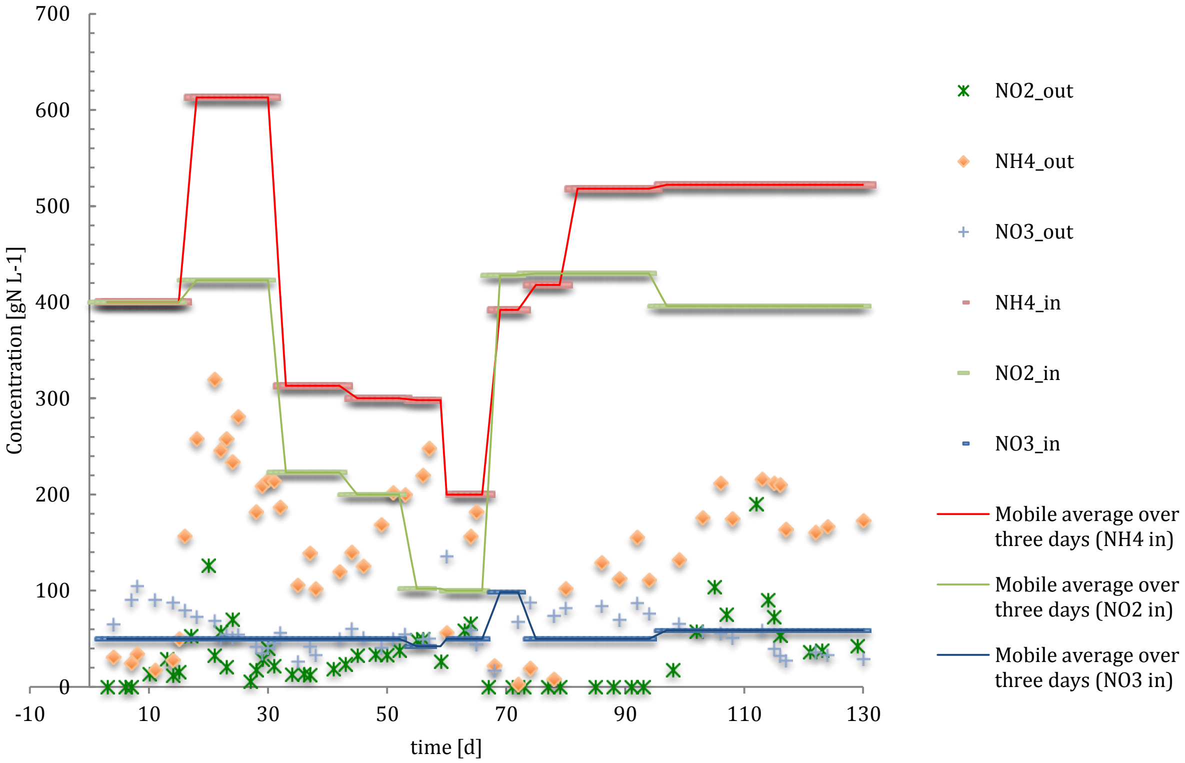
[15] The efficiency mentioned in the text is referred to nitrite removal rates as ammonium was given in excess

in Graph 10 and 11. This reduction in the activity demonstrated that the biomass could not cope with shocking changes in the influent.



Graph 9 Nitrite, nitrate and ammonium concentration in the effluent and in the influent during the course of the experimentation.

Because of this drop, in phase 3 it was tried to restore the activity by feeding the same synthetic wastewater used in phase 1 along with the bio-augmentation mentioned before (day 66). The bio-augmentation was preferred to the re-inoculation because it was thought that the biomass that had survived in the reactor might had undergone some form of adaptation. Therefore, the concentration of biomass in the reactor was increased by the 48% by addition of fresh anammox granules. Values are shown in **Table 23** in paragraph 6.3.1.

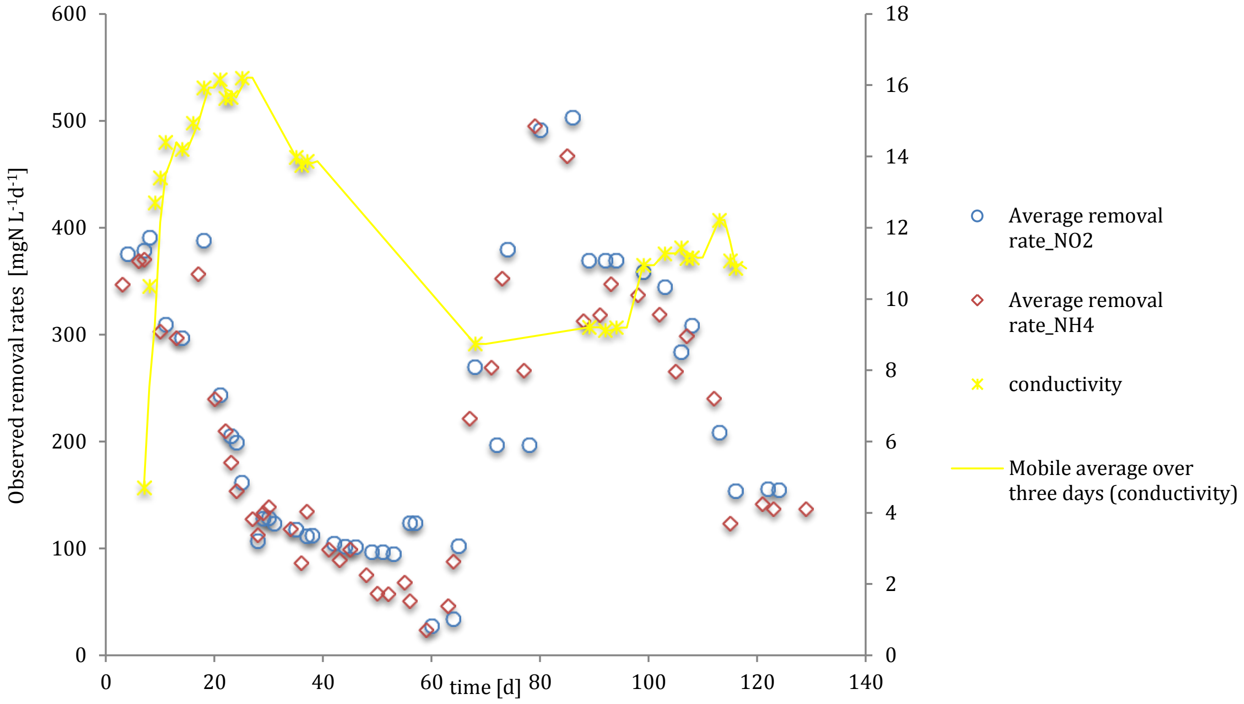
This action allowed the recovery of the activity. In the following phase 4 (days 66-93) a diluted (1:2) wastewater was treated. Under these feeding conditions, the reactor performed well: from day 67 to day 93 nitrite was never found in the effluent (nitrite removal efficiency = 98% ± 2%). This is a clear signal that the Anammox biomass could cope with the wastewater and the applied NLR.

Finally, in phase 5 from day 94 to 129 (end of the experimentation), there was a further reduction in the wastewater dilution factor. Despite observing the appearance of nitrite in the effluent, although at low concentrations, and a decrease in the activity, still the latter remained stable (removal efficiency 93% ± 12%).

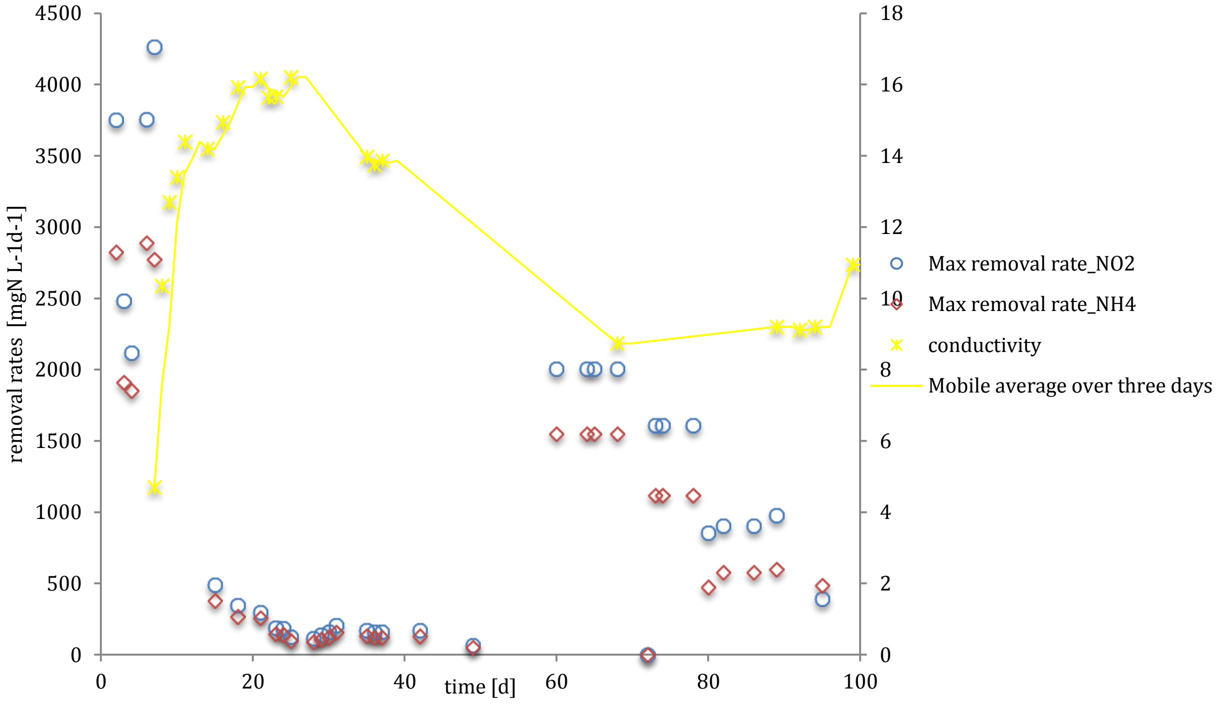
The following Graph 5 and Graph 6 detail the observed NRR and NRR_{max} throughout the whole experimental period along with the conductivity of the effluent.

The average NRR in phase 1 was 350 mg N $–NO_2^-$ $L^{-1}d^{-1}$. During phase 2, the NRR decreased constantly from 350 ± 44 mg N $–NO_2^-$ $L^{-1}d^{-1}$ to 151± 39 mg N $–NO_2^-$ $L^{-1}d^{-1}$ corresponding to an overall 57% reduction and no relevant recovery was observed during phase 3 (synthetic wastewater).

After bio-augmentation (day 66), and during the following phase 4 (diluted real wastewater), the NRR was slightly higher (370± 95 mg N $–NO_2^-$ $L^{-1}d^{-1}$) than the one measured with the synthetic wastewater (350± 44 mg N $–NH_4^+$ $L^{-1}d^{-1}$). This increase can be explained with the higher amount of biomass that was in the reactor after bio-augmentation rather than with an enhancement of the treatment capacity of the reactor, in fact those ratios are volumetric. Because of the technical difficulties in assessing the real biomass concentration, the specific reactor activity cannot be provided apart from the values at day 0 and at day 66, when the VSS of the granular suspension were reliably measured. The former value was 368 mg_N L^{-1} g_{vss}^{-1} while the latter was 150 mg_N L^{-1} g_{vss}^{-1}. During phase 5, when the influent was the 70% raw wastewater, there was a further reduction in the NRR that was steadily around 165± 24 mg N $–NO_2^-$ $L^{-1}d^{-1}$, corresponding to a 55% reduction if compared with the precedent phase.



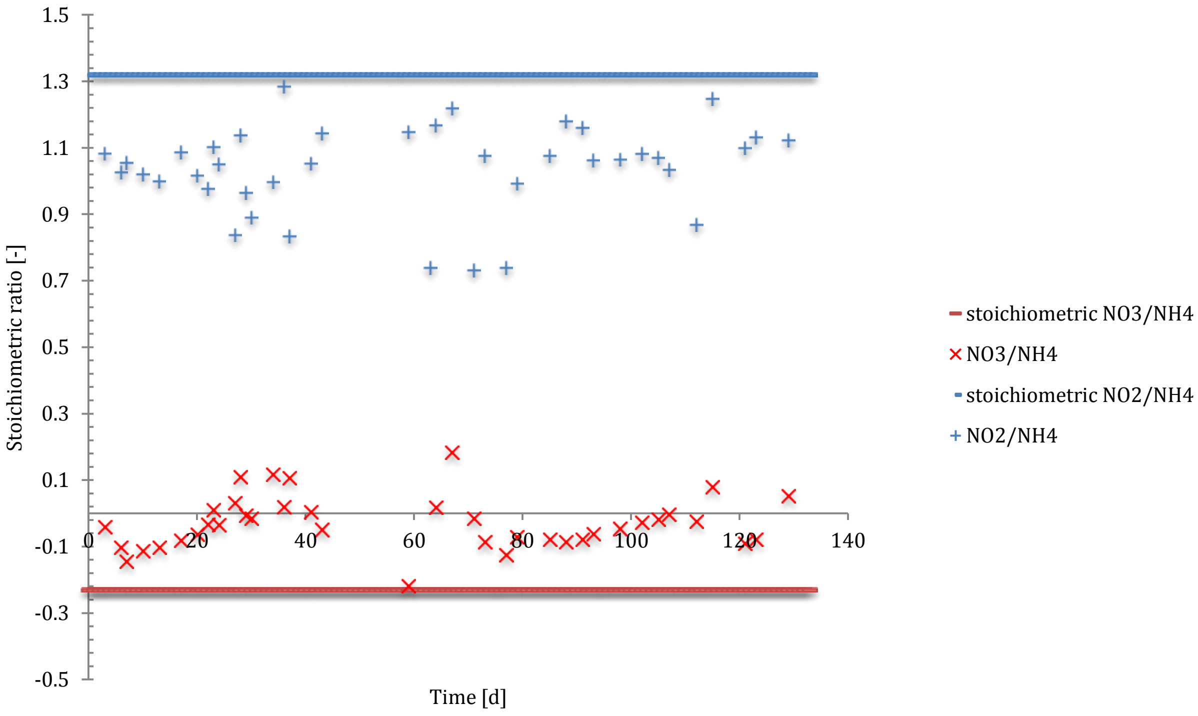
Graph 10 observed NRR in time and with different conductivity values on the secondary axis



Graph 11 The maximum nitrogen removal rate throughout the whole experimental period. Conductivity is also reported on the secondary axis.

In **Graph 10** the trends of the maximum removal rate (NRR_{max}) assessed during in-reactor batch tests are reported. This value quantifies the maximum volumetric removal capacity of the Anammox reactor and, during stable operation, is normally higher than the observed nitrogen removal rate (i.e. that reported in Graph 4). The graph is stopped at day 100 because, in phase 5, the maximum removal rate was not computed anymore since the average removal rate was more representative of the slow activity that was being observed. By comparing Graph 4 and 5, once can see that the tendency of the maximum removal rate is the same as the average removal rate. It can also be noticed that after a short transitory the activity reached a stable value in every experimental phase.

The NRR_{max} in phase 1 was 3027± 922 mg N $–NO_2^-$ $L^{-1}d^{-1}$ and it decreased to 195 ± 79 mg N $–NO_2^-$ $L^{-1}d^{-1}$ in phase 2, the 95% reduction was a clear signal that the biomass had been inhibited by the sudden switch. For this reason, after restoring the activity it was decided to decrease stepwise the dilution factor in order to allow the biomass to adapt. The strategy was successful as the NLR_{max} achieved in phase 4 was 1036± 634 mg N $–NO_2^-$ $L^{-1}d^{-1}$. Since both NRR and NRR_{max} were maintained stable for 30 operational day, it was decided to switch to a less diluted wastewater in phase 5.



Graph 12 Observed stoichiometric ratio throughout the whole experimental period. Along with punctual values, theoretical stoichiometric values are also provided

According to anammox stoichiometry, nitrite should be taken up in a ratio of 1.32 ($gN\text{-}NO_2^-/gN\text{-}NH_4^+$) over ammonium, and nitrate should be simultaneously produced according to a stoichiometric ratio of 0.26 ($gN\text{-}NO_3^-/gN\text{-}NH_4^+$). Graph 6 details the ratios between observed removal rates of the various nitrogen forms, that is to say a measure of how close the observed NRR are to the stoichiometry of the anammox reaction, represented by the blue and red lines. These data indicate that the observed nitrite removal rate was smaller than the theoretical one and there were moments when nitrate was not even produced. Average values for the observed removal ratio in each experimental phase are summarised in Table 18

Table 19 Summary of the average observed stoichiometric removal ratios

Phase	NO_2^-/NH_4^+ [-]	NO_3^-/NH_4^+ [-]
1	1.04 ± 0.03	0.08 ± 0.43
2	1.01 ± 0.10	0.09 ± 0.31
3	1.24 ± 0.30	0.18 ± 0.39
4	1.07 ± 0.14	0.08 ± 0.34
5	1.08 ± 0.10	0.09 ± 0.33

The deviation from the stoichiometry can be explained with the simultaneous occurrence of heterotrophic activity.

The fact that the nitrite to ammonium ratio is lower than the Anammox stoichiometric ratio may be due to heterotrophic activity that is responsible for the production of nitrite from nitrate thanks to the organic matter available in the influent. The topic was examined in depth in the work of Kumar and Lin (2010).

The heterotrophic conversion explains both the observed lower nitrite removal as well as the lower nitrate production. The estimation of this heterotrophic activity can be made by subtracting the observed NRR to the Anammox stoichiometric rate. The obtained nitrite production rate and nitrate removal rate should be close to one. Calculations are shown in Table 4. As a countercheck, the rate between COD removal and nitrate removal was examined. Indeed, in order to reduce NO_3^- to NO_2^- an external electron acceptor is needed. The calculation of the necessary amount of COD for heterotrophic metabolism is

$$COD_{request} = \frac{1.14}{1 - Y_H} gCOD/gN$$

Where 1.14 $g_{COD}\ g_{NO3}^{-1}$ is the value obtained from catabolism only, and Y_H is the cellular heterotrophic yield (0.67 $g_{COD\ g}\ g_{COD}^{-1}$ according to the default value assumed in the ASM1 model), thus obtaining 3.1 $g_{COD}\ g_{NO3}^{-1}$.

In order to give an estimation of the relevance of the heterotrophic activity, values are provided in Table 20, where observed COD removal rates are compared to the theoretical ones, and the errors are of 19% and 7% respectively.

Table 20 Calculation of the stoichiometry and estimation of the heterotrophic activity

Experimental day	**Observed values**			**Heterotrophic Activity**	
	NO_2^-/NH_4^+ [-]	NO_3/NH_4^+ [-]	COD r [g_{COD} L $^{-1}$d-^{1}]	NO_3^-/NO_2^- [-]	Theoretical COD [g_{COD} L $^{-1}$d-^{1}]
102	1.08	-0.03	312	1.08	253
107	1.03	0.00	243	0.99	261

8.2 Batch activity tests

8.2.1 Activity in the manometric batch tests

The results of the manometric test performed are shown in Table 20

Table 21 Results of the manometric tests carried out

Run	**Tested influent**	**Average SAA [mg N d^{-1} gVSS^{-1}]**	**Inhibition [%]**	**Conductivity influent [mS cm^{-1}]**	**Conductivity storage tank [mS cm^{-1}]**
1	Control	0.58	-	3.8	3.5
	Raw wastewater (sampling #0)	0.14	76	12.0	3.5
	High conductivity synthetic wastewater	0.21	63	11.4	3.5
2	Control	0.13	-	3.6	11.3
	High conductivity synthetic wastewater	0.055	56	12.3	11.3
	50 % raw wastewater (sampling #3)	0.12	**19**	7.5	11.3
	Raw wastewater (sampling #3)	0.049	62	11.6	11.3
3	Control	0.27	-	3.7	11.5
	Very high conductivity synthetic wastewater	0.019	91	16.0	11.5
	70 % raw wastewater (sampling #4)	0.11	50	9.3	11.5
	Raw wastewater (sampling #4)	0.077	**65**	11.6	11.5

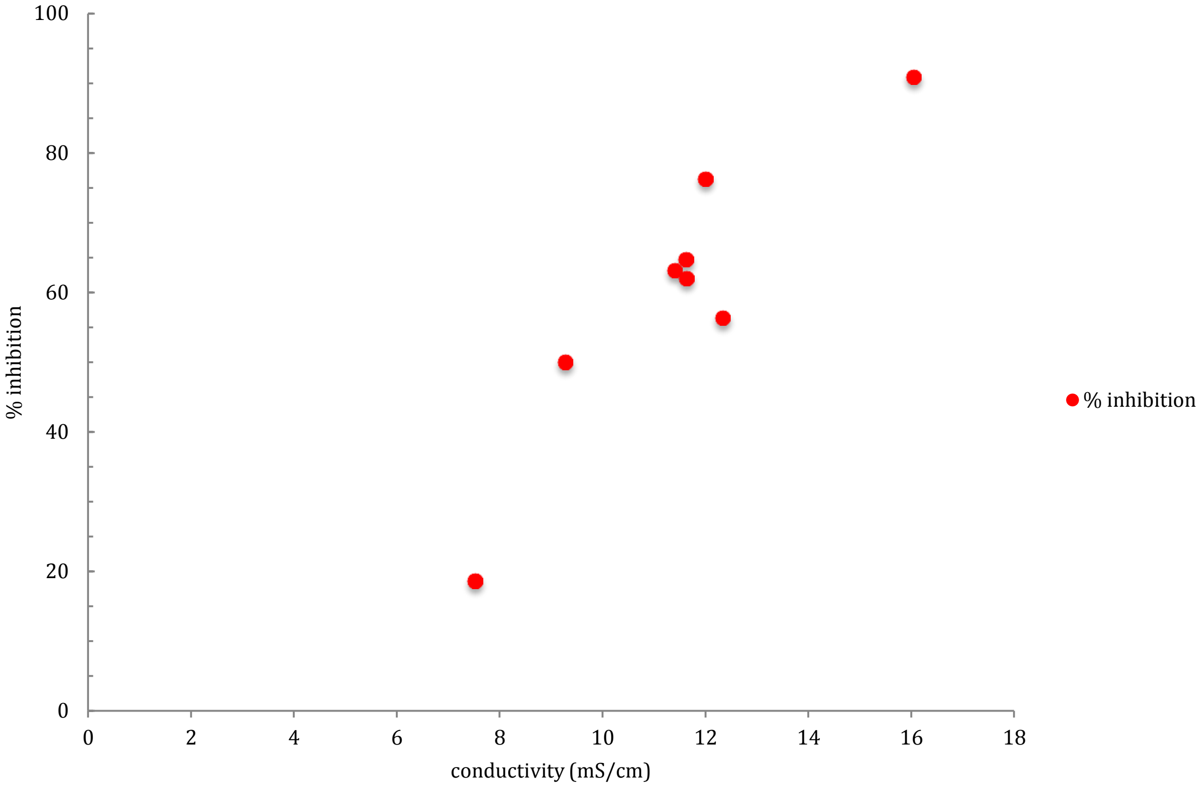
Batch test outside the reactor environment should be more reliable in providing information about the potential short term inhibition of an influent than the observed values in reactor operation.

Obtained results suggest that inhibition is strongly dependent upon conductivity of the influent. Indeed, the higher the salinity of the tested influent, the lower the measured SAA.

It should be considered that the anammox biomass used as inoculum for the inhibition tests in the first run was adapted to a low conductivity, while runs 2 and 3 were performed by using a biomass that was adapted to a higher conductivity by constantly increasing it in the storage tank (from 3.5 to 11.5 mS cm^{-1}). Interestingly, those different inocula responded differently when exposed to the same conductivity level. Specifically, the inhibition response decreased from 76 to 62% by using the adapted inoculum.

This is a very interesting result because it opens the possibility of adapting the biomass to the influent. However, above 16 mS cm^{-1} anammox activity was completely stopped despite the microorganism being adapted.

The correlation between conductivity and inhibition is shown in the following Graph 13

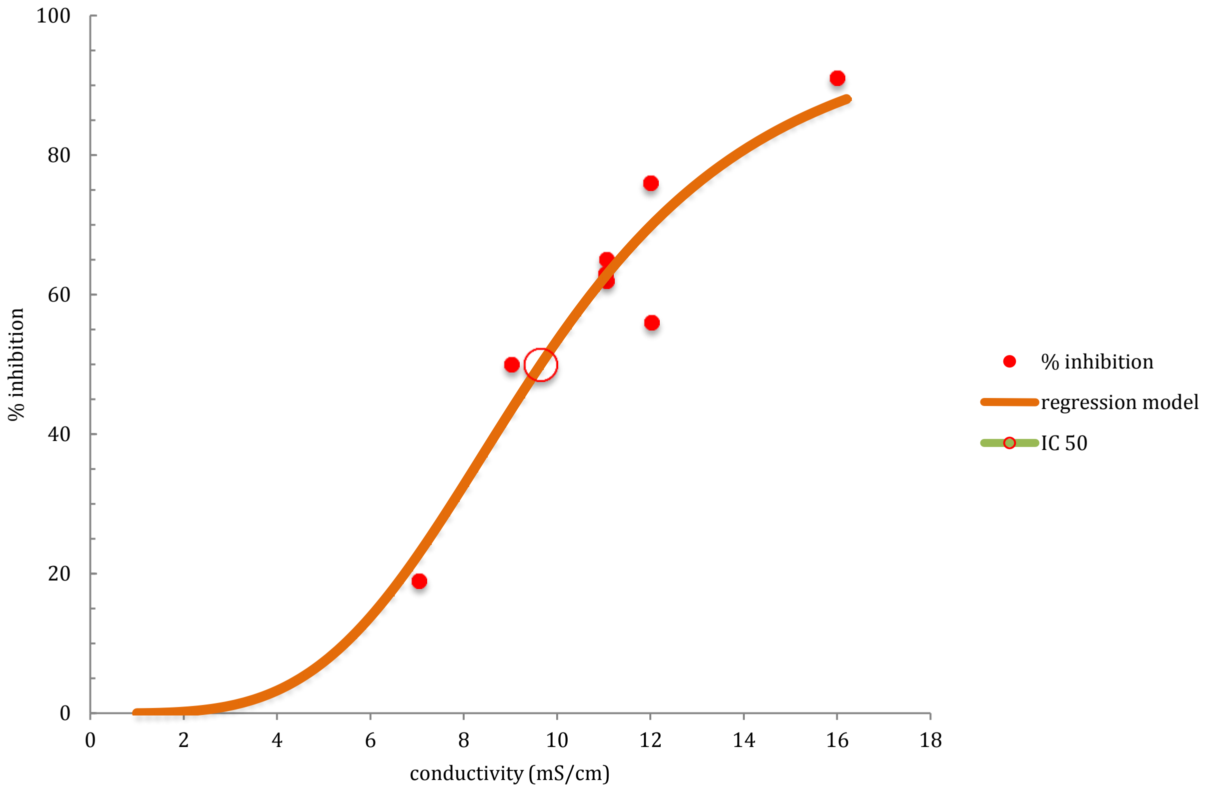


Graph 13 The observed inhibition values as function of the conductivity. An increases in conductivity immediately reduces the activity of the biomass. For a value higher than 16 mS cm^{-1} almost no nitrogen production was detected.

Conductivity is not the only parameter affecting the test. Indeed, the chemical composition of the wastewater is also expected to play a relevant role. This fact may explain why a completely different inhibition was observed for two tests with the same conductivities; hence a synthetic high conductivity wastewater and the raw influent induced a 56% (biomass adapted) and 76% (biomass no adapted) inhibition respectively.

It can be assessed that also other influent characteristics (content of metal, COD, general toxicity) are relevant and contribute to the potential inhibition of a wastewater.

Moreover, in the lasts batch tests the adapted biomass reacted well to the exposition of a low conductivity influent as the control. Apparently, no significant osmotic shock was suffered by anammox granules when instantly moved from a solution highly saline to a lower salinity one.



Graph 14 The fitting of the data with a non-competitive inhibition model. The value of IC_{50} I, for which the activity of the biomass is halved, is also reported in the graph. The interpolation was achieved a no-linear regression on the experimental data.

Finally, since the correlation between inhibition and conductivity of the influent appeared to be evident, it was tried to fit the data with a competitive inhibition model through a no-linear regression:

$$y = \left(1 - \frac{1}{1 + \left(\frac{x}{k_1}\right)^{k_2}}\right) * 100$$

Where:
y is the percentage inhibition [%]
x is the conductivity of the influent [mS cm^{-1}]
k_1 and k_2 are model parameters to be estimated by fitting experimental data

Particularly important is the value of k_1 because it represents the IC_{50}. Those values are respectively 9.7 mS cm^{-1} and 3.9. The R^2 of the model is 0.90 thus suggesting a good fitting of the data.

This results corroborates the empirical evidence and gives a quantitative indication of the expected inhibition given the conductivity. This model describes the short term response of the anammox biomass to an increased salinity. Potential long-term adaptation may be expected and would be observed during the long-term operation of a continuous anammox reactor.

8.2.2 Comparison between the obtained values

The inhibition measured in batch tests is in accordance with the reduction of the maximum removal rate observed when moving from the synthetic to the real wastewater. The raw wastewater was tested before being fed to the reactor.
However, the phenomenon of inhibition in the short term was stronger in the reactor where it was observed a ten folds reduction in the maximum removal rate if compared with the synthetic wastewater fed in the precedent phase 1, 95% less as discussed before in 6.1.
As for the average NRR, it underwent a 57% reduction in the reactor from phase 1 to phase 2 that can be compared with the obtained values in batch tests of 62% – 65% inhibition.
The inhibition found for the 50% diluted wastewater in the manometric batch test was lower than what seen in operation in the reactor for the NRR_{max}, those values were respectively 20% and 68%. In the long term, there are more factors influencing the reactor performance that in the ideal environment of the batch test cannot be assessed.
However, the average NRR was just the 6% less, for the reasons explained before of the increase in the biomass concentration at the beginning of phase 4.

If the biomass concentration could have been determined more often, data could be calculated on the specific activity of the reactor, thus providing a better comparison with the batch tests.

8.2.3 Literature review, a comparison

To our best knowledge, no published results exist of the application of autotrophic nitrogen removal on the liquid fraction of the AD OFMSW. Nonetheless, this study can be compared with previous experiences on the treatment of similar influents such as leachate, synthetic high salinity wastewaters and wastewaters from the food industry.

Increased salinity means an increase in the osmotic pressure in the medium outside the cells, thus affecting transport phenomenon inside the membrane and therefore it may cause a metabolic stress to the anammox biomass.

The following Table 22includes a summary of applying anammox to the treatment of similar wastewaters with high salinity levels. Sometimes comparisons are difficult because the influent, the experimental set-up or the duration of the experimentation differ from each other and from those applied in this work.

Within the literature experiences, the one of Ruscalleda et al. (2010) appears to be more similar to the present study. Indeed, these authors treated diluted urban landfilling leachate, thus a wastewater originated from bio-wastes, having a conductivity value very similar to that of the present study. In their work, they experienced a drastic reduction of the NRR when reducing the dilution factor and hence increasing the conductivity from 10.2 to 17.0 mS/cm. Eventually, the average achieved NRR was 0.21 g_N L^{-1} d^{-1} with a NLR of 0.24 gN L^{-1} d^{-1}. The NRR appears to be very similar to the value obtained in this study (i.e. 0.29 gN L^{-1} d^{-1} when operating with a dilution factor of 0.43).

Those values compare well with what obtained by Dapena Mora that achieved to remove from 0.44 and 1.19 gN L^{-1} d^{-1}, however the removal efficiency was just the 68%.

However, the adaption capacity shown in this study, along with the discovery of anammox bacteria in marine environment (Arrigo 2005) appear to be clear signals that high conductivity wastewaters can be treated with an autotrophic nitrogen removal process. As reported in Table 22, several authors (Liu, Kartal, Windey, Figureao, Zhang, Hwang, Karakashew, Ahn, Yamamamoto, Yang) could successfully cope with the treatment of highly saline wastewater (10-80 mS cm^{-1}) achieving NRR between 0.08 and 1.67 gN L^{-1} d^{-1}.

Table 22 Experiences in literature of similar influents treated with the anammox process in different configurations. Stoichiometric removal ratios are reported along with nitrogen loads. Conductivity is reported when available. When not reported in the study, it was calculated through the following relation: cond [mS cm^{-1}] =$[NaCl]_{conc}$*1.58 + 0.39 [16]

Author	Influent for the reactor	Experimental setup	Salt employed	Concentration [g L^{-1}]	Conductivity [$mScm^{-1}$ @20°C]	NLR treated [$gNL^{-1}d^{-1}$]	NRR [$gNL^{-1}d^{-1}$]	NO_2^-/ NH_4^+	NO_3/ NH_4^+
Dapena Mora 2006	Fish canning effluent	SBR	NaCL	19	16.6	0.44 - 1.09	0.34[17]	1.67	0.28
Liu et al., 2009	Synthetic wastewater	Fixed bed reactor	NaCl	30	48.2	1.70	0.3	1.29	0.24
Kartal, 2006	High salinity wastewater	SBR	90% NaCl, 10% K Cl	30	48.2	0.65	-	1.3	-
Kartal, 2006	High salinity wastewater	SBR		45	72.0	Irreversibile inhibited	-	1.6	-
Kartal, 2006	High salinity wastewater	Batch		45	72.0	SAA reduced of the 10%[18]	-	1.6	-
Windey 2005	High salinity wastewater	RBC(OLAND)	NaCl	20	36.0	0.85	0.59	-	-
Windey 2005	High salinity wastewater	RBC(OLAND)	NaCl	30	48.2	0.53	0.43	-	-
Windey 2005	High salinity wastewater	RBC(OLAND)	NaCl	30	48.2	0.73	0.61	-	-
Windey 2005	High salinity wastewater	RBC(OLAND) -short term	NaCl shock load	30	48.2			-	-
Figureao 2012	Diluted swine slurry	CANON	-	-	-	0.61	0.46	-	-
Ruscalleda et al., 2010	Diluted urban landfill leachates	SBR	-	-	from 10.2 to 17.0	0.24	0.21	1.40	-
Zhang et al, 2010	High salinity waste water	CANON biofilm reactor	NaCl	10	16.6	0.07		-	-
Zhang et al, 2012	Swine digester liquor	SBBR (CANON)	-	-		0.26	0.10	-	-
Hwang et al. 2006	Piggery waste	SHARON /ANAMMOX	-	-		0.62	0.08-0.42	-	-
Karakashew et al., 2008	Pig manure	OLAND	-	-		0.40	-	-	-

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[17] average value

[18] this is a manometri batch test and not a reactor operation

Author	Influent for the reactor	Experimental setup	Salt employed	Concentration [g L^{-1}]	Conductivity [mScm^{-1} @20°C]	NLR treated [gNL^{-1}d^{-1}]	NRR [gNL^{-1}d^{-1}]	NO$_2^-$/ NH$_4^+$	NO$_3$/ NH$_4^+$
Ahn et al., 2004	Piggery waste	UASB	-	-		0.10	-	-	-
Yamamoto et al., 2008	Swine wastewater	PN/ANAMMOX	-	-		0.66	-	-	-
Yang 2010	Synthetic wastewater	Up flown column reactor	NaCl	30	53.6	-	-	-	-
This study	Liquid fraction from AD of OFMW (1:2 dilution)	SBR	-	-	9.2	0.84	0.72	1.07	0.08
This study	Liquid fraction from AD of OFMW (70:30 dilution)	SBR	-	-	11.3	0.60	0.30	1.08	0.09
This study	Liquid fraction from AD of OFMW (raw)	SBR	-	-	13.5	0.52	0.30	1.01	0.09

8.3 Analysis of the biomass in the reactor

8.3.1 Biomass concentration

Average values are provided in **Table 21**.

Table 23 Biomass concentration at different operational time

Operational Day	TS [g/L]	VS [g/L]	% VS/TS [-]
0	19.8 ± 0.08	7.7 ± 0.2	38.8
57	19.3 ± 0.06	6.2 ± 0.2	32.2
66	28.2 ± 0.3	10.3 ± 1.0	36.7

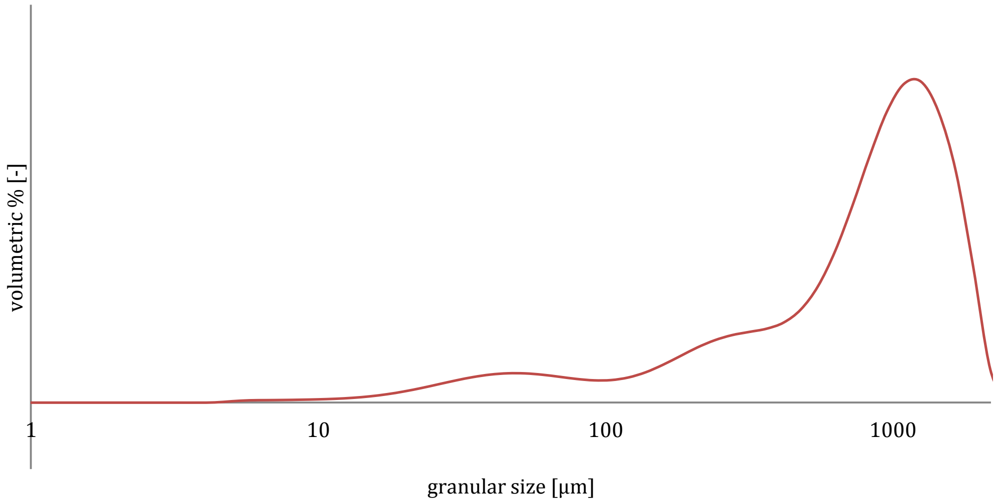
It can be noticed a decrease in the relative importance of VS on TS on day 57. This phenomenon can be due to mineralization of the biomass in the operation with the raw wastewater, thus involving a growth in the inorganic content of the granule.

The 20% reduction in VSS concentration in the reactor was one of the reasons to perform the bio-augmentation along with the reduction in the activity, as explained before. The biomass used to perform it on day 66 was inoculated from the tank, therefore the global TS decreased and it resulted in an increase in the ratio VS/TS.

8.3.2 Granules diameter

Granules diameters were determined as in 3.4.6.

The diameter of biomass used as inoculum in this study was determined with the Mastersizer 2000 as explained before. The granules volumetric distribution is showed in Graph 8.



Graph 15 The distribution of the particle size. The unimodal distribution is a clear index that the granular system was not affected from the wastewater

The measured average was 811± 547 µm. Those are typical values for a granular system, well above the 200 µm that discriminate with flocculent biomass.

8.3.3 Settling velocity

Settling velocity was determined as described in 3.4.7
The obtained value is 136.3 m/h.
The good settling properties if compared with the reference given in Table 15 were a further confirmation of a granular system.

9 Conclusions

The main objective of this work was to evaluate the feasibility of the application of an innovative treatment such as the biological autotrophic nitrogen removal to the liquid fraction of the digestate with the final purpose of promoting the energetic valorization of the organic fraction of urban wastes through its anaerobic digestion.
To the author best knowledge, this was not investigated before.

The operation and monitoring of the two reactors (partial nitritation and anammox) performances allowed assessing process stability as well as treatment capacities.

The partial nitritation process (WP1) was performed in the air-lift reactor of the Universitat Autonòma de Barcelona. This process achieved the oxidation of half the ammonium influent ammonium load to nitrite and a significant organic matter removal, reaching in the final part of the experimental period an effluent suitable for a subsequent anammox reactor. Biomass granulation was maintained throughout the whole experimental period (101 days). Moreover, NOB activity was effectively suppressed, as confirmed by FISH analysis. Yet, a longer experimentation would have allowed decreasing the dilution of the influent in order to assess the possibility of treatment of the raw wastewater.
In the final experimental period, the effluent stoichiometric ratio was 1.46 ± 0.48 with a NLR of 0.65 ± 0.1 mg $N - NH_4^+$ d^{-1} L^{-1} that is very close to stoichiometric value of 1.32. However, the maximum capacity of the reactor was higher and the system would be more performing with an automatic control loop adjusting the inflow rate to achieve the oxidation of just half of the ammonium present.
Moreover, in this last phase the 44% removal of TOC was achieved at a TOC loading rate of 0.61 ± 0.09 g_{TOC} $L^{-1}d^{-1}$, with an effluent TOC/N ratio of 0.57 g_{TOC} g_N^{-1}.

Moreover, the partial nitritation reactor was highly stable and NOB bacteria activity was successfully suppressed.

In the WP2 carried out in Milano, the kinetics of the anammox process were investigated as well as possible inhibition of the biomass in the short, medium and long term.

The stepwise decrease in the dilution factor allowed the determination of a correlation between conductivity in the influent and nitrogen removal rates. Despite a consistent reduction in the activity of the reactor was observed, still it remained stable. The long experimental period allows concluding that the liquid fraction of the digestate is not toxic for the anammox biomass, no in the short nor in the long period.

Indeed, adaption phenomena could be observed. This was not unexpected since anammox bacteria have been identified in marine ecosystems where salinity is higher than the one studied in this work. The reactor was not satisfactorily performing when feeding the raw wastewater (phase 2). However, better performances were observed by feeding a 50% diluted wastewater (phase 4). In this phase, the average applied NLR was of 0.84 g N d^{-1} L^{-1} and the achieved NRR for nitrite and ammonium were 0.37± 0.1 mg N $–NO_2^-$ $L^{-1}d^{-1}$ and 0.35 ± 0.04 mg N $–NH_4^+$ $L^{-1}d^{-1}$ respectively. The more concentrated wastewater fed in phase 5 with an average NLR of 0.6 mg N d^{-1} L^{-1} decreased the activity of the 59%, but still the anammox biomass was not inhibited, and the NRR was steadely around 165± 24 mg N $–NO_2^-$ $L^{-1}d^{-1}$.

In manometric batch tests, the lowest inhibition was observed when using a biomass inoculum previously adapted to high conductivity levels, which responded similarly to the SBR biomass. This suggests that adaptation of the anammox biomass to conductivity is possible and was taking place in the SBR.

Also, the IC_{50} for conductivity was determined through a no linear regression model on data obtained from the batch inhibition tests. The founded value of 9.7 mS cm^{-1} is a useful information to predict the short term response of an anammox reactor to increasing conductibvity levels in the influent.

The overall conclusion is that autotrophic nitrogen removal through the partial nitritation/anammox process might be a feasible alternative to the current more expensive treatment of nitrification/denitrification. However, further investigations are needed to elucidate the adaption phenomena and potential other inhibiting factors beyond conductivity, along with considerations for the scale up of the process. Indeed, the experimentation is still ongoing.

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11 Appendix

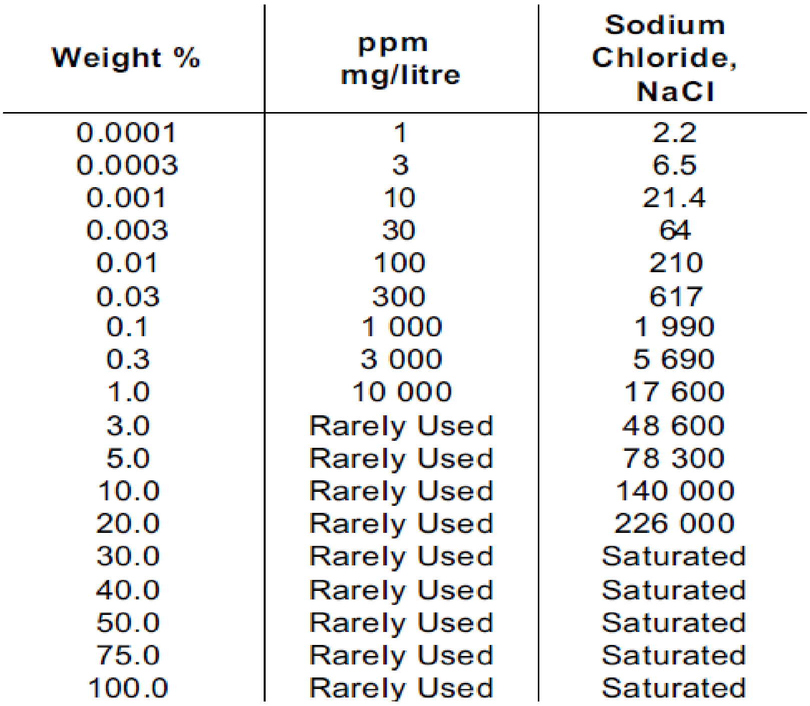
11.1 Solutions employed

*In the super high conductivity wastewater the following compounds and micronutrients were present (concentrations are expressed in g L^{-1}): $CaCl_2H_2O$, 0.3; KCl, 3.78; NaCl, 5; $NaHCO_3$, 2.014; $MgSO_4$*$7H_2O$, 0.91; $NaNO_3$, 0.607.*

In the control solution instead the following compounds and micronutrients were present (concentrations are expressed in g L^{-1}): ; K_2HPO_4, 0.025; $NaHCO_3$, 1; Ca^{2+}, 0.625; Mg^{2+} , 3.5; Fe^{2+}, 2; trace elements (solution 2), 1.25. The pH was set at 7.5 by dosing NaOH.

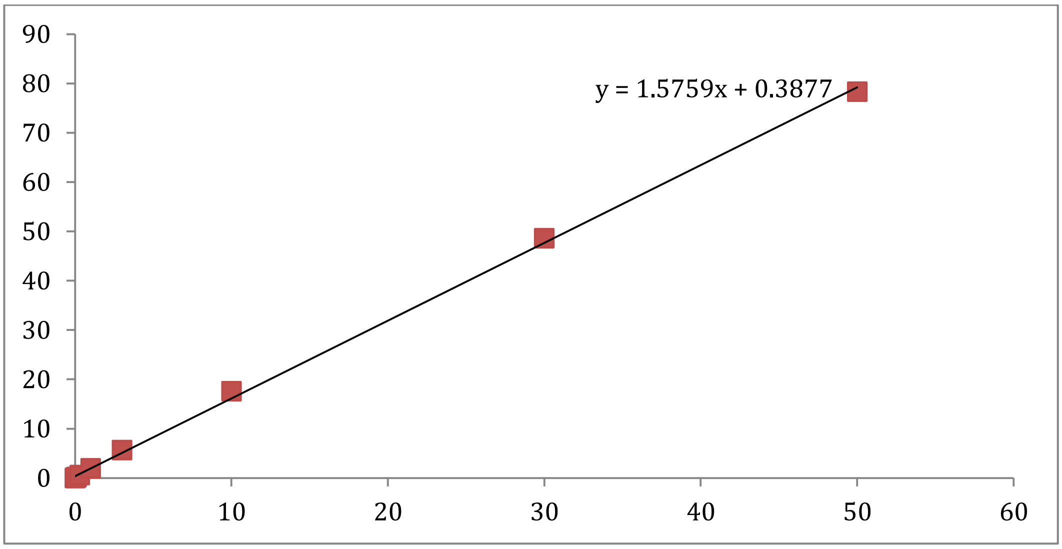
11.2 Regression for the conversion of salinity to conductivity

Table 24 A typical correlation between salt concentration and conductivity @ 25°

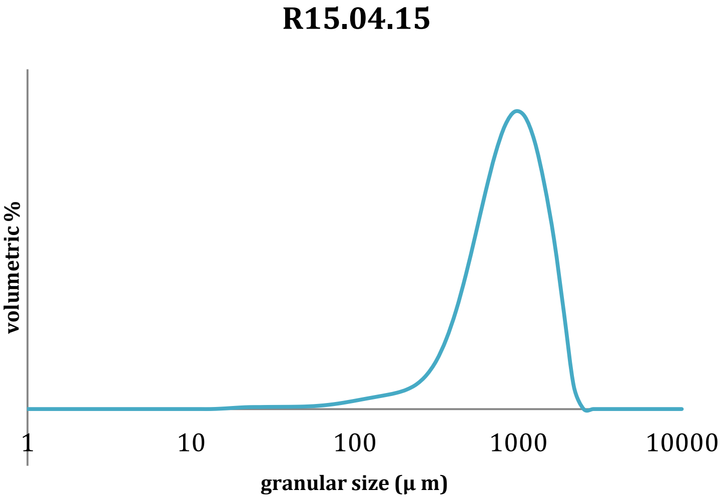


Weight %	ppm mg/litre	Sodium Chloride, NaCl
0.0001	1	2.2
0.0003	3	6.5
0.001	10	21.4
0.003	30	64
0.01	100	210
0.03	300	617
0.1	1 000	1 990
0.3	3 000	5 690
1.0	10 000	17 600
3.0	Rarely Used	48 600
5.0	Rarely Used	78 300
10.0	Rarely Used	140 000
20.0	Rarely Used	226 000
30.0	Rarely Used	Saturated
40.0	Rarely Used	Saturated
50.0	Rarely Used	Saturated
75.0	Rarely Used	Saturated
100.0	Rarely Used	Saturated

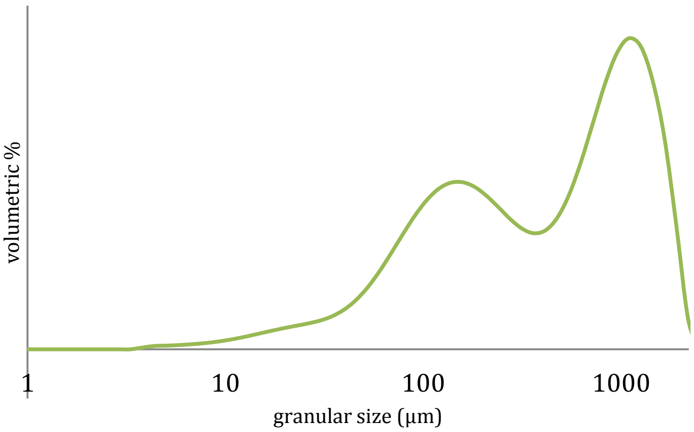
For the sake of simplicity, the influence of temperature was not taken into account.
Through a linear interpolation, the following relation was found:
$Y=1.58x+0.39$



11.3 Granulometric distribution in the PN reactor



R12.05.15



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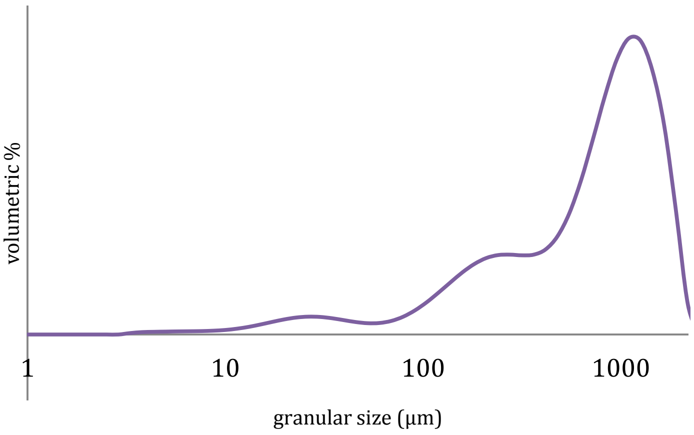
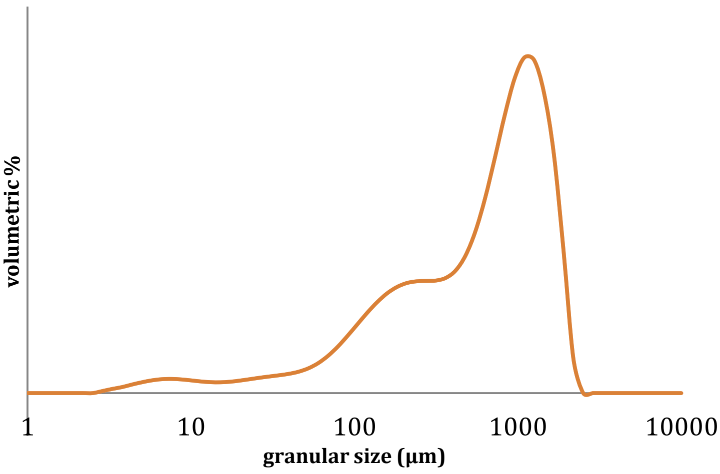
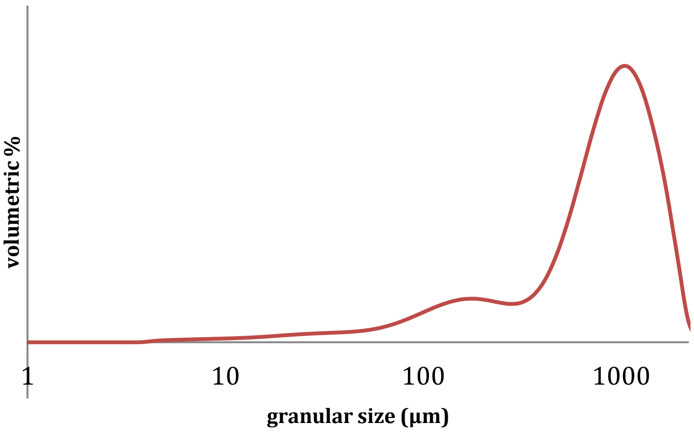
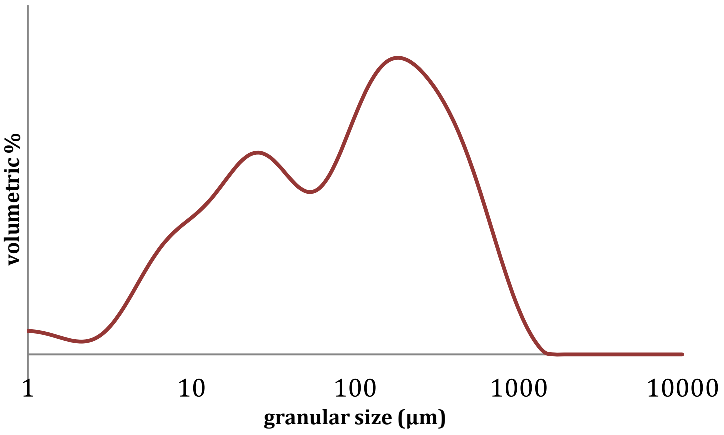
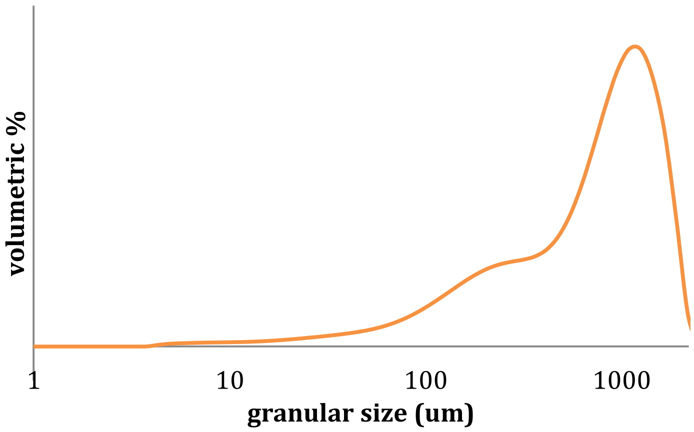


R8.06.15




R18.06.15


Effluent 18.06.15 [19]


R26.06.15

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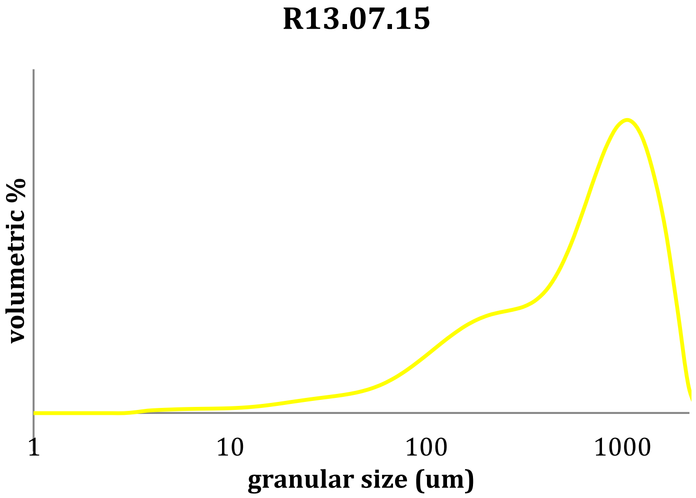
[19] For the effluent of the PN reactor, only one granulometric distribution is reported because of the relative minor importance. From the graph it is evident that granulation was not being lost.



11.4 N_2O emission rate determination

The concentration of N_2O in the off-gas should be corrected for the amount of oxygen consumed for oxidizing ammonium, since the air used by the stoichiometry (and thus not exiting the reactor) should not be taken into account in N_2O emission calculation.

For this calculation, it can first be calculated the oxygen uptake rate of the reactor (OUR) expressed as the amount of oxygen consumed daily in the reactor. In this calculation, the theoretic value of the NLR was used. Therefore, using the partial nitritation stoichiometry:

$$OUR = \frac{\eta_{oxidation} * NH_{3load} * \frac{3}{2}\frac{mol\ O_2}{mol\ NH_3} * \frac{16g\ O_2}{mol\ O_2}}{14\frac{gNH_3}{molNH_3}}$$

$$= \frac{0.5 * 1000\ \frac{mgNH_3}{L_{reactor} * d} * \frac{3}{2}\frac{mmol\ O_2}{mmol\ NH_3} * \frac{16mg\ O_2}{mmol\ O_2}}{14\frac{mgNH_3}{mmolNH_3}} = \frac{1714mg\ O_2}{L * d}$$

As the air inflow was regulated as liter of air per minute, its value should further computed considering the ideal gas law, the process temperature, O_2% in air and the reactor specific volume:

$$Air_{needed} = \frac{OUR * \frac{22.4L\, O_2}{mol\, O_2}}{\frac{16g\, O_2}{mol\, O_2}} * \frac{(273{,}15\, K + 20[20] K)}{273{,}15K} * 2.6L_{reactor} \frac{mL\, air}{0.21\, mL\, O_2} * \frac{d}{60 * 24\, min} =$$

$$= \frac{1714 * \frac{22.4L\, O_2}{mol\, O_2}}{\frac{16mg\, O_2}{mmol\, O_2}} * \frac{(273{,}15\, K + 30K)}{273{,}15K} * 2.6L_{reactor} \frac{mL\, air}{0.21\, mL\, O_2} * \frac{d}{60 * 24\, min}$$

$$= 3348 \frac{mL\, O_2}{d}$$

As the air inflow was regulated as liter of air per minute, its value should further computed considering O_2% in air:

$$Air_{needed} = 3463 \frac{mL\, O_2}{d} * \frac{mL\, air}{0.21\, mL\, O_2} * \frac{d}{60 * 24\, min} = 11.1 \frac{mL\, air}{min}$$

This accounts for about the 4% of the air blown and it can be neglected in the following calculations.

N_2O daily production can then be determined without subtracting the air used for oxidation:

$$N_2\, O_{emission} = 0.000075 \frac{mL\, N_2O}{mL\, air} * 280 \frac{mL\, air}{min} * \frac{60 * 24\, min}{d} = 30.4 \frac{mL\, N_2O}{d}$$

A better understanding of emissions can be given by making this value specific to the working volume of the reactor and expressing it as nitrogen:

$$N_2\, O_{specific\, rate} = 24.2 \frac{mL\, N_2O}{d} * \frac{mmol\, N_2O}{22.4mL\, N_2O} * \frac{28\, mg\, N_2O}{mmol\, N_2O} * \frac{1}{2.6L} = 14.6 \frac{mg\, N}{L * d}$$

11.5 Pyrosequencing analysis

Identification of the microbial population at the end of the reactor operation (day-380) was performed using next-generation sequencing. Total genomic DNA of the aerobic granular biomass was extracted and purified using a PowerBiofilmTM DNA Isolation Kit (MoBio Laboratories, USA); in accordance with the manufacturer's instructions. Paired-end sequencing of the extracted DNA was performed on Illumina MiSeq platform by Research and Testing Laboratory (Lubbock, Texas, USA).

The analysis was performed to determine the microbial population at class and genus level of the aerobic granules at the beginning and at the end of the experimental period. For the first experimental phase, Alphaproteobacteria,“No Hit” and Betaproteobacteria were the

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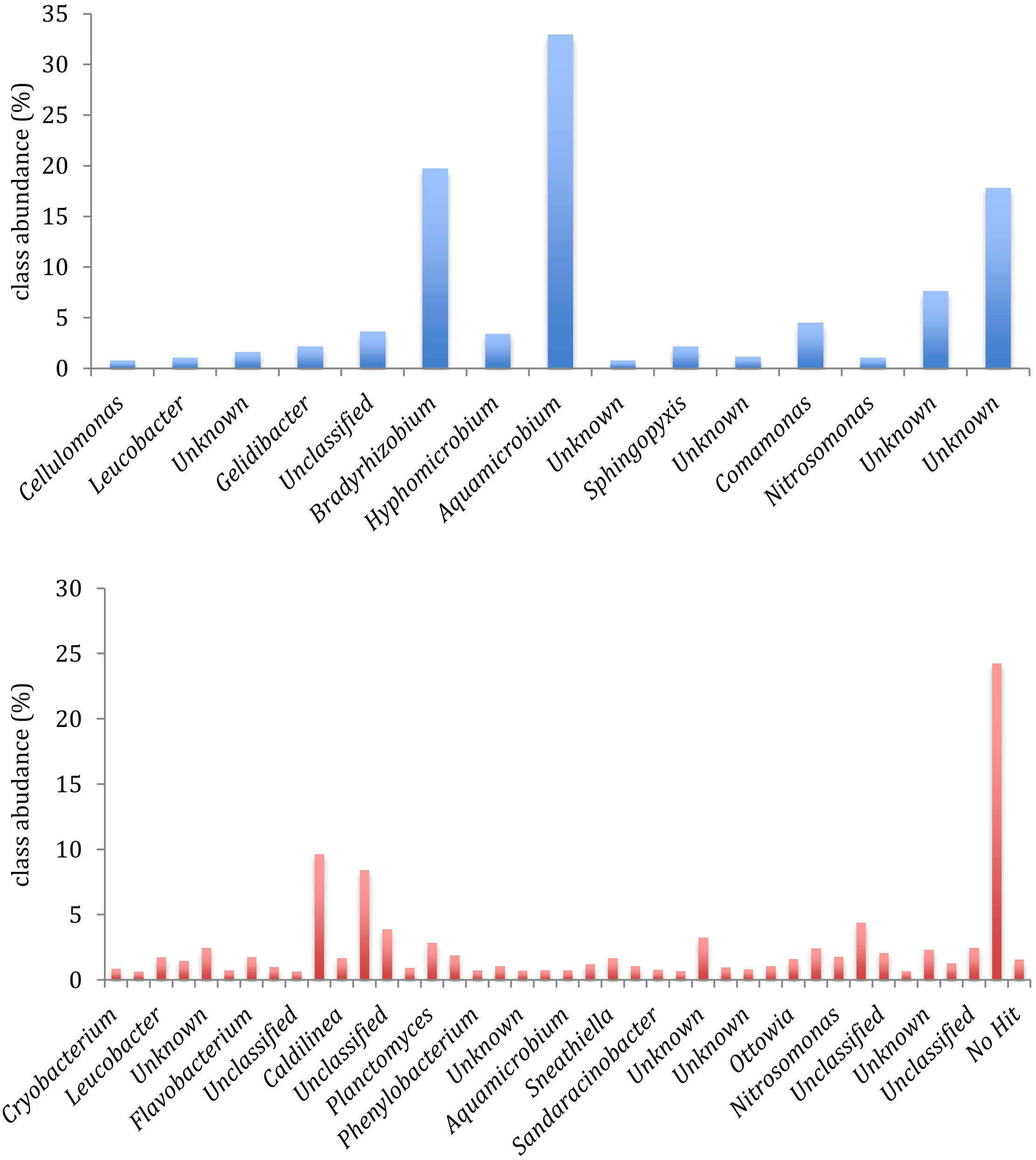
[20] SInce the rotometer was outside the reactor the temperature is the ambient temperature

main classes identified; representing 90% of the reads. At genus level, Bradyrhizobium (20%), Aquamicrobium (33%), “No Hit” (18%) and Comamonas (5%) were mainly detected.

At the end of experimentation instead, the main classes were Alphaproteobacteria, “No Hit”, Betaproteobacteria and Gammaproteobacteria, accounted for the 57% of the reads. At genus level, “Unknown” (24%), Sphaerobacter (8%),Planctomyces (3%), “Unclassified” (4%)were detected. From the comparison of these results, it can be concluded that most of the main classes and especially the genera changed.



Graph 16 Relative class abundances for the two experimental phases