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NOVEL BIOLOGICAL SUSTANABLE SOLUTIONS TO OPTIMIZE BIORESOURCE RECOVERY AND ENERGY EFFICIENCY FROM DOWNSTREAM OF ANAEROBIC DIGESTION

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Table of Content

List of Figure

List of Table

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Abstract

The main activities during three years of research, focused on the investigation, optimization as well as the validation beyond bench-scale to pilot reactor, of **advanced via-nitrite biological processes** for the side stream treatment of anaerobic effluents. The addressing of technical barriers and providing engineering solutions is also fundamental to understand the potential transferability and the commercial interest for large scale applications of such technologies and the strategies for how to exploit it. Therefore, the experimental activities have been carried out using real substrates (e.g. anaerobic supernatant, municipal sewage sludge, OFMSW, municipal domestic wastewater), thus reflecting the real environmental and operating conditions.

The anaerobic processes have gained high attention during the last years, because of the advantages connected with the production of green energy from the biogas utilization. Compared with the consolidated aerobic applications for the treatment of wastewater, the anaerobic processes allow less energy consumption, less sludge production, elimination of the off-gas air pollution and a potential for lower carbon footprint. The upflow anaerobic sludge blanket (UASB), is one af the most upgraded technology for the anaerobic treatment of the municipal wastewater. However, the effluents from the anaerobic processes still contain high amount of nutrients (nitrogen and phosphorus), which can be font of eutrophication for the water bodies. In addition, the effluents derived from the anaerobic digestion or co-digestion of the sewage sludge with organic biowaste (e.g. solid fraction of the municipal solid waste) represent an high strength nitrogenous stream which should be treated before recycled back into the main stream of the wastewater treatment plant (WWTP). Nutrients removal processes via nitrite are recognized to be a sustainable option to treat low and high strength nitrogenous effluents, such as municipal wastewater and the anaerobic supernatant from digested sewage sludge respectively. The application of innovative bioprocesses that will increase the on-site biological valorization of wastewater and sewage sludge is the major challenge.

The via-nitrite processes were investigated in a sequencing bacth reactor (SBR) for the treatment of anaerobic effluents from a UASB reactor. The municipal anaerobic UASB effluent with low carbon to nitrogen ratio (C/N), was treated through the completely autotrophic nitrogen removal process. The activities of anammox biomass was increased up to 161% compared with the initial inoculum, which correspond with nitrogen removal rate of 2.27±1.31 mgN $(gVSS\ h)^{-1}$ at 30 °C. The latter did not change significantly when the volumetric NLR was decreased but lower

heterotrophic denitrifying activities was observed and the nitrogen conversion ratios approached to 1.32 gNO_2-N/gNH_4-N removed. The Fluorescent In Situ Hybridisation analyses (FISH) confirmed the presence of anammox bacteria and different filamentous bacteria favoured by the long solid retention time (SRT). Although nitrogen was removed effectively from anammox biomass, phosphorus cannot be significantly eliminated by a complete autotrophic biomass. For this reason, the applicability of the integrated upflow anaerobic sludge blanket (UASB) with a short-cut nutrient removal was demonstrated feasible by the co-treatment of domestic sewage and biowaste at decentralized level. The occurrence of the simultaneous removal of nitrogen and phosphorus via-nitrite in a robust process was investigated using the best available carbon source produced by the acidogenic fermentation of domestic organic waste (DOW) and vegetable and fruit waste (VFW). Complete nitrite accumulation (NO_2-N/NO_x-N>97%) was observed operating at low dissolved oxygen (<0.8 $mg \cdot L^{-1}$) together with high vNLR (= 0.19-0.21 $kgN \cdot m^{-3} \cdot d^{-1}$). Moreover, the presence of propionic and butyric acid in the carbon source enhanced the denitrification and phosphorus uptake rates up to 6.33±1.92 $mgP \cdot (gVSS \cdot h)^{-1}$. The specific phosphorus uptake rate (sPUR) via nitrite route was not adversely affected by nitrite concentrations up to 50-70 $mgNO_2\text{-}N \cdot L^{-1}$, and was partially inhibited for concentrations of 100-120 $mgNO_2$-N L^{-1}.

Coupling the short-cut SBR with a fermentation unit of biowaste was the innovative side stream scheme investigated and optimized for the treatment of he high stenght anaerobic digestate. The anaeobic superantant from the co-digestion of secondary sludge and OFMSW was treated in a pilot SBR (2.8 m^3 of working volume), which accomplished the via-nitrite biological nutrients removal. Successful start-up operation was achieved in 20 days, which were enough to inhibit the growth of nitrite oxidizing bacteria (NOB) operating at high level of free ammonia (FA) (up to 6 mgN L^{-1}). The change of the bacteria population was observed by the sequencing the major bands from the DGGE analyses, which indicated mainly the presence of bacterial groups related to β Proteobacteria and Bacteroidetes. Among them, some of the main bands were closely related to the AOB genera, such as Nitrosomonas sp. and Nitrosospira sp. The maximal treatment potential observed was 0.8 $kgN\ m^{-3}d^{-1}$. The specific ammonium oxidizing bacteria (sAUR) was observed constant in the range 18-20 $mgN\ (gMLVSS\ h)^{-1}$ during the experimental period. Low temperature of the anaerobic digestor, as well as excess of polyelectrolyte residual in the anaerobic supernatant after dewatering process, were commonly observed during the experimental activities, resulting in wide fluctuations of the volumetric NLR (vNLR) applied

and significant leakage of flocculated active biomass from the scSBR. Although the specific ammonium uptake rate (sAUR) was maintained constant, when the vNLR was higher than the maximal biomass nitrifying capacity reduced the nitrogen removal efficiency of the system. From an economic point of view, alternative carbon sources derived from the acidogenic fermentation of biowaste (e.g. organic fraction of mucipal solids waste, cattle manure, maize silage, sewage sludge) could be a good option to replace synthetic originated carbons source (e.g. acetic acid, methanol, ethanol, glycerol). In addtition, the life cycle assessment analyses showed environmental benefits adopting the fermentation of the biowaste compared with the use of synthetic carbon source (0.28 kg PO_4^{-3} m^{-3}). Moreover, high denitrifying biological phosphorus removal via-nitrite was observed under anoxic conditions using the fermentation product. The specific phosphorus removal via nitrite was 0.27 kgPO_4-P $(kgMLVSSd)^{-1}$ when the fermentation liquid of OFMSW was dosed, resulting in a hyper-accumulation of phosporus in the activated sludge close to 50 mgP $gMLSS^{-1}$. During the via-nitrite processes large amount of N_2O could be generated which is a strong greenhouse gas and therefore off-set the promising energy and costs saving. In this work, low oxygen and high nitrite concentration in the mix liquor have a negative effect on the production of N_2O. Less production the of N_2O from the short-cut SBR was observed operating at DO = 1.5 mg L^{-1} and vNLR = 0.81 kgN $m^{-3}d^{-1}$ resulted in much lower nitrous oxide emissions (0.24% of influent nitrogen load) compared to the operation at lower DO (0.95 mg L^{-1}) and higher vNLR=1.08 kgN $m^{-3}d^{-1}$.

When other biowaste are not available in the WWTP, the fermentation of sewage sludge (primary and secondary sludge) is the best candidate to enhance nitrogen and phosphorus removal. The alkaline fermentation of sewage sludge was tested in a pilot sludge fermentation with membrane separation by the addtion of NaOH. The maximum VFA production increased from 200 mgCOD $gTVS^{-1}$ to 300 mgCOD $gTVS^{-1}$.when the pH was increased between 8 to 10. However, it was observed that the addition of NaOH adversely affect the separation by the membrane from the use of soda during the fermentation process. To this end, soda was replaced with wollastonite (Ca_2SiO_4) to improve the dewaterability of the final fermentation product, which increased the flow by 24% (12.5 LMH). The case studies analysed represented a successfull validation of these technology. The experience point out that the use of sludge fermentation liquid for the via nitrite nutrient removal resulted in high nitrogen and phosphorus removal (22.4 mgN $(gVSS\ h)^{-1}$ and 3.4 mgP $(gVSS\ h)^{-1}$. High presence of nitrite concentration (> 100 mg L^{-1}) during can interfere with phosphorus uptake mechanism of PAOs, and 65% of

the total P removed was attributed to biomass growth. The application of fermentation containing a mixture of SCFAs enhanced the efficiency and the rate of denitrification and P removal.

Finally, the thesis rised the possibility to integrate the side stream nitrogen removal via nitrite from anaerobic supernatant with the selection of polyhydroxyalkanoates (PHA) storing biomass. The main goal was to demonstrate the feasibility of the recovery of green added value products from the treatment of wastewater. After the phase of enrichment, the good capacity of the biomass to store PHA was highlight by the high VFAs uptake rate of 134.6±32 mgCOD $gVSS^{-1}h^{-1}$ and a PHA production yield which increased during the experimental activities from 0.26±0.2 to 0.38±0.03 $gCOD_{PHA}$ $gCOD_{VFA}^{-1}$. Preliminary results of the bioplastics produced showed similar characteristic with conventional biopolymer already present in the market. However, higher effort should be given for the final recovery of the bioplastics, since the extraction of the PHA from the biomass is an important bottleneck, which limited the diffusion of large scale application. Furtrher investigations for suistanable and green methods for PHA extraction are required in order to prevent negative environmental impact due for the use of organic solvents.

Riassunto

Le attività principali durante i tre anni di dottorato, si sono concentrate sull'ottimizzazione e la validazione in reattore sequenziale (SBR) dimostrativo, di processi avanzati per la rimozione biologica di azoto e fosforo via-nitrito da effluenti anaerobici. Capire gli ostacoli tecnici e fornire soluzioni ingegneristiche è di fondamentale importanza per capire da subito il potenziale di trasferibilità e l'interesse commerciale per l'applicazione in piena scala di queste tecnologie innovative. Le attività sperimentali sono state pertanto eseguite utilizzando substrati reali (ad esempio surnatante anaerobica, fanghi di depurazione comunale, FORSU, acque reflue domestiche municipali), riproducendo così le reali condizioni ambientali e operative.

I processi anaerobici hanno ottenuto un elevato interesse negli ultimi anni, dovuto principalmente ai vantaggi connessi alla produzione di "energia verde" tramite l'utilizzazione del biogas. Rispetto ai consolidati trattamenti aerobici, i processi anaerobici permettono minor consumo energetico, minore produzione di fanghi, eliminazione dell'inquinamento dovuto alle emissioni gassose e un impatto ambientale minore. I reattori anaerobici UASB sono tra le tecnologie più evolute per il trattamento anaerobico delle acque reflue municipali. Tuttavia, gli

effluenti anaerobici contengono ancora elevate quantità di nutrienti (azoto e fosforo), ed essere fonte di eutrofizzazione per i corpi idrici. Inoltre, gli effluenti derivati dalla digestione o co-digestione anaerobica dei fanghi con rifiuti organici (ad esempio, la frazione organica dei rifiuti solidi urbani, FORSU), rappresentano un flusso concentrato di azoto e fosforo che dovrebbe essere trattato prima di essere ricircolato nella linea principale degli impianti di depurazione. I processi biologici via-nitrito per la rimozione dei nutrienti sono riconosciuti per essere una scelta sostenibile per il trattamento degli effluenti anaerobici a basso e alto carico poiché consentono aerare il 25% in meno e utilizzano il 40% in meno di fonte di carbonio rispetto ai processi convenzionali. L'applicazione di bioprocessi innovativi per ridurre i costi di trattamento e valorizzare le acque reflue tramite il recupero di risorse è quindi la fida attuale.

Il processo via-nitrito è stata studiato in un reattore sequenziale discontinuo (SBR) per il trattamento di effluenti anaerobici proveniente da un reattore UASB. Le acque reflue domestiche a seguito di un processo anaerobico, sono state trattate prima tramite un processo completamente autotrofo (Anammox). Durante la sperimentazione, l'attività specifica dell'inoculo è stata incrementata del 161%, che corrispondeva ad un tasso di rimozione dell'azoto pari a 2.27 ± 1.31 mgN (gVSS h)-1 a 30 °C. Questo valore è diminuito con il diminuire del carico di azoto volumetrico, portando comunque a ridurre anche l'attività dei batteri eterotrofi denitrificanti, osservando un avvicinamento dei rapporti di conversione tipici della biomassa anammox (1.32 NO2-N / NH4-N rimosso). L'analisi d'ibridazione fluorescente (FISH) ha confermato la presenza di biomassa Anammox e di batteri filamentosi, favoriti dall'elevato tempo di ritenzione dei solidi. Sebbene la biomassa anammox rimuova efficacemente l'azoto, il fosforo non può essere eliminato significativamente da una biomassa completamente autotrofa. Per questo motivo, è stata studiata l'applicazione dello schema UASB-SBR accoppiato al co-trattamento decentralizzato delle acque reflue e dei rifiuti organici domestici, al fine di promuovere la rimozione dell'azoto e del fosforo via-nitrito. La rimozione simultanea di azoto e fosforo via nitrito è stata studiata usando la migliore fonte di carbonio disponibile prodotta dalla fermentazione acidogenica dei rifiuti domestici organici (DOW) e rifiuti di frutta e di verdura (VFW). L'accumulo dei nitriti (NO2-N/NOx-N> 97%) nel reattore SBR è stato osservato operando con basso tenore di ossigeno disciolto (<0.8 mg L-1) e relativamente elevato vNLR (0.19-0.21 kgN m-3d-1). Inoltre, la fonte di carbonio dosata conteneva quantità di acido acetico, propionico e butirrico che ha incrementato le velocità di denitrificazione via nitrito ed il contemporaneo iperaccumulo di fosforo (fino a 6.33±1.92 mgP (gVSS h)-1) da parte della

biomassa fosforo accumulante e denitrificante (DNPAOs). Tuttavia, l'attività della biomassa DNPAOs non è stata influenzata negativamente dalle elevate concentrazioni di nitriti nell'ordine di 50-70 mgNO2-N L-1, mentre si notava inibizione da concentrazioni di 100-120 mgNO2-N L-1.

Accoppiando un reattore SBR per la rimozione biologica dei nutrienti via-nitrito con la fermentazione di rifiuti organici di scarto, è stato implementato e ottimizzato un sistema innovativo per il trattamento dei digestati anaerobici. In questa tesi, il surnatante anaerobico proveniente dalla co-digestione dei fanghi secondari e della FORSU è stato trattato in un reattore SBR pilota (con volume utile pari a 2.8 m3), realizzando la rimozione biologica via-nitrito dei nutrienti. Le operazioni di avviamento del reattore sono durate circa 20 giorni, i quali sono stati sufficienti per inibire la crescita dei batteri nitrito ossidanti (NOB) operando ad alte concentrazioni di ammoniaca libera (FA) (fino a 6 mgN L-1) nel reattore. La variazione della popolazione batterica è stata osservata anche tramite il sequenziamento delle principali bande ottenute dall'analisi DGGE, la quale indicava la presenza di gruppi batterici sono connessi a β Proteobacteria e Bacteroidetes. Tra questi, alcune delle bande principali erano strettamente legate ai generi AOB, come Nitrosomonas sp. e Nitrosospira sp. La massima capacità di trattamento osservata è stata di 0.8 kgN m-3d-1. La velocità specifica di ossidazione dell'ammoniaca (sAUR) è stata osservata costante nel tempo, variando in ristretto range compreso tra 18-20 mgN (gMLVSS h)-1 durante il periodo sperimentale. Basse temperature del digestore anaerobico, nonché eccessi di polielettrolita residui nel surnatante anaerobico a seguito del processo di disidratazione, sono stati comunemente osservati durante le attività sperimentali, causando ampie fluttuazioni della carico volumetrico di azoto applicato e significative fughe di biomassa attiva flocculata dal reattore SBR. Sebbene il tasso di ossidazione dell'ammoniaca sia rimasto costante, quando il vNLR era superiore alla massima capacità di trattamento della biomassa le efficienza di rimozione dell'azoto del sistema diminuivano.

Da un punto di vista economico, le fonti di carbonio alternative derivanti dalla fermentazione dei rifiuti organici (come la frazione organica dei rifiuti solidi urbani, liquami zootecnici, insilati, fanghi di depurazione) potrebbe essere una buona opzione in sostituzione a quelle di origine sintetica (ad esempio acido acetico, metanolo, etanolo, glicerolo). Inoltre, l'analisi del ciclo di vita ha mostrato i benefici ambientali connessi alla fermentazione di rifiuti organici rispetto all'uso di una fonte di carbonio di origine sintetica (0.28 kg PO4-3 m-3). Elevate velocità di

denitrificazione via-nitrito con simultaneo accumulo di fosforo è stato osservato in condizioni anossiche, utilizzando il prodotto di fermentazione come fonte di carbonio. Dosando la frazione liquida della FORSU fermentata, la velocità specifica di rimozione del fosforo via nitrito è stata pari a 0.27 kgPO4-P (kgMLVSS d)-1, con il conseguente iper-accumulo di fosforo nei fanghi attivi, ottenendo una concentrazione fino a 50 mgP gMLSS-1. Durante il processo via-nitrito, elevata quantità di gas serra come l'N2O potrebbero essere generate, e quindi venire meno dei vantaggi connessi al risparmio in aerazione. In questa tesi, è stato osservato come il basso tenore di ossigeno accompagnato da un'elevata concentrazione di nitriti nel reattore, incrementano la produzione di N2O. Una riduzione delle emissioni di N2O (0.24% del carico di azoto influente) dal reattore SBR via-nitrito è stata osservata operando a concentrazioni di ossigeno (OD) pari a 1.5 mg L-1 e vNLR pari a 0.81 kgN m-3d-1, rispetto a concentrazioni di OD minori (0.95 mg L-1) e alti carici di azoto applicati (vNLR = 1.08 kgN m-3d-1).

Quando in un impianto di depurazione non sono presenti rifiuti biodegradabili, la fermentazione dei fanghi di depurazione (come i fanghi primari e secondari) è il miglior metodo candidato per migliorare le efficienze di rimozione dell'azoto e del fosforo. La fermentazione alcalina dei fanghi di depurazione (primari e secondari) con l'aggiunta di soda, è stata testata in un fermentatore pilota (del volume utile di 500 L) accoppiato alla separazione della frazione liquida su membrana tubolare. Operando a pH compresi tra 8 e 10 è possibile aumentare le rese di produzione degli acidi organici volatili da 200 fino a 300 mgCOD gTVS-1. Tuttavia, l'aggiunta di soda per l'aumento del pH di fermentazione ne pregiudica anche la disidratabilità, rendendo difficoltosa la separazione. Per questa ragione, l'idrossido di sodio è stato sostituito con wollastonite (Ca2SiO4) che consente di mantenere pH relativamente elevati (pH circa 8.5) e di migliorare la disidratabilità del prodotto di fermentazione finale, aumentandone il flusso di permeato del 24% dalle membrane (12.5 LMH). I casi di studio analizzati e riportati in questa tesi, confermano la validità della tecnologia studiata. Il prodotto di fermentazione derivante dai fanghi primari e secondari consente buone cinetiche di denitrificazione e l'accumulo del fosforo via-nitrito (rispettivamente pari a 22.4 mgN (gVSS h)-1 e 3.4 mgP (gVSS h)-1. Tuttavia, l'elevata concentrazione di nitriti (> 100 mg L-1) può interferire con i meccanismi di rimozione del fosforo da parte della biomassa PAOs: in questo caso il 65% del totale P rimosso è stato attribuito al normale metabolismo di crescita della biomassa. L'applicazione della fermentazione consente pertanto di produrre una miscela ottimale di acidi grassi a catena corta, la quale migliora e incrementa l'efficienza di rimozione di azoto e del fosforo via-nitrito.

In ultima analisi, in questo lavoro di tesi è riportata come è possibile integrare la rimozione dell'azoto da un flusso ad alto carico ammoniacale (come i surnatanti anaerobici) con i processi di selezione di una biomassa con elevate capacità di stoccare poliidrossialcanoati (PHA), precursori delle bioplastiche. L'obiettivo principale era di dimostrare la fattibilità per il recupero di prodotti con valore aggiunto mediante il trattamento di acque reflue. Dopo la fase di arricchimento compiuta in condizioni di "feast and famine", la buona capacità della biomassa a stoccare PHA è indicata dalle velocità di assorbimento degli acidi organici di 134.6 ± 32 mgCOD gVSS-1h-1, nonché dalle rese di produzione di PHA che sono aumentate nel corso delle attività sperimentali da 0.26±0.2 a 0.38±0.03 gCODPHA gCODVFA-1. I risultati preliminari delle bioplastiche prodotte hanno mostrato caratteristiche simili con biopolimeri convenzionali già presenti sul mercato. Tuttavia, lo sforzo maggiore dev'essere concentrato ai processi di recupero delle bioplastiche poiché ad oggi l'estrazione dei PHA dalla biomassa è un collo di bottiglia che ne sta limitando la diffusione e l'applicazione su vasta scala. Ulteriori ricerche saranno necessariamente focalizzate su metodi più sostenibili e più "green" che consentano di estrarre PHA evitando impatti ambientali negativi a causa dell'utilizzo di dei solventi organici.

1 Chapter. Introduction

This chapter includes a general introduction to this research, where the potential values of the current biotechnologies applied for waste and wastewater treatment are described. The paragraph has been written according with the 5th Edition of the book titled "Wastewater Engineering. Treatment and Resource Recovery. (Metcalf & Eddy, 2013).

1.1 Challenge statement

Proper management of wastewater and organic waste is essential for the human health, economic development and it is the major challenge for developing countries. Waste and wastewater are produce from every size and type of community and when are not correctly treated they accumulate creating septic conditions, malodorous gas emissions and scattered pollution and eutrophication in the water bodies (Metcalf & Eddy, 2013). In the annual Report by the National Observatory on Wastes on waste management in Italy (APAT-ONR, 2006), a production of 4.7 Mt of waste deriving from wastewater treatment plants (CER code 19.08) is reported for 2004. Considering that wastewater treatment sludge (WTS) represents about 90% of above figure, it follows that sludge yearly production is about 4.3 Mt, corresponding to about 1 Mt of dry solids at a solids concentration of 25%, with an increase of about 10% with respect to years 2001-2003. The U.S. EPA estimated that more than 7 million tons of dry solids disposed in landfill are generated from more than16000 municipal wastewater treatment plant. Nitrogen and phosphorus are abundantly contained in the wastewater and they take part negatively to the growth of aquatic plants leading to a complete depletion of oxygen in the water body, which causes a reduction in specific species of fish and other animals. The application of the European Directive 91/271/EEC concerning urban wastewater promoted the construction of new technologies for nutrients removal in the sensitive areas. The Directive requires the appropriate collection of sewage and regulates discharges of wastewater by specifying the minimum type of treatment to be provided and setting maximum emission limit values (Table 1.1) of the major pollutants (organic load and nutrients).

Table 1.1. Limits required to discharge from urban WWTPs in sensitive area subjected to eutrophication

Parameter	**Concentration**	**Removal Efficiency (%)**
BOD5 ($mgO_2\ L^{-1}$)	25	70-90
COD ($mgO_2\ L^{-1}$)	125	75
Total Suspended Solids (mg TSS L^{-1})	35	90

Total Nitrogen (mgN L^{-1})	15 (10'000-100'000 p.e.) 10 (>100'000 p.e.)	70-80
Total Phosphorus (mgP L^{-1})		80

The most developed techniques at the level of urban wastewater treatment plants are intensive biological processes (EPA, 2006). Large amounts of energy are required to manage the physical, chemical and biological processes needed to treat industrial and domestic wastewater. Their principle is to operate with reduced space and to intensify the natural phenomena of degradation of organic matter and nutrient removal. For centralized or centralized level, the most widespread type of wastewater treatment is the aerated activated sludge system, which requires stable electricity supply and professional staff for operation and maintenance. In this case, the energy demand for a typical wastewater treatment plant is 0.6 kWh m^{-3} of wastewater treated, where up to 50% is related for the aeration basin (McCarty et al., 2011). However, the chemical energy content of untreated wastewater could be estimated to be 2.11 kWh m^{-3} but through the conventional nitrification/denitrification process with carbon oxidation, followed by the anaerobic digestion of the sewage sludge only 15-35% of this energy potential is captured (Figure 1.1, Metcalf & Eddy, 2013, McCarty et al., 1981). Anaerobic processes are less energy intensive than aerobic processes as they do not required aeration for the bulk removal of bCOD.

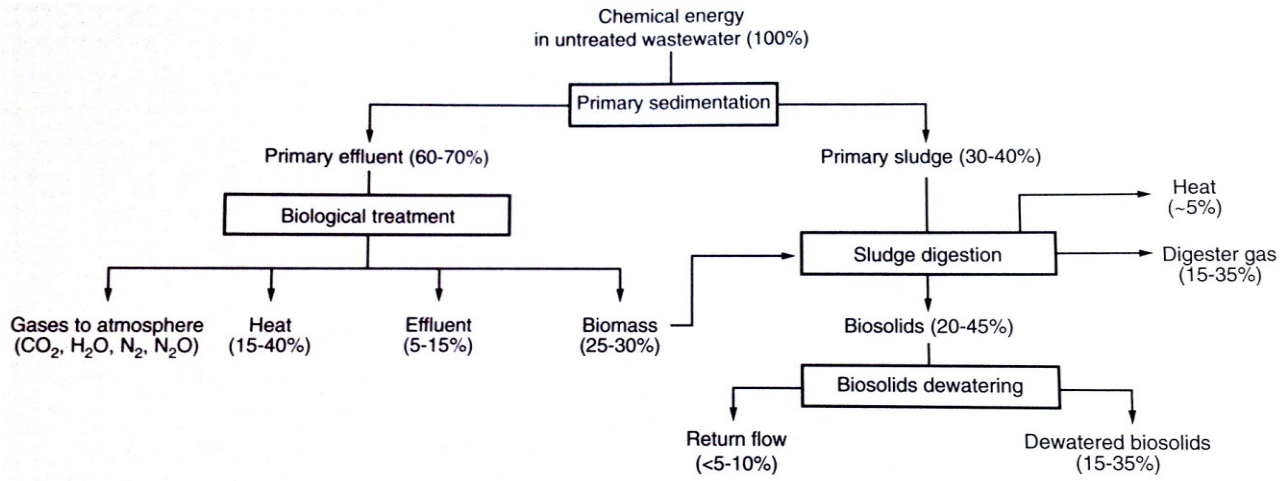

Figure 1.1. Fate of chemical energy in wastewater treatmentwith activated sludge and anaerobic sludge digestion (Metcalf & Eddy, 2013).

Furthermore, anaerobic treatment of waste and wastewater should be the core technology that

can be employed for energy recovery through the biogas production (Foresti et al., 2006). Additionally, less sludge is produced during anaerobic processes, which makes sludge handling and disposal costs greatly reduced (Lettinga et al., 1983). In this approach, complete anaerobic treatment of domestic waste and wastewater could contribute for the achievement of a net energy production in the WWTPs, opening the opportunity to substitute the conventional aerobic activated sludge processes (Figure 1.1). A number of examples are reported in literature that well demonstrate how the overall energy for wastewater treatment could be reduced through efficiently practice, with energy and fertilizing nutrients recovery (McCarty et al., 1981).

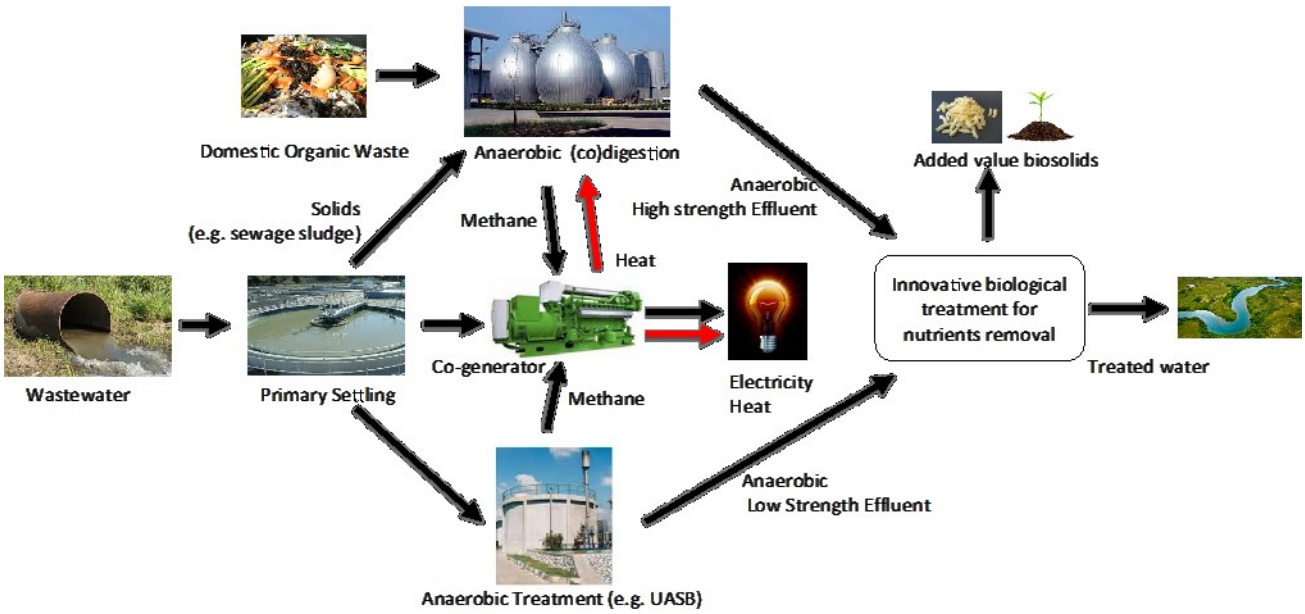

Figure 1.1. Implementation of anaerobic treatment for waste and wastewater

Despite all the advantages, low and high strength effluents from anaerobic reactors treating domestic wastewater or sewage sludge never comply with the effluent standards because nitrogen and phosphorus are still present (Foresti et al., 2002). On the other hand, the reject water from the anaerobic digestion of biowaste (e.g. sewage sludge, OFMSW) represents a high concentrated stream of nitrogen and phosphorus, which commonly is recycled back in the mainstream (Gustavsson et al., 2011). In both cases, a post-treatment stage is required and the via-nitrite nutrients removal routes is actually available for a more efficient bioprocesses.This strategy, compared with the conventional nitrogen removal, allows 25% less oxygen and 40% less external carbon source, thus could retrofit/upgrade existing treatments systems or result in the development of new plants with smaller footprints, maximizing nutrients removal

capabilities.

Autotrophic nitrogen removal using anammox bacteria presents a promising option, as it required up to 60% less of oxygen and does not require organic carbon and allows for maximum COD removal and energy recovery in the UASB reactor (Hendrickx et al., 2012; Malamis et al., 2014). However, the completely autotrophic nitrogen removal process suffers from two main drawbacks: (i) it does not remove phosphorus and struvite precipitation is also applied to recover phosphorus, which increases the overall cost to 2-3€ kgN^{-1} removed, (ii) the anammox bacteria are very sensitive and can be easily inhibited by several operating and environmental parameters (DO, nitrite, temperature).

Another option is the application of the short cut nutrients removal with addition of an available external carbon source, considered from many authors a bioprocess more reliable and less sensitive for the environmental and operating parameters. In this case, if the reactions step of anaerobic/aerobic and anoxic sequence are properly operated, and an optimal carbon source is added, effective nitrification, denitrification and biological phosphorus removal can occur in the same cycle (Callado and Foresti, 2001, Frison et al., 2013). Controlled hydrolyses and fermentation of organic material from sewage sludge and/or domestic organic waste, could be a possible source of volatile fatty acids and a valid substitute for synthetic and costly carbon sources (e.g. acetic acid, methanol, ethanol).

1.2 Conventional biological processes to treat wastewater

Basic to the design of a biological treatment processes, or to the selection of the type of biological processes to be used, is understanding of the biochemical activities of microorganisms. The classification of microorganisms by source of cell carbon, electron donor, electron acceptor and end products is summarized in (Table 1.2). The two major topics considered in this section are (1) general nutritional requirements of the microorganisms commonly encountered in wastewater treatment, and (2) the nature of microbial metabolism based on the need for molecular oxygen.

Table 1.2. Classification of microrganisms by electron donor, electron acceptor , source of cell carbon, and end products

Type of bacteria	**Common reaction name**	**Carbon source**	**Electron donor**	**Electron acceptor**	**Products**
Aerobic heterotrophic	Aerobic oxidation	Organic compounds	Organic compounds	O_2	CO_2, H_2O
Aerobic autotrophic	Nitrification	CO_2	NH_3, NO_2^-	O_2	NO_2^-, NO_3^-
	Iron oxidation	CO_2	Fe (II)	O_2	Ferric Ion, Fe (III)
	Sulphur oxidation	CO_2	H_2S, S°, S_2O_3	O_2	SO_4^{-2}
Facultative heterotrophic	Denitrification anoxic reaction	Organic compound	Organic compounds	NO_2^-, NO_3^-	N_2,CO_2, H_2O
Anaerobic heterotrophic	Acid fermentation	Organic Compounds	Organic Compounds	Organic Compounds	Volatile Fatty Acids (acetate, propionate, butyrate)
	Iron reduction	Organic Compounds	Organic Compounds	Fe(III)	Fe(II), CO_2, H_2O
	Sulphate reduction	Organic Compounds	Organic Compounds	SO_4^{-2}	H_2S, CO_2, H_2O
	Methanogenesis	Organic Compounds	Volatile Fatty Acids (VFAs)	CO_2	Methane

The objectives of the biological treatment of the wastewater are to transform (i.e. oxidize) dissolved and particulate biodegradable constituents into acceptable end product, capture and

incorporate suspended and nonsettleable colloidal solids into a biological floc or biofilm, transform or remove nutrients, such as nitrogen and phosphorus and in some cases, to remove specific trace organic constituents and compounds.

1.2.1 Aerobic Carbon Oxidation

The removal of dissolved and particulate carbonaceous BOD and the stabilization of organic matter found in wastewater is accomplished biologically using a variety of microorganisms, principally bacteria. Carbon, Oxygen, Hydrogen together with Nitrogen and Sulphur are normally presents in the organic matter of the wastewater, which consist of proteins (40-60 %), carbohydrates (25-50%), oils and fats (8-12%) and urea. Microorganisms are used to convert the dissolved and particulate carbonaceous organic matter into simple end products and additional biomass, as represented by the following equation for the aerobic biological oxidation of organic matter (Metcalf & Eddy, 2013).

Eq. 1 $3C_6H_{12}O_6+8O_2+2NH_3->2C_5H_7NO_6+8CO_2+14H_20$

In the

Eq. 1, oxygen (O_2), ammonia (NH_3), and phosphate (PO_4^{-3}) are used to represent the nutrients needed for the conversion of the organic matter to simple end products (i.e., carbon dioxide (CO_2) ad water).

The substrate utilization rate in biological system can be modeled with the following expression for soluble substrate. Because the mass of substrate is decreasing with the time due to substrate utilization, a negative value is shown in substrate mass balances.

Eq. 2 $$r_{su} = \frac{kXS}{K_s + S}$$

where: r_{su} is rate of substrate concentration change due to utilization (g $m^{-3}d^{-1}$); k is the maximum specific substrate utilization rate, (g substrate / g of microrganisms per day); X is the biomass (microorganism) concentration, (g m^{-3}); S is the growth limiting substrate concentration is solution, (g m^{-3}) and K_s is the half velocity constant, thus the substrate concentration at one half the maximum specific substrate utilization rate (g m^{-3}).

The concentration of the biomass in a biological reactor is commonly express as volatile suspended solids (VSS). Cell debris is also measured as VSS and contribute to the total VSS concentration measured in the reactor mixed liquor. The rate of production of cell debris is directly proportional to the endogenous decay rate, as follows:

Eq. 3 $$r_{Xd} = f_d(k_d)X$$

where r_{Xd} is the rate of cell debris production, (gVSS $m^{-3}d$) and f_d is the fraction of biomass that remains as cell debris (0.10-0.15 gVSS $gVSS^{-1}$). Thus, the net biomass growth rate is:

Eq. 4 $$r_X' = \frac{kXS}{K_s + S} - f_d(k_d)X$$

The observed yield accounts for the actual solids production that would be measured for the system and is shown as follows:

Eq. 5 $$Y_{obs} = \frac{r_X'}{r_{su}}$$

where Y_{obs} is the observed yield, express as gVSS produced per g of substrate removed.

1.2.2 Biological Nitrogen Removal

The biological nitrogen removal is carried out by two separated type of process: nitrification and heterotrophic denitrification. Furthermore, also the assimilation of nitrogen in the cells is involved during the biological nitrogen removal from the wastewater treatment. The latter could be assimilated as ammonium by the cell, or, if limited, as nitrate or nitrite. In a wastewater treatment plant, the nitrogen removed through the assimilation activities may range between 8 to 30%, depending on the organic loading rate and the sludge retention time of the system. However, if the sludge daily produced is digested, part of the nitrogen assimilated is recycled back in the biological reactor with the reject water.

Nitrification

The nitrification is the biological oxidation of ammonia to nitrite and then to nitritate. This process was discovered out by two types of chemiolithoautotrophic bacteria: ammonia oxidation is catalysed by ammonia-oxidizing bacteria (AOB, e.g. *Nitrosomonas, Nitrospira, Nitrosococcus*) whereas nitrite oxidation is catalysed by nitrite-oxydizing bacteria (NOB, e.g. *Nitrobacter, Nitrococcus, Nitrospira*). Compared with heterotrophic organisms, growth of nitrifying bacteria is slow and scarce, even in optimal condition. It is generally accepted that ammonia (NH_3) rather than ammonium (NH_4^+) is the substrate for AOB. The ammonia oxidation using a representative measurement of yield and oxygen consumption for AOB and NOB are as follows:

$$55\mathrm{NH_4^+} + 76\mathrm{O_2} + 109\mathrm{HCO_3^-} \rightarrow 2C_5H_7NO_2 + 54\mathrm{NO_2^-} + 57\mathrm{H_2O}\ (Eq.2)$$

$$400\mathrm{NO_2^-} + \mathrm{NH_4^+} + 195\mathrm{O_2} + 4H_2CO_3 + \mathrm{HCO_3^-} \rightarrow C_5H_7NO_2 + 400\mathrm{NO_3^-} + 3\mathrm{H_2O}\ (Eq.3)$$

The overall equation for nitrification is as follow:

$$\mathrm{NH_4^+} + 1.83\mathrm{O_2} + 1.98\mathrm{HCO_3^-} \rightarrow 0.021C_5H_7NO_2 + 0.98\mathrm{NO_3^-} + 1.041\mathrm{H_2O} + 1.88H_2CO_3(Eq.4)$$

In these equations, growth yields for AOB and NOB are 0.15 mgCells mgNH_4-N^{-1} oxidise and 0.02 mgCells mgNO_2-N^{-1} oxidise, respectively. Oxygen consumption ratios in the equation are 3.16 mgO_2 mgNH_4-N^{-1} oxidised and 1.11 mgO_2 mgNO_2-N^{-1} oxidized, respectively. Also, it can be calculated that 7.07 mg of alkalinity as $CaCO_3$ is required per mg of ammonia nitrogen oxidised.

For nitrification system operated at temperature below 28 °C ammonia oxidation versus nitrite oxidation kinetics are rate limiting, so that designs are based on saturation kinetics for ammonia oxidation are given below, assuming DO is available.

Eq. 6
$$\mu_n = \left[\frac{\mu_m N}{K_n + N}\right] - k_{dn}$$

where μ_n is the specific growth rate of nitrifying bacteria (g new cell/g cell per day), μ_m is the maximum specific growth rate of nitrifying bacteria (g new cell/g cell per day), N is the nitrogen concentration (g m^{-3}), and k_{dn} is the endogenous decay coefficient fro nitrifying organisms, gVSS $gVSS^{-1}d^{-1}$. A wide range of specific growth rate have been reported as a function of

temperature (Randall et al., 1992). At 20 °C, reported μ_m varies from 0.25 to 0.77 gVSS/gVSS. However, the μ_m values for nitrifying organism are much lower than the corresponding heterotrophic organism, requiring much longer solid retention time (SRT) values for nitrifying activated sludge system. Typical design SRT values may range from 10 to 20 d at 10°C to 4 to 7 d at 20°C. Above 28°C, both ammonia and nitrite oxidation kinetics should be considered. At elevated temperature, te relative kinetics of NH_4-N and NO_2-N oxidation change, and NO_2-N will accumulate at lower SRT values. Nitrification rate are effected by the liquid DO concentration in activated sludge, which increases up to DO concentration of 3 to 4 mg L^{-1}. To account for the effects of DO, the expression of DO, the expression for the specific growth rate is modified as follows:

Eq. 7
$$\mu_n = \left[\frac{\mu_m N}{K_n + N}\right]\left[\frac{DO}{K_0 + DO}\right] - k_{dn}$$

where DO is the dissolved oxygen concentration (g m^{-3}) and K_0 is the half-saturation coefficient for DO (g m^{-3}).

Denitrification

The term of denitrification is to indicate the biological reduction of nitrate to nitric oxide, nitrous oxide and nitrogen gas. The biological denitrification involves the biological oxidation of many organic substrates in wastewater treatment using nitrate or nitrite as the electron acceptor instead of oxygen. The electron donor is typically one of three sources: (1) the biodegradable soluble COD (bsCOD) in the influent wastewater, (2) the bsCOD produced during endogenous decay and (3) an exogenous source such as methanol or acetate. Reaction stoichiometry for different electron donos shown as follows, where $C_{10}H_{19}O_3N$ is often used to represent the biodegradable oranic matter in wastewater (U.S. EPA, 1993):

Wastewater: $C_{10}H_{19}O_3+10NO^{-3} + 10NO^{-3} \rightarrow 5N_2+10CO_2+3H_2O+NH_3+10OH^-$

Acetate: $5CH_3COOH+8NO^{-3} \rightarrow 4N_2+10CO_2+6H_2O+NH_3+8OH^-$

During the denitrification process, one equivalent of alkalinity is produced per equivalent of NO_3-N reduced, which equates to 3.57 g of alkalinity (as $CaCO_3$) production per g of nitrate

nitrogen reduced, so about one-half of the amount destroyed by nitrification can be recovered (Metcalf & Eddy, 2013).

The oxygen equivalent of using nitrate and nitrite can be determined from the oxidation half reactions. The oxygen equivalent is a useful design factor when calculating the total oxygen required for nitrification-denitrification biological treatment systems. The oxygen equivalent of nitrate is 2.86 gO_2 gNO_3-N^{-1}, while similarly, for nitrite as electron acceptor, the oxygen equivalent of nitrite is 1.71 gO_2 gNO_2-N^{-1}. For cell synthesis, $bsCOD_{syn}$ is calculated from the net biomass yield and the ratio of 1.42 gO2 per gram of VSS. The oxygen equivalent of the biomass is equal to the bsCOD incorporate into biomass by this equation: $bsCOD_{syn}$ =1.42 Y_n bsCOD utilized. As consequence, the bsCOD needed to reduce one gram of nitrite or nitrate is calculated by the follow:

Nitrite to nitrogen (NO_2^-->N_2): Eq. 8 $$\frac{bsCOD}{NO_3 - N} = \frac{1.72}{1 - 1.42Y_n}$$

Nitrate to nitrogen (NO_3^- ->N_2): Eq. 9 $$\frac{bsCOD}{NO_3 - N} = \frac{2.86}{1 - 1.42Y_n}$$

When the nitrate is used as an electron acceptor instead of oxygen, the maximum specific substrate utilization rate (k) may be lower than the rate with oxygen as the electron acceptor. Furthermore, dissolved oxygen can inhibit nitrate reduction by repressing the nitrate reduction enzyme. A dissolved oxygen concentration of 0.2 mg L^{-1} and above has been reported to inhibit denitrification for a *Pseudomonas* culture (Skerman and MacRae, 1957) and activated sludge treating domestic wastewater (Dawson and Murphy, 1972). The effect of nitrate and DO concentration on the biokinetics for the substrate utilization rate during the denitrification is express in the form of two saturation terms as follows:

Eq. 10 $$r_{su} = \left[\frac{kXS}{K_n + S}\right]\left[\frac{NO_3^-}{K_{S,NO_3^-} + NO_3^-}\right]\left[\frac{DO}{K_0' + DO}\right]$$

where K_0 is the DO inhibition concentration for nitrate reduction (mg L^{-1}), $K_{s,NO3}$ is the half velocity coefficient for nitrate limited reaction (mg L^{-1}). The value of K_0 is system-specific. Values in the range from 0.1 to 0.2 mg L^{-1} have been proposed for K_0 and 0.1 mg L^{-1} for $K_{s,NO3}$ (Barker and Dold, 1997).

Biological phosphorus removal

Phosphorus is important cellular energy transfer mechanisms via adenosine triphosphate (ATP) and polyphosphates. As energy is produced in oxidation reduction reactions, adenosine diphosphate (ADP) is converted to ATP with 7.4 kcal/mole of energy captured in the phosphate bond. In the wastewater treatment plants, biological phosphorus removal is performed from phosphorus accumulating organisms (PAOs). These bacteria accomplish the phosphorus removal based on two fundamental steps (Figure 1.2):

1- anaerobic conditions: the acetate and other volatile fatty acids (VFA) are produced by the fermentation of bsCOD and assimilate easily by the biomass. Depending of the hydraulic retention time of the anaerobic zone, some colloidal and particulate COD is also hydrolysed and converted to acetate (VFA). Using energy available from stored polyphosphates, the PAOs assimilate acetate and produce intracellular polyhydroxyalkanoates (PHA), which are storage compounds. The uptake of the VFAs and the increasing of PH, the release of orthophosphate ($O\text{-}PO_4$) can occurs with the magnesium, potassium and calcium cations.

2- Aerobic/anoxic conditions: the PHA stored are metabolized, providing the energy for new cell growth, producing also some glycogen from the metabolism. The energy released from the PHA oxidation is used to form new bonds in cell storage so that soluble orthophosphate ($O\text{-}PO_4$) is removed from the solution and incorporated into polyphosphates within the bacteria cell. Cell growth also occurs due to PHA utilization and the new biomass with high polyphosphate storage accounts for phosphorus removal. The removal of phosphorus is really obtained from the wastage of sludge from the bioreactor.

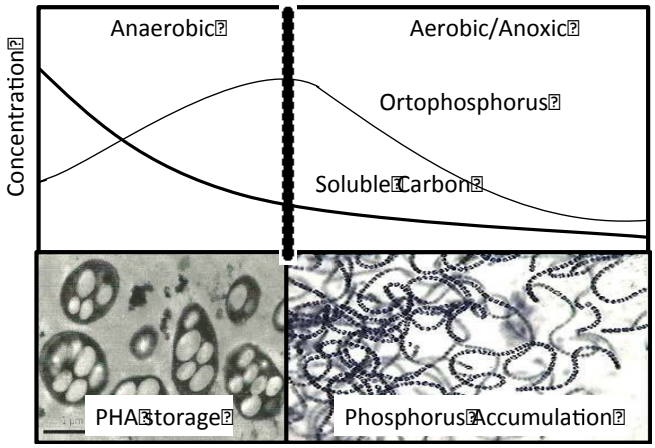

Figure 1.2. Fate of soluble carbon (i.e VFA) and phosphorus in a biological nutrients removal reactor.

The amount of phosphorus removed by biological storage can be estimated from the amount of biodegradable soluble COD (bsCOD) that is available in the wastewater influent, because most of it is converted later in volatile fatty acids (VFAs), thus acetic acid. In the literature is reported that about 10 g of bsCOD will be required to remove 1 g of phosphorus by the biological storage mechanism. Other biodegradable COD (bCOD) removal in the activated sludge will result in addition phosphorus removal by normal cell synthesis.

For common heterotrophic bacteria the typical phosphorus composition is 1.5 to 2 percent. However, many bacteria are able to store phosphorus in their cells in the form of energy-rich polyphosphate, resulting in phosphorus content as high as 20 to 30 percent by dry weight. Biological phosphorus growth kinetics are within the same order of magnitude of other heterotrophic bacteria. Mamais and Jenkins (1992) showed that biological phosphorus removal could be maintained in anaerobic/aerobic system as SRTs greater than 2.5 days at 20°C. Biological nutrient removal systems with longer SRTs are less efficient for BPR than shorter SRT designs. First, because the final amount of phosphorus removed is proportional to the amount of biological phosphorus storing bacteria wasted, the phosphorus storing production biomass is lower so that less phosphorus can be removed. Second, at long SRT the biological phosphorus bacteria are in a more extended endogenous phase, which will deplete more of their intracellular storage compounds.

However, the biological phosphorus removal is very site-specific and depends on the wastewater characteristics and the plant process design and operation. Methods to improve performance for overall phosphorus removal include the following:

1- Provide supplemental soluble and biodegradable carbon source by direct purchase (e.g., methanol, acetic acid) or by the fermentation of organic substrate directly available within the WWTP (e.g., primary sludge, OFMSW);

2- Reduce the sludge retention time of the biological system;

3- Reduce the amount of nitrate/nitrite and/or oxygen entering the anaerobic zone.

Fermentation and oxidation under under anaerobic conditions

Anaerobic fermentation and oxidation processes are used primarily for the treatment of waste sludge and wastewater, thus more or less concentrated biowaste. The final result of the anaerobic fermentation processes is the biogas, which contains a significant amount of methane (generally from 60%) that is produce from the biological conversion of the organic matter. Three basic steps are involved in the overall anaerobic oxidation of a waste:

1) hydrolyses, where particulate material is converted to soluble compounds that can be hydrolysed further to simple monomers that are used by bacteria that perform fermentation;

2) fermentation (acidogenesis and acetogenesis) where amino acids, sugar and some fatty acids are degraded further, resulting in the production of precursors of methane formation (methanogenesis);

3) methanogenesis, that is carried out by a group organisms involved in methane production.

Below, (Figure 1.3) the anaerobic schematic processes of hydrolyses, fermentation, and methanogenesis is reported.

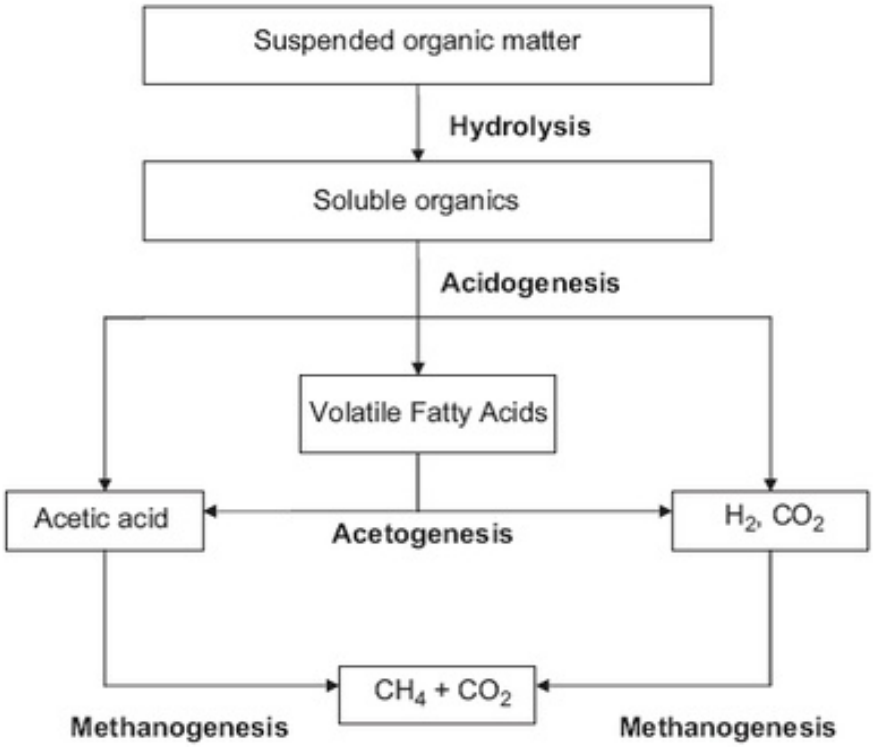

Figure 1.3. Fundamental steps of the anaerobic oxidation and methanogenesis of organic matter.

A limited number of substrate are used by the methanogenic organisms (Madigan et al., 1997): hydrogen, formic acid, carbon monoxide, methanol, methylamine and acetate (Eq. 6).

$4H_2+CO_2->CH_4+2H_2O$

$4HCOO-+4H+->CH_4+3CO_2+2H_2O$

$4CO+2H_2O->CH_4+3CO_2$

$4CH_3OH->3CH_4+CO_2$

$4(CH_3)_3N+H_2O->9CH_4+3CO_2+6H_2O+4NH_3$

$CH_3COOH->CH_4+CO_2$

From the stoichiometric balance of the COD, the volume of methane at standard conditions (0°C and 1 atm), equivalent of COD converted under anaerobic conditions is 0.35 L $gCOD^{-1}$ oxidized. Because of the relatively low free energy change for anaerobic reactions, growth yield coefficients are considerably lower than the corresponding value for aerobic oxidation. Typical synthesis yield (Y_{an}) and endogenous decay coefficients (k_d) for fermentation and methanogenic anaerobic reactions are 0.10-0.04 gVSS $gCOD^{-1}$ and 0.04-0.02 gVSS $(gVSS \cdot d)^{-1}$, respectively.

During the anaerobic digestion, the nitrogen contained in organic matter, specifically proteins, gets released when the organic compounds are degraded. This results in high concentration of ammonia nitrogen in the final effluent, which combines with CO_2 and H_2O to form alkalinity as $NH_4(HCO_3)$. The typical concentration of the alkalinity is in the range of 3000 to 5000 mg L^{-1} of $CaCO_3$.

1.3 Advanced biological processes to treat high strength wastewater

The high strength wastewaters in the context of nitrogen removal are considered such a streams, which contain ammonia in range of hundreds or even thousand mg of nitrogen per liter. These very high concentrated streams are typically founded in the anaerobic supernatant effluent from the anaerobic digestion, in landfill leachate, in many industrial final effluents or from the feedstock activities (Table 1.3). Usually, these effluents are also characterized by a low

BOD_5/TKN ratio, which means that it cannot favour an effective heterotrophic denitrification and the addition of a suitable external carbon source is required (Andreottola et al., 2012). Most cases, these high concentrated streams derive from processes with elevated or warm temperature giving us good advantage to apply certain efficient technologies, which are ardour applicable for low strength wastewater (i.e. municipal wastewater).

Table 1.3. Typical characteristics of high nitrogenous stream originated from different activities

Type of wastewater	COD (mg COD L^{-1})	BOD5 (mg COD L^{-1})	Total Nitrogen (mgN L^{-1})	Phosphorus (mgP L^{-1})	Reference
Reject Water					
	700-1000		1250-1700 (NH4-N)		Wett et al., (1998)
	810	230	1000 (NH4-N) 1053 (TKN)	27	Hellinga et al., (1999)
	-		657±56 (NH4-N); 0.4±0.7 (NO2-N); 0.2±0.7 (NO3-N)	7.3±5.9 (PO4-P)	Fux et al., (2002)
	1044±855	-	840±99 (NH4-N); 1177±163 (TKN)	-	Fux et a., (2003)
	119-530	9.5-86.7	403-997 (NH4-N) 0-2.25 (NO2-N); 0-0.2 (NO3-N)	41-92 (PO4-P	Caffaz et al., (2005)
Piggery wastewater					
	3969	1730	1650 (NH4-N); 1700 (TKN)	171	Obaja et al., (2003)
	2940±1100	-	970±50	93±26 (total P) 53±8 (PO4-P)	Hwang et al., (2005)
	-	2912	707	55	Chen et al., (2004)
Landfill leachate					
	14600-70800	-	1275-5500 (NH4-N)	-	Vilar et al., (2007)
	9660-20560	-	780-1080	20-51	Kalyuzhnyi and Gladchenko (2004)
	-	45	310	-	Ilies and Mavinik (2001)
	1300-1600	-	160-270		Jokela et al., (2002)
	2000-5000	1500-4000	500-1000	20-50	Chung et al., (2003)

1.3.1 Nitritation and Denitritation

The nitritation and denitritation or short-cut nitrogen removal process is accomplished bypassing the oxidation of ammonia to nitrite, which is directly converted to nitrogen gas through

heterotrophic denitritation (Figure 1.4). During the oxidation of ammonia under conventional environmental conditions (low strength wastewater, ambient temperature, neutral pH and oxygen concentration not limited) the AOB limit the reaction, while the NOB oxide quickly the nitrite to nitrate. However, in order to accomplish a stable short-cut nitrogen removal, thus to obtain the nitrite accumulation, selective pressure should be maintained in order to favour the growth of AOB and promoting the washout of the NOB. Compare with the conventional nitrogen removal, Turk and Mavinic 1986 reported that the biological nitrogen removal via nitrite leads an economical benefit because required less 25% of oxygen for aeration and 40% less of the carbon demand. Furthermore, it decreases sludge production by 30% and carbon dioxide emissions by 20% (Gustavsson et al., 2010).

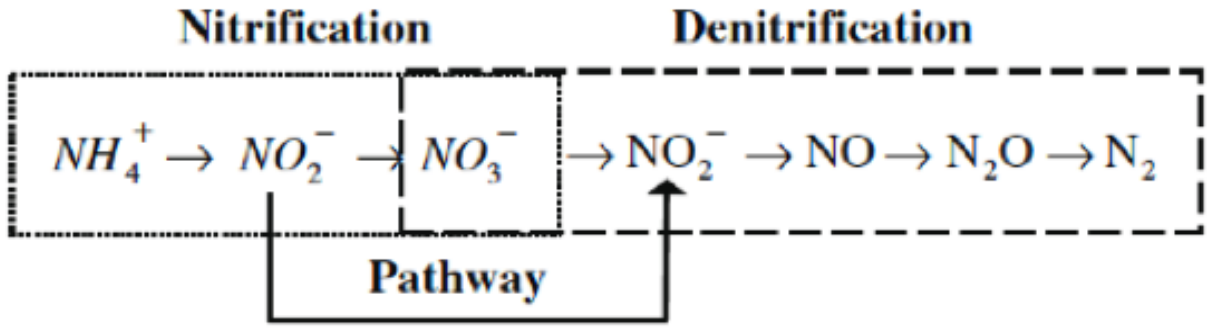

Figure 1.4. Biological nitrification-denitrification via nitrite pathway (short cut process)

Currently, several authors reported different control strategy (Figure 1.4) to achieve an effective partial nitrification (Ta), which could be classified as followed:

1) High temperature (T) and low sludge retention time (SRT): it has been demonstrated that partial nitrification is obtained operating with high temperature (30-40 °C) coupled with a dilution rate that is less than the growth rate of NOBs but greater than the AOB growth rate (about 1–0.5 days-1) (Grunditz and Dalhammar, 2001). On the other hand, these operating conditions were used in the SHARON process (Helling et al., 1998), that is a patented and well-known technology that accomplishes the ammonia removal over nitrite. However, other experiences reported by Pollice et al., (2002), Fux and Siegrist, (2004) good partial nitrification working at high sludge retention time.

-*Dissolved oxygen concentration (DO)*: low DO concentration exhibit limiting growth condition for the NOB more than AOB. Picioreanu et al., (1997), observed an half saturation constant for the AOB of 0.2-0.4 mg L^{-1}, which is lower of the values founded for the NOB (1.2-1.5 mg L^{-1}). The same authors observed a stable nitrite accumulation in a biofilm airlift suspension reactor working at low dissolved oxygen concentrations. However, this strategy alone was not enough to block completely the NOB activity, thus the nitrate formation, according with the findings of Van Loosdrecht and Salem (2005)

- *Free Ammonia (FA) and Free Nitrous Acid (FNA) concentrations*: free ammonia (FA) is a competitive inhibitor of nitrite oxidoreductase activity, which is located on the cell membrane of NOB (Yang and Alleman, 1992). Concentration of FA between 1 and 5 mgN L^{-1} inhibit the NOB but not the AOB (Abeling and Seyfried, 1992). The concentration of Free Ammonia (FA) and Free Nitrous Acid (FNA) can be calculated as a function of pH, temperature, and Total Ammoniacal Nitrogen (TAN) for FA, or Total Nitrite (TNO_2) for FNA, according to Anthonisen et al. (1976) (Eq. 7).

Eq. 11
$$FA = \frac{17}{14} \times \frac{[NH_4^+ - N] \times 10^{pH}}{k_a/k_w \times 10^{pH}}$$

Here, FA is free-ammonia concentration, $[NH_3]$ is the ammonium concentration, K_a and K_w are ionization constants of ammonia and water, respectively. In the Table 1.4, the effects of the main control strategy on the AOB and the NOB biomass are reported

Table 1.4. Main control strategy adopted to achieve partial nitrification

Operation conditions	**Range**	**The impact of operation conditions on the activity of nitrifying bacteria**		**References**
		AOB	**NOB**	
Temperature (°C)	11-16	Partial inhibited, µAOB < µNOB	Partial inhibited	Gu et al., (2012)
	20-30	No inhibition, µAOB > µNOB	No inhibition	Gao et al., (2010)
	30-35	No inhibition, µAOB > µNOB	No inhibition	Hellinga et al., (1998) Kim et al., (2006)
DO (mg/L)	>2	No inhibition	No inhibition	Gu et al., (2012)
	0.5-1.0	No inhibition	Partial inhibited	Blackburne et al., (2008) and Ma et al., (2009)
pH	6.85–7.85	No inhibition	No inhibition	Gu et al., (2012)

	<6.45 or >8.95	Complete inhibition	Complete inhibition	Ruiz et al., (2003)
FA (NH_3 mg/L)	0.31-0.69	No inhibition	No inhibition	Gu et al., (2012)
	4-10	No inhibition	Partial inhibited	Vadivelu et al., (2007)
	10-150	Partial or complete inhibited	Partial or complete inhibited	Anthonisen et al., (1976)
FNA (HNO_2-N mg/L)	0.001-0.005	No inhibition,	No inhibition	Gu et al., (2012)
	0.01-0.02	No inhibition	The anabolic activity was inhibited	Vadivelu et al., (2006)
	0.02-2.8	Complete inhibition	Complete inhibition	Anthonisen et al., (1976)
Periodic anoxic disturbances	Anoxic time 0.5 h	No inhibition	No inhibition	Gu et al., 2012
	Anoxic time 1.5-12 h	No inhibition	Partial inhibition	Kornaros et al., 2010
Toxic substance (salt inhibition) g/L	0.2-0.4	No inhibition	No inhibition	Gu et al., (2012)
	10	Partial inhibition	Complete inhibition	Cui et al., (2006)
SND% during aeration phase	25-30%	No impact	The growth process was limited in some extent	Gu et al., (2012)
	Above 20%	No impact	The growth process was limited	Yuan et al., (2008)
Real-time aeration control	Based on BF	No impact, μAOB > μNOB	No impact, but no chance to grow	Gu et al., (2012)
	Based on pH, OUR, DO	No impact, μAOB > μNOB	No impact, but no chance to grow	Buitron et al., (2005); Lemaire et al., (2008); Yang et al., (2007)

1.3.2 Nitritation and Anammox

An alternative biological process that has recently attracted attention is the complete autotrophic nitrogen removal accomplished through the anaerobic ammonium oxidation (anammox) process. The anammox process is the anoxic oxidation of ammonium using nitrite as electron acceptor. When coupled with nitritation, anammox has the advantages of much lower energy requirements (57% lower oxygen requirement) and very low demand for external carbon source (reduced by 86%) compared to conventional nitrification/denitrification (Figure 1.5).

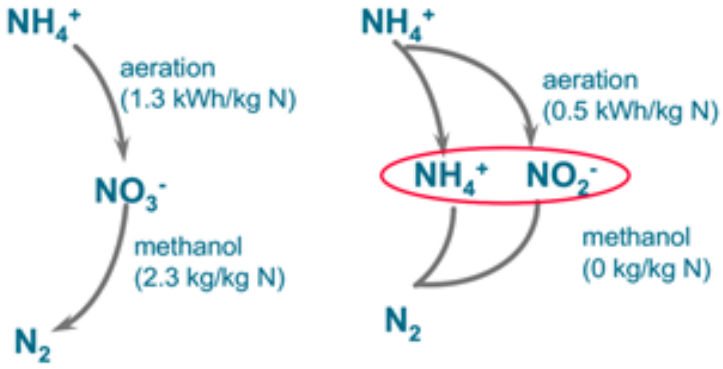

Figure 1.5. The advantages of Anammox technology (Right) than a conventional process (Left)

Effluents like reject water, landfill leachates or industrial wastewater after anaerobic treatment, are usually very poor in carbon, therefore the addition of an external carbon source is required to be able to treat them. For a long time, it was thought that ammonia oxidation could only take place aerobically. Since 1977 Broda predicted, using thermodynamic calculation, the existence of chemolitoautotrophic bacteria capable to oxidize ammonium using nitrite as electron acceptor, following this reaction:

$NH_4 + NO_2 \rightarrow N_2 + 2\ H_2O$ $-\Delta G=335$ KJ/mol NH_4.

That prediction was experimentally confirmed two decades later by Mulder (1995) in a denitrifying pilot plant, treating wastewaters from a yeast plant. An ammonium loading rate of 0.4 g NH_4-N $L^{-1}d^{-1}$ was applied in this system (Mulder et al., 1995). These authors called the process ANAMMOX (ANaerobic AMMonium OXidation).

Strous et al. (1998) and Van de Graaf et al. (2000) optimized the process condition and found the global equation of the process:

$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 0.26\ NO_3^- + 1.02\ N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$ ($\Delta G^{0'}= -357$ KJ/molNH_4+)

As shown from the free energy of Gibbs, the process is spontaneous. Based on the stoichiometry, there is a small production of nitrate: around 11% ammonium is converted to nitrate. The Anammox process is chemolitotrophic, which usually means that microorganisms are characterized by low growth rates and yields (0.066 molC/mol NH_4), due to the low Gibbs free energy of the reaction. In this case, the Gibbs free energy and the activation energy calculated by Strous (1998) is 357 KJ(mol $NH_4^+)^{-1}$ and 70 kJ (mol $NH_4^+)^{-1}$, respectively.

1.3.3 Microbiology of the Anammox biomass

In order to optimize the process, fourth classes of bacteria have to be balanced: (i) Aerobic ammonium oxidizing bacteria (aerobic AOB) produce nitrite which is the substrate for Anammox. The nitrite concentration must be maintained low because if there is excess, it becomes toxic (Jin et al., 2012). Also alkalinity can limits the growth of these bacteria (van der Star et al, 2007); (ii) Anaerobic ammonium oxidizing bacteria (Anammox) produce molecular nitrogen from ammonium and nitrite. The bacteria are characterized by low growth since the doubling time is 11 d; Nitrite oxidizing bacteria (NOB) are bacteria that must be inhibited or washed out; (iii) The heterotrophic biomass generally plays a secondary role due to the scarcity of organic carbon present in anaerobic supernatants. However, they are responsible for the reduction of nitrate to nitrite, which can be used again by the Anammox biomass (Wett B. et al, 2010).

Till now, ten anammox species have been identified, including seven that are available in laboratory enrichment cultures. All have the taxonomical status of *Candidatus*, as none was obtained as classical pure cultures. Known species are divided over five genera: (1) *Kuenenia*, represented by *Kuenenia stuttgartiensis*, (2) *Brocadia* (three species: *B. anammoxidans*, *B. fulgida*, and *B. sinica*), (3) *Anammoxoglobus* (one species: *A. propionicus)*, (4) *Jettenia* (one species: *J. asiatica)*, and (5) *Scalindua* (four species: *S. brodae*, *S. sorokinii*, *S. wagneri*, and *S. profunda).* Representatives of the first four genera were enriched from sludge from wastewater treatment plants; *K. stuttgartiensis*, *B. anammoxidans*, *B. fulgida*, and *A. propionicus* were even obtained from the same inoculum.

The sequence identities of the anammox 16S rRNA genes range from 87 to 99%, and phylogenetic analysis places them within the phylum *Planctomycetes*. Within the *Planctomycetes*, anammox bacteria deeply branch as a monophyletic clade. Their phylogenetic position together with a broad range of specific physiological, cellular, and molecular traits give anammox bacteria their own order *Brocadiales*.

1.3.4 Factors that affect the Anammox process

In order to optimize the process it is important to ensure a good balance between the anammox and denitrifying activities, since the bacteria compete for readily available carbon. Both processes, anammox and denitrification occur simultaneously in the reactor and denitrification becomes dominant increasing the OLR (>100 mg COD/Lday) (Molinuevo et al., 2009). In particular the Anammox biomass is affected by many parameters and shows a very slow growth rate (the doubling time is anywhere from 7–22 days). The most important parameters that must be controlled are: the chemical oxygen demand (COD), the pH, the temperature, the DO, the concentration of nitrite and free ammonia as shown is Table 1. 5.

Table 1. 5. Inhibition of Anammox biomass based on Jin et al., (2012).

Inhibitory substance	**Concentration**	**Effects**
Oxygen	Saturazione >18%	Irreversible inhibition
Carbon source (Metanol)	>0.05mmol/L	Irreversible inhibition
Free ammonia	35-40 mgN/L	Decreasing activity until total inhibition
Nitrite	>70 mgNO_2/L	Decreasing activity
pH	< 7	Disappearing of Anammox population
Temperature	>45 °C	Irreversible inhibition
Salinity	7.45 g/L KCl 7.1 g/L Na_2SO_4	Irreversible inhibition
Heavy metals (ex $HgCl_2$)	>1 mmol/L	Total inhibition

1.3.5 Market penetration of advanced biological nitrogen removal

Various patented industrial applications have been developed for the side stream treatment of high strength wastewater (Table 1.6). Most of them are already patented processes, including the deammonification (DEMON®), the completely autotrophic nitrogen removal over nitrite (CANON), the oxygen-limited autotrophic nitrification-denitrification (OLAND), the SHARON (Stable reactor nsystem for High Ammonia Removal Over Nitrite, Figure 1.6b) coupled with an anammox process, the BABE® (Biological Augmentation Batch Enhanced, Figure 1.6a) and in Sequencing Bacth Reactor (SBR, Figure 1.6c).

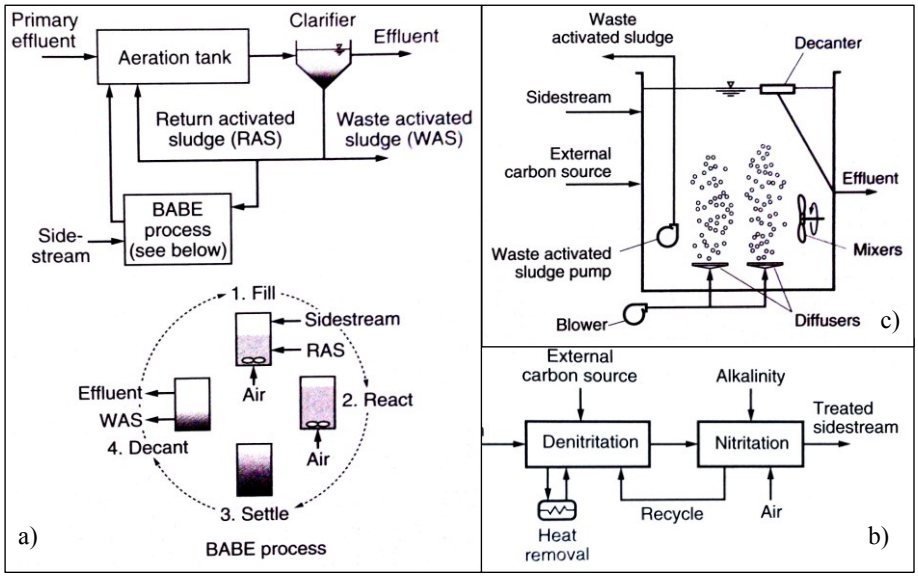

Figure 1.6a)BABE® process; b) SHARON® process; c) Sequencing Batch Reacto (SBR)

The BABE® process is an integrated sidestream-mainstream configuration to provide a source of nitrifier-enriched sludge for bioaugmentation of the mainstream process, but operating in such condition of oxygen limitation that limit the growth of the nitrite oxidizing bacteria. The BABE® reactor can be a sequencing batch reactor operated with intermitted aeration or a plug-flow reactor. The main feature that distinguish the BABE® reactors the addition of a portion of mainstream return activated sludge which serves to integrate the BABE® reactor with the mainstream process and control the BABE reactor temperature at or below 25°C. Sidestream ammonium is oxidized to a mixture of nitrite and nitrate, which are subsequently reduced through endogenous denitrification with the return of activated sludge and the addition of an external carbon source.

Nitritation-denitritation can be achieved in a SBR configuration through pH-controilled or time based intermittent aeration with the DO concentration controlled at 1mg/L or less during the aerated periods (Wett et al., 1998). An external carbon source such as primary sludge is added during the anoxic phase to promote denitrification. For sidestream with a COD/N approaching 1, an intermittent feeding strategy may be beneficial to minimize the external carbon demand. The SBR typically operates at temperature above 30°c die to biological heat generation and may require heat removal through the addition of dilution water to maintain the reactor temperature below 38°C. A total solid retention time (SRT) in range of 5 to 10 d is typical.

The SHARON® process consist of anoxic-aerobic continuous stirred tank reactor (CSTR) in a series configuration, without suspended solids retention. The process is operated at high

temperature (35 to 38°C) with the aerobic zone design according with an SRT of 1.5d and the aeration is controlled intermittently. (Hellinga, 1998). The anoxic and aerobic volume are based on the sidestream design flow and their respective design SRT values. The internal recycle flow of 13 times the feed flow is provided to supply nitrite to the anoxic zone.

Cyklar -Stulz is the company that has focused its research on the elimination of nitrogen in wastewater based on patent DEMON® (Figure 1.7). This process aims on deammonification with suspended biomass operating in a single stage SBR (Sequencing Batch Reactor). A control system for a single sludge SBR system has been developed in order to provide boundary conditions for sufficiently accurate adjustment of three impacts: ammonia inhibition, nitrite toxicity and inorganic carbon limitation. The control system includes time, pH and DO monitoring: (i) Time control defines operation cycles of 8 hours each, involving a fill/react phase, a settling period and a discharge period. During the react period of about 6 hours of the SBR cycle both deammonification processes – partial nitritation and anaerobic ammonia oxidation – are operated; (ii) These two successive processes conversely impact pH. The partial nitritation reaction depresses the pH and the anaerobic ammonia oxidation reaction elevates the pH. The actual duration of aeration intervals are ruled by the pH-signal, which characterizes the current state of reactions (pH control). (iii) The set-point of dissolved oxygen (DO control) control is specified at a low range, close to 0.3 mg/l in order to prevent rapid nitrite accumulation and to maintain a continuous repression of the second oxidation step of nitrite to nitrate.

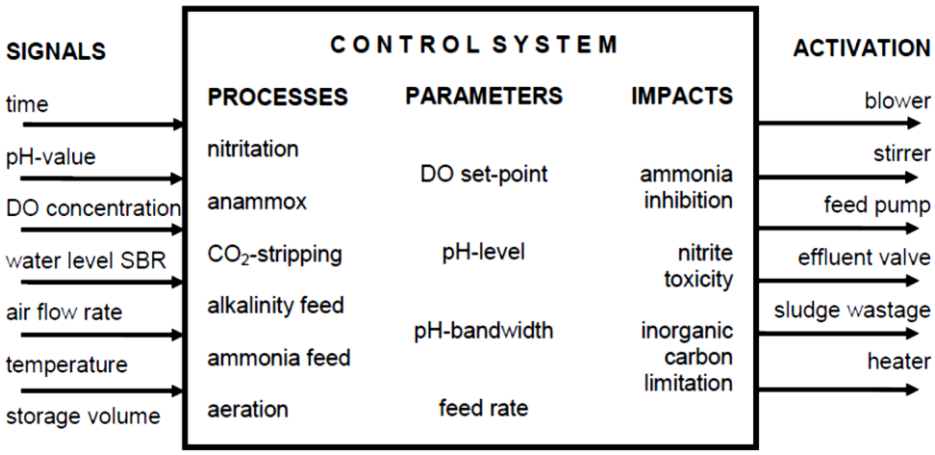

Figure 1.7. Control scheme of the DEMON process – parameter selection aims to optimize process performance considering ammonia inhibition, nitrite toxicity and inorganic carbon limitation (Wett et al., 2007)

The described deammonification process has been implemented at the WWTP Strass (Austria), in SBR tank with a maximum volume of 500 m^3 and at loading rates up to 340 kg of ammonia nitrogen per day. The aeration system is activated only within a very tight pH-bandwidth of 0.01. Due to oxygen input nitritation runs at a higher rate than anaerobic ammonia oxidation and H+ production drives the pH-value to the lower set-point and aeration stops. While dissolved oxygen is depleted all the nitrite that has been accumulated during the aeration interval is used for oxidizing ammonia. In the course of this biochemical process some alkalinity recovers and additionally alkaline rejection water is fed continuously to the reactor until the pH-value reaches the upper set-point and aeration is switched on again. In terms of stoichiometry the oxygen demand for nitritation/denitritation is 25% less than for conventional nitrification/denitrification and is reduced to 40% in case of deammonification. Therefore the specific energy demand decreased from a mean value of 2.9 kWh per kg of eliminated ammonia nitrogen down to below 1.0 kWh and leveled off at an annual average value of 1.16 kWh per kg N.

In the CANON (Completely Autotrophic Nitrogen removal Over Nitrite) system both types of bacteria can co-exist in one reactor due to oxygen and oxygen-free zones within the biofilm depth (Szatkowska et al., 2007). Ammonia is partially oxidized under oxygen-limited conditions to nitrite and next nitrite together with remaining ammonia is converted to dinitrogen gas by the Anammox bacteria. The technology can be applied for treatment of an ammonium-rich supernatant coming from dewatering of the digested sludge. As ammonium and hydrogen carbonate are the main ions (on a molar basis) affecting ionic charge in a supernatant and they both undergo transformations during partial nitritation and Anammox, it was possible to use conductivity measurements as a parameter to follow the nitrogen removal processes.
The concept of DEAMOX (DEnitrifying AMmonium Oxidation,) is to combine the recently discovered anammox (anaerobic ammonium oxidation) reaction with autotrophic denitrifying conditions using sulphide as an electron donor for the production of nitrite from nitrate within an anaerobic biofilm (Figure 1.8). To generate sulphide and ammonia, a Upflow Anaerobic Sludge Bed (UASB) reactor was used as a pre-treatment step. The UASB effluent was split and partially fed to a nitrifying reactor (to generate nitrate) and the remaining part was directly fed to the DEAMOX reactor where this stream was mixed with the nitrified effluent. (Kalyuzhnyi et al 2006).

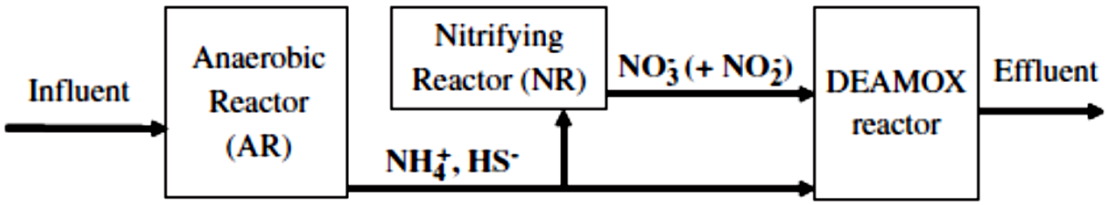

Figure 1.8. The DEAMOX process

Research is still ongoing. It can be applied to the treatment of *wastewater* with high *nitrogen* concentrations and high *organic* carbon levels, e.g. landfill leachate and *wastewater* from digested animal waste (e.g. large anaerobic digestion reactor).

PAQUES was the first industry to patent a purification system with Anammox biomass and the first to build a plant in full scale with this patent.

The first full-scale ANAMMOX® plant started up in the Netherlands in 2002. Ten years later there are 11 full-scale ANAMMOX® references operational. Paques technology's uses CSTR reactor (Continuous Stirred-Tanck Reactor) that consists of a reservoir feded with a constant flow of reagents and equipped with a stirring system. In this case, the nitrification phase is separated from the phase operated by the Anammox bacteria, in fact the nitrification phase takes place in the aerated reactor, connected with the Anammox reactor only mixed (anoxic phase). The reactors ANAMMOX ® are equipped with a ventilation system and a system of retention of biomass.

In the process ANITA™ Mox, the company Veolia Water uses AnoxKaldnes ™ MBBR technology. The ANITA™ Mox process (Figure 1.9), using a single MBBR (Moving Bed Biofilm Reactor) reactor with a specific control strategy, allows an ammonia removal higher than 90% and a total nitrogen removal in the range of 75 to 85% without having to add any external carbon source and at a very low energy cost compared to conventional nitrification-denitrification. The ANITA™ Mox process is performed in 2 steps: aerobic nitritation and anoxic ammonia oxidation performed by anammox bacteria. The two steps are taking place in a one-stage biofilm process in different layers of the biofilm: nitritation (aerobic) in the outer layer of the biofilm, anammox (anoxic) in the inner layer. This can be achieved within a single MBBR reactor, equipped with specially designed plastic carriers for the growth of the biofilm. A key element of the MBBR technology is the AnoxKaldnes carriers.

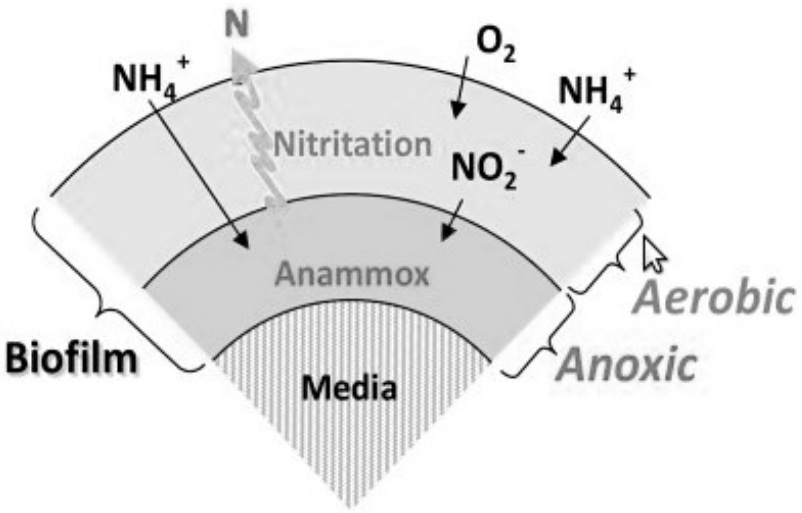

Figure 1.9. Structure of the biofilm biomass in the ANITA^{TM}MOx process

In the reactor, specific conditions are maintained in terms of pH, temperature and oxygen level for the ammonia-removing bacteria to develop as a biofilm on the carriers, thereby preventing washout of the bacteria from the reactor. This type of process can also be used for the treatment of the reject water coming from centrifugation of the digested sludge, that are usually send back to the main wastewater flow. The use of the ANITA™ Mox process on reject water can reduce dramatically the nitrogen load on the existing biological treatment line, reducing costs.

Table 1.6. Main application of advanced biological technology for high strength wastewater

Company	Position	Reactor type	WW treated	Operative condictions	Reference
Paques	Rotterdam	Airlift	Anaerobic supernatant	NLR (calculated)= 7.14 kgN/m3d	Wouter R.L. van der Star et al 2007
Cyklar	Strass	SBR	Anaerobic supernatant	NLR applied (calculated)= 0.68 kgN/m3d NLR removed (calculated)=0.5 kgN/m3d	Wett et al. 2010
Cyklar	Glarnerland	CSTR	Anaerobic supernatant	NLR applicato (calculated)=0.63 kgN/m3d	Wett et al. 2010
Paques	Angel Yeast-Cina	CSTR	industrial wastewater: production of yeast	NLR = 2kgN/m3d	www.paques.nl/page id=/ANAMMOX®.html
Paques	Waterboard Hollandse - Olanda	CSTR	Anaerobic supernatant		www.paques.nl/page id=/ANAMMOX®.html
Paques	Meihua -Cina	CSTR	industrial wastewater: production of aminoacids	NLR(calculated)=1.69 kgN/m3d HRT (calculated)=0.35 d	www.paques.nl/page id=/ANAMMOX®.html www.meihuagroup.c

					om/en/
Paques	Waterstromen (AVIKO)-Olanda	CSTR	industrial wastewater: production of fertilizers		www.paques.nl/pageid=/ANAMMOX®.html LIFE02 ENV/NL/000114 LAYMAN'S REPORT
Veolia	BIOFARM-Sjolunda	CSTR	Anaerobic supernatant	NLR(calculated)=0.94 kgNH4/m3d	www.veoliawaterst.com/anita/en

1.4 Get extra green values from the wastewater treatment plant

The anaerobic technologies have a central role within the cycle of the wastewater treatment, since it is fundamental for the development of the biorefinery concept (Verstraete, 2013). Indeed, certain of its process steps can help upfront to produce valuable products, thus represent the central operational platform from where is convenient the application of innovative technologies to clean the wastewaters and for recovery of added value materials (Figure 1.10). In this approach, it is predicted that will become soon the central process of the sewage factory where to harvest high value products such as biopolymers (Verstraete, 2013). However, the recovery and extraction of resources from the wastewater is not a new practice. The use biosolids in combination with the biogas production from the anaerobic digestion is a common place in the wastewater treatment industry. However, the recovery of new useful bioproducts from the treatment of wastewater is a challenge, focused on both the understanding of current resource markets and technologies and also identifying the future commodities and value added products that will drive future directions in the industry (WERF, 2010).

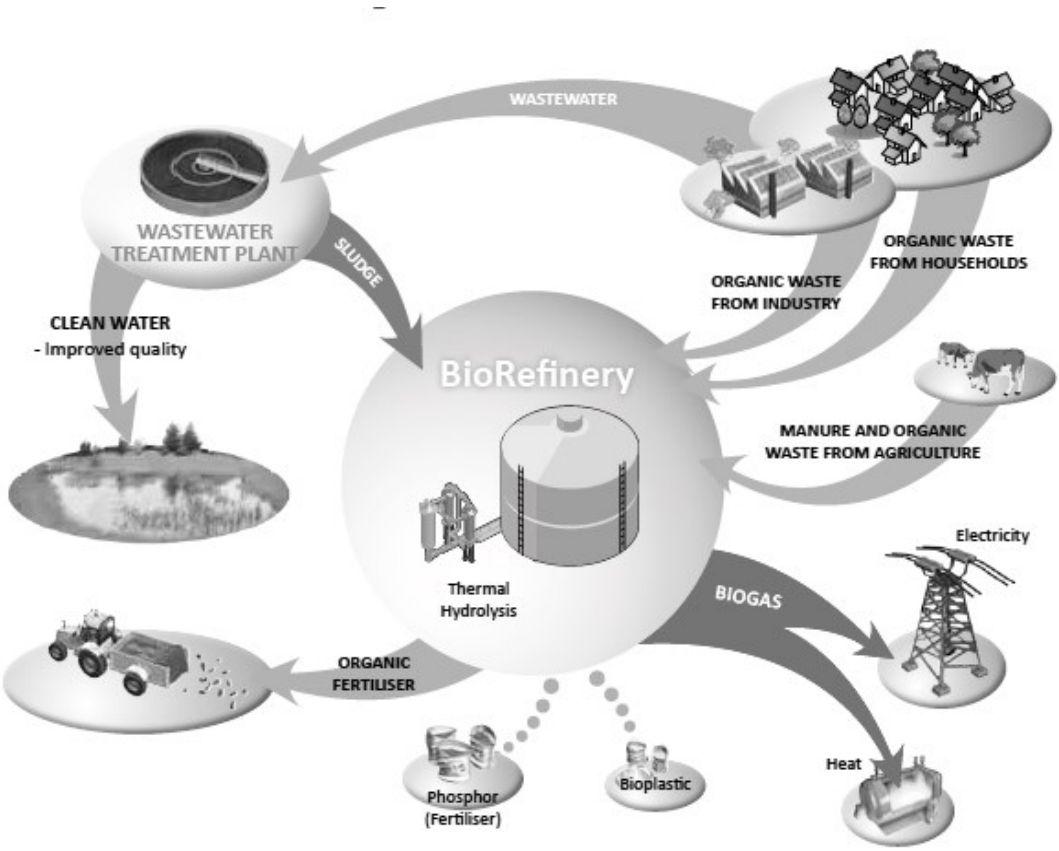

Figure 1.10. Green technologies for cleaning water clean, phosphorus fertilizer recovery, biogas and bioplastic production from wastewater and organic waste (www.billundbiorefinery.com).

New categories biotechnology processes have been emerged during the last years, aiming the extraction from wastewater treatment streams of specific chemical compounds with high market value. Among them, the production of high quality slow-release phosphorus fertilizer or biologically-deriving thermoplastic (polyhydroxyalkanoates, or PHA) from biological nutrient removal has been demonstrated by several authors (Morgan Sagastume et al., 2010). Polyhydroxyalkanoates (PHAs) are completely biodegradable polymers, that are produced from several types of bacteria, which are selected directly from the municipal sewage sludge. PHAs attracted high attention due to their thermoplastic and elastomeric properties, with the possibility of producing them from renewable resources (Dias et al., 2006, Gumel et al., 2013). Furthermore, their characteristics make them suitable for a wide range of applications (Figure 1.11).

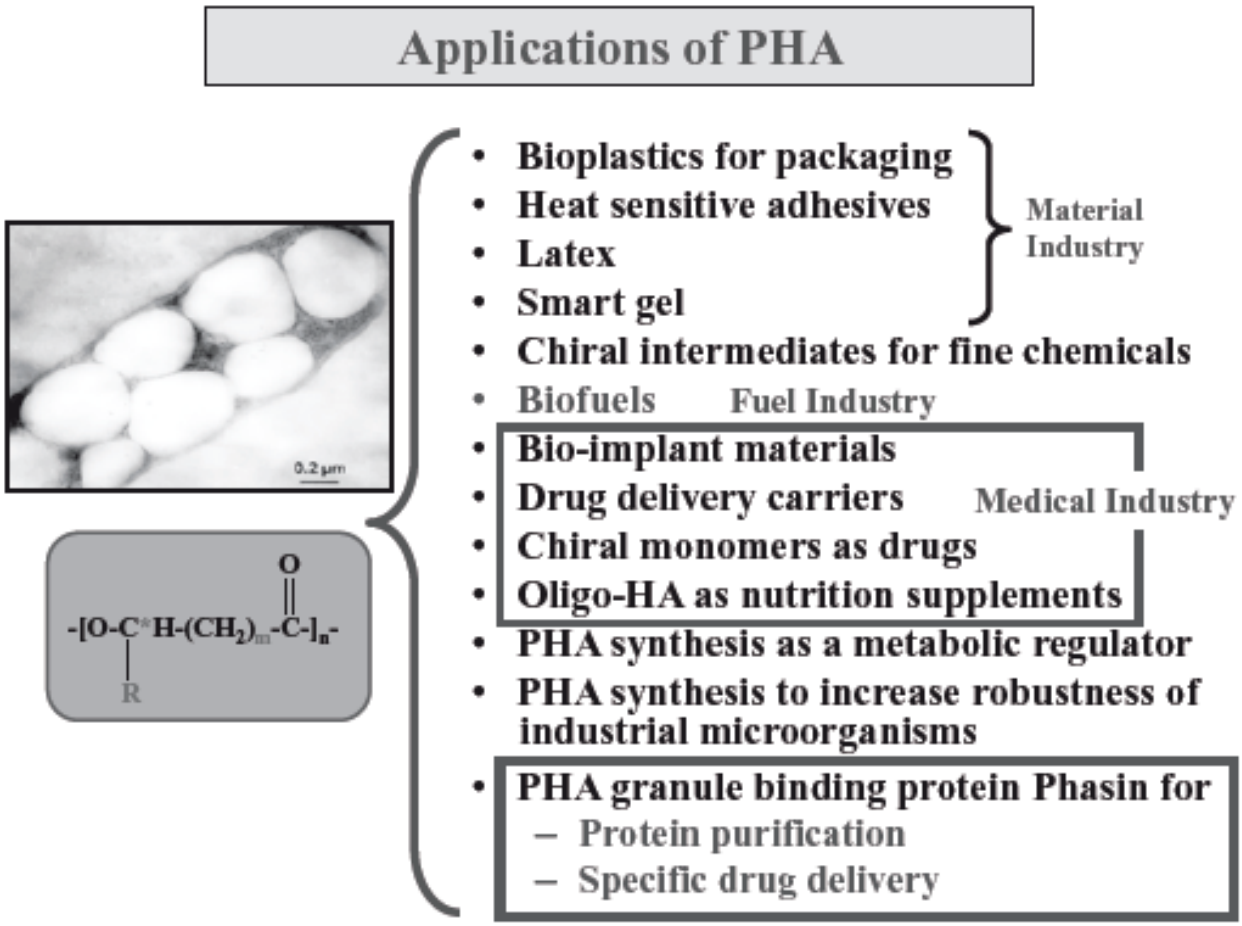

Figure 1.11. Main application for polyhydroxyalkanoates produced from bacteria

Thus, the application of biotechnology processes applied for the treatment of wastewater can be regarded as promising for sustainable development, because can provide a wide range of green bioproducts. The selection of the appropriate biowaste stream as a feedstock for biotechnological purposes mainly depends on the global region where the production plant will be constructed. To save costs for transportation, facilities for the production of biopolymers, biofuels and biochemical should be integrated into existing production lines, where the feedstocks directly accrue as waste streams. In Europe and North America, surplus whey from the dairy industry is available in large quantities, whereas huge amounts of non-wood lignocellulosic materials from rice, corn and sugar cane plants are found in many different countries worldwide. The enormously increasing production of biofuels provides a range of by-products such as glycerol and low-quality fatty acid esters from biodiesel production or distillery residues from bioethanol factories. However, the occurring of large scale implementations need that the recovery should be cost effective. Demonstration of benefits of existing technologies to improve their

implementation industry-wide is therefore an important component of this challenge. However, the utilization of waste streams for production of value-added products not only could enhances the economics of such products, but also provides industry with a strategy to overcome disposal problems of the biowaste.

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2 Chapter. Objectives

The problem definition and the research objectives of this thesis are defined in this chapter.

2.1 Research objectives

The aim of this thesis is to study the biological nutrients removal via-nitrite in Sequencing Bacth Reactor, as a possible post-treatment for anaerobic effluents. The general approach of the work was to optimize all the processes at pilot scale size in order to validate also the transferability for full-scale installation. Furthermore, the thesis moves around the anaerobic processes which are the a core the application of advanced and sustainable innovative schemes for nutrient removal and recovery of high added value biosolids.

In the existing scenario, anaerobic technology for waste and wastewater treatment has become widely accepted by the environmental industry as a cost-effective alternative to the conventional aerobic process. In addition, with the intrinsic advantages of energy saving, reduced sludge yield, and production of biofuel, anaerobic process will be the favoured green treatment technology for sustainable environment. Despite all these advantages, the anaerobic effluent still contain nutrient (N and P) and a post treatment should be require in order to satisfy the standard limits of discharge. In the decentralized anaerobic treatment of municipal wastewater from small communities generates a low strength stream that must to be treated in a post treatment stage to reach the discharge or reuse standard required by the legislation. On the other hand, the reject water derived from the digestion or co-digestion of biowaste (e.g. sewage sludge, OFMSW) is a high strength wastewater, which is commonly returned back in the mainstream of the WWTP. However this practice could have negative effect for the final effluent of the WWTP, since the total loads from this concentrated stream may represent but up to 25% of nitrogen and 30% of phosphorus incoming in the mainstream.

In both cases, the application of novel sustainable technologies based on nutrients removal via-nitrite could be relatively new options for nitrogen and phosphorus removal from low and high strength wastewater.

To better comprehend the overall features of this work, a schematic representation of the thesis is given in Figure 2.1.

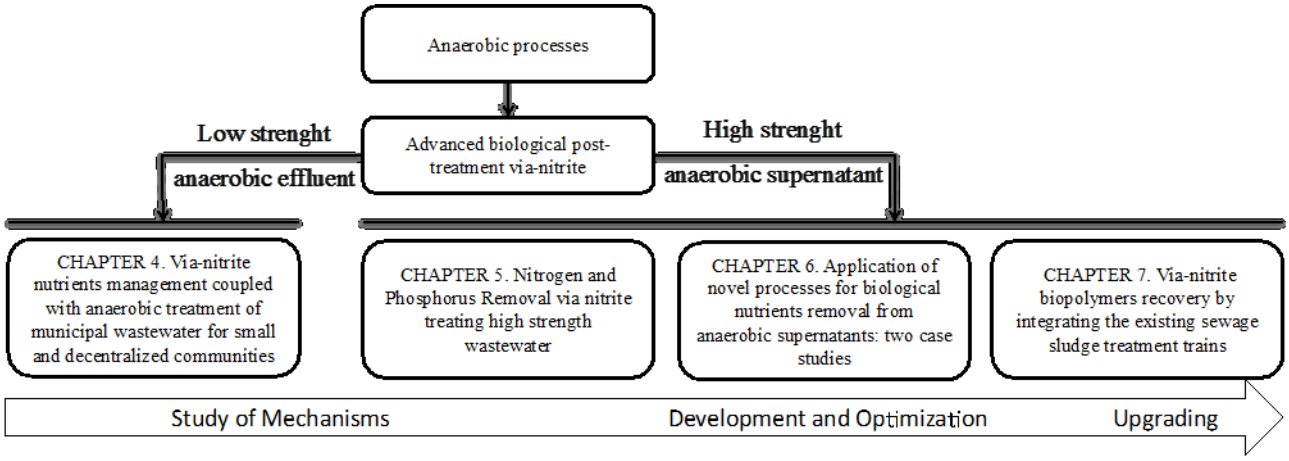

Figure 2.1. Structure of the thesis

Following the introductory chapters, the main body of the research activities falls between the chapter 4 and the chapter 7.

CHAPTER 3. Material and Methods. This section reports the experimental facilities, ginvin effort on the description of the pilot and lab scale reactors used to perform the experimental activities. . The methods, techniques and the analytical devices are also reported. The operating conditions of the reactor are detailed in the paragraph "Material and Methods" of each chapter.

CHAPTER 4. Anaerobic treatment and via-nitrite nutrients management for small and decentralized communities. In this chapter, the implementation of UASB coupled with a SBR for domestic wastewater treatment was examined. Firstly, the work focuses on the development of anammox using low activity anammox inoculum and the effect of organic loading rate (OLR) and nitrogen loading rate (NLR) on anammox. Secondly, the co-treatment of domestic wastewater and organic waste, at decentralized level, was investigated and optimize through the long-term via nitrite and enhance biological phosphorus removal. The effect of various operating conditions (e.g. the volumetric nitrogen loading rate) on the-via nitrite process was examined for the treatment of UASB effluent. The impact of the type and concentration of the applied external carbon source and of the nitrite levels on via nitrite biological phosphorus removal was investigated.

CHAPTER 5. Nitrogen and Phosphorus Removal via nitrite treating high strength wastewater. In this chapter, the short cut nutrients removal was examined in a pilot scale

Sequencing Batch Reactor, treating real anaerobic supernatant from the co-digestion of WAS and OFMSW. The best strategy to reduce the start-up operation was evaluated in order to achieve stable the via-nitrite nitrogen removal. The process was analysed under transient operating conditions, including also important variations in the NLR applied and the external carbon source dosed. The operating conditions of the via-nitrite SBR have also effect on the greenhouse gas (GHG) emissions produced, such as CO_2, N_2O, CH_4 and others. The quantification of the GHG emitted could suggest the best practice for minimizing the environmental impact due for the via-nitrite treatment of anaerobic supernatant. Finally, a life cycle assessment (LCA) was used as a tool in order to evaluate the environmental profile of the short cut nutrients removal using alternatives carbon sources derives from fermentation of biowaste.

CHAPTER 6. Application of novel processes for biological nutrients removal from anaerobic supernatants: two case studies. The aim of this chapter is to describe the integration of the novel schemes for nutrients removal from the anaerobic supernatant in two different municipal sewage treatment plants. This wastewater treatment plants were considered as two Italian case studies adopting the anaerobic digestion of only sewage sludge or co-digestion with wet fraction deriving from separate collection municipal solid wastes. As results, different strategy to produce the best carbon source were evaluated and the effect on the via-nitrite nutrients removal was investigated.

CHAPTER 7. Via-nitrite biopolymers recovery by integrating the existing sewage sludge treatment trains. The aim of this study was to evaluate the technical potential of an innovative scheme for the enrichment of PHA storing organisms coupling the nitrogen removal via-nitrite from the anaerobic supernatant. Aerobic-Feast and anoxic-famine condition were applied for the selection of PHA storing biomass using the fermentation liquid from sewage sludge as external carbon source. In addition, the stability and the response of the feast and famine process on the PHA accumulating capacity, and PHA properties were studied.

CHAPTER 8. General conclusion and recommendation. In this chapter, the general conclusion of the thesis are given extracted from the results reported in the previous chapter. Moreover, some suggestions for further investigation are proposed.

3 Chapter: Material and Methods

This chapter report the chemical and respirometric methods used along the experimental periods

3.1 List of abbreviations

-q(VFA): VFA Uptake Rate	PS: Primary sludge
AD: anaerobic digestion	q(PHA): PHA Storage Rate
AMO: ammonia monooxygenase	rbCOD: rapidly biodegradable chemical oxygen demand
AOB: Ammonium oxidizing bacteria	rDNA: rDNA
AUR-AOB: ammonium utilization rate (by ammonium oxidizing bacteria)	rRNA: rDNA
AUR-NOB: nitrite to nitrate oxidation rate	sAA: specific Anammox activity
AUR: ammonium uptake rate	SAF: sewage sludge alkaline fermentation liquid
BNR biological nutrients removal	sAUR: specific ammonium uptake rate
COD: chemical oxygen demand	SBR: sequencing batch reactor
CSTR: (continuous stirred tank reactor)	SCFAs: short-chain fatty acids
DGGE: denaturing gradient gel electrophoresis	SCND: short-cut nitrification–denitrification:
DNA: DNA	SCNR: short-cut nitrogen removal
DNPAOs denitrifying phosphorus accumulating organisms	sCOD: soluble chemical oxygen demand
DO: dissolved oxygen	scSBR: short-cut sequencing batch reactor
DPRN: denitrifying phosphorus removal via nitrite	SND: simultaneous nitrification-denitrification
EBPR: enhanced biological phosphorus removal	sNLR: specific nitrogen loading rate
Ed: Efficiency of denitrification	sNUR: specific nitrite uptake rate
Edd: Efficiency of denitrification upon the denitrificable incoming nitrogen	sOUR: Specific Oxygen Utilization Rate
En: Efficiency of nitrification	sPRR: specific phosphorus release rate
Enn: Efficiency of nitrification upon the nitrificable incoming nitrogen	sPUR: specific phosphorus uptake rate (under anoxic or aerobic condition)
FA: free ammonia	SRT: solids retention time
FNA: free nitrous acid	TCOD: Total Chemical Oxygen Demand
GAOs: glycogen accumulating organisms	Td-trans: decomposition temperature
HAO: hydroxylamine oxidoreductase	Tg: glass-transition temperature
HRT: Hydraulic retention time	TKN: total Kjeldahl nitrogen
MLSS: mixed liquor suspended solids	Tm: melting temperature
MLVSS: Mixed Liquor Volatile Suspended Solids	TN: Total Nitrogen
Mn: number average molar mass	TP: Total Phosphorus
MWCO: Molecular weight cut off	TS: Total Solids
N-SBR: Nitritation SBR	TSS: total suspended solids
NOB: Nitrite oxidizing bacteria	UASB: upflow anaerobic sludge banket
NOR: nitrite oxidoreductase	VFA: Volatile Fatty Acids
NUR: nitrite uptake rate	VFW FL vegetable and fruit waste fermentation liquid
NVZ: zones vulnerable to nitrites	vNLR: volumetric nitrogen loading rate
OC organic carbon	vNLR: volumetric nitrogen loading rate

OFMSW FL: organic fraction of municipal solids waste fermentation liquid	vOLR: Volumetric Organic Loading Rate
OFMSW: organic fraction of municipal solid waste	VSS: volatile suspended solids (g/L)
OLR: organic loading rate	WAS: waste activated sludge
ORP: oxidation reduction potential	WSF: wollastonite sludge fermentation
P&ID: piping and instrumentation diagram	WSFL: wollastonite sludge fermentation liquid
PCR: polymerase chain reaction	WWTP: wastewater treatment plant
PDI: polydispersity index	X: active biomass
PHA: polyhydroxyalkanoates	Y_H: heterotrophic biomass yields
PHB: Polyhydroxybutyrate	Y_{obs}: observed biomass yields
PHV: Polyhydroxyvalerate	$Y_{PHA/VFA}$: Storage PHA yield
PLC: programmable logic controller	$Y_{X/VFA}$: Growth biomass Yield
PO_4-P: Orthophosphates	ΔH_m: melting enthalpy

3.2 Lab scale reactors

3.2.1 Upflow Anaerobic Sludge Blanket (UASB)

The Upflow anaerobic sludge blanket (UASB) reactors (Figure 3.1a) consisted of a plexiglass cylindrical reactor having a height of 1 m and an internal diameter of 0.152 m. The reactor had a working volume of 16 L and it was fed with low strength synthetic wastewater simulating municipal wastewater, using a continuous mode peristaltic pump. The three phases separator was realized by a submerge hood, in order to separate the granular sludge and the gas the final treated effluent. The gas was collected with a tygon tube till a milligas counter (Ritter, type MGC-1 PMMA) in order to evaluate the daily volume of biogas produced. Twice a week, the biogas produced was also diverted in a gas-bag in order to be sampled for the off-site determination of the percentage of methane.

3.2.2 The lab scale Sequencing Batch Reactor

Two twins Sequencing Batch Reactors (Figure 3.1b and Figure 3.1c) were used for the experimental activities.

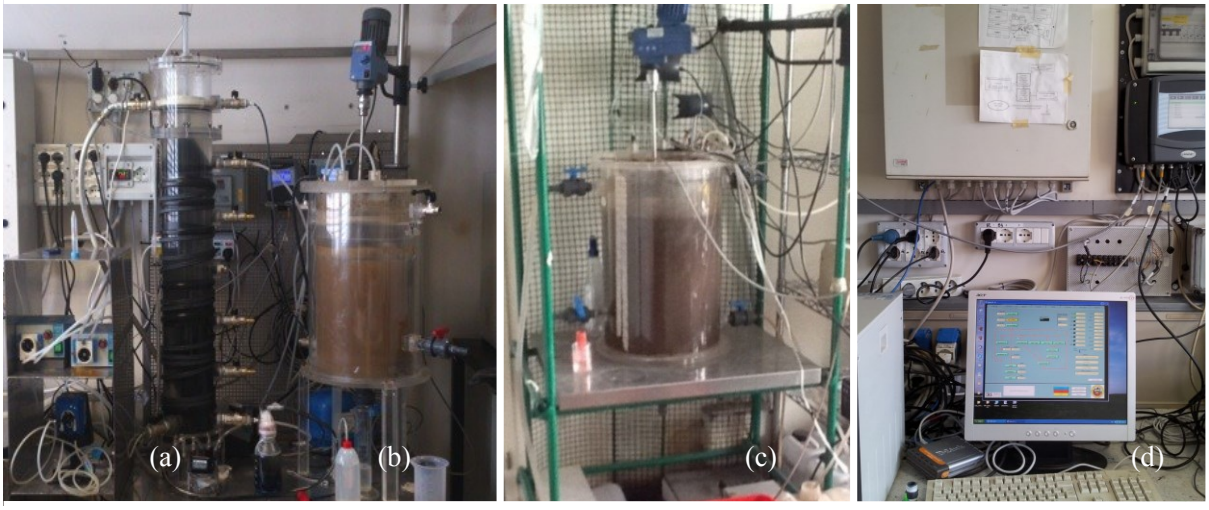

Figure 3.1. Lab scale facilities used for the experimental activities: a) upflow anaerobic sludge blanket, b) and c) sequencing batch reactors twins, d) Bench control station for the lab scale reactor.

Both system consisted of a cylindrical plexiglas reactor having a working volume of 28 L. The system was equipped with suitable submerged probes, such as dissolved oxygen (DO, LDO, Hach-Lange), pH (pHD, Hach-Lange), oxidation reduction potential (ORP, Hach-Lange) and conductivity (3798-S, Hach-Lange). The calibration of each sensor where carried out in off-line mode using the controllers sc-100 and sc-200 (Hach-Lange) and the head office standard solutions (Hach-Lange). The probe signals were processed and stored by a programmable logic controller (PLC, Compact Field Point CF-2200, National Instruments). Continuous mixing during the reaction phases (anaerobic, aerobic and anoxic) was provided by mixer IKA®RW 20 digital equipped with a flat-blade radial turbine as impeller. The proper aeration was controlled using three blowers that were switched on and off based on the DO consumption of the biomass in the reactor and the DO fixed set point. The diffusion of the oxygen in the mixed liquor was guarantee by ceramic porous diffusers, installed on the bottom of the reactor. The influent of the reactor was collected before the feeding in a equalization tank with 100 L of volume. Two peristaltic pump were used respectively during the feeding and the discharging operation. Chemicals (i.e. NaOH) and the carbon sources produce were stored in a 5 liters tank and each fed with their own peristaltic pump.

3.2.3 The sewage batch fermentators

The batch reactors used for fermentation experiments had a working volume of 1.0 L each and made of plexiglass. The reactors were maintained continuously under agitation using a magnetic stirrer a speed rate of 100 rpm. During the experimentation, the temperature was maintained constant at 37 or 55 °C, keeping the batch reactors in a oven with forced heated air recirculation.

3.3 Pilot scale reactors

3.3.1 The short cut Sequencing Batch Reactor

The pilot-scale SBR (Figure 3.2) had a reaction volume of 2.8 m^3 and processed up to 6-7 m^3 d^{-1} of real anaerobic supernatant. The latter was pumped manually by a mono pump 2-4 times a week in an equalization tank, which had 15 m^3 of volume. From this tank the anaerobic supernatant was fed into the pilot scale SBR using a mono pump (1.5 kW, flow rate 0.8–2 m^3 h^{-1}). Peristaltic pumps could provide suitable dosage of external carbon source and an acid/base for pH adjustment. Agitation was accomplished using a Rushton turbine (1.5 kW) with a disk-type radial-flow impeller. Three blowers (8.7 m^3 h^{-1} with 0.2 kW each) were used for the reactor's aeration. The on/off operation of the blowers was automatically controlled on the basis of the DO and/or pH real-time measurements, properly processed by the programmable logic controller (Compact RIO-9075, National Instruments). The pilot SBR was equipped with on-line submerged probes of dissolved oxygen (DO - LDO Hach-Lange), oxidation redox potential (ORP, Hach-lange), pH (pHD, Hach-lange), conductivity (3798-S, Hach-Lange), NOx -N (Nitratax plus, Hach-Lange) coupled with a sample filtration system Filtrax-Hach-Lange), NH_4-N (NH_4Dsc, Hach-Lange), mixed liquor suspended solids (Solitax, Hach-Lange) and temperature (PT100). During the winter or in the colder days, the heating system ensured the maintenance of temperature above 15 °C.

3.3.2 Pilot plant for fermentation of biowaste

The pilot completely stirred reactor (Figure 3.2(c)) for the fermentation of the OFMSW and the CM with MS was 200 L of working volume reactor. The reaction temperature was maintained stable at 37±1 °C by an electric water heater

3.3.3 Pilot plant for fermentation of sewage sludge coupled with tubular membrane reactor

The pilot completely stirred reactor (Figure 3.2(c)) for the fermentation of sewage sludge (mixed primary and secondary sludge) had a working volume of 500 L. The reaction temperature was maintained stable at 55±1 °C by an electric water heater.

Figure 3.2. a) Pilot Sequencing Batch Reactor with 2.8 m of working volume for the treatment of anaerobic effluents; b) Graphic interface to monitor and control the pilot SBR; c) fermentation

reactor 200 L of volume for OFMSW and CM with MS and CM; d) pilot plant for fermentation of sewage sludge coupled with tubular membrane reactor for the solid/liquid separation.

The separation of the fermented sewage sludge was carried out by employing two tubular cross-flow ultrafiltration (UF) membrane modules operating in the inside-outside filtration mode (MO P13U 1 m, Berghof, Germany). The membrane modules were made of polyvinylidene fluoride (PVDF) with internal diameter of 8 mm and molecular weight cut off (MWCO) of 15 kDa. The length of the membrane was 1m and each module had a total filtration area of 0.32 m^2. The maximum pressure of the module was 600 kPa and the maximum operating temperature was 40 °C. The pressure and flow rates were controlled by a centrifuge pump, two ball-type back pressure valves and two pressure gauges (Endress+Hauser type Cerabar M) installed in the inlet and outlet of the membrane unit. The flow rates in the filtration modules were recorded by an Endress+Hauser Promag 50 flow meter, while Riels FHKU flow meter was used to measure the permeate flow rate.

3.4 Calculations

3.4.1 COD, Nitrogen and Phosphorus mass balances

The mass balance for the carbon compounds were carried out always based on the following equation (Eq. 3. 1):

Eq. 3. 1 $$Qin \times CODin - Qout \times CODout - M(COD)f,ex - \Delta O2,COD - 1.72(2.86)\Delta N_{den} = 0$$

where:

COD_{in} is the concentration of COD (mgCOD L^{-1}) influent into the reactor;

COD_{out} is the concentration of COD (mgCOD L^{-1}) effluent from the reactor;

$M(COD)_{f,ex}$ is the mass COD in the waste activated sludge;

$O_{2,COD}$ is the mass of oxygen used for the oxidation of the COD;

ΔN_{den} is the mass of nitrogen (nitrite or nitrate) denitrified during the anoxic phase.

The nitrogen and phosphorus mass balances were calculated according with Battistoni et al., (2006), in order to evaluate the nitrification and denitrification performances as well as the efficiency of phosphorus removal. The procedure in order to evaluate the nitrifying efficiency (En) referring to the total incoming nitrogen and the nitrogen removal efficiency referring either to the total incoming nitrogen (Ed) is the followed:

Eq. 3. 2 $$LTN_{den}=LTN_{in}- LTN_{qw}\ LTN_{out}$$

Where: LTN_{den} is the total denitrified nitrogen mass loading; LTN_{in} is the total nitrogen mass loading in the influent (kg/day); LTN_{qw}= total nitrogen mass loading in the waste activated sludge (kg/day); and LTN_{out}= total nitrogen mass loading in the effluent (kg/day).

Eq. 3.3 $$Ed(\%) =(LTN_{den}/LTN_{in})x100$$

Where Ed is the denitrifying efficiency upon the denitrificable nitrogen, LTN_{den} is the total denitrified mass loading and LTN_{in} is the total nitrogen mass loading influent to the reactor.

Eq. 3.4 $$En(\%)=(LTNnit/LTNin)x100$$

where En is the nitrifying efficiency upon the total nitrogen incoming, LTN_{nit} is the total nitrified nitrogen mass loading and LTN_{in} is the total nitrogen mass loading in the influent to the reactor.

Eq. 3.5 $$Enn(\%)=[(LTN_{nit}/(LTKN_{in}+LN_{qw}-LN_{nb,org,out})]x100$$

Where Enn is the nitrifying efficiency upon the nitrificable incoming nitrogen, LTN_{nit} is the total nitrified nitrogen mass loading, $LTKN_{in}$ total Kjeldahl nitrogen mass loading in the influent of the reactor, LN_{qw} total effluent nitrogen mass loading in the wasted biological sludge and $LN_{nb,org,out}$ non biodegradable organic nitrogen mass loading in the final effluent.

Eq. 3.6 $$Edd(\%)=[(LTN_{den}/(LTKN_{den}+LNO_x-N_{out})]x100$$

Where Edd is the denitrifying efficiency upon the denitrificable nitrogen, LTN_{den} is the total denitrified nitrogen mass loading while LNO_x-N_{out} is the mass loading of NOx in the effluent.

The determination of the biological and non-biological contribution in the phosphorus removal mechanism was based on mass balances according with Eq. 3.7 and Eq. 3.8:

Eq. 3.9 $$LP_{in}=LP_{out}+LP_{w}$$

Eq. 3.10 $$LP_{w}=LP_{chem.prec}+LP_{growth}+LP_{anoxic}+LP_{aerobic}$$

Where:

LP_{in} is the phosphorus loading rate that is applied in the system;

LP_{out} is the phosphorus loading rate related to the phosphorus concentration in the effluent;

LP_{w} is the total mass of phosphorus that is removed daily per unit volume of reactor;

$LP_{chem.prec}$ is the mass of phosphorus that is removed daily due to chemical precipitation per unit volume;

LP_{growth} is the total mass of phosphorus that is removed daily and per unit volume of reactor due for the metabolic growth activities of the biomass;

$LP_{aerobic}$ is the mass of phosphorus that is removed by PAOs due to EBPR under aerobic conditions;

LP_{anoxic} is the mass of phosphorus removed by DPAOs due to EBPR under anoxic conditions;

LP_{growth} represents the mass phosphorus used for the cell synthesis of the sludge and is calculated using the general biomass formula $CH_{1.8}O_{0.5}N_{0.2}P_{0.015}$ (corresponding to 1.3659 g COD/gTS) as reported by Zeng et al., 20013 and the average MLVSS concentration of the relevant period;

$LP_{chem.prec}$ is determined experimentally using the cold perchloric acid (PCA) extraction procedure.

3.4.2 Free Nitrous Acid (FNA), Free Ammonia and Simultaneous Nitrification and Denitrification (SND)

The FNA (Eq. 3.11) was calculated using the formula (Anthonisen et al., 1976, Zhou et al., 2011):

$$FNA(HNO_2 - N) = \frac{NO_2 - N}{10^{pH} \cdot e^{\left(\frac{-2300}{273+T}\right)}}$$

Eq. 3.11

where T is the temperature in °C.

The FA (Eq. 3.12) was calculated using the formula:

$$FA(NH_3 - N) = \frac{[(NH_3 - N) + (NH_4 - N)] \cdot 10^{pH}}{e^{\left(\frac{6344}{273+T}\right)} + 10^{pH}}$$

Eq. 3.12

SND (Eq. 3.13) was determined using the equation (Gu et al., 2012):

$$SND\ (\%) = \frac{(NH_4 - N)_{oxidized} - (NO_x - N)_{produced}}{(NH_4 - N)_{oxidized}} . 100$$

Eq. 3.13

Where $(NH_4\text{-}N)_{oxidized}$ is the total amount of ammonium that is oxidized during the aerobic reaction phase and $(NO_x\text{-}N)$produced is the sum of nitrite and nitrate that is produced during the aerobic reaction phase.

3.5 Biomass activity test

The biomass activities, thus the kinetics of nitrogen and phosphorus release and uptake, were determined mainly by in situ experiments and occasionally by ex situ respirometric tests. The activity of biomass is been examined by determining the specific ammonium uptake rate (sAUR), the specific nitrite (or nitrate) uptake rate (sNUR), the specific phosphorus anaerobic release and anoxic and/or aerobic uptake rate (sPRR and anoxic sPUR and/or aerobic sPUR) and the specific oxygen uptake rate (sOUR) using an automatic multiple analysis programmable titration analyser (MARTINA), provided by SPESS Scpa and the specific Anammox activity (sAA). The ex situ sNUR, sAUR, sAA were conducted in batch reactors. The maximum sOUR was

determined using the MARTINA instrument. The tests were performed according to Kristensen et al. (1992).

3.5.1 Specific Oxygen Uptake Rate (sOUR)

The OUR (Oxygen Uptake Rate) indicated the oxygen consumption of the biomass while it is called maximal OUR when the biomass is in presence of a biodegradable substrate. Normally we refer to specific OUR, namely compared to the concentration of MLVSS and it is measured as mgO_2 consumed per hour and per gram of MLVSS. The OUR test is measured using the automatic respirometer MARTINA (SPES ScpA, Fabriano, Italy). Different types of respirometric tests can be conducted, based on the presence or absence of the substrates or to the types of substrate that can be more or less biodegradable, more or less toxic, for example, sOUR in presence of substrates: the depletion of O2 is due to the energy necessary for the oxidation of the substrates and for the cellular synthesis. One standard substrate that is added for the test is sodium acetate. The substrate is injected in non-limiting amount. A sufficient amount of biomass characterized by a concentration of solids equal to 3-4 gMLVSS L^{-1} is put under aeration until oxygen saturation (DO>4 mg·L-1). Using MARTINA (Multiple Analysis pRogrammable TItratioN Analyser) instrument, you measure the decrease of oxygen concentration in intervals of 15-30 seconds until the concentration values of 1 mgO_2 L^{-1}.

Figure 3.3. Multiple Analysis pRogrammable TItratioN Analyser (MARTINA).

OUR value is calculated from the slope of the line that shows the decreasing trend of dissolved oxygen over time. In order to evaluate only the oxygen consumption related with the

heterotrophic biomass, the activity of the nitrifying organisms were block spiking in the assay 10 mg L^{-1} of alliltiourea (ATU).

3.5.2 The Specific Ammonia Utilization Rate (sAUR)

This test allows determine the nitrification rate of the ammonia oxidizing bacteria, by monitoring the depletion of ammonium and the increase in nitrite with time under complete aerobic conditions. With the AUR test we can check the NOB activity, measuring the production of nitrate. To determine the nitrification rate (sAUR), 500 mL of activated sludge characterized by a concentration of solids equal to 3-4 gMLSS L^{-1}, was collected during the aerobic phase of the SBR and was placed in Erlenmeyer flasks under continuous aeration (DO>4 mg L^{-1}). After 30 min, the biomass was spiked with fixed ammonium concentration and the time profile of ammonium, nitrite and nitrate were recorded for 180 minute. The slope of ammonia, which indicates the depletion of ammonia nitrogen during the time, divided by the MLVSS, indicated the sAUR (Eq. 3.11)

Eq. 3.14
$$sAUR = \frac{\Delta NH_4 - N/\Delta t}{MLVSS}$$

3.5.3 Specific Nitrogen Uptake Rate (sNUR)

The denitritation rate (NUR) was determined to assess the denitrification capacity of the biomass in presence of nitrite (NO_2) or both nitrite and nitrate (NO_3). 500 mL of activated sludge was collected at the end of the anoxic phase (i.e. DO<0.05 mg·L^{-1}) and was placed in Erlenmeyer flasks, under mild agitation. The top part of the Erlenmeyer flask covered with aluminium foil to avoid direct contact with air. Subsequently, the biomass was spiked with fixed concentration of nitrite. Then, the biomass was spiked with an excess of external carbon source then, the nitrite and nitrate profile were followed for 180 minutes. The slope of the nitrite and/or nitrate (NOx= NO_3+NO_2) which indicates the depletion of ammonia nitrogen during the time, divided by the MLVSS, indicated the sNUR (Eq. 3.11).

Eq. 3.15

$$sNUR = \frac{\Delta NO_x - N/\Delta t}{MLVSS}$$

All batch activity tests were conducted at room temperature (25±2°C) and the pH was maintained in the range of 7.4±0.3.

3.5.4 Specific Anammox Activities (sAA)

Ex situ anammox activity experiments were also conducted occasionally to check the in situ measurements using the method developed by Dapena-Mora et al. (2007). In this method the gaseous nitrogen emissions were determined based on pressure differential. The heterotrophic denitrification rate was determined by considering the difference of NO_3-N and NO_2-N concentrations in the reactor compared to the stoichiometric value given by the anammox reaction. The SAA is calculated from the N_2 gas production rate divided by the biomass concentration in the vial X (g VSS L^{-1}) (Eq. (3)):

$$\mathrm{SAA} = \frac{\mathrm{dN_2/dt}}{\mathrm{XV_L}} \times \frac{\mathrm{28gN}}{\mathrm{molN_2}} \times \frac{\mathrm{1440min}}{\mathrm{d}}$$

Eq. 3.16

where VL is the volume of the liquid phase (L).

The amount of substrate dosed in the batch glass vessel was higher of the affinity constant of the anammox bacteria for ammonia and nitrite respectively, since the values were much higher than the affinity constant of the anammox bacteria (10 and 5 μM for ammonia and nitrite respectively).

3.5.5 Specific Phosphorus Release and Uptake Rate

These batch assays included test to evaluate the activity of the phosphorus accumulating organism (PAOs), thus:

- The maximal phosphorus release capacity of the biomass;
- The maximal phosphorus uptake under aerobic and anoxic conditions.

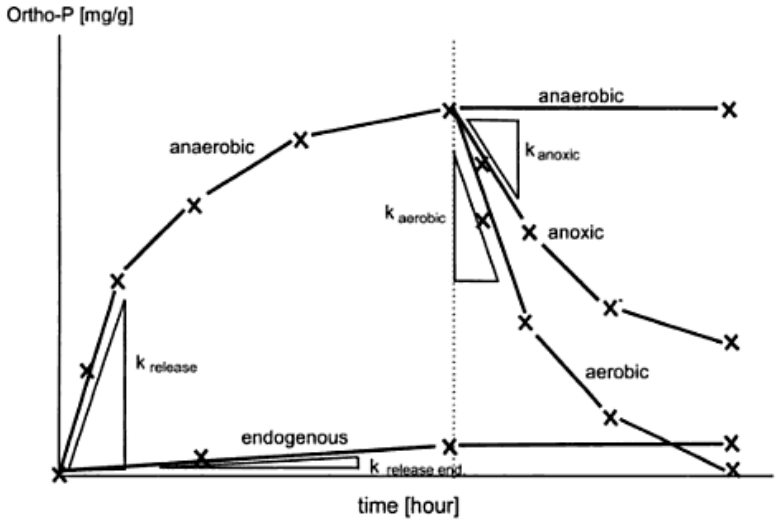

Figure 3.4. Typical profile of the phosphate concentration during the release and during the aerobic or anoxic uptake.

The results obtained might be compared based on the following classification (Table 3.1):

Table 3.1 Classification of the biomass based on its capacity to release and uptake phosphorus (Janssen et al., 2002)

Release or uptake rate (mg P/g VSS. hour)	Classification
<3	Moderate
3–7	Good
>7	Very good

Specific phosphorus release rate (sPRR). 500 ml of preaerated biomass was taken from the parent SBR and placed in a covered Enrlenmayer glass flask. An excess of carbon source was added under complete anaerobic environment: Metcalf & Eddy (2013) reported that 10 mg VFA are necessary to remove 1 mg of PO_4-P. After the addition of the carbon source, the biomass was sampled every 10 to 30 minutes and its background liquid was characterized by phosphate concentration. The duration of this assay was up to 3 hour. During this period, the polyphosphate accumulating organisms (PAOs) took up the VFAs to produce polyhydroxyalkanoates (PHA), and consequently they release phosphorus as phosphate in the mixed liquor. The specific

phosphorus release rate was calculated from the slope of the phosphate, which indicated the maximal increase phosphorus, and then divided by MLVSS.

Specific phosphorus aerobic uptake rate (aerobic sPUR). The biomass after an anaerobic period in presence of the carbon source (about 0.3-0.4 $mgPO_4$-P $mgCOD^{-1}$ as acetate, Janssen et al., 2002) was subjected to continuous aeration, provided by a small blower and a porous diffuser. After the start of aeration, the biomass was sampled every 10 to 30 minutes and its background liquid was characterized by phosphate concentration. The duration of this assay was up to 180 minutes. The maximal slope of the phosphate during the time normalized by the MLVSS indicated the specific phosphorus aerobic uptake of the biomass.

Specific phosphorus anoxic uptake rate (anoxic sPUR). After the anaerobic period described before, an electron acceptor was added to the biomass, such us nitrite or nitrate, without any presence of oxygen. The Erlenmayer glass flask was covered with a covered aluminium in order to avoid any transferring of oxygen from the external side. Furthermore, some nitrogen gas in the mixed liquor was flushed during the test in order to conserve the complete anoxic conditions. After the start of nitrite and/or nitrate, the biomass was sampled every 10 to 30 minutes and its background liquid was characterized by phosphate concentration. The duration of this assay was up to 180 minutes. The maximal slope of the phosphate during the time normalized by the MLVSS indicated the specific phosphorus anoxic uptake of the biomass.

3.5.6 Normalization with Arrhenius equation

All the kitetics obtained with the above methods, before any comparison were normalized to the reference temperature of 20°C using the Arrhenius temperature correction equation (Eq. 3.13).

Eq. 3.17
$$Kd^{20°C} = \frac{Kd}{1.026^{T-20}}$$

3.6 Analytical methods

Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), partial and total alkalinity, Chemical Oxygen Demand (COD), soluble COD (sCOD), total Kjeldahl nitrogen (TKN), ammonium (NH4-N), phosphorous (P) were determined according to standard methods. For nitrogen, the macro-Kjeldahl method was used (4500-Norg) and for phosphorus the ascorbic acid method (4500-P).31 The sCOD was measured in the filtrate obtained after the filtration of the sample through Whatman 0.45 μm membrane filters. The mixed liquor was analyzed for total and volatile suspended solids (MLSS, MLVSS), COD, nitrogen and phosphorus according to standard methods. Nitrite, nitrate and phosphate were determined by ion chromatography (Dionex ICS-900 with AS14 as column). The volatile fatty acids (VFAs) in the fermentation liquid (C2-C5) were analyzed by liquid chromatography (Dionex ICS-1100 with IonPac ICE-AS1 as column). The biomass collected during the sampling was removed from the supernatant through centrifugation and then freeze dried with a lyophilisation unit (Lio 5P). Then, the lyophilised biomass was analysed for the PHA content in the sludge samples with the method developed by Lanham et al., (2013). The content of polyhydroxyalkanoates (PHA) in the sludge was measured according with the method developed by Lanham et al., (2013). The fraction of the PHA in the biomass were calculated according with Eq. 3.18:

Eq. 3.18 $$PHA(\%)=W_{PHA}/(W_X+W_{PHA})\ X\ 100$$

where the PHA(%) is the percentage of PHA in the biomass, W_{PHA} in the biomass, while W_X represent the active biomass.

A polymer extraction procedure was adopted to extract the polymer from the final biomass after the PHA accumulation. Chloroform was used as solvent according with a ratio of 50 ml/g of freezed dry biomass weighted, and methanol was used after to precipitate the extracted PHA. In the extracted biopolymers, the weight average molecular and number average molecular weights and polydispersity indices were determined using the size exclusion chromatography (SEC,type of the instrument), the glass transition temperature (Tg), melting temperature (Tm), and the melting enthalpy (DHm) were analysed using a differential scanning calorimetry (DSC, type of the instruments).

3.6.1 Determination of the gaseous emissions

The gaseous emissions from the surface of the SBR tank were determined on line by the static chamber method, using the Bruel and Kjaer photo-acoustic analyzer (Type 1302, Copenhagen, Denmark) Specifically, a 0.2 m high floating container (chamber) was placed on the surface of the tank and the time variation of the gas concentration inside the chamber was measured on line, in order to determine its emission rate (Figure 3.5).

Figure 3.5. View of the SBR and floating container for the on line gas measurement, (b) instrument for the on line measurement of gas emissions and (c) cross section and side view of the floating container.

The gas concentration inside the chamber increased with time as a result of the gaseous emissions from the surface of the reactor (Sommer et al., 2004; Eklund, 1992). For each gas, a concentration versus time plot was developed and an interval of linear increase of the concentration was determined; the emission rate was calculated as follows (Chiumenti et al., 2007):

$$E_R = \frac{C_i - C_0}{t_i - t_0} \frac{V_{ch}}{A_{ch}}$$

Eq. 3.19

Where:

E_R (mg/m^{2}h) is the emission rate of the gas;

t_i (h) and t_0 (h) represent the time edges of the linear portion of the concentration plot;

C_i (mg/m^3) and C_0 (mg/m^3) represent the gas concentration at times t_i and t_0 respectively;

V_{ch} is the volume of the chamber, 0.01116 m^3 and;

A_{ch} is the area of the emitting surface covered by the chamber, 0.07789 m^2.

This way, ER was determined for NH_3, N_2O, CO_2, CH_4 and methyl mercaptan (CH_3SH) at various times of the SBR process, during aeration reaction, anoxic reaction and sedimentation. Therefore, a plot of ER versus time was obtained for each gas for an SBR cycle. To determine the total amount of gas emitted during one cycle, the area below this graph was determined using the following formula:

Eq. 3.20 $$GM=\sum(ERASBR\Delta t)$$

Where:

G_M (mg/cycle) is the amount of the emitted gas per cycle;

A_{SBR} (m^2) is the surface area of the pilot SBR, 1.5·1.5 = 2.25 m^2;

Δt (h) is the time interval during which the gas emissions were recorded.

3.6.2 Fluorescent in situ hybridization (FISH)

The fluorescent in situ hybridisation (FISH) techniques was performed following the procedure described by Amman (1995) using the probes reported in table (Table 3.2). The percentage area of the cells was quantified via image analysis using the software ImageJ. Thirty to forty images were taken for the quantification. FISH preparations were visualised with a Leica DM6000 B laser scanning microscope. Microscopic examinations for the characterization of biomass were performed according to Eikelboom and van Buijsen (1981) and Jenkins et al. (1993) employing a 0–5 scale for Filament Index (FI) and Specific Filament Index (SFI) determination. The Gram and Neisser staining procedures were also employed for identification purposes. All microscopic

observations were made at 200X and 1000X magnifications by using a phase contrast microscopy.

Table 3.2. FISH probes used in this work by ThermoFisher

Probe (ThermoFisher®)	**Detected Microorganism**
EUBmix	All Eubacteria
Nso190	All AOB β-Proteobacteria
Nso1225	All AOB β-Proteobacteria
Nit3	Nitrobacter ssp. (NOB)
Nitspa712	Nitrospira ssp. (NOB)
PAOmix	Accumulibacter phosphatis
GAOmix	Competibacter
Amx 368/Amx 820	Anammox/kuenenia
Cte	Comamonas sp., Acidovorax sp., Hydrogenophaga sp., Aquaspirillum sp.
Mz21	Thauera denitrifiers
ZRA23a	Zoogloea spp.

3.6.3 Polymerase Chain Reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE)

Molecular Techniques to Analyze the Bacterial Community Structure. PCR-DGGE analysis−Total DNA extraction from SBR sludge samples was carried out using the FAST DNA Spin Kit for Soil (MO BIO, Carlsbad, CA) according to the manufacturer's instructions. Approximately 0.5 mL of material was used per extraction and the extracted DNA was polymerase chain reaction (PCR) amplified and then analyzed by denaturing gradient gel electrophoresis (DGGE). Samples of biomass from the parent SBR, as well as from conventional activated sludge and from the anaerobic supernatant fed to reactor were analyzed. The whole 16S rDNA was first amplified using primers F8 and R11 (Weisburg et al., 1991). Then a nested PCR was performed starting from 16S rDNA amplicons obtained from the first amplification. In this second PCR reaction, the iper-variable V3 region of the 16S rRNA gene was amplified using

primers p3 (with a GC clamp) and p2 (Altschul et al., 1997). The PCR reactions master mix composition and temperature conditions for PCR were as described in Lampis et al. (2009). The PCR products were quantified using Low DNA Mass Ladder (Celbio, Italy) in a 2.0% agarose gel. DGGE analyses were carried out on V3 region amplicons. Gels (8% acrylamide/bisacrylamide 19:1, BioRad) were cast using a denaturing gradient of 30− 60%, with 100% denaturant defined as 7 M urea and 20% (v/v) formamide. Electrophoresis was performed at 50 V for 16 h at 65 ° C with the D code Universal Detection System (Biorad) and gels were stained with EtBr (1 mg/L). The PCR products corresponding to the different samples were loaded in the same DGGE gel. Equal masses of the PCR products were loaded in each lane to allow for a semiquantitative comparison among lanes. The interpretation of the DGGE gels with respect to the Similarity index was carried out by relying on the software SPSS 8.0 for the calculation of the Pearson coefficient, while the NTSYS software was used for the dendrogram formation, according to the UPGMA method.

Cloning and sequencing analysis. Major bands in the DGGE profiles were cut off from the gel, eluted, and reamplified using primers p1 (without GC clamp) and p2 (Altschul et al., 1997). The amplicons obtained were then cloned in the pGEM vector trough the pGEM-T Easy vector system (Promega, Italy), according to the manufacturer's instructions. Clones were then checked for their correspondence to the original bands by performing DGGE analysis, which compared the bands excised in the profile with those cloned. Afterward, cloned bands were sequenced (PRIMM Biomedical, San Raffaele, Milan, Italy) on both strands, and finally searched for similarity using the BLASTN database (Muyzer et al., 1993). The sequences were initially aligned using the multiple alignment program CLUSTAL_X 1.83 (Thompson et al., 1997). A phylogenetic tree was then constructed relying on the neighbor-joining method with the MEGA version 4.0 software package (Kumar et al., 2008). Bootstrap analysis was performed on the basis of 1000 replications.

3.7 References

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4 Chapter. Anaerobic treatment and via-nitrite nutrients management for small and decentralized communities

In this chapter, the implementation of Upflow Anaerobic Sludge Blanket coupled with a Sequencing Batch Reactor the treatment of decentralized domestic wastewater was examined.

4.1 Introduction

In decentralized communities, the separation of black and grey water and the treatment of the more concentrated black water by the upflow anaerobic sludge blanket (UASB) followed by suitable post treatment for nutrients recovery/removal is a sustainable treatment option (Latif et al., 2011, Khan et al., 2011). Technologies, such as vacuum toilets result in more concentrated black water streams resulting in enhanced biogas production by the UASB technology. Currently, the treatment of wastewater at EU level is achieved through the implementation of conventional aerobic-anoxic activated sludge systems, fact that results in significant carbon footprint. This trend must be changed by focusing the research on methods that promote the environmental and economical sustainability. The anaerobic treatment of the urban sewage through UASB process has been recognized as a key method for the advanced treatment of several wastewater streams (Latif et al., 2011). The latter leads several advantages for the treatment of the wastewater, including energy recovery through the utilization of the produced biogas, the small energy requirements for the system operation, the production of significant lower amount of sludge compared to that produced from the conventional aerobic processes, the production of stabilized sludge with good dewatering characteristics that can be directly disposed without the need for further treatment, the application of the system on either very small or very large scale, the ease of operation and construction, the low carbon footprint and the reduction of CO_2 emissions, the low nutrients and chemical requirements (Gomec, 2010; Latif et al., 2011). In spite of the well proven advantages of the UASB systems, the low quality of the treated effluent due to the partial removal of pathogens and nutrients creates the need for the post-treatment of the anaerobic effluent in order to remove the residual organic matter, nutrients and pathogens and to acquire the desired characteristics for reuse purposes. SBR could be employed as an effective post-treatment stage for the further treatment of effluent produced by the UASB system offering many advantages compared to the conventional activated sludge system especially with respect to environmental and economical aspects. SBR can be applied for the treatment of the UASB effluent, taking advantage of the time-based process cycle which provides high flexibility in terms of operation (i.e. adjusting phase time of each process and phase sequence), less footprint of the facilities, as opposed to conventional activated sludge system (CAS) where limited

flexibility of process control is possible. Such an integrated system is the upflow anaerobic sludge blanket (UASB) coupled to the completely autotrophic nitrogen removal process. This scheme has inherent benefits emanating from energy recovery through biogas by the UASB, minimization of CO2 emissions and elimination of external carbon source requirements. The completely autotrophic nitrogen removal process is implemented in two steps: initially ammonium is partially oxidized to nitrite by ammonium oxidizing bacteria (AOB) under aerobic conditions; subsequently the remaining ammonium is oxidized directly to gaseous nitrogen under anoxic conditions using nitrite as electron acceptor by the autotrophic anoxic ammonium oxidation (anammox) bacteria. Compared to conventional biological nitrogen removal processes, the application of the nitritation/anammox process can reduce the operating expenses by 60%, eliminates the need for external carbon source and the waste activated sludge is much lower. Furthermore, the process reduces the greenhouse gas emissions by 90% since CO2 is consumed and there are limited N_2O emissions (Hu et al., 2010; Kartal et al., 2010). The application of the anammox process for autotrophic nitrogen removal has focused on elevated nitrogenous (>300 mgNH_4-N L^{-1}) effluents (Furukawa et al., 2009; Joss et al., 2009; Vázquez-Padín et al., 2009; Ganigué et al., 2012) but limited informations are available on the application of anammox on low strength effluents (de Clippeleir et al., 2011; Hendrickx et al., 2012) with a low C/N ratio, such us the anaerobic effluent from UASB reactors. At such an environment, anammox can outcompete the denitrifiers (Güven et al., 2005) but the disadvantage is the low specific growth rate, which makes the start-up period much longer compared to other nitrogen removal processes (Van Dongen et al., 2001; Lopez et al., 2008). The estimated doubling time of anammox is 11 d at 32–33 °C (Strous et al., 1998) and can be even higher at lower operating temperatures. Hendrickx et al. (2012) treated low strength UASB effluent (31 mgNH_4-N L^{-1}) using a gas lift reactor for the completely autotrophic nitrogen removal process and found an anammox doubling time of approximately 17 d at 20 °C. However, a common start-up strategy of anammox is to use inoculum collected from the few existing plants which apply patented processes, such as DEMON (Deammonification) SHARON (Single reactor High activity Ammonia Removal Over Nitrite)-Anammox, CANON (Completely Autotrophic Nitrogen removal Over Nitrite) and OLAND (Oxygen Limited Autotrophic Nitrification Denitrification) (Li et al., 2008). The nitritation/anammox process can be carried out in a single reactor or in two separate reactors. When dealing with high loaded wastewater with a significant amount of organic matter, the two step process is recommended in order to avoid heterotrophic growth in

the anammox reactor (Van Hulle et al., 2010). In the case of UASB reactors treating municipal wastewater, the majority of the biodegradable organic content has already been removed. Under these conditions, the growth of heterotrophic denitrifiers is limited by the low availability of easily biodegradable organic carbon, allowing the implementation of a single reactor for nitritation/anammox. Table 4.1 summarizes the findings of various studies concerning the effect of organic matter on Anammox process. Two different mechanisms have been identified to explain the organic matter inhibition on anammox; in the first mechanism, the heterotrophic bacteria outcompete the autotrophic anammox bacteria that also exist, while in the second mechanism the anammox bacteria are still the dominant species under high concentrations of organic matter, but perform different metabolic pathways (i.e., use organic matter rather than ammonium and nitrite as substrate) (Jin et al., 2012). However, when the separation of black and grey water is not practiced, the potential co-treatment of biodegradable organic waste (BOW) and domestic sewage by UASB can be a viable alternative for decentralized communities resulting in energy recovery. The UASB coupled to the sequencing batch reactor (SBR) for the nutrients removal is characterized by lower energy requirements than the conventional activated sludge process. UASB effluents require an external organic carbon source for denitrification; the use of BOW as carbon source can increase the sustainability of the treatment scheme. Organic carbon sources, which contain a mixture of short chain fatty acids (SCFAs) can improve nutrients removals (Ji et al., 2010; Frison et al., 2013). Biowaste fermentation results in the production of a liquid that is very rich in SCFAs and can serve as an ideal substrate for the via nitrite process. The acidogenic fermentation of source separated vegetable and fruit waste could transform 43% of the total chemical oxygen demand (COD) to soluble COD (sCOD) in the resulting fermentation effluent (Traverso et al., 2000). In via nitrite nitrogen removal process ammonium is oxidized to nitrite and is subsequently reduced to gaseous nitrogen. Nitritation/denitritation is achieved through the inhibition or washout of nitrite oxidizing bacteria (NOB) and the accumulation of ammonium oxidizing bacteria (AOB) (Gustavsson et al., 2010; Malamis et al., 2013). Nitritation/denitritation has been examined for strongly nitrogenous effluents (Fux et al., 2006; Ganiguè et al., 2007, 2012) and for low strength effluents (Yang et al., 2007, Blackburne et al., 2008a, Guo et al., 2009). The via-nitrite processes are more difficult to implement in low strength effluents (e.g. domestic wastewater) where the FA concentration in the reactor is usually low. The process has been tested for low and high DO reactor concentrations (Pollice et al., 2002; Blackburne et al., 2008b) high salinity (Ye et al., 2009)

various free ammonia (FA) and free nitrous acid (FNA) concentrations (Park et al., 2010) various solids retention times (SRTs) (Pollice et al., 2002)

Short-cut nitrogen removal (SCNR) through ammonium oxidation to nitrite and its subsequent reduction to gaseous nitrogen instead of the conventional nitrification/denitrification via nitrate pathway has gained increasing attention over the last years due to its inherent advantages (Peng and Zhu 2006). The via-nitrite processes are more difficult to implement in low strength effluents (e.g. domestic wastewater) where the FA concentration in the reactor is usually low. In such cases, effective real time process control is required.

Differently from nitrogen, phosphorus is a non-renewable resource. In general, the management of phosphorus is not necessary oriented towards the production of struvite as a slow-release fertilizer: in fact it is rarely used directly in agriculture (Hao et al., 2013). The development and application of bioprocesses could be a sustainable option to enhance phosphorous bioaccumulation and, if properly operated, the potential production of high added value products. The mechanism of phosphorus uptake can also be realized under anoxic conditions by denitrifying phosphorus accumulating organisms (PAOs) that can utilize nitrate or nitrite as electron acceptors (Kishida et al., 2006) Denitrifying PAOs require less carbon source compared aerobic PAOs (Carvalho et al., 2007). The via nitrite soluble phosphorus removal achieved was 97.6% when sludge fermentation liquid was applied and only 73.4% when acetic acid was dosed in an SBR treating synthetic wastewater (Ji et al., 2010). The rate of phosphate uptake can be higher in the presence of nitrite compared to nitrate (Lee et al., 2001). Exposure to high nitrite concentrations may result in severe inhibition of phosphate uptake (Saito et al., 2004). Various threshold nitrite concentrations have been reported concerning the inhibition of phosphate uptake, which vary from 3-93.7 mg NO_2-N L^{-1} (Peng et al., 2011; Lee et al., 2001; Meinhold et al., 1999; Zhou et al., 2007). The aim of this chapter is to compare and combined the UASB-SBR process was implemented to treat low strength wastewater at decentralized level. Firstly, the work focuses on the implementation of anammox process from low activity anammox inoculum in order to assess the effect of organic loading rate (OLR) and nitrogen loading rate (NLR) on anammox. Secondly, the via-nitrite nitritation and denitritation was evaluated as post-treatment process for the anaerobic effluent of UASB. The co-treatment of domestic sewage and biowaste at decentralized level was demonstrated. Biowaste fermentation results in the production of a liquid that is very rich in short chain fatty acids (SCFAs) and can serve as an ideal substrate for the via-nitrite nitrogen and phosphorus removal. In this case, the fermentation liquid produced

from the stored organic waste was employed as an external carbon source, increasing the attractiveness of the process

Table 4.1. Effect of organic matter on Anammox process for the treatment of various wastewater streams characterized by different COD/N ratios

Stream	**Reactor**	**Inoculum**	**Nitrogen forms ($mg \cdot L^{-1}$)**	**COD ($mg \cdot L^{-1}$)**	**Effect**	**Reference**
UASB post-digested pig manure effluent	semi-continuous UASB	50 mL anaerobic granular sludge from a potato factory	NH_4-N: 40, NO_2-N: 50 , NO_3-N: 50	< 142 > 237 (OLR 112 mgCOD/L-d)	No inhibition Complete inhibition	Molinuevo et al., 2009
UASB partially oxidized pig manure effluent	semi-continuous UASB	50 mL anaerobic granular sludge from a potato factory	NH_4-N: 3780, NO_2-N: 1700 , NO_3-N: 4001	< 242 > 290 (OLR 136 mgCOD/L-d)	No inhibition Complete inhibition	Molinuevo et al., 2009
Synthetic wastewater	UASB	40 mL from anaerobic granular sludge	NH_4-N: 40, NO_2-N: 50 , NO_3-N: 50	> 300 (COD/N ~ 2)	Anammox inactive	Chamchoi et al., 2008
Synthetic wastewater	UASB	Anammox inoculums NLR: 0.072kg NH_4–N /VSS-d	NLR: 13.9 kgN/m^3-d	700	2.1% NO_2-N consumption via anammox At COD/NO_2-N~2.92 denitrification was dominant	Tang et al., 2010
Synthetic wastewater	SBR	Inoculation with nitrifying, anammox & denitrifying bacteria (seed sludge from a landfill-leachate treatment plant)	NH_4-N: 200 NO_3-N: 17 NLR: 22.2, 44.4, 66.7 g/m^3-d	COD: 100 OLR: 11.1, 22.2, 33.3 g/m^3-d (COD/TN: 0.1)	85.5 % TN removal through partial nitrification with anammox (%) 7.3% denitrification	Lan et al., 2011

4.2 Material and Methods

4.2.1 Set-up of the Upflow Anaerobic Sludge Blanket (UASB)

The lab scale UASB reactor was set up in order to treat real municipal wastewater with an hydraulic retention time (HRT) between 0.25 and 0.42 days. The flow treated corresponded to a vOLR varying in a range of 0.7 till 1.5 kgCOD m^{-3} d^{-1}. The working temperature was always above 20°C and it was not control during the operation.

4.2.2 The SBR accomplishing autotrophic nitrogen removal

The SBR was inoculated with anammox biomass collected from a DEMON® plant, which was treating landfill leachate and winery wastewater. After the inoculum, the anammox biomass received UASB effluent and operated for 98 days, without wasting any sludge (solids retention time, SRT > 60 d). Warm water was circulated in the external chamber of the SBR in order to maintain the temperature constant at 30±1 °C. The SBR cycle was set-up in order to accomplish this sequence of the phases: feeding, aerobic, anoxic, settling, discharge. During the aerobic period, the concentration of DO was strictly controlled in the range from 0.2 to 0.3 mg L^{-1}. Table 4.2 shows the operating conditions maintained during the start-up (in total 98 days of operation), which can be divided into two main periods.

Table 4.2. Operating parameters for the two experimental periods of the start-up.

Parameter	Units	Period 1 (0-33)	Period 2 (34-98)
SRT	d	No Sludge discharge	No Sludge discharge
HRT	d	5.64±2.68	15.03±2.83
vNLR	kgN $m^{-3}d^{-1}$	0.24±0.11	0.10±0.02
sNLR	kgN $(kgVSS\ d)^{-1}$	0.17±0.11	0.06±0.005
MLSS	g L^{-1}	1.96±0.53	2.15±0.38
MLVSS	g L^{-1}	1.41±0.38	1.54±0.28

OLR	kgCOD $m^{-3}d^{-1}$	0.37±0.18	0.15±0.01

During days the first period (29 days), a short HRT (5.6 h) and relatively high vNLR and vOLR were applied. In the subsequent days (34–98) the HRT was increased up to 1.5 h in order to reduce further the residual organic matter and put more stress on the heterotrophic biomass.

Autotrophic Biomass Examination

The biomass was characterised using fluorescence in situ hybridisation (FISH) techniques following the procedure described by Amman (1995) using the probes Amx 368 (anammox) and NSO190 (all AOB). The percentage area of the cells was quantified via image analysis using the software ImageJ. Thirty to forty images were taken for the quantification. FISH preparations were visualised with a Leica DM6000 B laser scanning microscope. Microscopic examinations for the characterization of biomass were performed according to Eikelboom and van Buijsen (1981) and Jenkins et al. (1993) employing a 0–5 scale for Filament Index (FI) and Specific Filament Index (SFI) determination. The Gram and Neisser staining procedures were also employed for identification purposes. All microscopic observations were made at 200and 1000magnifications by using a phase contrast microscopy.

4.2.3 The short-cut SBR accomplishing the via-nitrite nutrient removal

The short-cut SBR (scSBR) was inoculated with the conventional activated sludge (CAS) originated from the municipal wastewater treatment plant of Treviso municipality (Northern Italy). Five different experimental periods were operated (Table 4.3). In the period 1, the solids retention time was not applied for the first 10 day while later the wastage of sludge was based on and SRT of 18 days.

Table 4.3 Operating conditions of the SBR (a) and main parameters of the system (b), during the experimental periods (*Start-up operation between days 0 to 45).

Parameter	Period 1 (0-58)*	Period 2 (59-73)	Period 3 (74-89)	Period 4 (90-172)	Period 5 (173-253)

HRT	0.79±0.03	0.79±0.03	0.31±0.04	0.29±0.0	0.35±0.03
vNLR ($kgN \cdot m^3\ d^{-1}$)	0.13±0.03	0.21±0.06	0.22±0.07	0.21±0.0	0.19±0.02
sNLR $kgN(kgVSS\ d)^-$	0.03±0.02	0.09±0.06	0.10±0.06	0.09±0.0	0.07±0.01
DO_{aer} ($mg\ L^{-1}$)	2	2	2	2	0.2-0.8
Carbon Source	DOW FL	Acetic acid	Acetic acid	VFW FL	DOW FL
MLSS ($g\ L^{-1}$)	5.35±1.10	5.40±1.07	4.65±0.86	4.12±1.4	3.19±0.53
MLVSS ($g\ L^{-1}$)	4.42±0.98	4.57±0.91	2.81±0.82	2.81±0.9	2.73±0.40
$F\ M^{-1}$	0.32±0.20	0.31±0.22	0.22±0.10	0.15±0.0	0.13±0.04

The DO set point was 2.0 mg L^{-1} for periods 1-4 and at 0.2-0.8 $mg \cdot L^{-1}$ for period 5. During periods 1-2, the vNLR was kept low (on average 0.13 kgN $m^{-3}d^{-1}$), while in periods 3-5 the vNLR was increased on average to 0.19-0.21 kgN $m^{-3}d^{-1}$. The sequence of the SBR cycle consisted of static filling, anoxic, aerobic, settling, discharge and idle during periods I-IV and static filling, anaerobic, aerobic, anoxic, settling, discharge and idle during period 5.

As the UASB effluent is very poor of biodegradable organic matter, an external carbon source was additionally supplied to promote the biological processes of denitritation and phosphorus removal. Three different types of external carbon source were used: DOW FL, acetic acid and VFW FL. The fermentation liquid originating from the organic fraction of municipal solid waste (DOW FL) was produced in the Treviso hall through the mesophilic (37°C) acidogenic fermentation of source separated DOW. The source-separated DOW was grinded and diluted with secondary effluent up to 6% TS and then fermented at an organic loading rate (OLR) of 20 $kgTVS \cdot m^{-3}d^{-1}$, which involved an HRT of 3 d. The pH during the acidogenic fermentation process was controlled in the range of 4.1-4.5. The fermentation liquid originating from vegetable and fruit waste (VFW FL) was produced in the lab by acidogenic fermentation following the procedure described above. This carbon source was enriched with butyric and propionic acid because of it was very poor in SCFA. The external carbon source was always added during the first 1-2 min of the anoxic and anaerobic phase. The acetic acid (HAc) used in periods 2 and 3 was commercially available (80% wt).

Analyses of short-cut biomass through microscopic examination.

During the short cut SBR operation, representative biomass samples were characterized using fluorescence in situ hybridisation (FISH) technique following the procedure described by

Amman (1995). NSO1225 (Ammonio-oxidizing-β-Proteobacteria), MZ1 (Thauera sp.), PAOmix (Accumulibacter phosphatis), GAOmix (Competibacter phosphatis, Glycogen accumulibacter organisms), Nit3 (Nitrobacter ssp. NOB), Nitspa712 (Nitrospira ssp. NOB), Bet42a (Betaproteobacteria), ZRA23a (Zooglea spp) and Cte (Comamonas sp., Acidovorax sp., Hydrogenophaga sp., Aquaspirillum sp.) probes were used for FISH analysis. The details concerning each probe (formamide percentage, sequence and target organism) are provided in the probe-base database (http://www.microbial-ecology.net/probebase). Fluorescence signals were recorded with an acquisition system (Coolsnap, Roper Scientific Photometrics) coupled to an Axioskop 2 epifluorescence microscope (Zeiss, Germany). The quantification of microbial populations was based on a biovolume fraction and was performed after the application of the FISH technique with the Daime software (Daims et al., 2006).

4.3 Results and discussion

4.3.1 The effluent from the UASB reactor

The main physicochemical characteristics of the UASB effluent (i.e., SBR influent) are summarized in
Table 4.4.

Table 4.4. Physicochemical characteristics of the effluent produced from UASB reactor (SBR influent) during the two experimental configurations.

Parameter	Average (Min-Max) Period UASB-Anammox	Average (Min-Max) Period UASB-scSBR
TSS ($mg \cdot L^{-1}$)	33± 15.0	55 ± 23.0
Total COD ($mg \cdot L^{-1}$)	88.4 ± 35.0	123.4 ± 38.6
sCOD ($mg \cdot L^{-1}$)	55.3 ±19.1	89.9 ± 21.1
TKN ($mg \cdot L^{-1}$)	58 ± 13.9	65.1 ± 20.7
NH_4-N ($mg\ L^{-1}$)	51 ± 21.5	56.2 ± 18.6
P ($mg \cdot L^{-1}$)	8 ± 3.7	11.4 ± 3.7
PO_4-P ($mg \cdot L^{-1}$)	6 ± 2.4	10.0 ± 7.9

The effluent is characterized by very low sCOD/TKN ratio (= 0.95). In practice, the readily biodegradable COD is low and the complete autotrophic nitrogen removal process seems to be a viable alternative that has the advantage of no carbon source requirements. In fact, the minimum (stoichiometric) amount of organic carbon required for conventional denitrification is 2.86 $mgCOD\ (mgN)^{-1}$ and for denitritation it is 1.72 $mgCOD\ (mgN)^{-1}$. It is clear that the effluent characteristics do not favour the conventional denitrification, neither the heterotrophic short-cut via-nitrite pathway. In this case, an external carbon source must be applied in order to promote the nitritation and denitritation process.

4.3.2 Operation of the UASB–Anammox configuration

Inoculum and start-up strategy

The inoculum was then placed in the SBR and was fed with UASB effluent. Although the origin of the inoculum was a complete autotrophic full-scale reactor, the anammox activity that was measured just prior the inoculation was low (0.87 mgN (gVSS h)$^{-1}$ at 30 °C). On the other hands, the activity of heterotrophic denitrifiers was very high and was found to be 38 mgN (gVSS h)$^{-1}$, suggesting that the anammox bacteria growth was suppressed by denitrifying community. The start-up strategy was to maintain a low vOLR and thus promoting the anammox bacteria decreasing the competition for nitrite with heterotrophic denitrifiers.

Activities of the biomass

As mentioned before, the inoculum was acclimatized to an environment rich is organic matter and consequently the activity of heterotrophic denitrifiers was very high. However, when it was placed in the SBR and was fed with UASB effluent, the denitrifying biomass activity decreased dramatically within hours due to the absence of readily available organic matter.

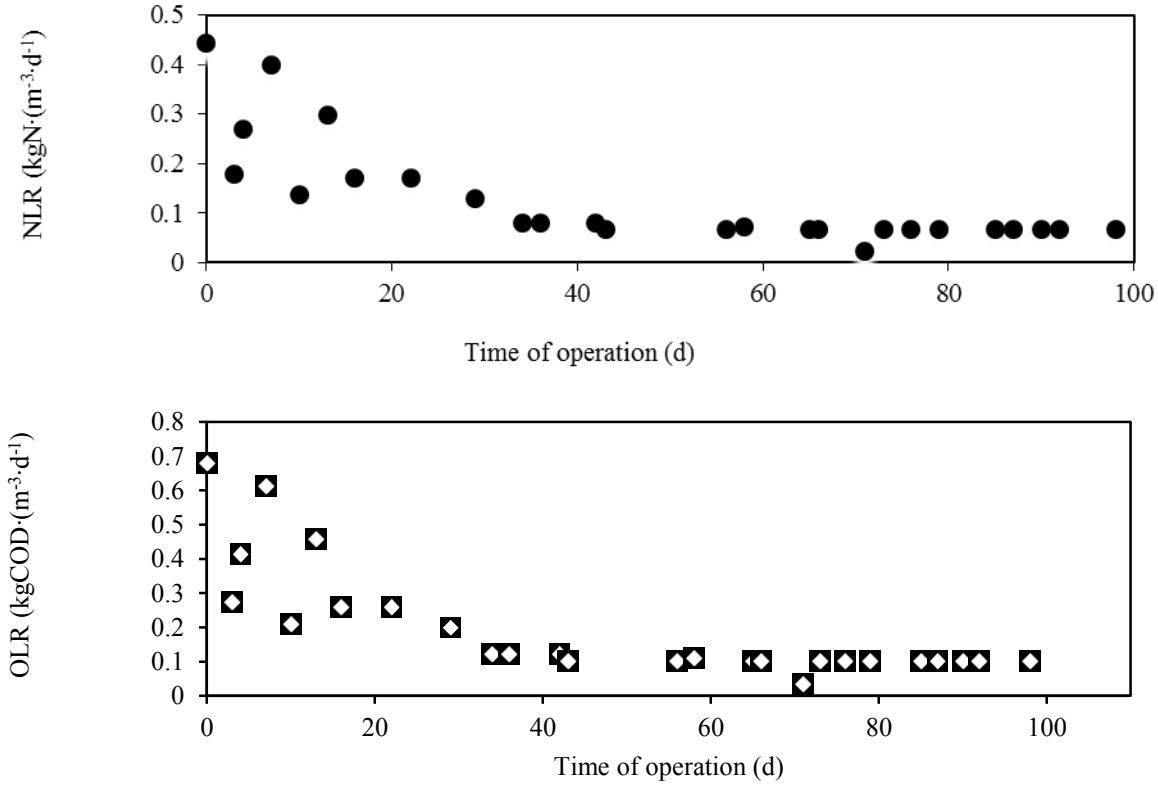

Figure 4 shows OLR and the vNLR that were maintained during the SBR operation and shows

the variation in the activity of the anammox bacteria (a) and of the heterotrophic denitrifiers with time (b).

The first in situ experiment that was conducted in day 1 showed that the anammox activity was very close to that of the inoculum (i.e., 0.92 mgN (gVSS h)$^{-1}$ at 30 °C), while the activity of denitrifiers decreased dramatically to 0.85 mgN (gVSS h)-1 at 30 °C.

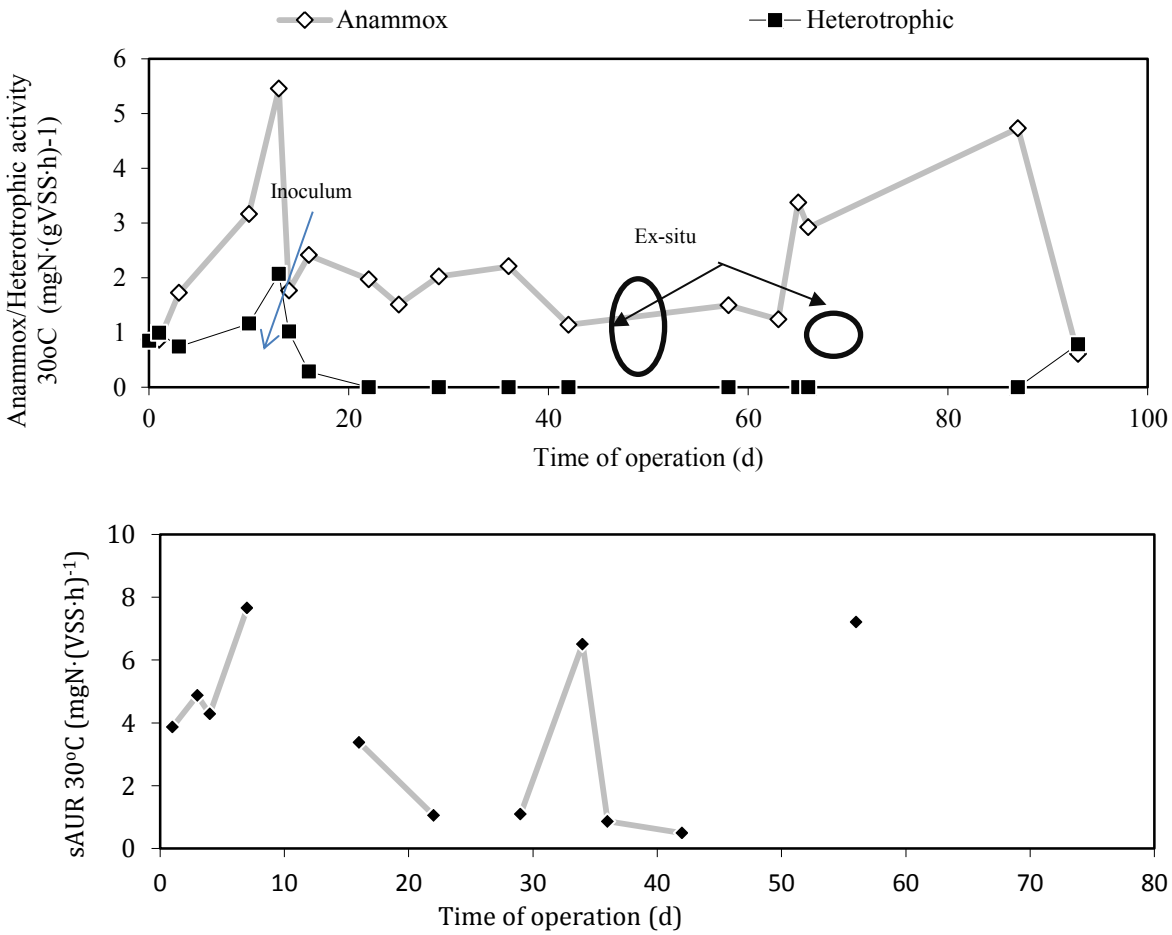

Figure 4.1 (a) Anammox and denitrifying heterotrophic biomass activity (b) specific ammonium uptake rate in the SBR treating effluent from low strength UASB effluent (in-situ activity tests).

In the first period the vOLR was variable and quite high and as a result some heterotrophic denitrification was observed. It seems that residual organic carbon present in the UASB effluent in combination with the applied vOLR maintained still a residual heterotrophic denitrification (0.79–2.07 mgN (gVSS h)$^{-1}$). During the first 13 days an increase in anammox activity was observed up to 5.52 mgN (gVSS h)$^{-1}$, which was the highest sAA value monitored throughout

the operation of the SBR. After day 13 the vOLR and vNLR were gradually reduced and this had a significant impact on the heterotrophic denitrifiers activity which decreased from 2.07 mgN $(gVSS\ h)^{-1}$ (day 13) to less than 0.1 mgN $(gVSS\ h)^{-1}$ (day 22). In the 2nd period (days 30–98) the vNLR was kept at low values (0.10 kgN $m^{-3}\ d^{-1}$) to maintain a low OLR (0.15 kgCOD $m^{-3}d^{-1}$) that would also favour the anammox bacteria development. In general, the process was quite sensitive with occasionally low anammox activity measured at days 42 and 93 and high rates of nitrogen removal obtained at days 13 and 87. Nevertheless, the sAA was almost always higher than the sAA of the inoculum. The average sAA was 2.27±1.31 mgN $(gVSS\ h)^{-1}$ at the temperature of 30 °C (0.08 kgN $m^{-3}d^{-1}$), which represents an increase of 161% compared to the inoculum anammox activity. The activity of heterotrophic denitrifiers was much lower (i.e., 0.53±0.50 mgN $(gVSS\ h)^{-1}$). In the first and second period the average sAA were similar (i.e., 2.39±1.35 mgN $(gVSS\ h)^{-1}$ in the first period and 2.22 ± 1.39 mgN $(gVSS\ h)^{-1}$ in the second period). On the contrary, the activity of denitrifiers decreased from 0.80 ± 0.66 mgN $(gVSS\ h)^{-1}$ in the first period to 0.22 ± 0.24 mgN $(gVSS\ h)^{-1}$ due to the decrease in the vOLR. Even though the denitrifiers activity was suppressed in the second period, this was not followed by an increase in the sAA. Actually, the obtained removal rate in that period, was 0.09 kgN $m^{-3}d^{-1}$ (including anammox and denitrifiers) which is very close to the applied vNLR of that period (0.10 kgN $m^{-3}\ d^{-1}$). Consequently, the decrease in vNLR was considered to limit the sAA. Nevertheless, the strategy of implementing an OLR lower than 0.20 kgCOD $m^{-3}d^{-1}$ was successful as the denitrifying heterotrophic biomass activity decreased to very low levels, while the anammox activity was not significantly affected. The obtained nitrogen removal rate (i.e., 0.09 kgN $m^{-3}\ d^{-1}$) is low compared to the rate given by Hendrickx et al. (2012) (0.26 kgN $m^{-3}\ d^{-1}$) for the treatment of low strength UASB effluent using a gaslift nitritation/anammox reactor, and to the rates given by De Clippeleir et al., 2011 (0.38–0.44 kgN $m^{-3}\ d^{-1}$) who operated a rotating biological contractor with oxygen-limited autotrophic nitrification/denitrification (OLAND). Chamchoi et al. (2008) found nitrogen removal rates ranging approximately from 2–5 mgN $(gVSS\ h)^{-1}$ after the first 10 days of operation of an anammox UASB reactor for the treatment of low-strength effluents, which are values similar to the ones found in this work. Anammox activity values higher than 10 mgN $(gVSS\ h)^{-1}$ and higher than 0.5 kgN $m^{-3}d^{-1}$ are often reported in literature (Fernández Rodríguez, 2010; Vlaeminck et al., 2009; Joss et al., 2009); however, these values are derived for very high influent ammonium concentrations and vNLR. Table 4.5 shows higher

percentage of nitrogen in the sludge as an indicator of higher presence of autotrophic species of bacteria.

Table 4.5 Physicochemical characteristics of sludge during the periods

Period	COD (%SS)	N (%SS)	P (%SS)	MLSS ($g \cdot L^{-1}$)	MLVSS ($g \cdot L^{-1}$)	MLVSS/MLSS (%)
(0-29)	72 (68-75)	5.1 (4.4-5.5)	1.8 (1.7-2.1)	2.1 (1.7-2.5)	1.6 (0.9-2.0)	73.5 (51.6-87.3)
(30-98)	71 (67-72)	6.2 (5.6-6.3)	1.2 (0.9-1.3)	2.0 (1.8-2.4)	1.4 (1.2-1.6	69.8 (58.7-79.4)

Table 4.6 shows the NO_2-N/NH_4-N, NO_3-N/NH_4-N and NO_3-N/NO_2-N conversion ratios that were determined by monitoring the various nitrogen forms under anoxic conditions in the reactor.

Table 4.6. Removal ratios of the substrates during the operation of the anammox reactor

Day	NO_2-N/NH_4-N	NO_3-N/NH_4-N	NO_3-N/NO_2-N
1	1.05	-0.14	-0.13
4	2.32	-0.19	-0.08
10	2.01	-0.01	-0.01
13	1.35	-0.07	-0.05
14	1.35	-0.07	-0.05
16	1.56	-0.14	-0.09
29	1.23	-0.23	-0.19
Average Period 1	1.56	-0.12	-0.09
34	1.33	-0.21	-0.20
42	1.28	-0.17	-0.13
65	1.34	-0.23	-0.17
73	1.34	-0.20	-0.15
87	1.35	-0.24	-0.17
93	1.74	-0.10	-0.06
Average Period 2	1.35	-0.19	-0.15
Stoichiometric Anammox (Strous et at., (1998)	1.32	-0.26	0.20

The ratios in most cases were close to the stoichiometric values of the anammox reaction. However, deviations were observed particularly in early days of the first period. The NO_2-N/NH_4-N conversion ratio was higher than the anammox stoichiometric value since nitrite was also denitrified by heterotrophs. Furthermore, the absolute value of the conversion ratio of NO_2-N/NH_4-N was lower than the stoichiometric one, also reflecting the reduction of nitrate by heterotrophs. In the second period where the heterotrophic activity was suppressed, all ratios approached the stoichiometric values of the anammox reaction. The NO_3-N/NO_2-N conversion ratio (as absolute value) was very low also reflecting the reduction of both nitrite and nitrate by heterotrophic biomass. The sAUR was variable having an average value of 3.94±2.18 mgN (gVSS h)$^{-1}$ in the first period and 3.24±2.90 mgN (gVSS h)$^{-1}$, in the second period at 30 °C. Despite the decrease in vNLR in the second period, this was not followed by a significant decrease in activity. The variability in sAUR (i.e., high standard deviation) could be partly attributed to the low DO that was maintained in the reactor during the aerobic conditions. In certain days low sAUR were monitored (<1 mgN (gVSS h)$^{-1}$).

Microscopic Examination

The microscopic observations showed that the biomass exhibited rather moderate to high FI values, starting from 2.5 in the first days and increasing up to 3.5. As a result of the presence of filamentous bacteria, flocs exhibited an open structure with irregular shapes and significant bridging. Most of the filamentous bacteria were present inside the flocs and the presence of protozoa and higher forms of microorganisms was rather low. Flocs size was relatively low with a length distribution of: 70% <150 μm, 20% between 150–500 μm and the 10% >500 μm. The dominant filamentous microorganisms during the first days of operation were Type 0092, Type 0675, N. Limicola and Type 021 N, whereas a change in the relative filamentous abundance was recorded gradually. During the latest days of operation, the dominant filamentous microorganisms in descending order were Thiothrix, Type 021 N, GALOs, M. parvicella and Type 1701 (i.e.,Figure 4.2).

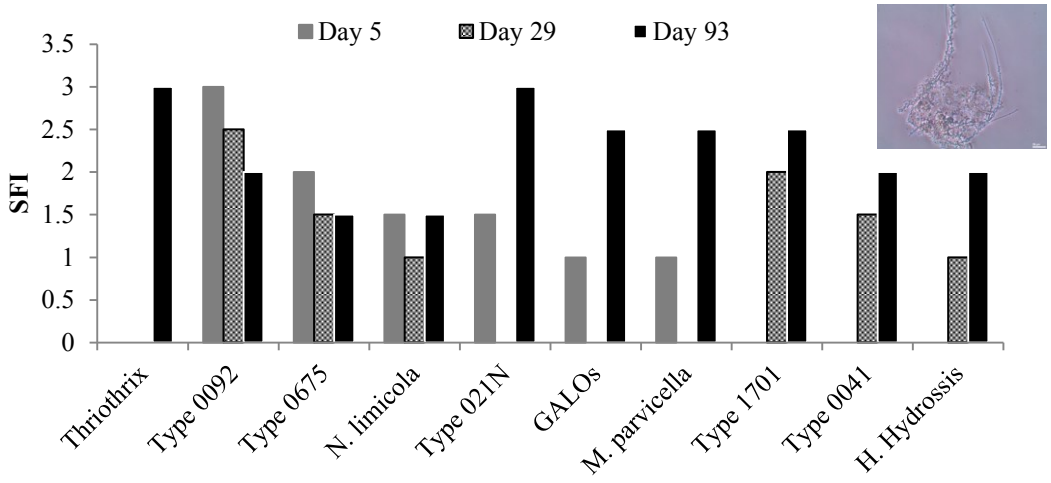

Figure 4.2. Dominant filamentous microorganisms in the biocenosis of the experimental system; microscopic observation (upper-right side) of a typical open structure floc with significant presence of filamentous bacteria (phase contrast 200X)

The presence of filamentous microorganisms should be attributed to the operational conditions of the experimental systems. More specifically, it is well known that the growth of the filamentous bacteria Thiothrix, Type 021 N and Type 1701 is favoured under low DO conditions while the bacteria GALOs, M. parvicella and Type 0041 are typical low Food:Microorganism (F/M) filamentous microorganisms (Wanner, 1994; Noutsopoulos et al., 2011). Therefore, the relatively moderate to high FI values should be attributed to the microaerophilic conditions that prevailed in the SBR system (DO concentrations equal to 0.2–0.3 mg/L), as well as to the long SRT adopted (i.e., >60 d). Despite the presence of filamentous bacteria, sludge settling problems were not encountered (average sludge volume index SVI = 120 mL $(gSS)^{-1}$). The significant presence of filamentous bacteria in anammox reactors is also reported by Li et al. (2009). The FISH analysis of the microbial community was conducted for the inoculum and biomass samples for days 29 and 66. FISH analysis confirmed the presence of anammox bacteria as well as of ammonium oxidizing bacteria (AOB) in the mixed liquor. The FISH image response of the total bacteria and anammox bacteria are given in Figure 4.3, while the semi-quantified analysis of bacterial populations is given in Table 4.7.

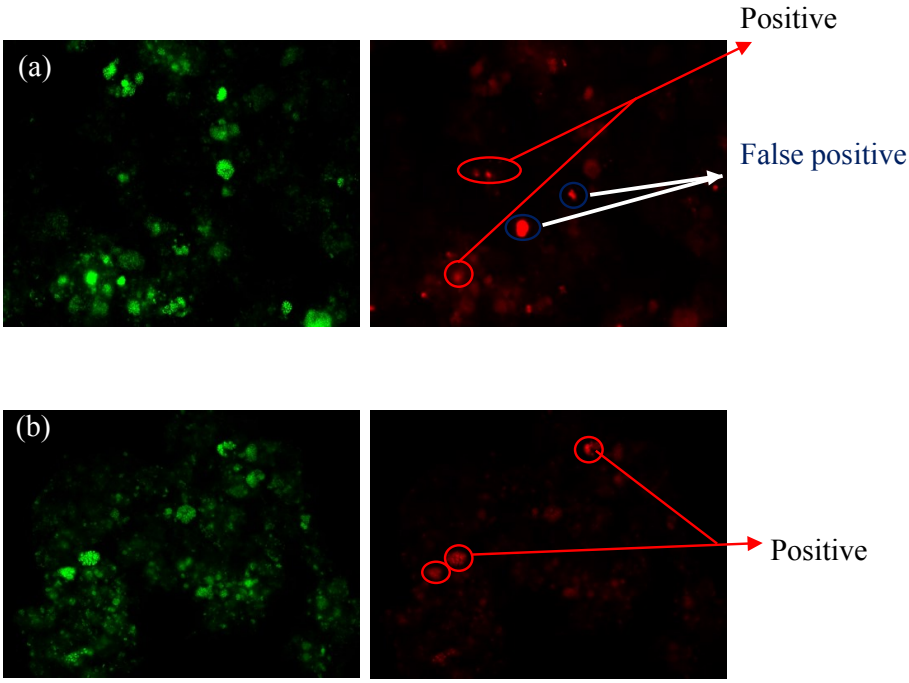

Figure 4.3. FISH images total bacteria (green - probe EUBmix) and anammox bacteria (red - probe Amx 368) for (a) day 7 and (b) day 29 (40X)

Table 4.7 FISH quantification results of Anammox and AOB

Day	vOLR ($kgCOD \cdot m^{-3} \cdot d^{-1}$)	Anammox activity ($mgN \cdot (gVSS \cdot h))^{-1}$	Bacterial Population Average	
			AOB (%)	Anammox (%)
	Inoculum	0.87	9.78 (1.75)	8.59 (1.28)
Period 1	0.20	2.02	8.56 (1.77)	12.26 (1.37)
Period 2	0.14	2.92	7.25 (1.11)	12.58 (2.47)

Although consistently present, the anammox bacteria did not dominate the mixed liquor. An increase of the percent anammox population in both the first and the second period was observed compared to the inoculum. This finding also agrees with the anammox activity tests. In the anammox start-up of Wang et al. (2011) much greater domination of the anammox bacteria was obtained (58.1%) for low strength effluent compared to this work; however, in that work, a synthetic solution was used as feed which did not contain any organic matter and had an optimum ratio of NH_4-N/NO_2-N; in turn this allowed the application of a high vNLR (0.41 $kgNm^{-3}d^{-1}$). The AOB decreased in the first and second period compared to the inoculum since the biomass treated low strength effluents.

4.3.3 The short-cut SBR process treating UASB effluent

Carbon source supplied during the operation

The DOW FL contained significant concentrations of butyric and propionic acid, thus presenting favourable VFA composition for the subsequent biological nutrients removal (Frison et al. 2013; Ji and Chen 2010). According with the results of Traverso et al. (2000), the acidogenic fermentation of VFW from supermarkets at mesophilic temperature resulted in the production of a liquid that is rich in acetic acid (>80%) and lactic acid, but with very low content in butyric acid and propionic acid. This is related to the type of waste fermented as it only consisted of vegetables and fruit waste. Both fermentation liquids and particularly the DOW FL contained high concentrations of organic nitrogen, ammonium and phosphate (Table 4.8). The fermentation process resulted in the hydrolysis of organic nitrogen and phosphorus to ammonium and phosphate respectively. The presence of high nitrogen and phosphorus in the waste derived carbon source is clearly a disadvantage since it increases the nutrients load that must be removed in the SBR process.

Table 4.8 External carbon source characteristics

Parameter	Carbon source		
	DOW FL (Period I)	VFW FL (Period VI)	DOW FL (Period V)
pH	4.2 ± 0.2	5.3 ± 2.1	4.2 ± 0.2
Alkalinity (mS/cm)	-	4920 ± 1876	-
COD (mg/L)	45009 ± 14288	34390 ± 15904	43273 ± 7351
sCOD (mg/L)	34789 ± 14549	27755 ± 14423	28514 ± 1042
TKN (mg/L)	1680 ± 570	730 ± 537	818 ± 198
NH4-N (mg/L)	337.0 ± 121.9	108.7 ± 68.16	198 ± 47.4
NO_3-N (mg/L)	59.4 ± 29.8	5.8 ± 6.6	28.1 ± 11.2
NO_2-N (mg/L)	-	1.8 ± 2.0	1.9 ± 0.2
TP (mg/L)	156.3 ± 44.7	91.0 ± 47.6	292 ± 55.1
PO4-P (mg/L)	137.9 ± 80.3	54.5 ± 37.8	208.7 ± 35.0

Nitrite accumulation over the long-term operation

The nitrogen removal via nitrite process was difficult to maintain during periods 1 and 2 due to the low vNLR (=0.13 $kgN \cdot m^{-3}d^{-1}$), which resulted in low FA concentration in the reactor.

Specifically, at the beginning of the aerobic reaction phase the FA was always <0.7 $mgNH_3\text{-}N\cdot L^{-1}$ and it was usually within the range of 0.08 - 0.44 $mgNH_3\text{-}N\cdot L^{-1}$ for the pH range of 7.3-8.0 of the mixed liquor. At the end of the aerobic period, the FA was lower than 0.01 $mgNH_3\text{-}N\cdot L^{-1}$ due to the oxidation of ammonium. During these two periods some nitrite accumulation was observed only at the early stages of reactor operation. The latter was attributed to the presence of some residual ammonium concentration at the end of the aerobic phase. After day 35, the complete nitrification pathway occurred and nitrite accumulation could not be sustained (Figure 4.4). The increase of vNLR to 0.21 $kgN\cdot m^{-3}\cdot d^{-1}$ (periods 3 and 4) resulted in some accumulation of nitrite with the $NO_2\text{-}N/NO_x\text{-}N$ ratio (at the end of the aerobic phase) usually ranging from 35-65% from day 99 up to day 185. The vNLR increase allowed for a higher concentration of FA in the reactor (=0.19-1.52 $mgNH_3\text{-}N\cdot L^{-1}$ at the beginning of the aerobic phase) for the given pH range (7.2-8.1) and temperature (22±3 °C) of the mixed liquor. The FA inhibition has been observed to start at 0.1 $mgNH_3\cdot L^{-1}$, with complete inhibition obtained at FA higher than 1 $mgNH_3\cdot L^{-1}$ (Anthonisen et al., 1976). At the end of the aerobic reaction phase, the FA was low, but some residual FA was actually present (around 0.05-0.10 $mgNH_3\text{-}N\cdot L^{-1}$). To increase further the nitrite accumulation, the target DO concentration during the aerobic phase was set at 0.3-0.7 $mg\cdot L^{-1}$ in period 5. Low DO concentration can provide a competitive advantage of AOB against NOB. As a result, the nitrite accumulation gradually increased up to $NO_2\text{-}N/NO_x\text{-}N$=100% (Figure 4.4). During the last 33 days of operation in period 5 the $NO_2\text{-}N/NO_x\text{-}N$ was steadily above 97%. Previous work has shown that stable nitrite accumulation can occur in domestic wastewater through the real time control of the aerobic phase of the SBR in order to favour only the first step of nitrification (Guo et al., 2009). This work showed that a relatively high vNLR when coupled with low DO in the reactor during the aerobic period can achieve complete and stable nitrite accumulation.

The range of FNA concentrations affecting NOB activity have been found to begin from 0.011-0.07 $mgHNO_2\text{-}N\cdot L^{-1}$. Complete inhibition was observed at 0.026-0.22 $mgHNO_2\text{-}N\cdot L^{-1}$ (Anthonisen et al., 1976; Vadivelu et al., 2006; Zhang et al., 2010). However, in our case the FNA concentration was very low to cause any inhibition of the NOB for the operating pH (7.2-8.1) and temperature (22±3°C). Specifically, the FNA concentration was around 0.0015 $mgHNO_2\text{-}N\cdot L^{-1}$ at the end of the aerobic phase. The highest value of FNA was 0.0048 $mgHNO_2\cdot L^{-1}$, value which is lower than the lowest HNO3-N value at which NOB inhibition starts. High temperatures (30-40°C) can directly provide a competitive advantage to NOB over

AOB (Malamis et al., 2013). However, the operating temperature in our case was much lower (22±3°C). The temperature also indirectly affects the NOB and AOB activity by changing the ammonia equilibrium. Higher temperatures result in a shift towards more ammonia and less ammonium. However, in our case the SBR operated always at ambient temperature (22±3°C). This temperature variation was taken into consideration when calculating FA.

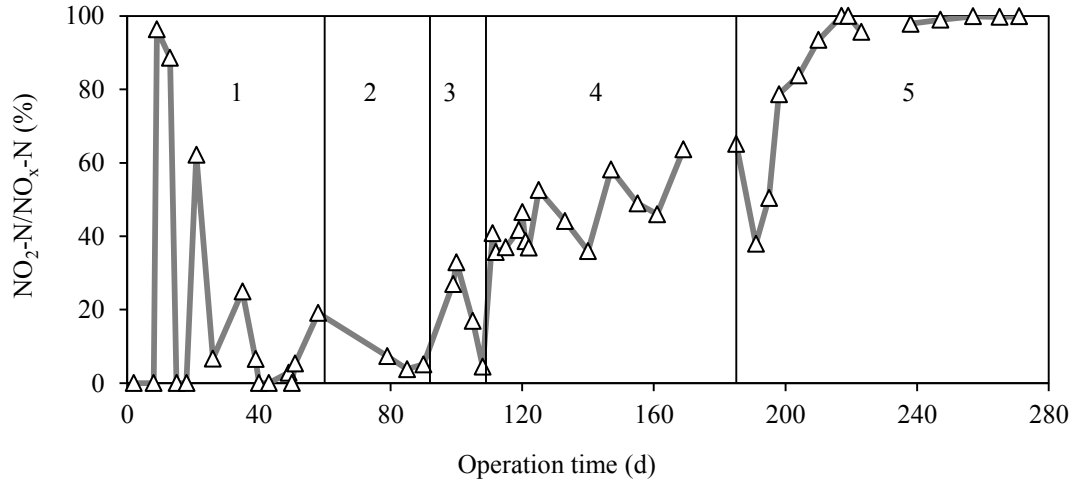

Figure 4.4. Variation of NO_2-N/NO_x-N ratio (end of the aerobic phase)

Effect of operating parameters on nitrification

The nitrification rate was significantly affected by the vNLR that was applied. The low vNLR (0.13 kgN·m^{-3}·d^{-1}) resulted in low sAUR (2.04±1.02 mgNH_4-N·(gVSS·h)$^{-1}$), while the increase in vNLR to 0.21 kgN·m^{-3}·d^{-1} resulted in higher and more stable sAUR, which was consistently above 4.5 mgNH_4-N·(gVSS·h)$^{-1}$ (Figure 4.5).

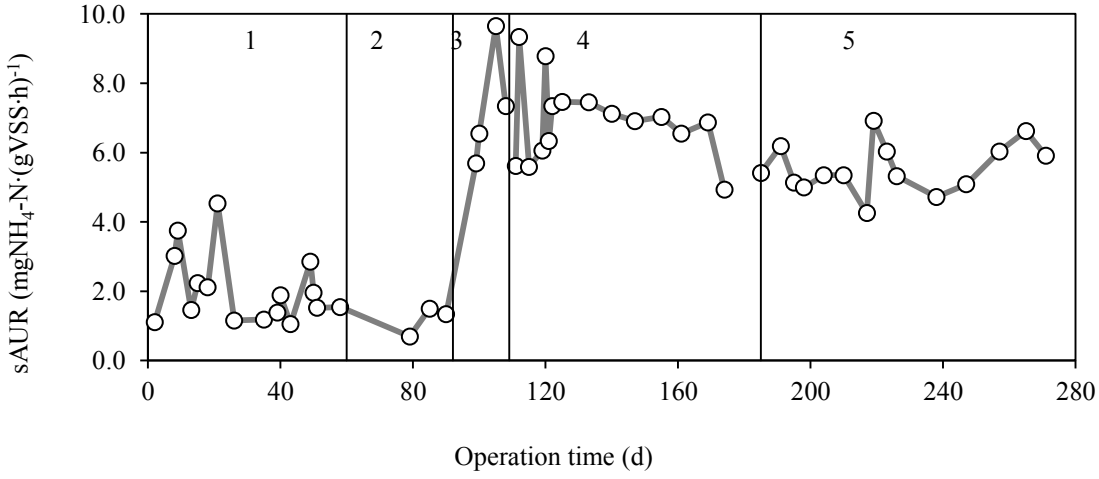

Figure 4.5. sAUR during the SBR operation

As evidenced by Table 4.9, the average in situ sAUR increased to the value of 6.97±1.72 $mgNH_4\text{-}N\cdot(gVSS\cdot h)^{-1}$ in period 4.

Table 4.9 Nitrification/denitrification and phosphorus removal rates for in situ activity tests

Periods	Carbon Source	NLR $kg\cdot m^{-3}\cdot d^{-1}$	sAUR $mgN(gVSS\cdot h)^{-1}$	Aerobic sPUR $mgP(gVSS\cdot h)^{-1}$	sNUR $mgN(gVSS\cdot h)^{-1}$	Anoxic sPUR $mgP(gVSS\cdot h)^{-1}$	sPRR $mgP\cdot(gVSS\cdot h)^{-1}$
Period 1	DOW FL	0.12	2.04±1.02	3.92 ± 1.82	5.05 ± 1.46	3.89 ± 3.12	3.06 ± 2.69
Period 2	Hac	0.12	1.09±0.57	0.19 ± 0.20	2.96 ± 1.37	0.62 ± 0.81	0.22 ± 0.47
Period 3	Hac	0.21	7.31±1.70	0.38 ± 0.18	2.34 ± 1.32	0.53 ± 0.53	0.30 ± 0.45
Period 4	VFW FL	0.21	6.97±1.72	0.76 ± 0.59	3.11 ± 0.86	1.02 ± 0.87	0.96 ± 0.79
Period 5	DOW FL	0.21	5.57±1.45	2.33 ± 0.82	6.81 ± 1.99	6.33 ± 4.28	5.95 ± 2.21

In periods 2 and 3, the same carbon source was used (i.e. acetic acid), but different vNLR was applied; the increase of vNLR resulted in a significant increase in sAUR as the higher availability of ammonium in the reactor increased the in situ biological activity of nitrifiers. Higher sAUR was also obtained in the ex situ experiments for the biomass acclimatized to higher vNLR compared to the biomass acclimatized to lower vNLR, despite the fact that both experiments were conducted with excess ammonium in the mixed liquor (Table 4.10). This shows the importance of biomass acclimatization to the experimental conditions. The sAUR was usually higher when it was measured as NH_4-N decrease with time compared to NO_x-N (=NO_2-N+NO_3-N) increase with time. This is normally attributed to the uptake of some ammonium by bacteria for synthesis. However, in several cases the sAUR measured as NH_4-N was significantly higher than the one calculated as NO_x-N; this could not be justified only by bacterial assimilation of ammonia. Specifically, SND occurred during the aerobic phase and thus, some NO_x-N was actually denitrified and "lost" during the aerobic operation of the SBR. In the last period, SND was more pronounced due to the low target DO (0.3-0.7 $mg\cdot L^{-1}$) in the aerobic phase that was practiced in order to inactivate NOB. This is reflected by the higher difference of sAUR of this period measured as ammonium oxidized compared to NO_x-N produced. The change in the type and composition of the external carbon source did not seem to affect the activity of nitrifiers. Similar sAUR values were obtained for periods 1 and 2 and for periods 3 and 4, despite the fact that different types of external carbon sources were applied (Table 4.9).

The concentration of DO in the mixed liquor during the aerobic reaction phase impacted on the nitrification rate. Comparing the in situ sAUR for periods 3-5, in which similar vNLR was applied, it seems that the lowest sAUR was obtained in the last period. This observation shows that the low DO concentration in the aerobic reaction phase of period 5 decreased the nitrification rate. During period 5, the in situ sAUR was lower than the ex situ one. In the ex situ tests (Table 4.10) of the same experimental period a high DO was maintained, thus increasing the ammonium oxidation rate by approximately 30% and the NO_x-N production rate much more (since the nitrate/nitrate was no longer denitrified under strictly aerobic conditions) compared to the in situ experiments.

Table 4.10 Nitrification/denitrification and phosphorus removal rates for ex situ activity tests

Period	sAUR $mgNH_4$-N·(gVSS·h)$^{-1}$	sAUR $mgNO_2$-N·(gVSS·h)$^{-1}$	sAUR $mgNO_3$-N·(gVSS·h)$^{-1}$	sNUR mgN·(gVSS·h)$^{-1}$	Aerobic sPUR mgP·(gVSS·h)$^{-1}$
I	2.86±1.77	0.83±0.76	2.09±0.07	4.50±1.51	4.31±0.85
IV	6.99±2.41	3.70±0.88	2.56±0.72	2.83±1.02	1.13±0.61
V	7.28±1.09	6.76±0.61	0.23±0.13	7.42±1.47	3.94±1.13

Effect of operating parameters on denitrification

Figure 4.6 shows the variation of the denitrification rate for the various experimental periods. The increase in vNLR did not affect the denitrification rate. This is more clearly demonstrated in periods 2 and 3, where acetic acid was added and despite the difference in vNLR similar sNUR was obtained.

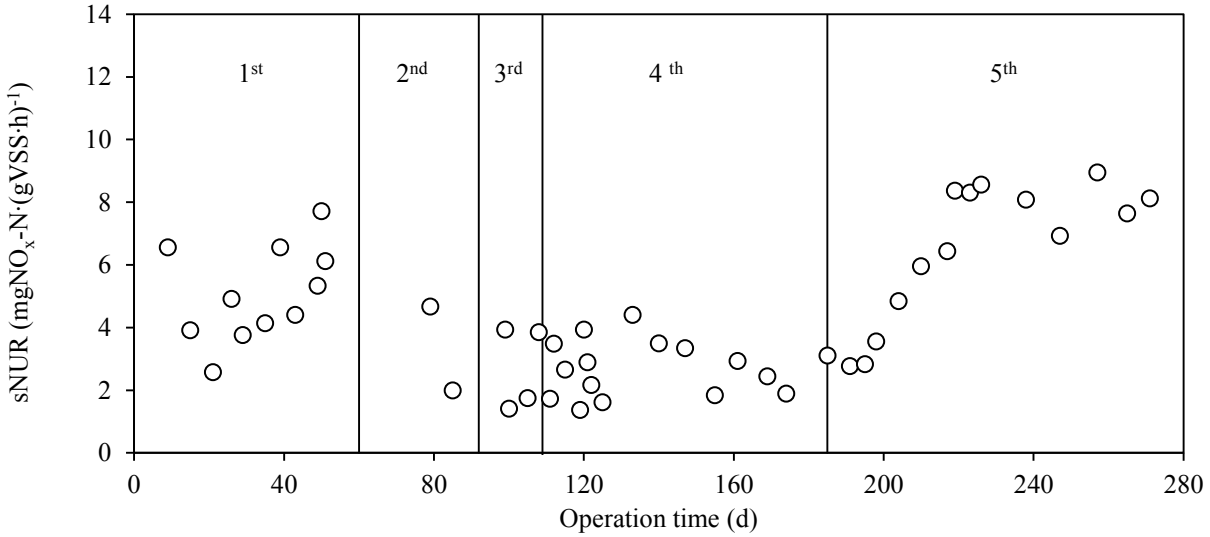

Figure 4.6. Variation of the in situ specific nitrogen uptake rate (sNUR) during the experimental period.

On the other hand, the type and composition of carbon source critically impacted on the sNUR. As seen in Table 4.9 and Table 4.10, the use of DOW FL resulted in significantly higher denitrification rate than the one achieved by VFW FL both in the ex situ and in situ tests. The DOW FL contained significant concentration of butyric acid and propionic acid, while the vast majority of VFAs in VFW FL was acetic acid (>90% of total VFAs). The presence of propionic acid and butyric acid can promote nutrient removal via nitrite (Frison et al., 2013). The highest denitrification rates were obtained in the last period, since the low DO of the aerobic period favoured the growth of denitrifiers, with significant SND occurring during the aerobic phase.

Denitrifying via nitrite biological phosphorus removal

Enhanced biological phosphorus removal (EBPR) was investigated under aerobic and anoxic (nitrite) conditions. The focus was on the impact of external carbon sources, the vNLR and the nitrite levels on the phosphorus removal bioprocess. In periods 1 and 5, significant phosphorus uptake was observed. The presence of propionic acid and butyric acid contained in DOW FL resulted in EBPR, providing PAOs with a competitive advantage over glycogen accumulating organisms (GAOs). The VFA composition plays a critical role in the selection of PAOs over GAOs. The latter is in agreement with previous research works, which have shown that carbon sources that contain a mixture of VFAs can improve denitrifying biological phosphorus removal via nitrite (DBPRN) (Ji and Chen, 2010; Frison et al., 2013). It has been demonstrated that propionic acid enhances the accumulation of polyhydroxyalkanoates (PHA) in sludge (up to 10-15% TVS), which allows the simultaneous denitritation and phosphorus removal in the presence of nitrite (Oehmen et al., 2005, 2006; Kishida et al., 2006). In periods 2-4 the sPUR under both anoxic and aerobic conditions was very low. In these periods, the DO concentration during the aerobic reaction was high (2.0 $mg \cdot L^{-1}$); so this did not cause a decrease in the activity of PAOs under aerobic conditions. Elevated nitrite levels in the mixed liquor can inhibit the aerobic and anoxic phosphorus uptake (Saito et al., 2004). In our case the nitrite levels at the end of the aerobic phase did not exceed 35 $mg \cdot L^{-1}$ and were usually in the range of 10-25 $mg \cdot L^{-1}$. Therefore, these nitrite levels did not inhibit the DBPRN. This was also confirmed by ex situ tests in which the biomass was subjected to different initial nitrite concentrations and $sPUR_{anoxic}$ was monitored.

The $sPUR_{anoxic}$ via nitrite was not adversely affected by nitrite concentrations up to 50-70 $mgNO_2\text{-}N \cdot L^{-1}$ and was partially inhibited for concentrations of 100-120 $mgNO_2\text{-}N \cdot L^{-1}$.

In period 1 the phosphorus uptake and release rates were significant. However, in period 2, $sPUR_{anoxic}$, $sPUR_{aerobic}$ and sPRR dropped to very low levels. The only difference between these two periods was the type of external carbon source that was dosed. Specifically, in period 1 DOW FL was applied and in period 2 acetic acid. The DOW FL contained a mixture of volatile fatty acids (VFAs), including propionic acid, butyric acid and acetic acid. Previous findings suggest that propionate may be a more effective substrate as compared to acetate for EBPR (Chen et al., 2004, Oehmen et al., 2006, 2007). Laboratory scale experiments showed superior EBPR when propionate was dosed instead of acetic acid during long-term enrichment experiments (Hood and Randall, 2001, Chen et al., 2004, Oehmen et al., 2005, 2007). The literature suggests that propionate may provide a competitive advantage of phosphorus accumulating organisms (PAOs) over glycogen accumulating organisms (GAOs) (Oehmen et al., 2007). Other works have also shown that fermentation liquids, which contain a mixture of SCFAs can improve denitrifying phosphorus removal via nitrite removal (Ji and Chen, 2010; Frison et al., 2013). It seems that the addition of acetic acid in periods 2-3 and VFW FL (which contained mainly of acetic acid) in period 4 resulted in low phosphorus uptake.

During periods 1-4, the $sPUR_{aerobic}$ was similar to the $sPUR_{anoxic}$. In period 5, $sPUR_{anoxic}$ was much higher than $sPUR_{aerobic}$; it seems that the low DO concentration in the aerobic phase of that period negatively impacted on the aerobic phosphorus uptake since the aerobic heterotrophs, nitrifying bacteria and PAOs all competed for the available oxygen. On the contrary, in period 5 the $sPUR_{anoxic}$ increased compared to period 1 reaching an average of 6.33 ± 1.92 $mgP \cdot (gVSS \cdot h)^{-1}$.

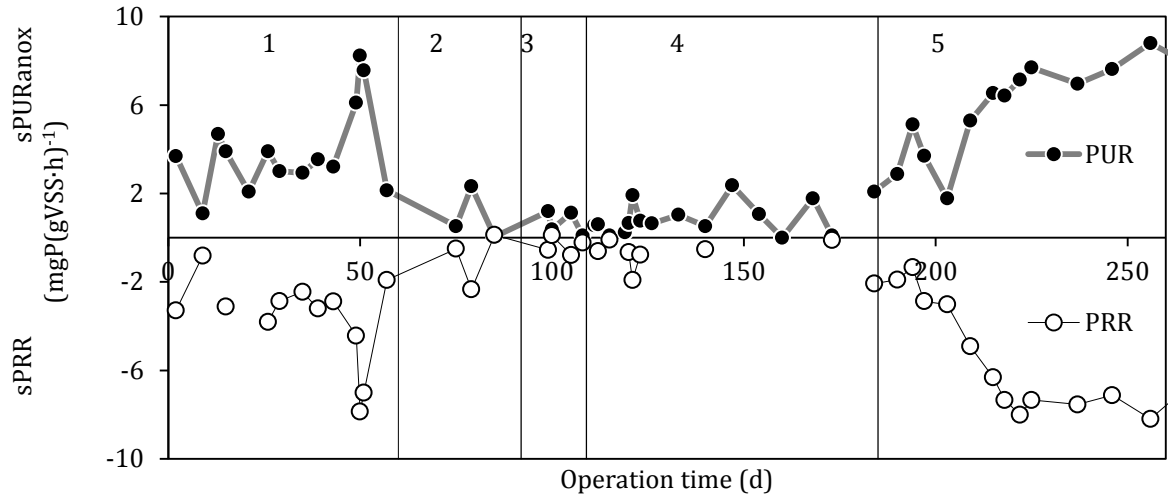

Figure 4.7. Profile of anoxic sPUR and anaerobic sPRR during the short cut SBR operation

This value was the highest average $sPUR_{anoxic}$ that was recorded in the five experimental periods. Figure 4.7 shows the gradual increase of the $sPUR_{anoxic}$ from 2.88 $mgP\cdot(gVSS\cdot h)^{-1}$ at day 190 up to 8.80 $mgP\cdot(gVSS\cdot h)^{-1}$ at day 256. The type and composition of the external carbon source critically impacted on the denitrification and phosphorus uptake.

The fermentation liquid derived from DOW resulted in high denitritation and phosphorus uptake via nitrite rates (i.e. 6.81±1.99 $mgN\cdot(gVSS\cdot h)^{-1}$ and 6.33±1.92 $mgP\cdot(gVSS\cdot h)^{-1}$) due to the addition of butyric acid and propionic acid, while denitrification was poor when VFW FL and acetic acid were used.

Profile of nutrients during the cycle of the scSBR

The inorganic nitrogen forms and the phosphate concentration were monitored within the SBR in each operating period. Figure 4.8 shows the typical variation of ammonium, nitrite, nitrate and phosphate within the reactor for periods 1, 4 and 5. In period 1 (Figure 4.8a), the fast depletion of NO_x-N due to fast kinetics in combination with the low vNLR that was applied resulted in a complete depletion of NO_x-N before the end of the anoxic phase. A net release of phosphate was observed at the end of the anoxic phase due to the complete depletion of nitrates and nitrites. In period IV (Figure 4.8b), the NO_x-N denitrification rate and the phosphorus uptake rate were much slower compared to period 1, since the external carbon source that was added (VFW FL) contained very low concentrations of butyric acid and propionic acid. On the contrary, the

nitrification rate was fast owing to the higher vNLR. In the last period (Figure 4.8c) the nitrate concentration was 0 mg L^{-1}, as the nitrite oxidizing bacteria (NOB) were completely inactivated. In this period, high sPUR and sNUR were obtained under anoxic conditions and this is reflected by the fast decrease of phosphate and nitrite during the anoxic reaction phase.

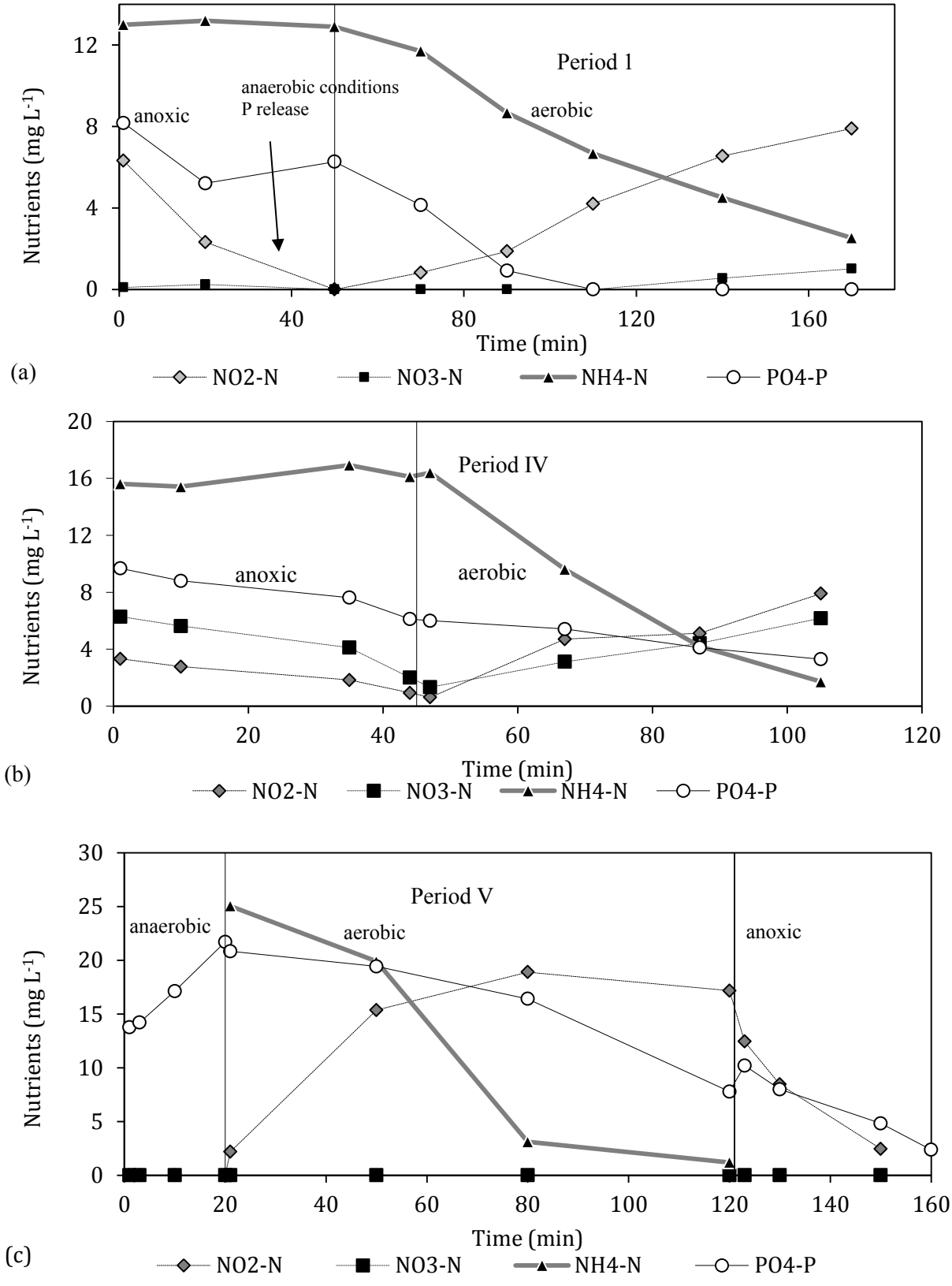

Figure 4.8. Typical profiles of ammonium, nitrite, nitrate and phosphate during the SBR cycle for periods 1(a), 4 (b) and 5 (c).

Nutrient removal efficiency

The evaluation of the SBR via nitrite process was carried out based on mass balances. Table 4.11 summarizes the calculated sAUR and sNUR obtained from mass balances.

Table 4.11. Nitrification/denitrification activity obtained from mass balances in the SBR reactor and deviations

Period	sAUR mgN·(gVSS·h)$^{-1}$	sAUR difference with in-situ test (%)	sNUR mgN·(gVSS·h)$^{-1}$	sNUR difference with in-situ test (%)	Difference in phosphorus mass balance (P_{in}-P_{out})/P_{in} (%)
Period 1	1.96	-3.9	4.29	-17.8	19.7
Period 2	1.27	-9.4	2.78	-6.6	26.8
Period 3	5.73	-27.5	4.09	42.8	20.2
Period 4	5.97	-16.7	2.87	-8.2	16.8
Period 5	5.83	4.5	6.98	2.4	39.0

The deviation between the calculated and measured sAUR and sNUR for the examined experimental periods was usually below 20%. In the case of phosphorus, the deviations in the mass balances were less than 30% in periods 1-4. Period 1 exhibited the highest removal efficiencies, which were 90% for ammonium oxidation, 85% for nitrogen and 88% for phosphorus removal. Period 5 was the most effective one in terms of kinetics, since it was characterized by high nitrogen and phosphorus removal rates via the nitrite pathway (i.e. 100% nitrite accumulation). However, the high vNLR resulted in lower removal efficiencies: the ammonium oxidation was 84%, the nitrogen removal was 82% and the phosphorus removal was 85%. More importantly, the total nitrogen concentration of the treated effluent was low (9.2±4.8 mgN·L-1), showing that nitrogen was effectively removed from domestic effluents and fermented biowaste. The combined application of high vNLR and acetic acid or VFW FL resulted in low nitrogen removal efficiencies in periods 3 and 4 (<70%). The use of acetic acid and VFW FL resulted in low kinetics.

Simultaneous nitrification/denitrification

In all the experimental periods, an excess of external organic carbon was dosed during the anaerobic/anoxic phases. Particularly during the first 20 days of SBR operation, significant overdose of organic carbon was practiced. As a result, during the aerobic phase, a lag time was

observed in the increase of the DO concentration to the target value. The low DO concentration during the aerobic phase, resulted in some SND occurred. After these 20 first days the carbon source dosage was decreased. The highest SND was observed in period I (SND=61.8 ± 18.0%) during which the quantity of organic carbon source that was added was high, and in period 5 (SND=57.8 ± 15.7%). In period 5, the target DO in the aerobic phase was low resulting in significant SND. In periods 2, 3 and 4 was 27.2 ± 12.8%, 26.7 ± 10.9% and 8.7 ± 4.4% respectively in which the carbon source dose was reduced and thus the lag time to reach the target DO value decreased. The SND can be of added value to the process as nitrogen is actually removed simultaneously with its oxidation due to the presence of micro-anoxic conditions in parts of the flocs. However, the simultaneous production and depletion of nitrite/nitrate can adversely affect the DBPRN. During periods 1 and 5, in which the highest SND was observed, the sPUR anoxic was high. Despite the SND, some nitrites were available as electron acceptors in the mixed liquor to allow for DBPRN at a high rate. In fact, our work showed that the type of carbon source was the critical parameter that affected EBPR under both aerobic and anoxic conditions.

Microbial populations

FISH images (Figure 4.9) show the AOB, NOB (*Nitrobacter, Nitrospira*) and PAOs for specific days during the system operation (supplementary material). In periods 4 and 5 more AOB and PAOs and less NOB were present in activated sludge.

The FISH images imply that the growth of PAOs was promoted during periods 1 and 5, while in period 4 the presence of PAOs was low. This is in agreement with the phosphorus activity tests. The bacterial semi-quantification (Figure 4.10) was also conducted for periods 1, 4 and 5. Although absolute comparisons cannot be made due to variation in the total bacterial population, nevertheless, a clear trend was identified showing an increase in the percentage of AOB in total biomass and a decrease of NOB from the shift of period 1 (i.e. low vNLR) to periods 4 and 5 (i.e. high vNLR). Specifically, the AOB increased from 8.04 ± 0.82 % in period 1 (day 51) to 11.61 ± 0.91 % in period 4 (day 121) to 16.22 ± 1.98 % in period 5 (day 205). *Nitrobacter* decreased from 5.04 ± 0.82 % in period 5 (day 51) to 1.78 ± 0.33 % in period 5 (day 205) and *Nitrospira* decreased from 3.98 ± 0.57% to less than 0.1%.

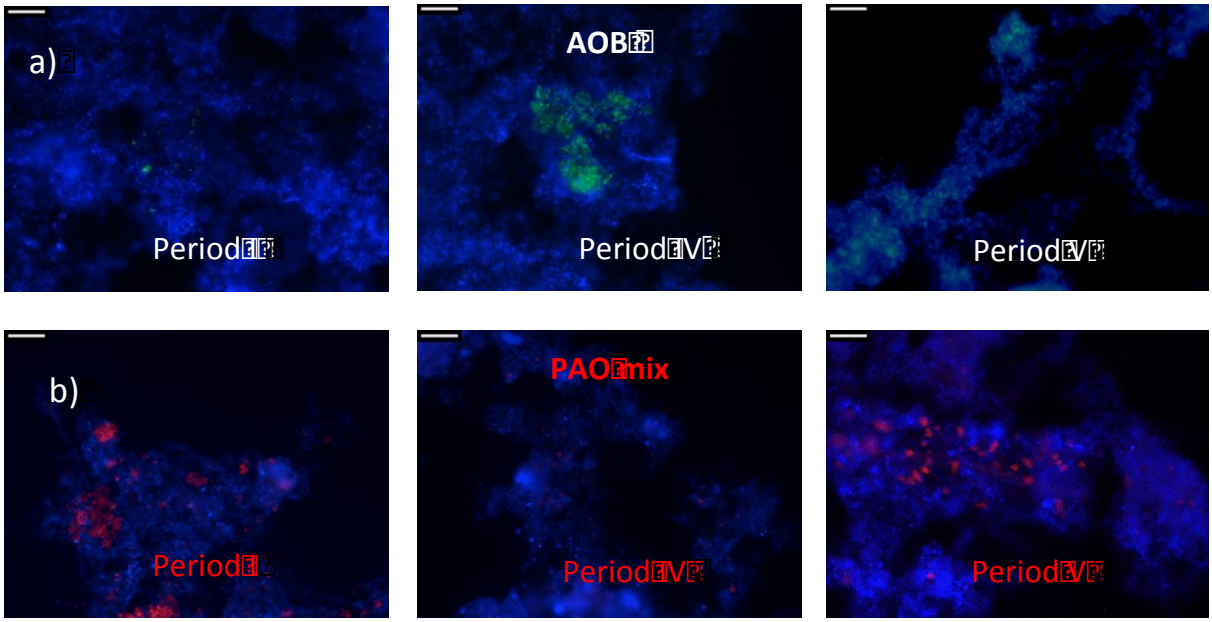

Figure 4.9. FISH images for (a) AOB Dapi (blue)+NSO190 (green) and (b) PAOs - Dapi (blue)+PAOsmix (red)

Table 4.12. FISH semi-quantified results of AOB, PAOs, NOB, GAOs and denitrifiers given by the % bacterial population average (mean & standard error)

Period (day)		AOB	PAOs	Nitrobacter	Nitrospira	Comamonas	Thauera	BET	Zoogloea
Period (51)	1	8.04 (0.82)	6.19 (0.68)	5.04 (0.86)	3.98 (0.57)	14.61 (1.12)	8.12 (0.88)	81.1 (1.9)	9.2 (1.7)
Period (121)	4	11.31 (0.94)	3.92 (0.51)	4.13 (0.69)	1.39 (0.39)	9.32 (0.71)	6.91 (0.49)	82.91 (0.3)	10.1 (1.4)
Period (181)	5	-	4.78 (0.62)	-	-	-	-	-	-
Period (205)	5	16.22 (1.98)	6.41 (1.1)	1.78 (0.33)	<0.1	17.13 (1.09)	9.44 (0.78)	79.82 (1.1)	11.3 (1.2)
Period (221)	5	-	7.83 (0.36)	-	-	-	-	-	-
Period (246)	5	-	7.61 (0.47)	-	-	-	-	-	-

Considering the decrease of MLVSS in periods 4 and 5 compared to 1, the decrease of *Nitrobacter* and *Nitrospira* in absolute numbers is even greater. In period 5, there was a gradual

growth in the population of PAOs which was evidenced by FISH analysis. In day 246 the PAOs were ubiquitous in the floc structure (7.61 ± 0.47 %), while in day 181 fewer PAOs were identified (4.78 ± 0.62 %). Significant decrease in the growth of denitrifiers was also observed in period 4, which is attributed to the change in the carbon source rather than in the change in vNLR. The FISH results were in accordance with the activity tests confirming that the increased vNLR was beneficial for promoting the via nitrite nitrogen removal and the use of VFW FL did not favour the development of denitrifiers.

4.4 Conclusion

The UASB-SBR process can successfully treat low strength wastewater through the completely autotrophic nitrogen removal process. Using low activity anammox inoculum an anammox rate of 2.27 ± 1.31 mgN (gVSS h)$^{-1}$ at 30 °C was obtained, which was 161% higher than the inoculum. The decrease in vNLR did not significantly impact of anammox activity, but resulted in a decrease of denitrifying heterotrophic biomass activity to very low levels. Thus, the nitrogen conversion ratios approached the stoichiometric values of anammox reaction. Several different filamentous bacteria were identified which were favoured by long SRT. FISH analysis confirmed the stable presence of anammox bacteria and AOB.

However, the phosphorus present in the wastewater cannot be removed through a complete autotrophic process. For these reason, we study the occurring of biological nutrients removal via-nitrite using the best available carbon source such as the fermentation liquid of DOW and VWF. The presence of propionic acid and butyric acid in the carbon source enhances the denitrification and phosphorus uptake rates. The application of high vNLR (0.19 kgN·m^{-3}·d^{-1}) in combination with a low dissolved oxygen during the aerobic period resulted in stable nitrite accumulation (NO_2-N/NO_x-N>97%) and high nitrogen and phosphorus removal rates. DOW FL was the best carbon source as it contained butyric acid and propionic acid. The addition of these compounds in VFW FL significantly improved the nutrient removal rates.

The type and concentration of external organic carbon source critically affected the via nitrite phosphorus uptake and release rates. The use of DOW FL as external carbon source resulted in the highest sPUR and sPRR since it contained mixture of acetic, propionic and butyric acids in suitable proportions and at much higher concentrations compared to the VFW FL. The enrichment of VFW FL with propionic acid and butyric acid resulted in an increase of the sPUR and sPRR. Initial nitrite concentrations up to 50-70 mg L^{-1} did not adversely impact of the via nitrite sPUR. However, higher nitrite concentration in the range of 100-120 mgNO2-N L^{-1} resulted in some sPUR inhibition.

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5 Chapter. Nitrogen and Phosphorus Removal via nitrite treating high strength wastewater

In this chapter, the short cut biological nutrients removal was applied using a demonstration Sequencing Batch Reactor for the treatment of anaerobic co-digestate of waste activated sludge (WAS) and the organic fraction of the municipal solids waste (OFMSW).

5.1 Introduction

The anaerobic digestion process for biogas production is a very common choice for the treatment of the waste activated sludge (WAS) produced after the biological treatment of the municipal wastewater. Energy recovery from waste is an attractive option, which has resulted in the construction and operation of several anaerobic digestion (AD) plants worldwide for the treatment of the organic fraction of municipal solid waste (OFMSW). Furthermore, OFMSW is often fed to anaerobic digesters treating waste activated sludge (WAS) to increase biogas production in wastewater treatment plants (WWTPs) (De Baere, 2006). During the AD process, the extensive ammonification of the organic matter, releases high amount of nitrogen and phosphorus which can be significant when the WAS is originated from biological nutrients removal (BNR) processes (Battistoni et al., 2006). Consequently, such effluents are characterized by high ammonium content and significant phosphate concentrations with low BOD/TKN ratio. Typically, the ammonium concentration in the anaerobic supernatant could vary between 500 to 2500 mgN L^{-1}, with the molar ratio alkalinity:ammonium about 1 (Gustavsson et al., 2010). In a wastewater treatment plant, although it represent less than 1% of the total flow influent, the anaerobic supernatant liquid resulting from dewatering of the digestate, could contain 12-25% of the total nitrogen and up to 40% of the total phosphorus influent (Cervantes et al., 2006). However, high concentration of ammonium and warm and stable temperature conditions of the anaerobic supernatant lead the potential for advantageous and efficient technologies for nutrients removal, which usually are difficult to apply for the treatment of low strength wastewater.

Even if the physical/chemical methods are of interest because nutrients are recovered as fertilizer, (Battistoni et al., 2006, 2005; Mace et al., 2002) it is reported that the biological methods are more cost-effective (Gustavsson et al., 2010, 2011; Siegrist et al., 1996). In addition, based on the life cycle assessment (LCA) the via nitrite processes (nitritation and denitritation or anammox processes) showed lower environmental impacts compared with the struvite precipitation process, due to the high electricity needed per kg of phosphorus recovered (Rodriguez-Garzia et al., 2014).

Nitritation and Denitritation process, also called as short-cut nitrogen removal, has gained increasing attention over the last years because decreases 25% the oxygen demand, requires 40% less the external carbon source, and decreases up to 30% the sludge production and CO_2 emissions compared to conventional nitrification and denitrification. Compared to complete autotrophic nitrogen removal, partial nitrification and heterotrophic denitritation may be more robust and reliable as it is less sensitive to environmental and operating parameters (Gustavsson et al., 2010). A wide number of experimentations and "key-factors" for achieving stable short cut nitrogen removal (Table 5.1) were reported in the literature. Fux et al., (2003) reported that high temperature (about 30 °C) and low solids retention time (SRT), in SHARON and SBR pilot scale process, favoured NOB wash-out because AOB grow faster than NOB at temperature above 20 °C. On the basis of full-scale results, (Joss et al., 2009) van Kempen et al., (2001) suggested to maintain the SRT between 1 day and 2.5 days. In fact, it is well-known that AOB growth can be favoured by an appropriate regulation of SRT in suspended-growth system, due to the different minimum required times. Recently, Mayer et al., (2009) observed stable nitritation−denitritation in pilot scale SBR treating real anaerobic supernatant of OFMSW and WAS: the authors concluded that the cause of this phenomenon was uncertain, while attributing the role of major drivers to low dissolved oxygen (DO) (<1 $mgDOL^{-1}$) in the reactor. The low concentration of readily biodegradable COD in the anaerobic supernatant is not favorable for the heterotrophic denitritation and a synthetic external carbon source (e.g. methanol and acetic acid), could increase drastically the operating cost of the process. Alternative carbon source produce from the fermentation of biowaste are reach in volatile fatty acids, like acetic, propionic and butyric (Frison et al., 2013).

The heterotrophic processes may contemporary allow for the short-cut nitrification denitrification (SCND) and phosphorus removal as long as they are coupled to certain short-chain carbon sources (Ji and Chen, 2010). The VFA composition is critical for the selection of DPAOs over glycogen accumulating organisms (GAOs) and can result in more efficient and reliable phosphorus removal (Oehment et al., 2006). Studies have shown that alkaline fermentation enhanced the composition of the VFAs in the fermented liquid, while fermentation at acidic or neutral pH resulted in lower VFAs production (Wu et al., 2010). Research studies have also documented the occurrence of denitrifying phosphorus-accumulating organisms (PAOs) that can utilize nitrate or nitrite as electron acceptors instead of oxygen (Carvalho et al., 2007). Denitrifying phosphorus removal via nitrite (DPRN) can reduce significantly the

requirements of organic matter and the sludge production through the simultaneous denitrification and phosphorus uptake. However, during the nitritation and denitritation process, several green house gas emissions may easily emitted in significant amount, such as CO_2, N_2O, CH_4 and others gases (Kampschreur et al., 2008). Furthermore, the use of sequencing batch reactor (SBR) technology, particularly when combined with the treatment of highly nitrogenous effluents such as the reject water can enhance nitrous oxide emissions (Desloover et al., 2012). Nitrous oxide is of particular environmental concern, since it has a global warming potential that is 298 times higher than that of CO_2. In terms of CO_2 equivalents (eq.) nitrous oxide contributes by 7.9% to the total anthropogenic greenhouse gas (GHG) emissions. It is reported that the nitrous oxide emissions from wastewater management are estimated to contribute by 26% to the total greenhouse gas (GHG) emissions of the water chain (Frijns et al., 2008). The implementation of strategies to mitigate N_2O emissions during the short-cut nitrogen removal processes can increase their sustainability. For example, the use of sludge fermentation liquid as alternative carbon source in the via nitrite processes can mitigate N_2O and NO emissions (Zhu and Chen, 2011).

In this work we examined the nitritation and denitritation in a pilot scale SBR for the treatment of anaerobic supernatant produced from the full scale co-digestion of the organic fraction of municipal solid waste (OFMSW) and waste activated sludge (WAS). The start-up operation to achieve the via-nitrite nitrogen removal was deeply described and the stability and behavior of the via-nitrite process were examined under transient conditions, imposed by the ordinary and extraordinary operation of the full-scale AD plant. Along the long-term operation, various important parameters were investigated including the nitrogen loading rate (NLR) and the best alternative external carbon source (acetic acid, glycerol, fermentation liquid, liquid drainage from OFMSW and fermentation liquid from cattle manure and maize silage) to achieve high activity in terms of nitrogen and phosphorus removal via nitrite. The type of carbon source is also a fundamental choice that assesses the economical sustainability of the overall process. For this reason, an economic evaluation of the supernatant treatment were estimated by taking into account the whole integrated plant, including the minor specific production of biogas or the incoming due for the treatment of solid waste.

Important greenhouse gas (GHG) emissions, such as CO_2, N_2O, CH_4 and others, were quantified in order to monitor and mitigate the emissions during the experimental cycle of the short cut SBR. Specific operating conditions (type of carbon source, nitrogen loading rate applied and the

alternation of aerobic and anoxic phases) were applied and the gaseous emissions were recorded. Finally, a life cycle assessment (LCA) was used as a tool in order to evaluate the environmental profile of the short nutrients removal using the alternative carbon sources for the treatment of the supernatant resulting from the dewatering of digested sludge.

Table 5.1 NLR: nitrogen loading rate; AUR-AOB: nitrite production rate; DNR: denitritation rate; SRT: sludge retention time; AeRT: aerobic retention time; AnRT: anoxic retention time.

Type of reactor	Type of feeding	Key-factors to achieve partial nitrification	Start-up length (days)	Maximum Nitrogen Loading Applied ($kgNm^{-3}d^{-1}$)	SRT (days)	DO ($mg\ L^{-1}$)	External carbon source	Reference
Bench scale chemostat (V= 5.65 L)	Reject water	FA and FNA	60	0.6	> 60 days	3.6±0.80	Ethanol	Zhang et al., 2012
Pilot scale SBR (3.6 m^3), SESA (Este)	Liquid digested	Low DO	>85	-	-	< 1	"Mash liquid"	Mayer et al., 2009
Pilot scale SBR, Werdhoelzi WWTP – Zurich	Sludge digester liquid from Werdhoelzli WWTP - Zurich	Low SRT; High T (29.2 °C)	60	AUR-AOB: 0.23; DNR: 1.0	3-5	< 3.1	Methanol	Fux et al., 2003
Pilot scale chemostat (SHARON), Werdhoelzi WWTP Zurich -	Sludge digester liquid from Werdhoelzli WWTP - Zurich	Low SRT; High T	70	AUR-AOB: 0.34; DNR: 0.55	-	-	Methanol	Fux et al., 2003
Pilot scale SBR (0.4 m^3), Partal Nitritation-ANAMMOX	Digester supernatant	Inoculum from other nitritation reactor; Low SRT	-	0.510	4 days	< 1	NA	Joss et al., 2009
Full scale chemostat (SHARON) (4500 m^3), Utrecht WWTP	Reject Water	Low SRT; High T	-	0.2	AeRT=2.5; AnRT=1.25	-	Methanol	Van Kempen et al., 2001
Full scale chemostat (SHARON) (1800 m^3), Rotterdam WWTP	Reject Water	Low SRT; High Temperature	-	0.46	AeRT=1; AnRT=0.5-1.4	-	Methanol	Van Kempen et al., 2001
Demonstration SBR – Treviso WWTP (2.8 m^3)	Reject Water	FA	20	0.8	> 20 days	1.5	Acetic acid	This Study

5.2 Material and Methods

5.2.1 The operating conditions of the scSBR

Five experimental periods including the startup were performed along 160 days of operation. Tabl5 5.2 includes a brief description of each period, while the operating characteristics of the scSBR in each period are shown in Table 5.3.

1st Period. The aim was to accomplish nitrite accumulation through the inhibition of NOB anabolism (Frison et al., 2012, Vadivelu et al., 2007). The reactor was initially inoculated with activated sludge originating from the full-scale reactor of the Treviso WWTP. After the inoculum, the initial concentration of suspended solids in the mixed liquor was 1.9 gMLSS L^{-1}, while the volatile fraction was 75%. The inoculated biomass had partly acclimatized to the treatment of anaerobic supernatant since the full-scale plant treated also the anaerobic recycles from the co-digestion reactor. This might have allowed a short start-up period (i.e. 1st period) during which aerobic conditions were maintained in the reactor. The biomass was allowed to acclimatize to the operating conditions for the treatment of highly nitrogenous anaerobic supernatant allowing the stability of the nitrifiers. During the start-up operation, the SBR was operated according with this sequence of phases: feeding of the anaerobic supernatant, aeration followed by 30 min of settling and discharging of the clarified fraction. Only the length of the aerobic phase was not fixed, since was controlled according with the concentration and the oxidation rate of the ammonia measured by an ion-selective probe (NH4D sc, Hach-Lange).

Table 5.2. Experimental periods (*AOB growth and NOB wash-out)

Period	Days	Description	External carbon source
1st	0-20	Start-up operation*	-
2nd	21-52	High vNLR = 0.8 kg N $m^{-3}d^{-1}$	Acetic acid
		(control process)	Glycerol (only days 36-37)
3rd	53-80	Very high vNLR = 1.1 kg N $m^{-3}d^{-1}$	Acetic acid (days 53-80)
		Significant polyelectrolyte residual in the anaerobic supernatant	

4th	81-120	Low vNLR = 0.2 kg N $m^{-3}d^{-1}$ Temperature decrease in the AD Low FA concentration Preliminary test of different carbon sources	Acetic acid (81-100) Drainage liquid (DL) from OFMSW (days 101-120)
5th	120-160	Added (NH_4HCO_3) in the supernatant to achieve vNLR = 1.1 kg N $m^{-3}d^{-1}$ Test of different carbon sources	DL from OFMSW (days 121-130) Fermentation liquid (FL) from OFMSW (days 131-150) FL from cattle manure & maize silage (CM&MS) (days 151-160)

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Table 5.3. Operating conditions during the experimental period (average (min-max))

Parameters	Units	1st period	2nd period	3rd period	4th period	5th period
Total vNLR	kg N $m^{-3}d^{-1}$	0.29	0.81	1.09	0.17	1.08
HRT	d	2.41 (1.09-4.10)	0.75 (0.74-0.76)	0.41 (0.39 -0.43)	0.49 (0.42-0.56)	0.52 (0.40-0.62)
SRT	d	-	>20	>20	>20	>20
Total OLR	kg COD $m^{-3}d^{-1}$	0.05	1.68	1.17	0.20	2.13
Total F/M	kg COD (kg MLVSS d)$^{-1}$	0	0.60	0.73	0.16	1.08
MLSS	g L^{-1}	2.38 (2.22-2.47)	3.62 (2.38-4.16)	2.15 (1.64-2.61)	1.61 (1.51-1.88)	2.65 (2.13 - 2.80)
MLVSS	g L^{-1}	1.59 (1.14-1.86)	2.78 (1.32-3.31)	1.61 (0.98-1.85)	1.28 (1.09-1.46)	1.98 (1.64-2.33)
Effluent pH	-	8.04 (7.95 - 8.14)	7.91 (7.73-7.96)	7.76 (7.39-7.93)	7.21 (7.35-7.25)	7.88 (7.29 - 8.02)
FA	mg NH_3-N L^{-1}	3.23 (2.15 - 4.33)	3.22 (4.89-2.28)	7.53 (10.44-2.73)	0.28 (0.03-0.42)	7.14 (10.03-1.26)
HNO_2	mg N L^{-1}	<0.01	<0.005	<0.005	<0.005	<0.005
DO	mg O_2 L^{-1}	1.53 (1.45-1.55)	1.48 (1.45-1.55)	1.48 (1.45-1.55)	1.52 (1.45-1.55)	1.48 (1.45-1.55)

2nd Period. After the 20 days of start-up, the sequence in one SBR cycle was 15 min filling, 80 min anoxic, 155 min aerobic, 30 min settling, 12 min discharge and 1 min idle and the maximum treatment potential (vNLR = 0.8 kgN $m^{-3}d^{-1}$ and sNLR = 0.29 kgN $(kgMLVSS^{-1}d)^{-1}$) was accomplished. The anaerobic supernatant was fed into the SBR during the mixing feeding state. Acetic acid (commercial solution 85% in weight) was added during the first 5 min of the anoxic phase of the SBR using a peristaltic pump controlled by the PLC. The feeding rate of the organic carbon was fixed approximately at 2 $kgCOD/kgNO_2$–N, according to the stoichiometric value for denitrification via-nitrite (1.72 $kgCOD/kgNO^2$–N removed).

3rd period. In this period the system's response at a vNLR higher than its complete nitrification capacity was tested (1.1 kgN $m^{-3}d^{-1}$); this period coincided with significant polyelectrolyte residual in the anaerobic supernatant. A cationic polyelectrolyte was used in the full-scale plant to facilitate the dewatering process of the digestate. The plant's operators used to irregularly and roughly adjust the dosage of the cationic polyelectrolyte due to variations in the dry content of the dewatered sludge. Such a careless, but almost common management of the dewatering process often led to polyelectrolyte residual in the supernatant. The polyelectrolyte residual resulted in some biomass escape in the treated effluent. As a result, in this period the biobiomass concentration was low and the sNLR was high (0.68 $kgN(kgMLVSS\ d)^{-1}$).

4th and 5th periods. The behaviour and stability of the process was studied even under highly variable AD conditions and anaerobic supernatant characteristics. Specifically, in the 4th period

the vNLR and sNLR decreased drastically due to an extraordinary decrease of temperature in the full-scale AD plant, caused by to extra-ordinary maintenance of digester-related heat exchanger. We took advantage of this drastic variability of the digestate to investigate the stability of the nitritation/denitritation process under variable loading conditions and at low FA concentration. In the 5th period the vNLR and sNLR were artificially increased to 1.1 kg N $m^{-3}d^{-1}$ and 0.54 kgN (kgMLVSS d)$^{-1}$ respectively by dosing NH_4HCO_3. The effect of different types of external carbon source on the performance of SCNR and DPRN was examined. We investigated the system's behaviour by restoring nutrients to ordinary levels.

The solids retention time (SRT) was above 15 days in all experimental periods. However, as some biomass escaped with the treated effluent (particularly in the 3rd period) it was not possible to determine the true SRT in each period. As seen in Table 5.2 five different external carbon sources were investigated according to their availability in AD plants. Acetic acid was selected since it is a carbon source that is widely applied and is commercially available. Glycerol can be generated as a waste material from biodiesel production, while the other three carbon sources (i.e. drainage liquid from OFMSW (DL OFMSW), fermentation liquid of OFMSW (FL OFMSW) and fermentation liquid of cattle manure and maize silage (FL CM&MS) can be generated from biowaste and can thus be available in full-scale AD plants. The applied external carbon sources were characterized by high concentration of short chain fatty acids including acetic acid, propionic acid and butyric acid. Studies have shown that these substances can decrease nitrate formation without affecting the AOB activity in activated sludge processes (Ji and Chen, 2010). Also, the type of carbon source applied can significantly affect denitrifying phosphorus removal (Carvalho et al., 2007; Patel et al., 2006).

5.2.2 Physical and chemical parameters of the anaerobic supernatant and the external carbon source

The Table 5.4 show summirize the characteristics of the anaerobic supernatant that was fed to the SBR for the examined experimental periods

Under ordinary operation of the full-scale anaerobic co-digestion unit, this liquid stream was characterized by high ammonium concentration, molar ratio of alkalinity to ammonium sufficient for the shortcut nitrogen removal and significant phosphorus concentration. The TSS

concentration was low indicating that the solid/liquid separation process was effective. However, the unstable operation of the AD unit and the variable loadings of the OFMSW fed to the AD system affected the characteristics of the anaerobic supernatant. In the 4th and 5th periods the temperature in the full-scale AD decreased significantly. Consequently, the hydrolysis of organic matter was much lower, resulting in lower ammonium and phosphate concentrations of the anaerobic supernatant compared to the other periods. Alkalinity and pH are parameters that indicate the stability of the AD process. The low pH and alkalinity of the anaerobic supernatant in the 4th period show that the AD process was problematic due to the low temperature in the AD reactor. In the 5th period the high ammonium concentration was restored through the intentional addition of NH_4HCO_3. The low COD/TKN ratio of the anaerobic supernatant means that adequate and sufficient external carbon source must be supplied to accomplish effective denitritation and DPRN.

Table 5.4 Anaerobic supernatant characteristics for each experimental period (average (min-max)).

Parameter	Units	1st Period	2nd Period	3rd Period	4th Period	5th Period
		0-20	21-52	53-80	81-120	121-160
pH	-	7.8 (7.7-7.9)	7.8 (7.7-7.9)	7.6 (7.5-7.7)	7.3 (7.1 -7.4)	7.9 (7.8-8.1)
TSS	mg L^{-1}	152.5 (133.4-169.5)	145 (122.5-167.3)	111 (88.4-144.5)	16.9 (15.5-17-8)	33.4 (29.6-37.9)
COD	mg L^{-1}	140.7 (103.5-155.4)	133.8 (118.4-162.8)	102.4 (95.1-122-9)	15.6 (14.0-22.2)	30.9 (21.5-56.6)
Soluble COD	mg L^{-1}	110.4 (77.1-127.7)	96.9 (83.6-130.6)	88.6 (23.9-109.5)	10.5 (10.2-15.9)	25.7 (18.6-31.9)
TN	mg L^{-1}	504.0 (412.2-542-8)	636.5 (578.8-850.2)	476.7 (627.5-185.6)	92.2 (18.2-231.4)	570.3 (430.0-670.1)
NH_4-N	mg L^{-1}	492.4 (387.1-631.5)	592.5 (544.5-631.3)	448.1 (180.4-615)	88.6 (15.1-227.5)	558.9 (410.5-650.0)
NO_3-N	mg L^{-1}	0.23 (0.11-0.67)	0.36 (0.30-0.45)	0.21 (0.18-0.25)	0.98 (0.85-1.23)	0.78 (0.52-1.23)
NO_2-N	mg L^{-1}	0.32 (0.08-0.75)	0.56 (0.22-0.61)	0.11 (0.05-0.55)	0.33 (0.22-0.68)	0.42 (0.35-0.69)
Conductivity	mS cm^{-1}	5.12 (4.85-5.27)	5.78 (5.21-6.21)	4.23 (3.98-4.77)	2.11 (1.01-2.76)	4.44 (3.88-5.21)
TP	mg L^{-1}	76.4 (77.2-79.3)	80.4 (71.1-88.9)	71.0 (70.3-72.5)	29.4 (24.5-32.7)	31.3 (25.6-33-6)
PO_4-P	mg L^{-1}	74.9 (55.3-88.1)	71.6 (55.2-83.5)	69.9 (38.2-77.6)	28.6 (14.5-34.5)	30.1 (12.0-32.1)
Alkalinity/NH_4^+	mol HCO_3^-/ mol NH_4^+	1.50	1.58	1.55	2.65	1.10

In all periods (except the start-up) an external carbon source was supplied (Frison et al., 2013). We chose the external carbon sources on the basis of the organic substrates commonly used in anaerobic co-digestion plants. Therefore, further to the acetic acid (Period 2 and 3), which was taken as baseline, we tested the glycerol (which is a waste from biodiesel production, and is often used to maintain the optimal organic loading rate to anaerobic co-digesters), and three biowaste-originated carbon sources (fresh liquid drainage from OFMSW piles, OFMSW fermentation liquid, and CM&MS fermentation liquid). The fresh liquid drainage from the full scale storage pit of the OFMSW was pumped to an accumulation tank located before the demonstration scSBR. As the actual hydraulic retention time of the accumulation tank was about

24 h, depending on the environmental temperature the fresh liquid drainage could be subject to non-controlled partial fermentation. Before to be fed in the pilot fermentation unit the source-separated OFMSW was grinded, diluted with secondary effluent of the main WWTP up to 6% TS and then fermented. As suggested by Pavan et al., (1998), the sequencing batch fermenter was fed according to an organic loading rate (OLR) of 20 kgTVS m^{-3} day^{-1}, which involved a hydraulic retention time of 3 days. The total VFA production was independent of HRT in the range 3–6 days (Pavan et al., 1998). On the other hand, the cattle manure (CM) and maize silage (MS) were mixed according to a dry weight ratio of 1:1, then diluted with secondary effluent up to dry content of 10% TS. The sequencing batch fermenter was fed according to OLR of 70 kgTVS m^3day^{-1}, while the HRT was 2 days. Then, the raw fermentation effluents was separated by a screw-press. Then, the liquid was fed as external carbon source to the scSBR, while the solid fraction was tested for the bio-methanization potential, so as to provide reliable experimental basis for the final cost analyses.

Table 5.5. Main chemical-physical characteristics of the external carbon sources investigated (12 samples analyzed over one year)

Parameter	Units	Acetic Acid	Glycerol	OFMSW Drainage liquid	OFMSW Fermentation liquid	CM&MS Fermentation liquid
TS	gTS kg^{-1}	-	-	6.0±0.5	25±4	48±5
TVS	gTVS kg^{-1}	-	-	5.0±0.4	22±2	44±2
TVS/TS	%	-	-	83±1	88±1	92±2
Total COD	gCOD L^{-1}	663	1160±25	58±6	62±6	85±5
sCOD	gCOD L^{-1}	663	1160±25	42±9	31±3	43±6
rbCOD	gCOD L^{-1}	663	-	38±9	29±4	28±5
NH_4-N	gN L^{-1}	-	-	0.11±0.03	0.31±0.06	0.93±0.04
TN	gN L^{-1}	-	-	2.2±0.4	1.1±0.3	5.0±0.6
TP	gP kg^{-1}	-	-	0.13±0.02	0.54±0.07	0.90±0.03
PO_4-P	gP L^{-1}	-	-	0.09±0.02	0.12±0.04	0.31±0.01
pH	-	-	-	4.5±0.1	4.1±0.2	4.6±0.4

Total VFA	gCOD L^{-1}	663	0	6.9±0.8	18±2	27±3
Acetic Acid	gCOD L^{-1}	100	-	4.4±0.2	13.3±0.7	21.5±0.8
Propionic Acid	gCOD L^{-1}	-	-	0.5±0.3	1.8±0.3	0.5±0.1
Butirric Acid	gCOD L^{-1}	-	-	0.8±0.4	2.9±0.4	3.4±1.2
VFA C5-C7	gCOD L^{-1}	-	-	1.2±0.4	0.2±0.1	1.1±1

All the investigated external carbon sources were analyzed and characterized by chemical–physical and respirometry analyses (**Errore. L'origine riferimento non è stata trovata.**). Therefore, we evaluated both the chemical forms and the degree of biodegradability of the organic matter (Jenkins et al., 1998). The fermentation liquids of OFMSW and MS&CM presented 50% of the total COD in the soluble form. On the other hand, the soluble COD in the raw drainage liquid represent up to 73% of the total COD due to lower content of organic suspended solids. In addition, the degree of biodegradability of the sCOD was investigated by respirometry tests: up tp 90% of the soluble COD is composed by RBCOD for the OFMSW-related carbon source and about 65% for CM&MS fermentation liquid. In particular, the acid fermentation involved specific VFA production ranging from 273 to 199 gCOD $kgTVS^{-1}$ for OFMSW and CM&MS, respectively. In agreement with (Traverso et al. 2000) and (Pavan et al. 1998), the main VFAs ranged between C2 and C3: specifically, content of acetic acid was up to 4.4 gCOD L^{-1} even in the fresh liquid drainage of OFMSW. However, the SCOD contains also lactic acid and several alcohols, as a consequence of alcoholic and lactic transformation of pyruvate (Traverso et al. 2000). In addition, the biofibers in the OFMSW and in CM&MS are not completely degraded during the acidogenic fermentation (. As a result, humic acids with a high molar mass according with 2.91 gCOD gHA^{-1} (Qi et al., 2003) may form and accumulate in the liquid fermentation up to 26% of the total COD (Tong et al., 2009). However, some authors demonstrated that humic acids might have effect to improve SCDN as well as DNPR (Tong et al., 2009; Ji and Chen 2010). Although ammonification is less significant in acid fermentation than in anaerobic digestion, nutrients solubilize during fermentation processes (Jiang et al., 2007), so fermentation liquids may involve major contraindications when used as external carbon source. In fact, the OFMSW fermentation liquid showed 100–382 gSCOD $gNH_4–N^{-1}$, while the CM&MS showed 45 gSCOD $gNH_4–N^{-1}$. Considering the specific COD consumption reported and later discussed (Table 5.7), the CM&MS fermentation liquid may influence the actual

nitrogen removal as much as 375 g of nitrogen added per kg of nitrogen removed, corresponding to about 67 gNH_4–N gN^{-1} removed. On the other hand, the phosphorus ranged from 258 to 138 gSCOD gPO_4–P^{-1} for the fermentation liquids, while 467 gSCOD gPO_4–P^{-1} was contained in the OFMSW drainage liquid.

5.2.3 Monitoring of the greenhouse gas emissions

The operating characteristics of the two experimental periods are summarized in Table 5.6 and the physicochemical characteristics of the anaerobic supernatant and the OFMSW FL are shown in Table 5.5.

Table 5.6: Operating conditions during the experimental period (mean values ± standard deviation)

Parameters	Units	Period 1	Period 2
vNLR	kgN $m^{-3}d^{-1}$	1.08 ± 0.20	0.81 ± 0.14
sNLR	kgN $(kgMLVSS{\cdot}d)^{-1}$	0.54 ± 0.10	0.29 ± 0.06
HRT	d	0.52 ± 0.15	0.75 ± 0.01
OLR	kgCOD $m^{-3}{\cdot}d^{-1}$	2.13	1.68
F/M	kgCOD $(kgMLVSS{\cdot}d)^{-1}$	1.08	0.60
MLSS	g L^{-1}	2.65 ± 0.47	3.62 ± 1.26
MLVSS	g L^{-1}	1.98 ± 0.49	2.78 ± 1.41
FA	mgNH_3-N L^{-1}	7.14 ± 6.20	3.22 ± 1.84
FNA	mgHNO_2-N L^{-1}	< 0.02	< 0.02
DO	mgO_2 L^{-1}	0.95 ± 0.12	1.48 ± 0.07

F/M: food to microorganism ratio, FA: free ammonia, FNA: free nitrous acid

The vNLR applied in period 1 was 30% higher than the system's nitrogen removal capacity. The vNLR that was applied in period 2 was close to its nitrogen removal capacity in order to reduce the ammonium and nitrite concentrations in the mixed liquor. The anaerobic supernatant was characterized by a very low COD/TKN ratio (<0.3 in both periods). As a result, the OFMSW FL was added to allow for effective denitritation. The OFMSW FL was characterized by a very high content of volatile fatty acids (VFA) and sCOD that can be used by heterotrophic denitrifiers.

5.2.4 Tools for the Life Cycle Assessment

The main objective of side stream technologies is the removal of eutrophying substances, namely N and P compounds. For this reason, we chose, as functional unit (FU), the reduction of the eutrophication potential (EP) as defined by the CML methodology v.2.05 (Guinée et al., 2002), 1 kg PO_4^{-3} equivalent removed. As seen in Rodriguez-Garcia et al. (2014), this FU reduces the effect influent quality has on the profile of a WWTP, giving more importance to the effort made by the plant than to the actual effluent quality. As a result, this FU allows a better comparison between carbon sources with different characteristics. Like those FU based on volume (e.g. 1 m^3), it does not show how a technology would behave with a different influent. Global warming (GWP), acidification (AP), EP, photochemical oxidation (POP) and toxicity-related impact categories are, according to Corominas et al. (2013), those most widely assessed. As such, we evaluated those impacts, excluding POP, since previous studies had shown that the effect of WWTPs in this category was negligible (Hospido et al., 2005). GWP, AP and EP were assessed using the last updated version (v.2.05, November 2010) of the CML methodology (Guinée et al., 2002). According to Corominas et al. (2013), this is the impact method most commonly used in LCA of WWTP. The toxicity-related categories of human toxicity (HT) and freshwater ecotoxicity (ETP) were evaluated using USEtox, including both recommended and interim substances (Rosenbaumet al., 2008). USEtox is considered a state-of-the-art methodology thanks to the large consensus achieved by model developers, including those from CML, during its design. The main assumptions considered were:

- For the glycerol provision in scenario 1, an average Italian manufacturing mix was calculated: 41.6% Glycerine from rape oil at esterification plant, 27.3% Glycerine from soybean oil at esterification plant, 25.9% Glycerine from palm oil at esterification plant, and 5.2% Glycerine from vegetal oil at esterification plant.

- The production of the liquid drainage from the OFMSW is considered to be as impact free since it is originated naturally during the storage of the OFMSW and collected by gravity in the pit area of MSW. This stream would otherwise be mixed with the influent entering the main line of the wastewater treatment plant.

- The production of the fermentation liquid form the OFMSW would have some environmental loads associated with the fermentation process itself. Because fermentation would take place

prior to anaerobic digestion regardless of whether is used as a carbon source or not, those items have not been included in the Life Cycle Inventory. Also, since the original use of OFMSW is as a co-substrate for the anaerobic digestion of the sludge, its use as carbon source reduces the electricity production associated to the biogas combustion. So, the system analysed has been expanded in order to include the provision of the no-produced energy by the average electricity mix.

5.3 Results and discussion

5.3.1 Experimental period for the short-cut SBR

Figure 5.1 shows the influent ammonium concentration and treated effluent ammonium, nitrite and nitrate concentrations of the SBR for the five experimental periods.

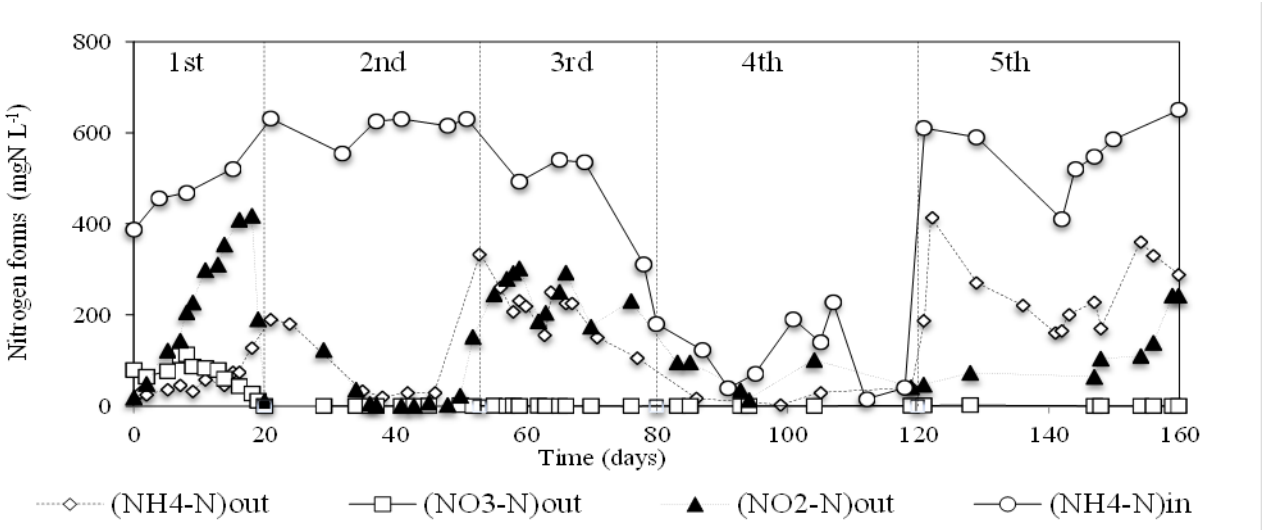


Figure 5.1. Nitrifying capacity of the system to the total nitrogen feed for the examined experimental periods

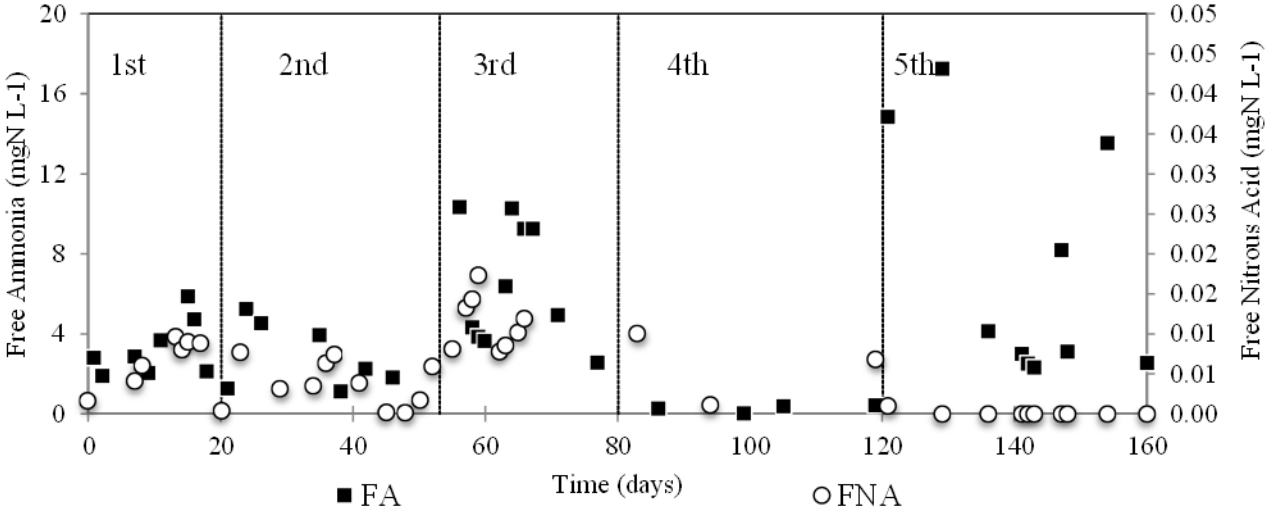


Figure 5.2. Level of FA and FNA during the five experimental periods

During the start-up period, a build-up of nitrites was observed up to 420 mgNO_2–N L^{-1} (day 20), while nitrate increased up to 110 mgNO_3–N L^{-1}. The accumulation of NO_x–N is reasonable since only aerobic conditions were applied during the start-up. So, the free ammonia during each cycle was high enough to inhibit NOB anabolism and contemporary promoting AOB growth. However, the concentration of the free nitrous acid (calculated according to Anthonisen et al. (1976) did not exceed the value of 0.01 mgHNO_2-N L^{-1} . As the growth of AOB and NOB is not influenced by FNA under that value, (Balmelle et al., 1998) the drastic enrichment of AOB, the nitrite accumulation and the stabel inhibition of NOB was caused by the sole effect of the free ammonia. After the first 20 days of operation, the nitrate in the treated effluent decreased to very low values (<1 mgNO_3–N L^{-1}) and stable via-nitrite operation was accomplished there after. As seen in Figure 5.2, the FA concentration in the mixed liquor was sufficient to inhibit the anabolic processes of Nitrobacter (Vadivalu et al., 2007). Specifically, during the 2nd, 3rd and 5th periods the FA level in the reactor was high (always > 1.2 mgNH_3–N L^{-1} and usually >2 mgNH_3–N L^{-1}). The FA levels were higher than the range of 0.1–1.0 mgNH_3 L^{-1} reported by Anthonisen et al. 1976 for the inhibition of Nitrobacter. In the 3rd and 5th periods, the high FA concentration (in some instances it exceeded 10 mgNH_3–N L^{-1}) did not adversely impact on AOB activity. Vadivelu et al. 2007 reported that an FA concentration up to 16 mgNH_3–N L^{-1} did not have any inhibitory effect on AOB. Anthonisen et al. 1976 found that AOB inhibition started at an FA concentration of 10 mgNH_3 L^{-1}. The FNA was not a critical parameter and did not affect AOB or NOB activity since its concentration in the reactor was always very low (as seen in **Errore. L'origine riferimento non è stata trovata.** < 0.02 mgHNO_2–N L^{-1}). An interesting finding is that complete and stable suppression of NOB activity was maintained even in the 4th period during which the FA concentration was low (it never exceeded 0.45 mgNH_3–N L^{-1}). This finding shows that other important parameters may suppress the NOB activity. Ji and Chen et al., (2010) attributed the NOB inhibition observed in two SBRs to the presence of humic acids which were present in sludge fermentation liquid that was dosed as external carbon source, rather than to the FA levels which were relatively low. However, the FA levels reported by the authors (i.e. 0.62 and 0.51 mgNH_3 L^{-1}) are above the threshold value of 0.1 mgNH_3 L^{-1} at which NOB inhibition starts (Anthonisen et al, 1976). Zhang et al. (2012) observed that an increase in the NLR resulted in the accumulation of nitrite in the reactor and thus concluded that this parameter is more important than FA concentration. In our case, FA, sNLR and vNLR were low in the 4th period. Furthermore, the influent anaerobic supernatant was characterized by a relatively high molar

ratio of alkalinity to ammonium. According to the results reported by Ganigué et al. (2012) showing the percentage of nitrate and nitrite as a function of the molar ratio of alkalinity/ammonium versus NLR, our conditions would promote the formation of nitrate. Interestingly, the ratio of NO_2–N/NO_x–N was maintained at 100% for the 40 days of SBR operation during the 4th period (i.e. days 81–120). Two potential reasons could account for this: (a) process dynamics and specifically the high NLR and FA of the previous period could be detrimental to subsequent NOB activity. It seems that the inhibition of the NOB anabolism for the 2nd and 3rd period was sufficient to have the stable suppression of NOB activity and (b) NOB inhibition caused by humic acids contained in DL OFMSW, which was dosed during days 101–120. Humic acids are known to inhibit NOB activity (Ji and Chen, 2010). The DL OFMSW had a concentration of humic acids ranging between 1140 and 1352 mgCOD L^{-1}. Humic acids could originate from fruit and vegetable residuals which consist of a large proportion of OFMSW and from solids contained in wastewater, thus increasing the lignocellulosic fraction of the influent fed to the SBR (Sun et al., 2002).

5.3.2 Nitrogen removal over the long term experimental period

Figure 5.3 shows the sAUR versus the vNLR for the examined experimental periods. At low to medium vNLR, an increase in the vNLR was accompanied by an increase in the specific nitritation rate, reflecting the increased availability of ammonium for nitrifying bacteria. During the start-up operation low to medium vNLR were applied (<0.5 kgN $m^{-3}d^{-1}$) and are represented by three different points in Figure 5.3. During the start-up operation, the average specific ammonium uptake rate was 4.8 mgN (gMLVSS h)$^{-1}$, while at the beginning of the second period (thus after the start-up period), the sAUR increased up to 18-20 mgN (gMLVSS h)$^{-1}$. A plateau was reached at vNLR of 0.8 kg $m^{-3}d^{-1}$, showing that during the 2nd period the maximum nitrifying capacity was reached. In this period, high ammonium oxidation (90%) and nitrogen removal (85%) were obtained under transient conditions. In this period period, the maximal denitritation rate was around 45− 50 mgN (gMLVSS h)$^{-1}$. In the 3rd and 5th periods, the sAUR was similar to that of the 2nd period, while the vNLR that was applied was higher than the maximum treatment capacity (i.e. 1.1 kgN $m^{-3}d^{-1}$). The sNLR was also very high in both periods (>0.5 kgN (kgMLSS d)$^{-1}$). High vNLR in combination with operational problems resulted in low

performance with respect to ammonium oxidation and nitrogen removal during the 3rd period. In fact in this period, residuals of polyelectrolyte in the anaerobic supernatant were often observed. The sNLR was also very high in both periods (>0.5 kgN (kgMLSS d)$^{-1}$). High vNLR in combination with operational problems resulted in low performance with respect to ammonium oxidation and nitrogen removal during the 3rd period. In fact in this period, residuals of polyelectrolyte in the anaerobic supernatant were often observed. Specifically, the polyelectrolyte overdose coupled with biomass aeration created a porous and floating floc structure resulting in partial washout (i.e. escape) of the biomass during its discharge. Thus, the concentration of the biomass in the mix liquor (MLVSS) underwent to drastic irregular drops from 3.3 to 1.4 (days 49.51) and 2.3 to 1.4 gMLVSS L^{-1} (days 59-64). Contemporary, a drastic decrease of the sludge volume index (SVI) for the non floating sludge flocs was observed (from 65 to 25 mL gMLSS^{-1}). Consequently, the decreased biomass concentration could not cope with the high vNLR and sNLR. Ammonia oxidation and nitrogen removal was also not complete in the 5th period. In the 4th period the vNLR and sNLR decreased and at the same time the Food:Microrganism ratio was also lowered in order not to waste the external carbon source and at the same time achieve effective denitritation. As seen inFigure 5.3, lower sAUR was obtained in the 4th period compared to the periods in which higher vNLRs were applied. Despite the low vNLR and sNLR a significant amount of nitrite was found in the effluent showing that the denitritation process was incomplete. Figure 5.4 shows the sAUR and sNUR with operating time. Despite the transient and unfavourable conditions that were experienced (i.e. flocculent residual in the anaerobic supernatant, decrease in anaerobic digestion temperature) steady sAUR was obtained in the periods when high vNLR was applied. The sNUR obtained when acetic acid was dosed (i.e. 2nd period) was comparable to the one obtained when FL CM&MS was used as external carbon source (i.e. end of 5th period). However, nitrogen removal efficiency was lower in the 5th period, implying that vNLR was a critical factor affecting the process efficiency. No significant difference was obtained in sAUR when the three different biodegradable waste-derived carbon sources were employed (i.e. 5th period). Several researchers have found that the presence of specific organic substances, such as propionic acid and butyric acid can partially inhibit NOB (Ji and Chen, 2010; Patel et al., 2006). In our case NOB activity was never observed and the results show that the different external carbon sources did not inhibit AOB growth.

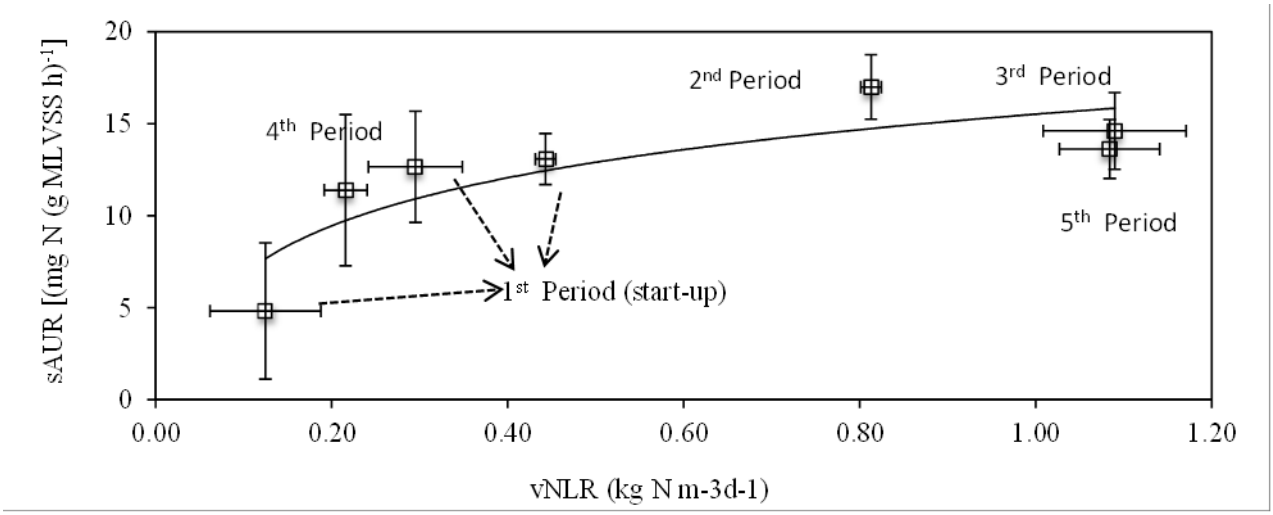


Figure 5.3. sAUR versus vNLR

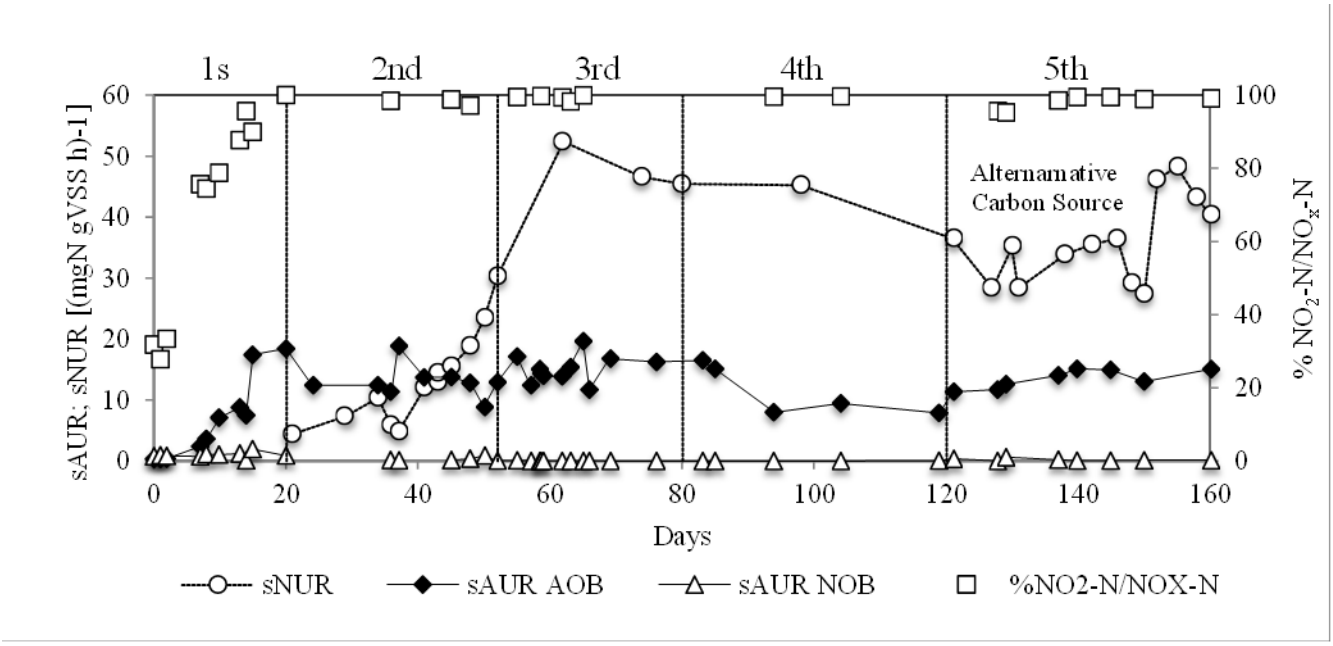


Figure 5.4. Specific ammonium uptake rate (sAUR) and specific nitrite uptake rate (sNUR) during the experimental periods

The sNUR was variable even within the same period. The sharp decrease of sNUR in days 36–37 of the 2nd period is attributed to the use of glycerol instead of acetic acid and was abandoned because of poor performance. The increase of sNUR during the 2nd period ‘‘matches’’ with the gradual decrease in NO_2–N concentration seen in days 21–40 (i.e.Figure 5.1). In the 3rd period the sNUR sharply increased owing to the high substrate (i.e. organic carbon–acetic acid) to bacteria ratio (i.e. high Food:Microrganism ratio). In the 4th and 5th periods the type of external

carbon source critically impacted on the performance of denitrifying bacteria with acetic acid and FL CM and MS resulting in very high specific denitritation rates, while the addition of the DL OFMSW and the FL OFMSW resulted in lower sNUR.

5.3.3 Occurring of denitrifying phosphorus removal via-nitrite

The occurring of phosphorus removal with the simultaneous nitrite denitrification was observed in the 4th and 5th periods. To investigate the biological DPRN, both in situ monitoring of sequencing cycles and ex situ batch tests were conducted to evaluate the DPRN. Although a specific anaerobic phase was not implemented, phosphorus release actually occurred during the settling period as well as during the first 5–10 min of the anoxic phase due to the addition of the external carbon source. Under these conditions the anaerobic cores within the flocs could have occurred. The phosphate concentration in the treated effluent was higher than the PO_4–P concentration in the mixed liquor at the end of the reaction phase. This indicates that minor phosphorus release occurred during sludge settling probably due to the development of anaerobic conditions in the settled sludge. Furthermore, after the initial dosage of anaerobic fermentation liquid in the anoxic phase, local anaerobic conditions in the suspended sludge could have occurred. When the anoxic conditions were homogeneously established, anoxic phosphorus uptake was clearly observed. Interestingly, our process did not need a proper anaerobic phase for carbon uptake. In fact, the high carbon gradient at the beginning of the anoxic phase probably allowed anoxic carbon storage, which led to high DPRN. In addition, DPRN consumes less carbon source than conventional aerobic or via-nitrate enhanced biological phosphorus removal. Therefore, residual carbon was even sufficient for aerobic phosphorus uptake in the following cycle. In Figure 5.6 (a,b,c) the phosphate and nitrite profiles are shown for ex situ, batch tests that were conducted in the laboratory with the addition of (a) DL OFMSW (b) FL OFMSW and (c) FL CM&MS.

Table 5.7. Specific denitrifying phosphate uptake rate and specific nitrite uptake rate for the different carbon sources that were added in batch reactors

Parameter	**Units**	**DL from OFMSW**	**FL from OFMSW**	**FL from CM&MS**
Specific phosphate uptake rate	kg PO_4-P (g VSS d)$^{-1}$	0.188	0.270	0.200
sNUR	kg NO_2-N (g VSS d)$^{-1}$	0.432	0.542	0.671

Phosphate to nitrite ratio	kg $(PO4\text{-}P)_{uptake}$ / kg $(NO_2\text{-}N)_{denitrified}$	0.300	0.430	0.298

In addition, Table 5.7 shows the specific phosphate uptake rate (sPUR) and sNUR for the three different carbon sources as obtained from Figure 5.6 (a,b,c). The FL OFMSW resulted in the highest sPUR and the FL CM&MS in the highest sNUR. As documented in the work of Frison et al. 2013, the FL OFMSW and FL CM&MS contain much more readily available short chain fatty acids (i.e. propionic acid and butyric acid) than the DL OFMSW, thus increasing phosphorus and nitrogen removal via nitrite. The presence of phosphate and cationic metals in the supernatant and the pH in the range 7.2–8 may lead to irregular precipitation of metal phosphates in both the storage basin and in the SBR. To assess the extent of chemical precipitation in phosphorus removal the PCA method was conducted in activated sludge samples. It showed that chemical precipitation was low (<15%) compared to the biological phosphorus uptake. Also, the smooth slopes of the orthophosphate and the nitrite profiles clearly show that we were considering only the biological DPRN. The results clearly demonstrate that the use of these organic carbon sources resulted in effective phosphorus removal via nitrite. Studies have shown that substrate storage occurred under anoxic conditions and played a major role in denitrification, being also strongly enhanced by the simultaneous presence of the diverse substrates with respect to single substrates Dionisi et al., 2006. However, inhibition of PHB storage has been found to correlate well with nitrite accumulation in the reactor Ciggin et al., 2009. In addition, it has been reported that FL promotes the denitrifying PAOs more than the glycogen accumulating organisms (GAOs). However, another important parameter that must be considered is the initial PO_4-P/NO_2-N ratio. As this work demonstrates, lower values of this ratio favour phosphate uptake by denitrifying PAOs. In these batch experiments the initial PO_4-P/NO_2-N ratio was higher for LD compared to FL (i.e. 0.24 compared to 0.17–0.18) and this may partly explain the lower phosphorus uptake. The ratio of $kg(PO_4\text{-}P)_{uptake}$/kg $(NO_2\text{-}N)_{denitrified}$ varied between 0.298 and 0.430 depending on the carbon source. The addition of FL OFMSW resulted in the removal of more phosphate for the same amount of denitrified nitrite. In several studies, it has been reported that nitrite are inhibitory for the growth of denitrifying PAOs (Peng et al., 2011; Saito et al., 2004; Meinhold et al., 1999). In this work, despite the high nitrite concentrations in the mixed liquor which at the beginning of the anoxic phase in the batch experiments were 117–169 $mgNO_2$–N L^{-1}, effective phosphate uptake occurred. The availability

of nitrites as electron acceptors resulted in high phosphorus removal. This shows that nitrite itself is not inhibitory for the growth of denitrifying PAOs.

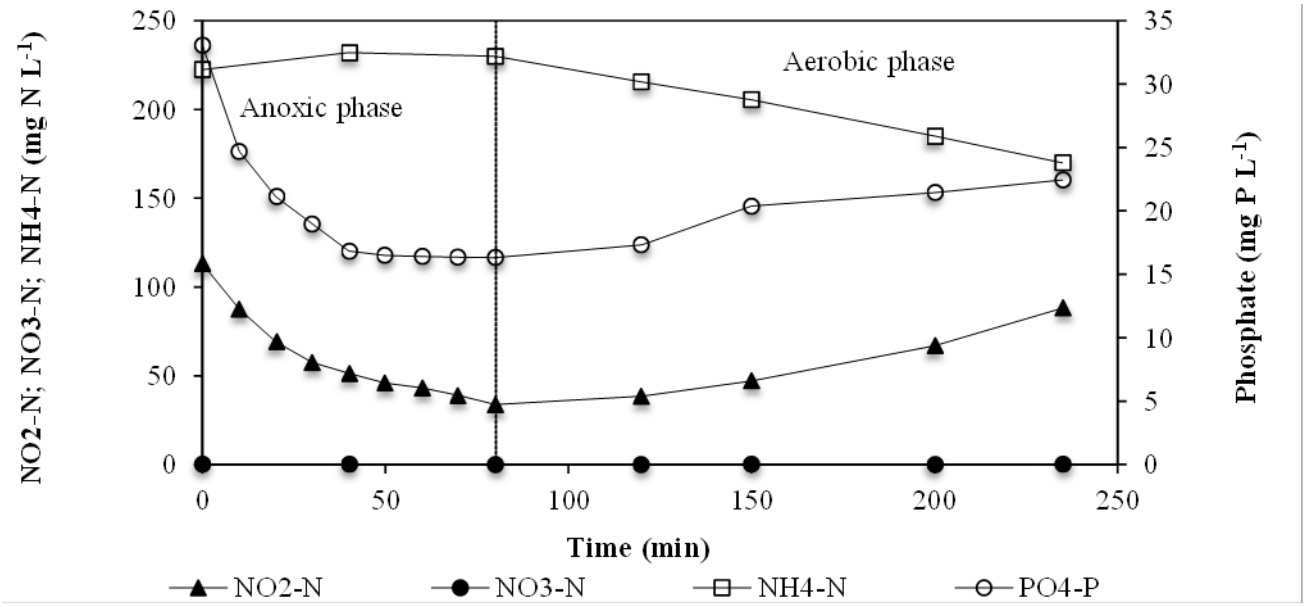


Figure 5.5. Ammonium, nitrite, nitrate and phosphate profile in the SBR reactor during the anoxic and aerobic phases of the 5th period

Most probably other derivatives such as FNA rather than nitrite inhibit the activity of denitrifying PAOs. As reported before, the FNA concentration was very low (<0.02 mgHNO_2-N L^{-1}) as pH was relatively high (pH = 7.2–8). Some researchers have reported phosphorus release under anoxic conditions and/or at the end of the phase when nitrite (or nitrate) is depleted (Patel et al., 2006; Zhang et al., 2010). In our work, phosphorus release was not observed during the late stages of the anoxic phase since nitrite concentration in the mixed liquor was usually significant. During the 5th period of the SBR operation, phosphate release was occasionally observed during the (nominal) aerobic phase Figure 5.5. In this period, the high loading decreased the DO level during the aerobic phase to values lower than in previous cycles (i.e. 1.0 mg L^{-1} compared to 1.5 mg L^{-1} for the other periods) and local micro-anaerobic conditions likely occurred. Furthermore, the polyelectrolyte residuals occurring in periods 3–5, resulted in much more dense flocs compared to the floc structure of the 2nd period. Thus, the relatively low DO in combination with the dense floc structure could result in some parts of the flocs interior exhibiting anaerobic rather than aerobic conditions (Patel et al., 2006). In the batch experiments presented in Figure 5.6, phosphate uptake was always observed under aerobic conditions.

However, these experiments were conducted in 2 L bench scale reactors where it is much easier to achieve complete aeration of the biomass. Further investigations are ongoing with concern to the forms of carbon stored and metabolized during the DPRN. In this way we will demonstrate whether in a single SBR it is possible to enhance at the same time the via nitrite nutrients removal and the production of poly-beta-hydroxyalkanoate (PHA) using fermentation liquid of biodegradable waste.

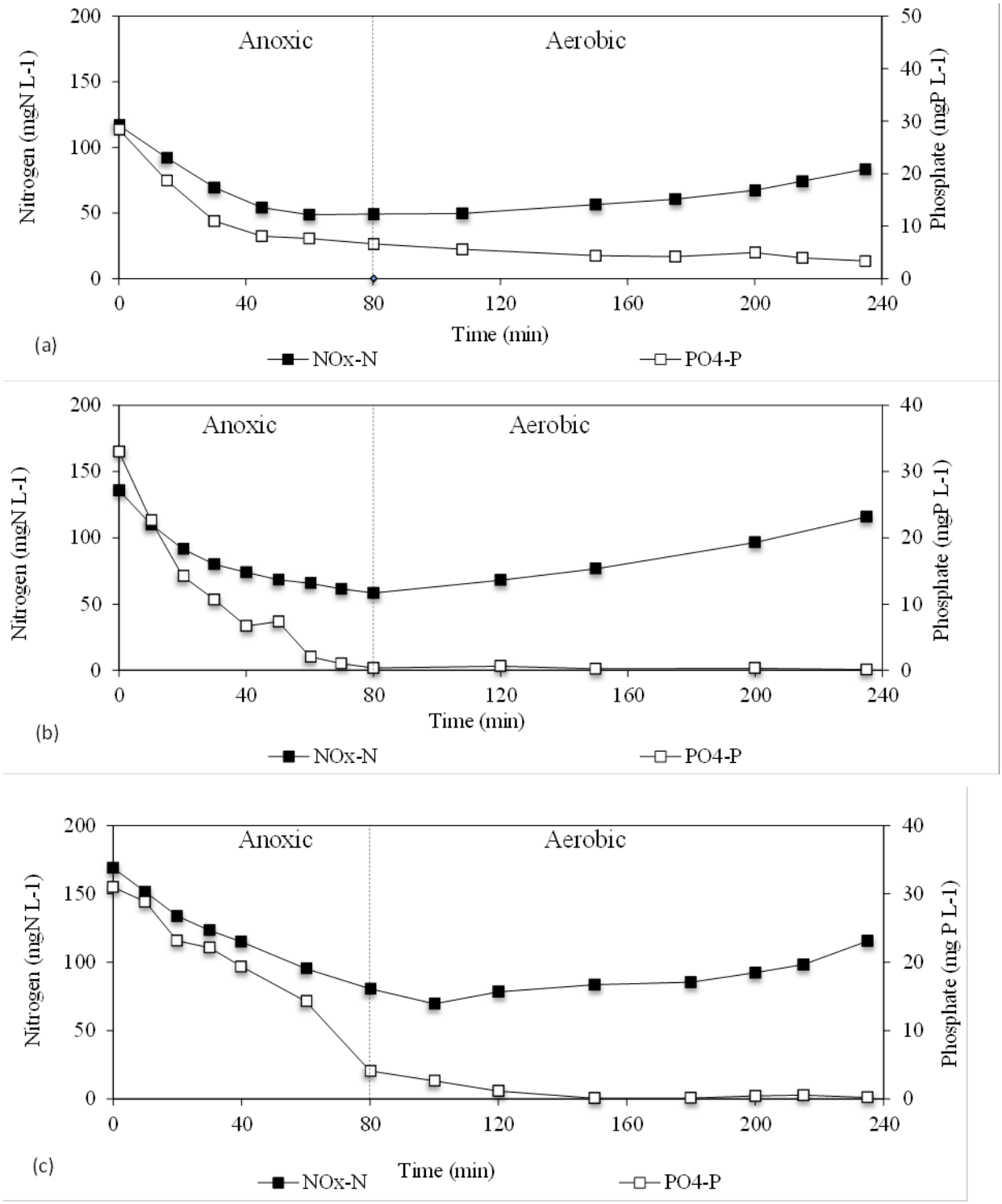


Figure 5.6. Phosphate and nitrite profile obtained in batch tests with the addition of (a) DL from OFMSW (b) FL from OFMSW and (c) FL from CM & MS.

5.3.4 COD consumption and sludge yield of heterotrophic denitrifiers

According to Mulder and Van Kempen (1996), the total consumption of COD per gram of nitrite converted to nitrogen gas is 1.72. Therefore, the heterotrophic sludge yield can be calculated as follows:

$$Y_{HD} = 1 - (1.72 \times NO_2 - N_{reduced})/COD_{consumed})$$

Abeling and Seyfried (1992) reported a specific COD consumption of 2.1 and 2.3 kgCOD $kgNO_2\text{-}N^{-1}$ denitrified using acetic acid and glycerol respectively. On the other hand, using OFMSW drainage or fermentation liquids we found In this work, the specific COD consumptions using OFMSW LD and OFMSW FL were 2.7 and 3.1 kgCOD $kgNO_2\text{-}N^{-1}$ denitrified respectively (Table 5.8). Generally, waste originated carbon sources lead to higher yields than pure carbon source in agreement with Hwang et al. 1994. Acetic acid and glycerol showed respectively yields of 0.18 and 0.24 kgCOD $kgCOD^{-1}$. In fact, the type and dose of organic carbon source influence the fraction, type and anabolism of the active biomass (Obaja et al., 2005). We observed lower yields when fermentation liquid of CM&MS was used (Y_{HD} = 0.31 kgCOD $kgCOD^{-1}$), while using OFMSW-originated the yield were comparable with the one reported by Mayer et al., 2009 using mash liquid from OFMSW

Table 5.8. Effect on COD consumption and sludge yields with different external carbon sources.

Paremeter	**Unit**	**Acetic acid (80%)**	**Glycerol**	**OFMSW LD**	**OFMSW FL**	**CM&MS FL**
Specific COD Consumption	kgCOD $kgNO_2\text{-}N^{-1}$	2.1	2.3	2.7	3.1	3.0
Sludge yields in anoxic phase (YHd)	kgCOD $kgCOD^{-1}$	0.18	0.24	0.36	0.45	0.31

5.3.5 Cost analysis of the carbon sources for the via-nitrite nutrients removal

The cost of the applied carbon source is critical, since it significantly affects the full scale application of the via nitrite process for nutrient removal from high and low strength anaerobic

effluents. An economic analysis was carried out to determine the specific cost required for nitrogen removal via nitrite in euro per kg of nitrogen removed using commercially available acetic acid and biowaste derived carbon sources. To conduct this analysis several parameters were taken into consideration; these include the specific consumption of the COD of the raw material per kg of nitrite removed, the deprived biogas (and thus energy) due to the diversion of the organic carbon source away from the anaerobic digestion process, the calorific value of methane (9200 kcal/m^3CH_4) and the COD content of the fermentation liquid (1 gCOD/gTS), the cost of the raw materials, and the cost (or gate fee) for the treatment of the OFMSW (75 €/ton OFMSW). In terms of acetic acid, a cost of € 700/t of 80% (wt) acetic acid was used (Frison et al. 2013), while for the waste-derived products no production cost was considered. The revenues from selling electrical energy produced from biogas were considered as 0.15 €/kWh. This is a realistic value, which considers the gradual reduction in the price that is taking place in several EU countries in which the specific cost used to be higher than 0.20 €/kWh. The cost of buying energy was taken as €/kWh. In the analysis the more adverse option of using both the heating and electrical energy was considered. Consequently, the use of OFMSW as carbon source deprives energy both in terms of heat and electricity. The combined heat and power unit was considered to produce 35% electrical energy and 55% thermal energy from biogas. The economic analysis did not consider the cost of a base required to increase the pH of the carbon sources, as this is also required for commercially available VFAs, nor the energy required to heat the fermenter, as this can be covered by the heat generated by the anaerobic digestion unit. Furthermore, the capital and operating expenses of the fermentation reactor were not considered in the analysis. Table 5.9 shows the amount of methane that is lost when the waste-derived carbon source is diverted from the anaerobic treatment process and is used in the SBR for nutrient removal.

Table 5.9 Economic evaluation of external organic carbon source addition for the vianitrite nitrogen removal (capital and operating expenses of the fermentation unit have been neglected). ([1]highly nitrogenous anaerobic effluent, [2] weakly nitrogenous anaerobic effluent)

Parameter	Aceti Acid	OFMSW DL	OFMSW FL	VFW FL	OFMSW FL
		HNAE	HNAE[1]	WNAE[2]	WNAE[2]
Savings from OFMSW gate fee (€/kgN removed)		-	0.93	0.96	0.75
Methane deprived (Nm^3CH_4/kgN removed)			1.09	1.12	0.88

Total energy deprived (kWh/kgN removed)		11.61	11.98	9.36
Deprived revenue (€/kgNremoved)		1.25	1.29	1.01
Net specific cost of carbon source use (€/kgN removed)	1.72*	0.32	0.33	0.26

*The cost was calculated by considering the COD/N ratio of 2.1.

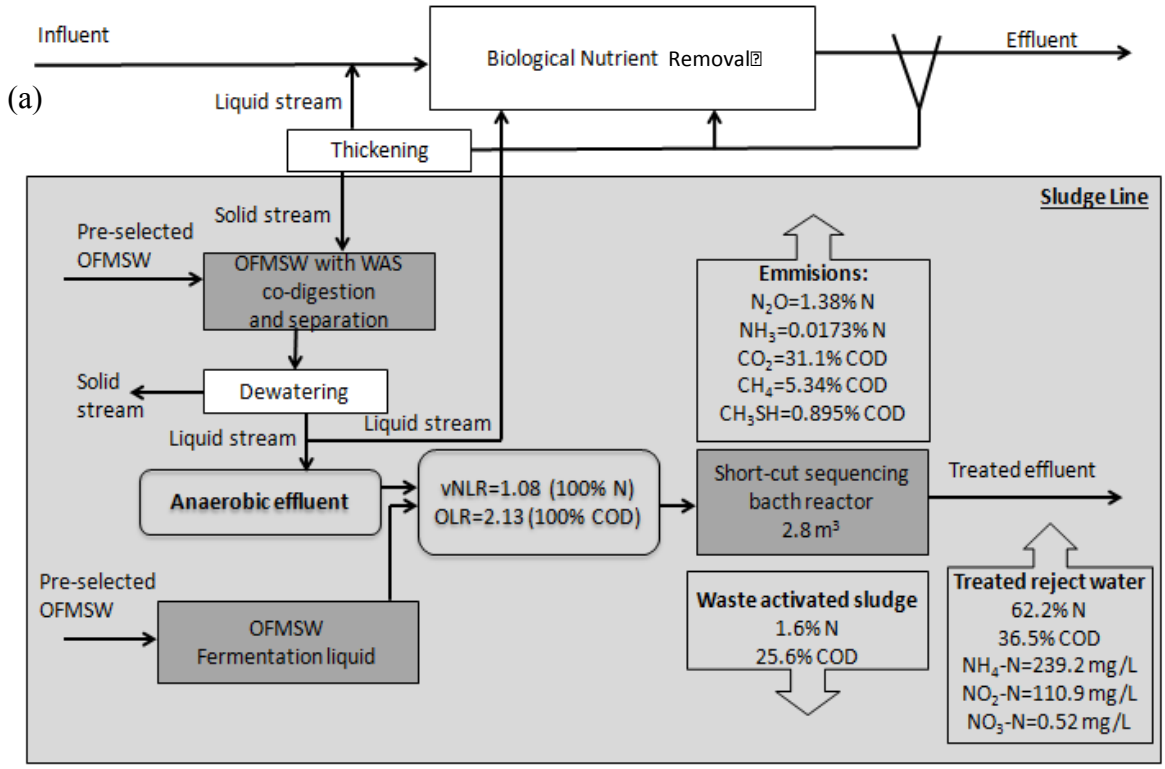
This amount is relatively similar for the different carbon sources ranging from 0.88 to 1.12 Nm^3CH_4/kgN removed. Furthermore, the diversion of OFMSW away from the usual treatment process has a net income, as the gate fee for the collection and treatment of OFMSW is not paid. The use of OFMSW FL results in much lower specific cost than acetic acid use, even when the revenues that are lost due to the diversion of OFMSW towards fermentation instead of anaerobic digestion are considered. However, this analysis does not consider the cost resulting from the operation and maintenance of the fermentation unit. Although the heat generated by the anaerobic digestion process could cover the heat requirements, energy is also required for the solid/liquid separation process. In the future, the use of OFMSWFL is expected to be an even more attractive solution, as the cost of petrochemically derived products increases, while the tariffs for selling electricity from the production of biogas through OFMSW anaerobic digestion decrease. These features are useful to estimate the payback time for investments in fermentation plants for the in situ production of best available carbon source.

5.3.6 Greenhouse gas emission and mitigation

Nitrous Oxide Emissions.

In the nitritation/denitritation process the formation of nitrate is inhibited. However, the three biochemical pathways that contribute to N_2O production can still occur, since they are not associated with the activity of NOB. Furthermore, the implementation of an SBR is expected to lead to higher nitrous oxide emissions than an activated sludge process due to the fluctuation and higher nitrite concentrations in the mixed liquor that are observed during the reaction phases. Figure 5.7 shows the mass balances for the two experimental periods.

(a)


Influent
Biological Nutrient Removal
Effluent
Liquid stream
Thickening
Sludge Line
Solid stream
Pre-selected OFMSW
OFMSW with WAS co-digestion and separation
Emmisions:
N_2O=1.38% N
NH_3=0.0173% N
CO_2=31.1% COD
CH_4=5.34% COD
CH_3SH=0.895% COD
Solid stream
Dewatering
Liquid stream
Liquid stream
Anaerobic effluent
vNLR=1.08 (100% N)
OLR=2.13 (100% COD)
Short-cut sequencing bacth reactor
2.8 m³
Treated effluent
Pre-selected OFMSW
OFMSW Fermentation liquid
Waste activated sludge
1.6% N
25.6% COD
Treated reject water
62.2% N
36.5% COD
NH_4-N=239.2 mg/L
NO_2-N=110.9 mg/L
NO_3-N=0.52 mg/L

(b)

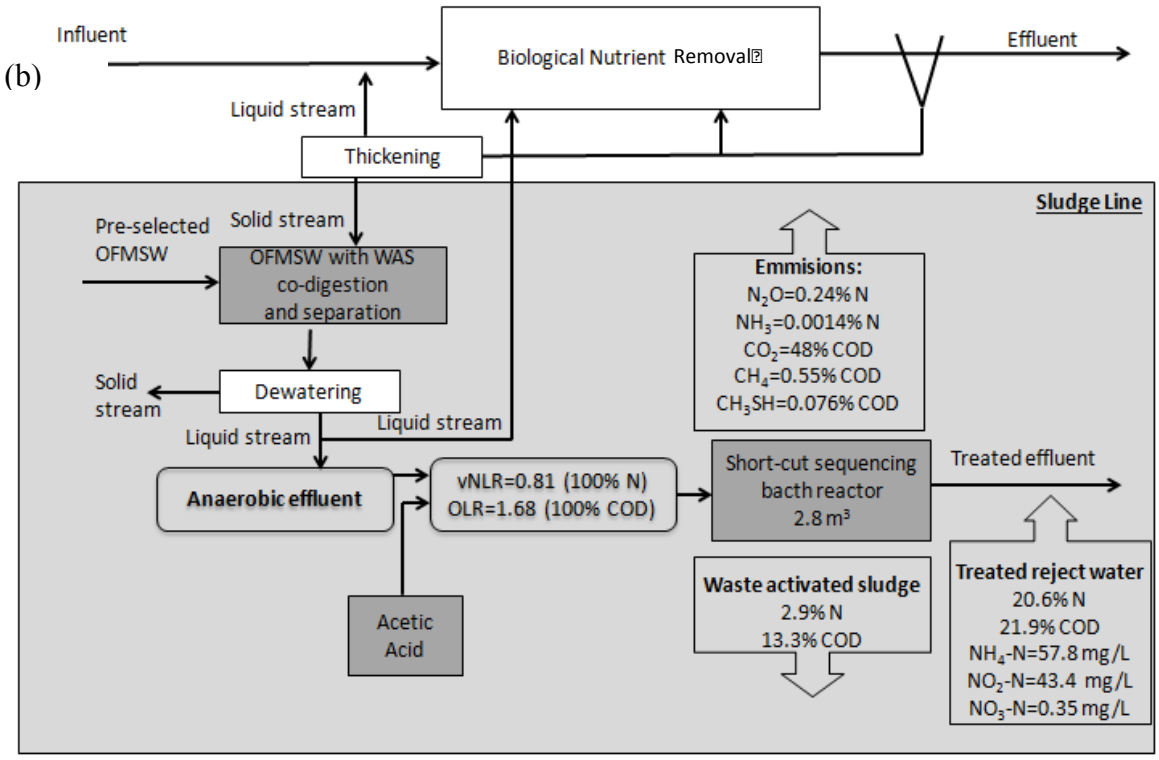

Influent
Biological Nutrient Removal
Effluent
Liquid stream
Thickening
Sludge Line
Pre-selected OFMSW
Solid stream
OFMSW with WAS co-digestion and separation
Emmisions:
N_2O=0.24% N
NH_3=0.0014% N
CO_2=48% COD
CH_4=0.55% COD
CH_3SH=0.076% COD
Solid stream
Dewatering
Liquid stream
Liquid stream
Anaerobic effluent
vNLR=0.81 (100% N)
OLR=1.68 (100% COD)
Short-cut sequencing bacth reactor
2.8 m³
Treated effluent
Acetic Acid
Waste activated sludge
2.9% N
13.3% COD
Treated reject water
20.6% N
21.9% COD
NH_4-N=57.8 mg/L
NO_2-N=43.4 mg/L
NO_3-N=0.35 mg/L

Figure 5.7 a). Nitrogen mass balance of the two experimental periods using OFMSW FL as external carbon source. b) Nitrogen mass balance of the two experimental periods using acetic acid as external carbon source

In period 2 the N_2O emissions from the treatment of the anaerobically co-digested supernatant were much lower than those of period 1 (i.e. 0.24% compared to 1.38% as per cent of influent nitrogen load). In period 1, the DO concentration during the aerobic reaction phase was quite low (i.e. 0.95 mg/L). The DO level is a critical parameter affecting the N_2O emissions during nitritation. Low DO levels favour nitrous oxide emissions due to nitrifier denitrification (Desloover et al., 2012). Another parameter that is important is the nitrite level in the mixed liquor. In period 2 the lower vNLR and the sequence change to aerobic/anoxic instead of anoxic/aerobic resulted in lower accumulation of nitrite, which also increased the emissions of N_2O. This is also reflected in the nitrite and ammonium concentrations of the treated effluent, which were on average 239 mgNH_4-N/L and 111 mgNO_2-N/L in period 1 and decreased to 58 mgNH_4-N/L and 43 mgNO_2-N/L during period 2. The range of reported N_2O emissions from full scale WWTPs is very wide and ranges from 0-14.6% of the nitrogen load for full scale WWTPs and 0-95% for lab scale investigations (Kampschreur et al., 2009). The treatment of sludge reject water is expected to result in higher nitrous oxide emissions than those of municipal wastewater treatment due to the higher nitrogen loads that are applied and potentially high nitrite/nitrate concentrations in the mixed liquor. The OFMSW and WAS co-digestion increases the nitrogen concentration in the resulting co-digestate; this further increases the potential N_2O emissions. As a result, the N_2O emissions are higher than those reported in several works concerning the operation of activated sludge processes with BNR for municipal wastewater treatment (Kimochi et al., 1998; Sommer et al., 1998; Sümer et al., 1995). Table 5.10 N_2O emissions for the treatment of sludge reject water and anaerobic co-digestatecompares the nitrous oxide emissions obtained in this work with previous works for the treatment of sludge reject water.

Table 5.10 N_2O emissions for the treatment of sludge reject water and anaerobic co-digestate

Wastewater	Process and conditions	N_2O emissions (% of N load)	Sampling details	Reference
Anaerobic supernatant produced from WAS &	Nitritation/denitritation High DO	0.24	On line Continuous	This study

OFMSW co-digestion Anaerobic supernatant produced from WAS & OFMSW co-digestion	Low nitrite accumulation Nitritation/denitritation Low DO High nitrite accumulation	1.38	1 day On line Continuous 1 day	This study
Sludge reject water	2 stage partial nitritation – anammox	2.3	Off line Continuous 4 days	(Kampschreur et al., 2008)
Sludge reject water	1 stage partial nitritation – anammox	1.3	Off line Grab samples 15 min/3×8 h	(Weissenbacher et al., 2010)
Sludge reject water	Nitritation	3.8	On line Continuous 12×6 h	(Gustavsson and Jansen, 2011)

In our work, the strategy of implementing higher DO and process optimization for effective removal of nitrogen decreased the nitrous oxide emissions by 83%.

The aerobic reaction phase contributed much more to the N2O emissions than the anoxic reaction phase. However, this does not mean that all the N_2O emitted during the aerobic phase is actually produced during aerobic conditions.

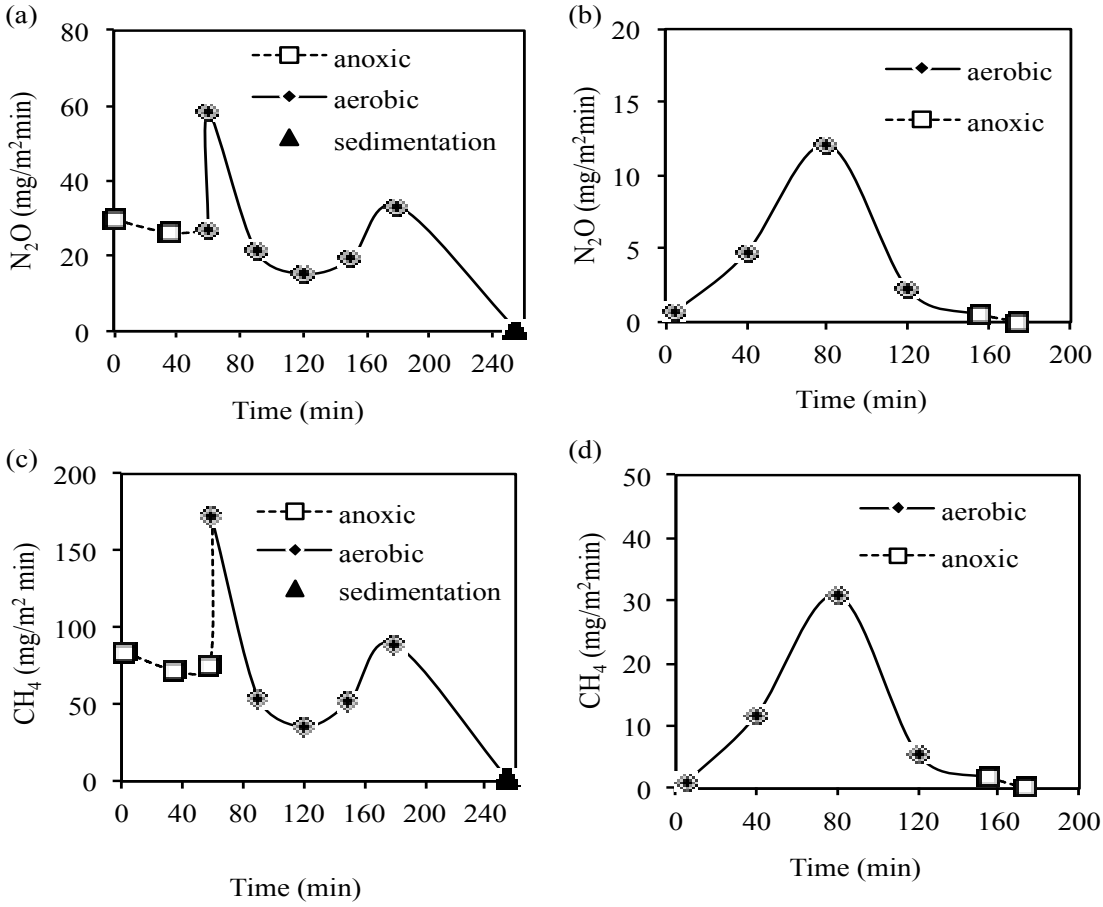


Figure 5.8. Variation of N_2O and CH_4 emissions for period 1 (a, c) and period 2 (b, d) during the SBR operation

It is also produced during the anoxic phase and can be subsequently stripped during the aerobic phase. The solubility of nitrous oxide in water is relatively high, so it may take some time for its stripping. This is characteristically represented with the peak that was observed at the beginning of the aerobic phase in Figure 5.8. In period 2, the aerobic phase took place just after the anoxic one, so the sudden introduction of air bubbles striped off the N_2O that was produced during the anoxic phase. Since the nitrous oxide that was formed during the anoxic zone could be stripped off during the aerobic zone, it was very difficult to determine the individual contribution of nitritation and denitritation towards nitrous oxide production.

Carbon dioxide, ammonia, methane and methyl mercaptan emissions

CO_2, NH_3, CH_4 and CH_3SH emissions from the SBR were also determined as a per cent of the influent nitrogen and COD load (Figure 5.7a). The CO_2 emissions were much higher during period 2 compared to period 1 (48% compared to 31%) since the use of acetic acid resulted in lower biomass yield (Y_H) compared to the use of fermentation liquid produced from OFMSW. (Figure 5.7b). Therefore, a higher percentage of the external carbon source was oxidized when acetic acid was used, leading to higher CO_2 emissions. Ammonia emissions were very low (<0.1% influent N load) in both periods, despite the fact that the mixed liquor contained significant free ammonia (FA) concentration (>2 mgN/L and often >4 mgN/L). An interesting finding is the significant levels of methane that were detected during period 1 (i.e. 5.34% of influent COD load). In this period, the relatively low DO levels in the mixed liquor probably created local micro-anaerobic conditions within the sludge flocs, resulting in the production of gaseous emissions that are produced under anaerobic conditions such as methane. Furthermore, in period 1 fermentation liquid derived from OFMSW was added as carbon source rather than acetic acid, increasing the potential for methane emissions under micro-anaerobic conditions.

As seen in Figure 5.1c, high methane peak was observed in period 1 at the initiation of the aerobic phase due to the stripping of the methane produced during the previous anoxic conditions (in which micro-anaerobic conditions also occurred within the interior of the sludge flocs).

Contribution of each stage and type of emission.

Figure 5.9 shows the contribution of the aerobic (nitritation) and the anoxic (denitritation) reaction phase on gaseous emissions. It is evident that the vast majority of gases were emitted during the aerobic phase.

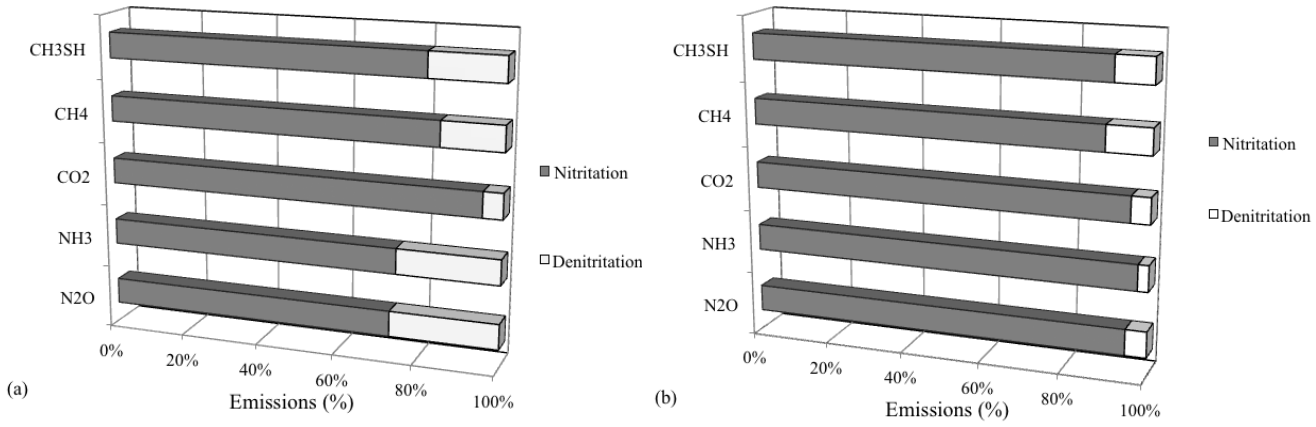


Figure 5.9. Contribution of aerobic and anoxic conditions to gaseous emissions for period 1 (a) and period 2 (b).

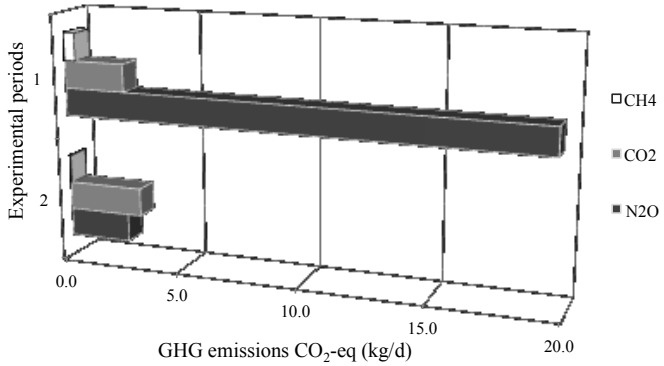


Figure 5.10. GHG emissions as CO_2-eq during experimental periods 1 and 2 respectively

Significant reduction of methane was also observed with also decreased the carbon footprint of the process. This work confirms the findings of previous researchers which, have shown that the aerobic phase is a major contributor of GHG emissions and particularly of nitrous oxide emissions (Kampschreur et al., 2008)

However, this only reflects the gases that are emitted and not the relative contribution of each phase in terms of gaseous production. The introduction of air enhances stripping of gases, which were formed under anoxic conditions. As mentioned previously this was clearly seen in period 1, where the aerobic phase took place just after the anoxic one; thus, at the initial minutes of the aerobic phase a very high peak of methane and nitrous oxide (was observed mainly due to stripping of the gases formed during the anoxic phase. The contribution of the denitritation stage was higher for N_2O, NH_3, CH_4, NH_3, CH_3SH in period 1 compared to 2. The total GHG

emissions as CO_2 equivalents were also calculated for periods 1 and 2 respectively. The total daily GHG emissions decreased from 22.5 kg CO_2-eq/d in period 1 to only 5.7 kg CO_2-eq/d in period 2, which represents a 75% decrease. As seen inFigure 5.10, this was mainly attributed to the dramatic decrease of nitrous oxide emissions.

5.1.1 Life Cycle Assessment

The adoption of alternative carbon sources o treat anaerobic supernatant via nitrite was evaluated based on a life cycle assessment approach (LCA). Thus, the major aim was to quantify the benefits or the negative impact on the environment when alternative or synthetic carbon source are used. The life cycle inventory for the short cut nutrients removal using the different carbon sources is presented per m^3 in Table 5.11. One of the main differences observed between the four operation strategies are the lower COD and phosphorus concentrations of the average influents for the scenarios where OFMSW liquids are used. These differences are, on the one hand, associated with the influent variability that is inherent to all WWTP, but, more importantly, due to variations in the amount and quality of the OFMSW that is fed to the digester.

Using OFMSW FL allowed higher saving in term of electricity compared with the other type of carbon sources. In addition, as reported by Frison et al. (2013), the COD consumption to denitrify the same amount of nitrite, changes depending of the carbon source, as we go from the synthetic acetic acid to the OFMSW FL. As results, higher amount of sludge produced avoided fertilizers and nutrient-related emissions (Rogriguez-Garcia et al., 2014).

Table 5.11. Inventory data for the four operating strategies of the short-cut nutrients removal.

	Unit	Acetic Acid	Glycerol	OFMSW DL	OFMSW FL
Influent - Supernatant from anaerobic digestion					
COD	gCOD m^{-3}	133.8	133.8	30.90	30.90
NH_4-N	gN m^{-3}	592.5	592.5	558.9	558.9
NO_3-N	gN m^{-3}	0.360	0.360	0.780	0.780
NO_2-N	gN m^{-3}	0.560	0.560	0.420	0.420
TP	gP m^{-3}	80.40	80.40	31.30	31.30
Electricity					
Used	kWh m^{-3}	3.026	3.027	3.035	3.043
Not produced	kWh m^{-3}	x	x	x	1.895
Chemicals					
Carbon source	kg m^{-3}	1.557	0.884	32.30	6.601
Polyelectrolyte	g m^{-3}	0.592	0.717	1.492	2.301
Transport	kg*km m^{-3}	31.155	17.70	0.030	0.046
Gaseous emissions from the reactor					

N_2O	g m^{-3}	10.62	10.62	10.02	10.02
NO	g m^{-3}	0.088	0.088	0.083	0.083
NH_3	g m^{-3}	0.508	0.508	0.579	0.504
CO_2 (biogenic)	g m^{-3}	-160.6	-179.1	–	–
CO_2 (fossil)	g m^{-3}	187.7	652.8	x	X
Effluent - Wastewater to further treatment					
COD	g O_2 m^{-3}	56.72	67.93	126.1	101.5
NH_4-N	g N m^{-3}	24.00	24.00	27.38	23.85
NO_3-N	g N m^{-3}	1.900	122.5	27.92	32.18
NO_2-N	g N m^{-3}	0.230	0.210	0.110	0.160
TP	g P m^{-3}	52.30	52.60	8.100	7.800
Gaseous emissions from the anaerobic digestion of the sludge					
CO (biogenic)	mg m^{-3}	0.804	0.975	2.028	3.127
CO_2 (biogenic)	mg m^{-3}	497.2	602.5	1253	1933
NMVOC	mg m^{-3}	0.012	0.015	0.030	0.047
CH_4 (biogenic)	mg m^{-3}	2.637	3.195	6.647	10.25
SO_2	mg m^{-3}	2.156	2.613	5.435	8.382
NO_2	mg m^{-3}	1.601	1.941	4.037	6.226
NH_3	mg m^{-3}	0.178	0.216	0.449	0.692
N_2O	mg m^{-3}	0.122	0.148	0.302	0.475
N_2	mg m^{-3}	8.027	9.727	20.23	31.21
Sludge application					
Transport	kg km m^{-3}	9.567	11.59	24.12	37.19
Application	L m^{-3}	0.478	0.580	1.206	1.860
Gaseous emissions from sludge application					
N_2O	mg m^{-3}	55.86	67.83	149.4	217.3
NH_3	mg m^{-3}	1222	1484	3268	4753
Emissions to water from sludge application					
PO_4^{3-} to groundwater	mg m^{-3}	83.06	99.78	183.4	266.8
PO_4^{3-} to river	mg m^{-3}	23.61	28.37	52.15	75.84
Emissions to soil due to sludge application					
Cd	µg m^{-3}	0.010	0.013	0.026	0.040
Cr	µg m^{-3}	469.5	569.0	1184	1825
Cu	µg m^{-3}	2.020	2.448	5.092	7.853
Hg	µg m^{-3}	0.007	0.009	0.019	0.029
Ni	µg m^{-3}	0.092	0.111	0.231	0.357
Pb	µg m^{-3}	0.772	0.936	1.946	3.002
Zn	µg m^{-3}	2.086	2.528	5.258	8.110
Avoided products					
N based Fertilizer	g m^{-3}	2.367	2.874	6.331	9.208
P_2O_5 based fertilizer	g m^{-3}	2.167	2.604	4.786	7.051

– No information available, x No flow

Compared with acetic acid, the organic fraction of municipal solid waste (OFMSW) gives lower environmental impacts in terms of eutrophication (0.28 kg PO_4^{3-} m^{-3}). However, they do present higher removal efficiency (85%). The rationale behind this is the lower concentration of P in the influent (Table 5.11), necessarily resulting in an even lower value in the effluent and thus presenting a reduced impact on the eutrophication category (Table 5.11).

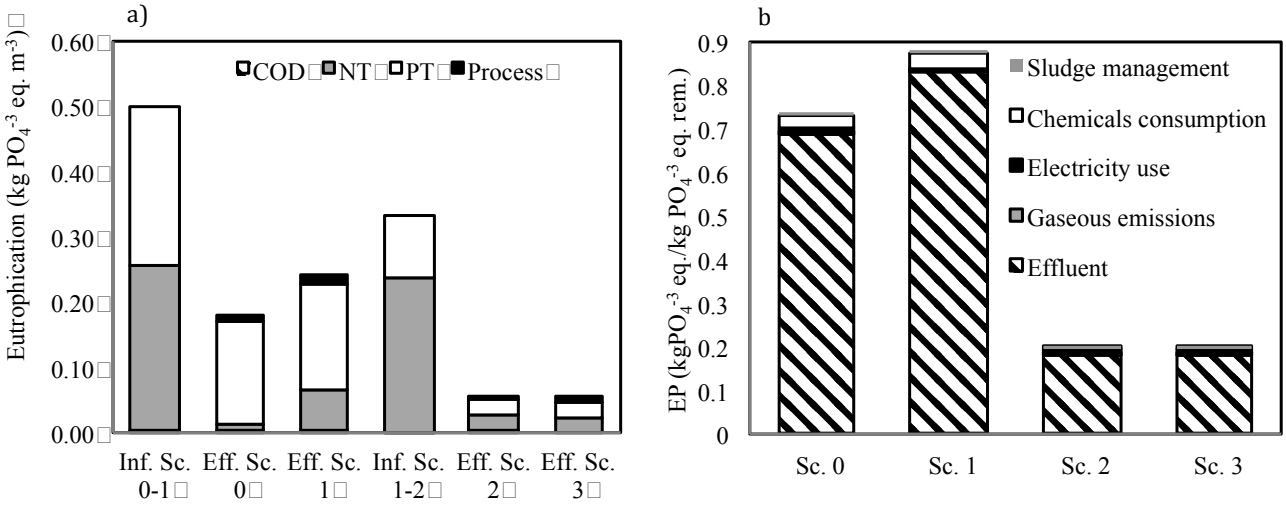


Figure 5.11 a) Eutrophication impact of the influent and the effluent + the process of the NSC reactor using acetic acid (AcOH), glycerol, the drainage produced by the organic fraction of municipal solid waste ($OFMSW_d$) and fermented ($OFMSW_f$) as alternative carbon sources (per m^3) and b) Eutrophication potential impact per FU (1 kg PO_4^{-3} eq. rem.)

In Figure 5.11 a) Eutrophication impact of the influent and the effluent + the process of the NSC reactor using acetic acid (AcOH), glycerol, the drainage produced by the organic fraction of municipal solid waste ($OFMSW_d$) and fermented ($OFMSW_f$) as alternative carbon sources (per m^3) and b) Eutrophication potential impact per FU (1 kg PO_4^{-3} eq. rem.)a it can be seen that in general terms, the carbon sources based on OFMSW present a lower environmental profile than those using synthetic ones, even when they produce a larger amount of sludge that results in higher load of N spread in the soil and thus higher NH_3 emissions (Figure 5.11 a) Eutrophication impact of the influent and the effluent + the process of the NSC reactor using acetic acid (AcOH), glycerol, the drainage produced by the organic fraction of municipal solid waste ($OFMSW_d$) and fermented ($OFMSW_f$) as alternative carbon sources (per m^3) and b) Eutrophication potential impact per FU (1 kg PO_4^{-3} eq. rem.)b). As for the acetic acid the presence of heavy metals in the sludge compensates the benefits from not using industrial fertilizers, being the sludge management almost irrelevant for all operation strategies when it comes to toxicity related impacts.

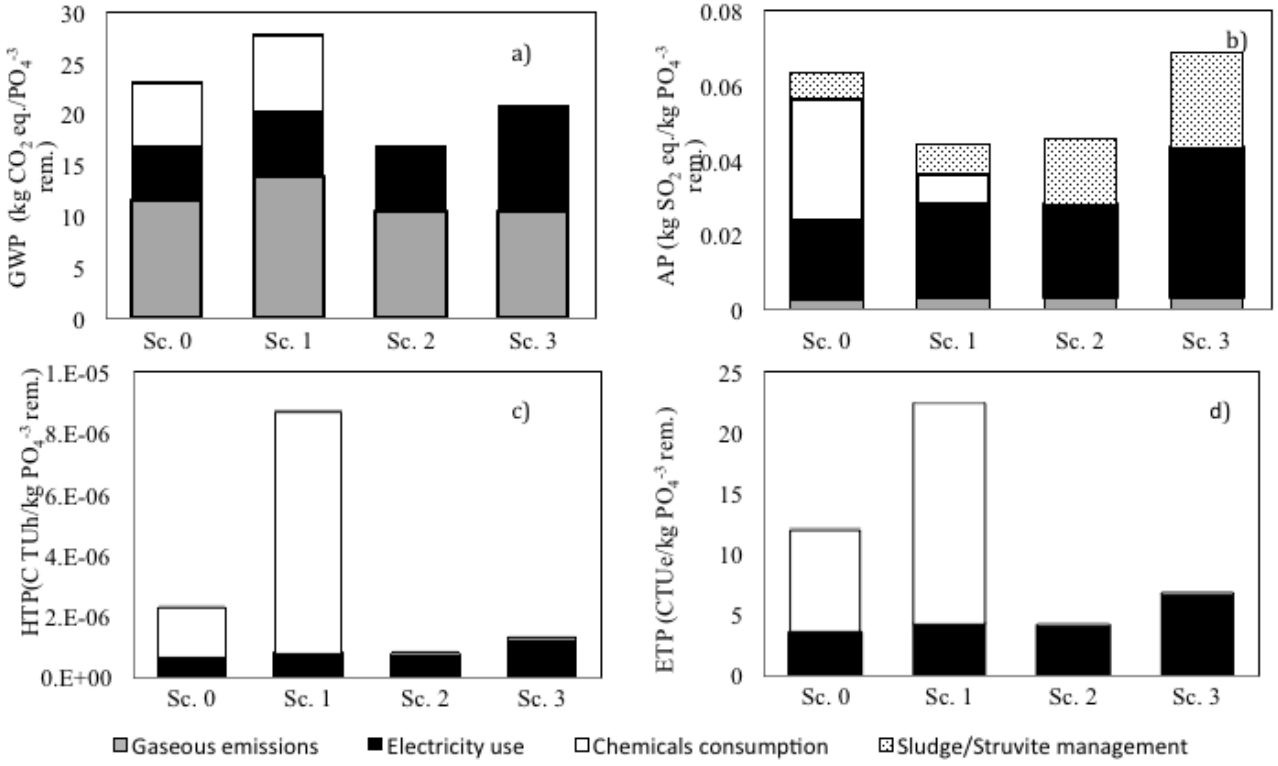


Figure 5.12.a) Global Warming, b) Acidification, c) Human Toxicity and d) Freshwater ecotoxicity potential impacts of the NSC reactor using acetic acid (HAc), glycerol, the drainage produced by the organic fraction of municipal solid waste ($OFMSW_d$) and fermented ($OFMSW_f$) as alternative carbon sources per FU (1 kg PO_4^{3-} eq. rem.).

The use of acetic acid results in a better environmental profile than the one using glycerol. The presence of nitrate not only affects the eutrophication impact of the former (Table 5.11) but it also reduces its eutrophication removal in comparison with the acetic acid scenario (0.27 kg PO_4^{3-} eq. rem./m^3 vs. 0.32) affecting its results in the other impact categories. The higher environmental impact of the glycerol based on the global toxicity categories source discourages its use as a carbon source (Figure 5.12). For the four carbon sources assessed in this study, the drainage liquid together with the fermentation liquid from OFMSW can be considered the best carbon source for the operation of the short-cut nitrogen removal. However, the availability of the liquid drainage rarely is constant and the characteristics could be not uniform since they depend on the amount and the origin of the OFMSW, that are not easily to controlled by the operators of the WWTP. Nevertheless, the liqeuid drainage could be good integrated with the OFMSW FL, with the advantage that more raw OFSMW is used for the biogas production. The

treatment of urban waste in a WWTP in not a very common practice and the most widespread practice is the just the digestion the sewage sludge (primary and secondary sludge). For these very common situations the acetic acid represents the easiest option because the availability and the characteristics of the substrate are sure. However, fermentation liquid of the sewage sludge could give the optimal carbon source for the application of the short-cut nutrients removal, as it is reported in Chapter 6.

5.1.2 Analyses of the microbial comunities

The composition of the bacterial communities selected during the fi rst and second stages (S1 and S2, respectively) were analyzed and compared with those present in the starting inoculum (STI) and in the inlet (IN). Results obtained for all of the samples analyzed indicated the presence of a heterogeneous microbial community, rich in different bacterial species.

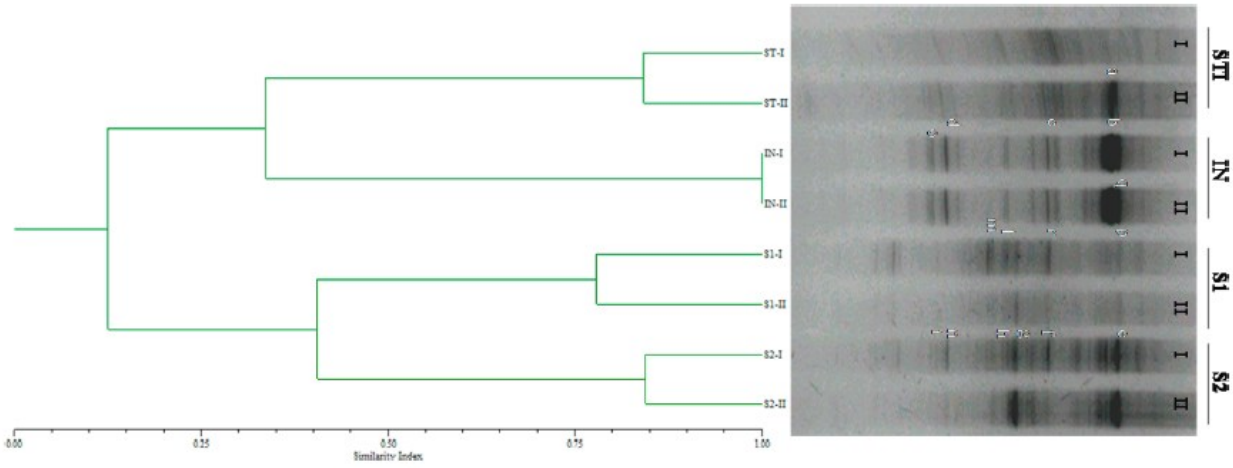


Figure 5.13. Right - DGGE fingerprints of the eubacterial communities. Samples collected from the starting inoculum and the inlet are marked with STI and IN, respectively. On the other hand, samples from different steps are indicated as S1 and S2. Roman numerals (I and II) correspond to different replicates. White letters in the gels indicated the major bands excised, cloned and sequenced. Left - Similarity dendrogram, obtained through UPGMA method, indicating relationships among different DGGE profiles.

However, we found clear evidence for the presence of a dominant, restricted bacterial population, represented by major bands migrating in the gel, especially in the shows a taxonomical grouping in two diff erent clusters where one of these is represented by the DGGE profi les obtained from S1 and S2 samples. Low similarity values (<0.25) between the ribotypes corresponding to S1 and S2 samples and those corresponding to STI and IN samples were registered. Then, major bands in the DGGE profiles were excised from the gel and sequenced. Sequencing data obtained were searched for similarity with the BLASTN database. Afterward, a phylogenetic tree was constructed relying on the neighbor joining method with the MEGA version 4.0 software package (Figure 5.13). As shown in Figure 5.14, the major bands selected at the end of the process (S2) and labeled with e, f, g, i, and n letters resulted to be mainly related to the Beta subclass of Proteobacteria and to the Bacteroidetes bacterial group. It is worth noting that the main AOB bacterial genera, such as Nitrosospira sp., Nitrosomonas sp., and Nitrosococcus sp. belong to the β-proteobacteria class. Nevertheless, even if bands i and n within S2 rybotipe resulted to be closely related to this genera, they showed also high similarity with Thauera sp. genus. Thus, further investigations, using primer sets specific for AOB bacterial populations, (Peng et al., 2008; Purkhold et al., 2000) will be performed in order to better understand the composition of AOB bacterial populations putatively selected.

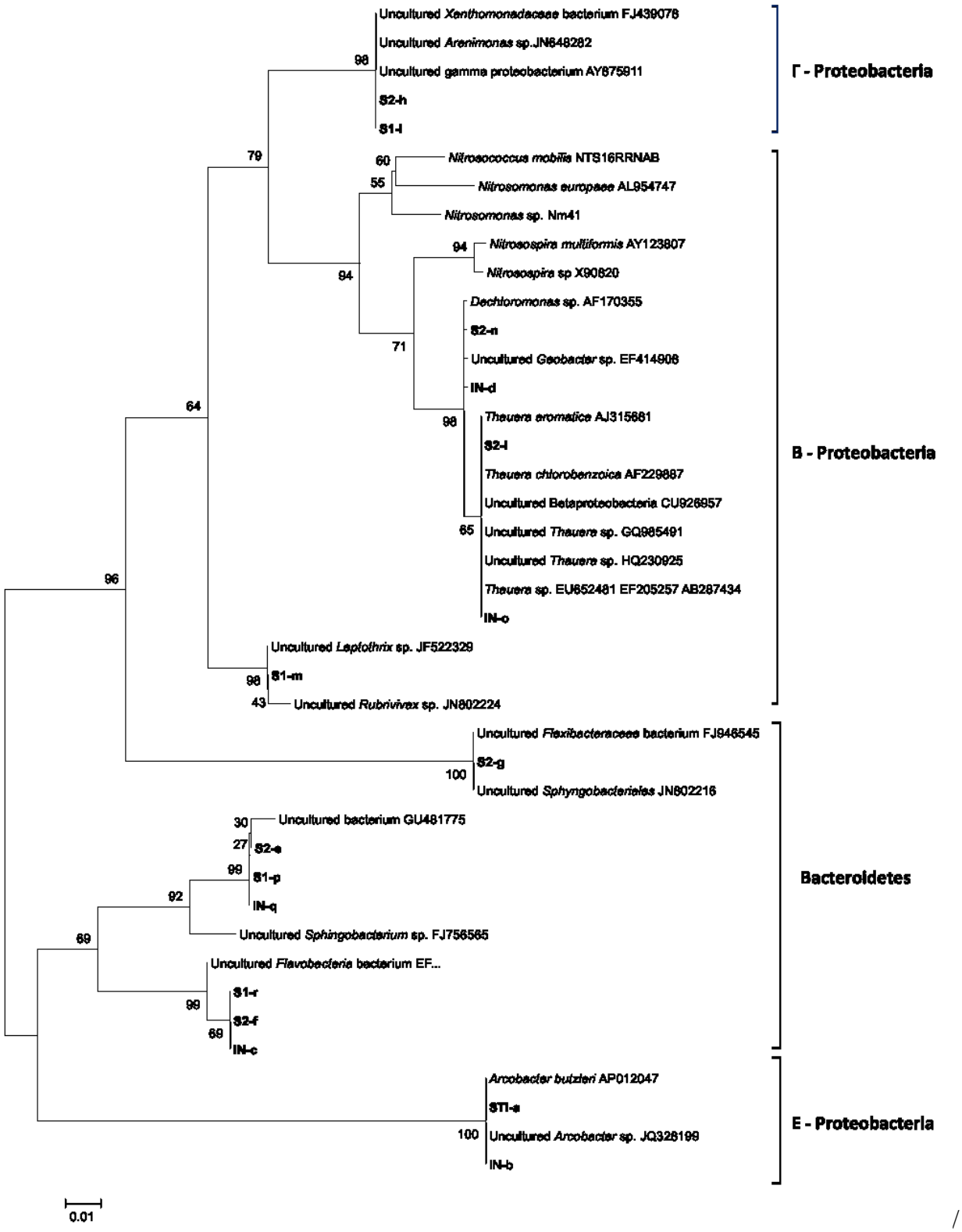


Figure 5.14. Neighbour-joining phylogenetic tree, based on the sequence of the hypervariable V3 region of the 16S rRNA gene for Eubacteria, showing the relationships of different DGGE bands

(indicated with letters in bold) and related species. Bootstrap values are given at branch nodes and are based on 1000 replicates. Bar, 0.01 substitutions per nucleotide position.

5.4 Conclusion

Successful operation of a complete short-cut nutrients removal was carried out in Sequencing Batch Reactor. Stable partial nitritation was easily achieved in 20 days treating supernatant from anaerobic co-digestion of WAS and OFMSW. Under these conditions, the concentrations of free ammonia and free nitrous acid are not toxic or inhibiting for the growth of ammonia oxidizing bacteria.

Operating at 15 ± 3 °C, the maximal treatment potential was as high as 0.8 kgN $m^{-3}d^{-1}$. The absence of nitrite oxidizer bacteria (NOB) was obtained at all times, even under transient conditions and when the FA concentration in the reactor was very low indicating that either other parameters apart from FA can inhibit NOB activity or the initial FA had inhibited the NOB anabolism for a sufficient time-length. The scSBR operation at a volumetric NLR higher than the maximal biomass nitrifying capacity reduced the nitrogen removal efficiency, while maintaining the same high and stable sAUR.

The DGGE analysis pointed out the presence of a restricted bacterial population selected at the end of the process (S2). Sequencing of the major bands present in the rybotipes evidenced the presence of bacterial species mainly related to β Proteobacteria and Bacteroidetes bacterial groups. Interestingly, some of the main bands hich have been selected in sample S2 were closely related to the AOB genera, such as Nitrosomonas sp. And Nitrosospira sp.

The best available external carbon sources for short-cut nitrification–denitrification (SCND) and denitrifying phosphorus removal via nitrite (DPRN) were investigated at demonstration scale to treat anaerobic supernatant showing that nitrite did not inhibit PAOs. High denitritation rates were obtained when FL CM&MS or FL OFMSW were used as external carbon sources (i.e. 0.67 and 0.43 $kgNO_2$-N gMLVSS $d)^{-1}$ respectively). In addition, significant phosphorus uptake via nitrite was observed under anoxic condition: the sPUR via nitrite obtained was 0.27 $kgPO_4$-P $(kgMLVSSd)^{-1}$ when FL OFMSW was dosed and 0.20 $kgPO_4$-P (kgMLVSS $d)^{-1}$ when FL CM&MS was used.

The short cut biological processes produce greenhouse gas emission, like nitrous oxide. This can be decreased providing enough aeration during the nitritation stage so that the DO is maintained at least at 1.5 mg L^{-1} and applying a vNLR that is not higher than the system's nitrifying and

denitrifying capacity, thus the accumulation of ammonium and nitrite is limited. Furthermore, we observed less nitrous oxide emissions applying the aerobic/anoxic sequence. Specifically, the SBR operation at DO = 1.5 mg L^{-1} and vNLR = 0.81 kgN $m^{-3}d^{-1}$ resulted in much lower nitrous oxide emissions (0.24% of influent nitrogen load) compared to the operation at lower DO (0.95 mg L^{-1}) and higher vNLR=1.08 kgN $m^{-3}d^{-1}$.

The life cycle assessment (LCA) is a good instrument to assess the environmental impact of the biological processes. The results based on the LCA, point out that the use of drainage liquid from OFMSW was considered the most suitable alternative, although the use of acetic acid was considered the best alternative for those WWTPs where no waste is treated. However, in this case the sewage sludge (primary and secondary) could be a good source to obtain carbon source to performed effective nitrogen and phosphorus removal from anaerobic supernatant.

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6 Chapter. Application of novel processes for biological nutrients removal from anaerobic supernatants: two case studies.

In this chapter, the integration of the novel schemes for nutrients removal were applied in two different municipal sewage treatment plants.

6.1 Introduction

A sidestream configuration is defined as "integrated" when mixed liquor suspended solids in the mainstream secondary and sidestream processes are interchanged (Metcalf & Eddy, 2013). As reported in chapter 5, the reject water from digested sludge contains high amount of nitrogen and phosphorus that could leads problem for the main WWTP. Typically, in the WWTP the flow from reject water is recycled back constitutes 10–30% of the total N-load (Cervantes, 2009; Gustavsson et al., 2011). Volcke et al., (2006) reported high load of nitrogen in the (more than 28%) when the reject water was recycled to the mainstream. New biological methods to treat high nitrogenous effluents with low ratio COD N^{-1} and relatively high temperature were developed during the last decades and were mainly based on partial oxidation of ammonia to nitrite, followed by heterotrophic or autotrophic nitrite removal (Abeling and Seyfried; 1992, Hippen et al, 1994; Mulder et al 1995). Compared to the physicochemical processes, these processes do not allow nitrogen recovery. Howerver, comparing with the biological process, the energy demand of the reference stripping varies from 100-150 MJ/kg N (aeration, heat, the chemicals, including the values of the chemicals) and is significantly higher than the energy demand of the nitrogen producing Haber-Bosch process combined with Anammox (total 60 MJ/kg N). This shows that nitrogen recovery is more expensive (1.9 – 3.2 EUR/kg N) than nitrogen removal using Anammox (0.8 EUR/kg N) (Maurer et al. (2003)). This is caused by the energy use and price and quantity of chemicals (NaOH or CaO and H_2SO). However, the choice for the best-separated treatment for the anaerobic supernatant depends frequently for the total costs of the treatment, but also for the stability and robustness of the process (Jenicek et al., 2007).

The sustainability of currently available biological nitrogen removal systems has been investigated by several authors (Mulder, 2003) and the full-scale applications for the biological treatment of reject water were also well documented in literature. Among them, examples of methods employing combinations autotrophic and heterotrophic processes are: bio-augmentation batch enhanced technology (BABE) (Salem et al., 2004), single reactor system high activity ammonium removal over nitrite process (SHARON) (Hellinga et al., 1998), anaerobic ammonium oxidation process (ANAMMOX) (Strous et al., 1997), SHARON-ANAMMOX (Van

Dongen et al., 2001), CANON (Szatkowska et al., 2007), OLAND (Kuai and Verstraete, 1998), and aerobic/anoxic deammonification process (Gut et al., 2006).

In contrast to nitrogen, phosphorus is a limited resource, which must be recovered and reused (Hao et al., 2013). However, phosphorus removal in reject water has not gained as much progress and focus as N removal. This is due to the fact that the main mechanism for P removal is by precipitation and the chemical processes could be complex and costly. In the seventies, phosphorus removal from rejects water concentrated on the chemical crystallisation of hydroxyapatite (HAP) (Momberg and Oellerman, 1992) or magnesium hydroxyapatite (MAP) through the addition of metal salts and or high pH level (Pitman, 1999). However, if the economic and life cycle costs are taken into account, phosphate recovery as struvite may not be the best approach, for the following reasons: (1) production of P-mineral with a high content of struvite from real wastewater is a difficult and costly process; and (2) struvite is not be superior to other phosphate based compounds in fertilization efficiency. Hence, phosphate recovery could be aimed at any accept forms of phosphate-based compounds by the fertilizer industry, depending on onsite circumstances. Accordingly, efforts should also be targeted towards the use of (composted) sludge for effective fertilization (Hao et al., 2013).

According with the main findings, this chapter report two different case studies of innovative scheme were considered in order to apply and optimize the novel integration of the biological nitrogen and phosphorus removal via-nitrite from the anaerobic supernatant. In the first case study the anaerobic supernatant is originated from the anaerobic co-digestion of the WAS and OFMSW. The second case study could be considered a conventional WWTP, where the reject water derived form anaerobic digestion of the sewage sludge (primary and secondary sludge).

6.2 Material and Methods

Case study 1: the integrated system with the co-digestion of WAS and OFMSW within the Treviso's WWTP

The pilot hall is located in the wastewater treatment plant (WWTP) of Treviso in Veneto Region (north Italy). This plant serves a population equivalent (PE) of 70000 and it was designed following the Johannesburg configuration, to accomplish effective biological nutrient removal (BNR) from municipal wastewater. Up to 10 t d^{-1} of source separated organic fraction of municipal solid waste (OFMSW) were mixed with 120 m^3 d^{-1} of thickened waste activated sludge (WAS) and were fed to the mesophilic anaerobic digester (37°C), having a reaction volume of 2000 m^3. The total organic loading rate (OLR) fed to the digester was 1.7 kgTVS $m^{-3}d^{-1}$, while the hydraulic retention time (HRT) was approximately 20 days, resulting in the production of 750 m^3 d^{-1} of biogas. The biogas consisted of approximately 60-65% CH_4 and was used in a co-generation unit of 200 kWe to produce heat and electricity. The anaerobic digestate was dewatered using a belt press from an initial total solids (TS) concentration of 2-4% to 20-25% TS. The anaerobic supernatant was sent to the mainstream line of the WWTP after an equalization basin of 15 m^3 that was used to feed to the pilot-scale short-cut SBR (scSBR). It was operated in the following sequence: 10-16 min anaerobic fill, 180 min aerobic reaction, 50 min anoxic reaction, 30 min settle and 9-17 min draw. Fermentation liquid produced from the OFMSW was supplied to the scSBR as an external carbon source; it was always fed during the initial 1-15 min of anoxic phase of the SBR operation. The solids retention time (SRT) was maintained at 15 d by discharging WAS at the beginning of the idle phase and replacing the same volume with anaerobic supernatant during the subsequent feeding phase.

In this work, the short cut nutrients removal was applied for the treatment of anaerobic supernatant derived from the co-digestion of secondary waste activated sludge and the organic fraction of the municipal solid waste (OFMSW) in a pilot scale SBR. The scheme shown in Figure 6.1, represents the flow chart of Treviso's WWTP, where a reactor for hydrolyses of the OFMSW was used to provide volatile fatty acids for the anaerobic and anoxic phase of the short cut SBR.

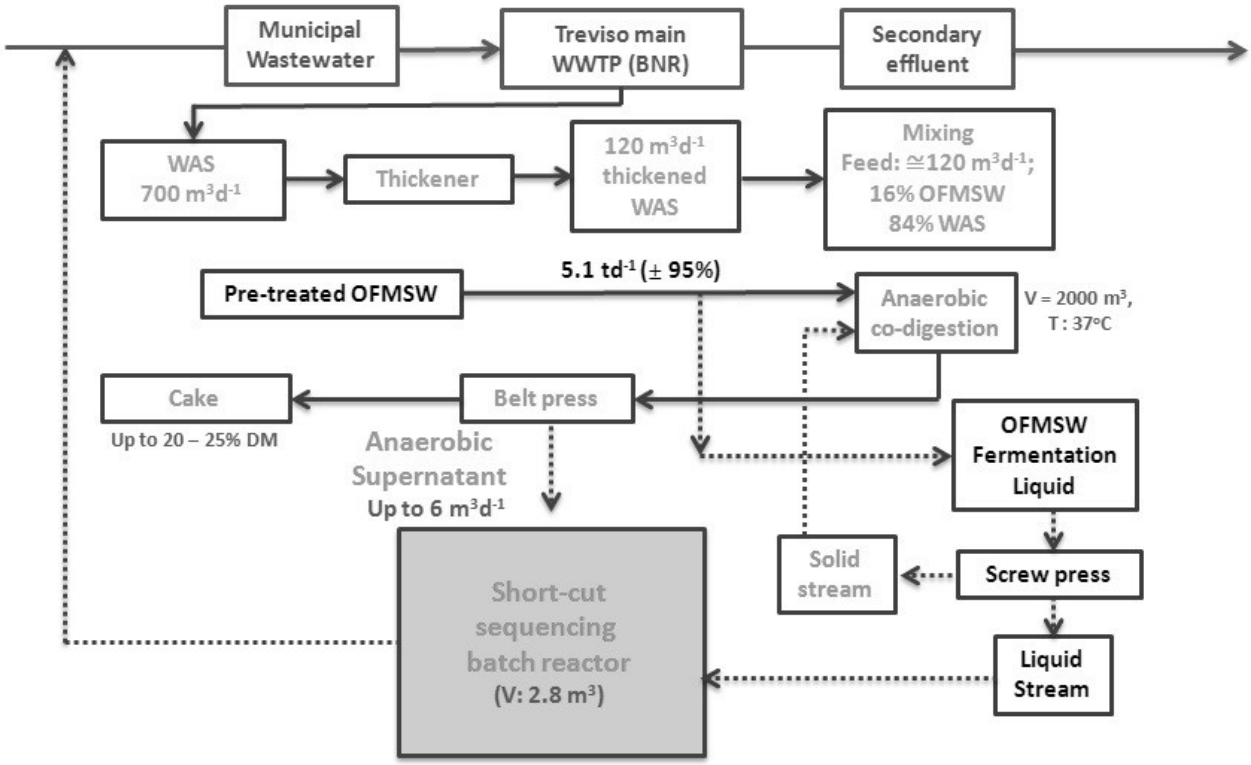

Figure 6.1. Flow diagram showing the various processes taking place in the Treviso wastewater treatment plant

Characteristics of the anaerobic supernatant from the co-digestion, OFMSW FL and the operating conditions of the scSBR

The scSBR was operated for a time period of 130 days with the operating conditions summarized in

Table 6.1.

Table 6.1. Operating conditions of the scSBR (average ± standard deviation)

Parameter	Unit	Start-up	Period 1	Period 2
HRT	d	0.84± 0.50	0.74 ± 0.08	0.35 ± 0.07
MLSS	g L^{-1}	4.83± 1.37	5.67 ± 0.37	7.04 ± 0.53
MLVSS	g L^{-1}	3.43 ± 0.99	4.17 ± 0.26	5.09 ± 0.40
F M^{-1}	kgCOD $kgVSS^{-1}d^{-1}$	0.29 ± 0.10	0.24 ± 0.08	0.25 ± 0.07
OLR	kgCOD m^{-3} d^{-1}	0.89 ± 0.23	0.88 ± 0.32	1.2 ± 0.30

The first 27 days were dedicated for the start-up and achievement of a stable via-nitrite nitrogen

removal. The first period of operation had a duration of 39 days (28-67), in which a relatively low specific phosphorus loading rate (sPLR = 14.27±4.51gP (kgVSS d)$^{-1}$) was applied. This was followed by period 2 with a duration of 61 days (69-130) and a much higher sPLR (=32.64±3.91 gP (kgVSS·d)$^{-1}$). The anaerobic supernatant fed during the 2 period had a relatively high TKN (378 ± 194 mg L^{-1} in period 1 and 246 ± 93 mg L^{-1} in period 2), high ammonium (291 ± 167 mgNH_4-N L^{-1} in period 1 and 194 ± 87 mgNH_4-N L^{-1} in period 2), and significant phosphorus concentrations (39 ±16 mg L^{-1} in period 1 and 54 ± 15 mg/L in period 2). The biodegradable fraction of the total COD was less than 20% while the ratio TKN/COD ratio was less than 1. Full characterization of the anaerobic supernatant is reported in the Table 6.2.

Table 6.2. Physicochemical characteristics of the anaerobic supernatant produced from the co-digestion of OFMSW and WAS (average ± standard deviation)

Parameter	Unit	Start-up	Period 1	Period 2
Flow	L cycle^{-1}	1300 ± 444	760 ± 154	1633 ± 192
pH	-	7.84 ± 0.51	7.7 ± 0.09	7.5 ± 0.20
Temperature	°C	26.6 ± 2.67	21.6 ± 3.10	16.9 ± 2.42
Conductivity	mS cm^{-1}	3.68 ± 1.48	3.11 ± 1.12	2.40 ± 0.75
TSS	mg/L	140 ± 372	127 ± 17	66 ± 11
Alkalinity pH=5.75	mg$CaCO_3$ L^{-1}	1860± 387	1597 ± 561	1163 ±473
Alkalinity pH=4.3	mg$CaCO_3$ L^{-1}	2433 ± 514	2034 ±833	1486 ±528
Total COD	mgCOD L^{-1}	240± 5	132 ± 59	57.5 ± 37
TN	mgN L^{-1}	361 ± 82	378 ± 194	246 ± 93
NH_4-N	mgN L^{-1}	356 ± 71	291 ± 167	194 ± 87
TP	mgP L^{-1}	34 ± 7	39 ±156	54 ± 15
PO_4-P	mgP L^{-1}	33 ± 5	37.3 ± 27.5	51 ± 12

Off course, the biodegradable organic carbon present in the anaerobic supernatant was not enough to sustain efficiently the heterotrophic denitrification. For this reason the fermentation liquid from OFMSW (OFMSW FL) was produced in a semi-continuous pilot fermentation reactor. The source-separated OFMSW was grinded and diluted with secondary effluent up to 6% TS and then fermented with an organic loading rate (OLR) of 20 kgTVS m^{-3}d^{-1} and an HRT of 3 days. The pH during the acidogenic fermentation process was in the range of 4.1-4.5. The fermentation product was separated from the solid fraction through a screw press and only the fermented liquid fraction was stored in 1 m^3 of storage tank. The solid fraction was fed into the full-scale anaerobic digestor. Chemical and physical characteristics of the liquid fermentation

were reported in Table 6.3.

Table 6.3. Characteristics of the OFMSW fermentation liquid applied as external carbon source to the scSBR (average ± standard deviation)

Parameter	Unit	Value
Total COD	mgCOD L^{-1}	38891 ± 7966
Soluble COD	mgCOD L^{-1}	33764 ± 7235
Total VFAs C2-C5	mgCOD L^{-1}	11332 ± 2686
rbCOD	mgCOD L^{-1}	33505
bCOD	mgCOD L^{-1}	34578
NH_4-N	mgN L^{-1}	556 ± 301
TKN	mgN L^{-1}	1450 ± 297
PO_4-P	mgP L^{-1}	359 ± 75
TP	mgP L^{-1}	418 ± 105
Conductivity	(mS/cm)	12915 ± 3526
F^-	mg L^{-1}	5404 ± 1091
Cl^-	mg L^{-1}	3062 ± 637
SO_4^{2-}	mg L^{-1}	1572 ± 1605
Na^+	mg L^{-1}	776 ± 112
K^+	mg L^{-1}	1228.0 ± 249
Mg^{2+}	mg L^{-1}	273.8 ± 98
Ca^{2+}	mg L^{-1}	1525 ± 422

The OFMSW FL that was applied as a carbon source was characterized by high COD concentration; more importantly, the OFMSW FL had significant VFA content, in particular acetic acid (HAc = 5585 ± 228 mgCOD L^{-1}), propionic acid (HPr = 907 ± 412 mgCOD L^{-1}) and butyric acid (HBut = 2200 ± 885 mgCOD L^{-1}). The proportion between the volatile fatty acids analyzed the OFMSW fermentation liquid is reported in the following diagram (Figure 6.2):

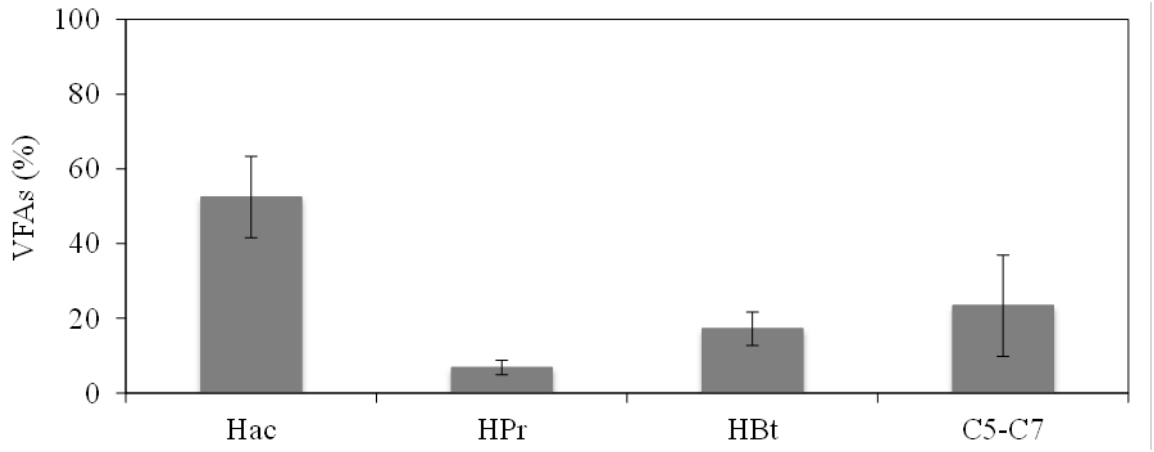

Figure 6.2. Contribution of acetic acid (HAc), propionic acid (HPr) and butyric (HBt) acid to the total VFAs in the OFMSW FL.

6.2.1 Case study 2: the scSBR integrated in the conventional sewage sludge treatment of Carbonera's WWTP

The wastewater treatment plant (WWTP) of Carbonera is located in Veneto Region (north Italy). Currently, the plant treats daily around 10000 m^3 d^{-1} of municipal wastewater, which correspond to a maximal treatment capacity of 40000 population equivalent (PE). The wastewater treatment line is composed of the following operation units: screening and degritting, primary sedimentation, activated sludge process (+ chemical P precipitation) and secondary clarifier, anaerobic digestion of the sewage sludge and dewatering. The biological processes are accomplished in a single basin Schreiber system, for the simultaneous nitrification and denitrification. The primary and the waste activated sludge (PS and WAS) are collected in the primary settler and then statically thickened up to 3.5±1.0%. Every day, around 60-65 m^3 d^{-1} of thickened primary sludge fed a mesophilic anaerobic digestor (reaction volume of 1800 m^3), which corresponds to a vOLR applied of 1.2 kgTVS $m^{-3}d^{-1}$. Currently, the biogas with 60-65% of CH_4 is used in a industrial boiler with a thermal power of 180 kW, mainly used to maintain more as possible stable the temperature of the digestor. The anaerobic digestate was dewatered using a centrifuge from an initial total solids (TS) concentration of 2-3% to 20-25% of TS, while 10 m^{-3} of anaerobic supernatant was equalized in two tanks (5 m^3 of volume each) and used during the experimental period. The rest part of the supernatant was discharge directly into the mainstream of the WWTP.

The pilot scSBR was installed in the pilot hall within the conventional municipal WWTP of Carbonera (Italy), where the carbon source was produced from the alkaline fermentation of the sewage sludge. In this case, the overall process consists with the following key processes (Figure 6.3): (1) alkaline sludge fermentation for the VFAs production; (2) ultrafiltration of the product from the fermentation unit by the pilot tubular membrane reactor (Chapter 3, paragraph); (3) short cut SBR for via nitrite nitrogen and phosphorus removal driven by the addition of fermentation liquid of sewage sludge.

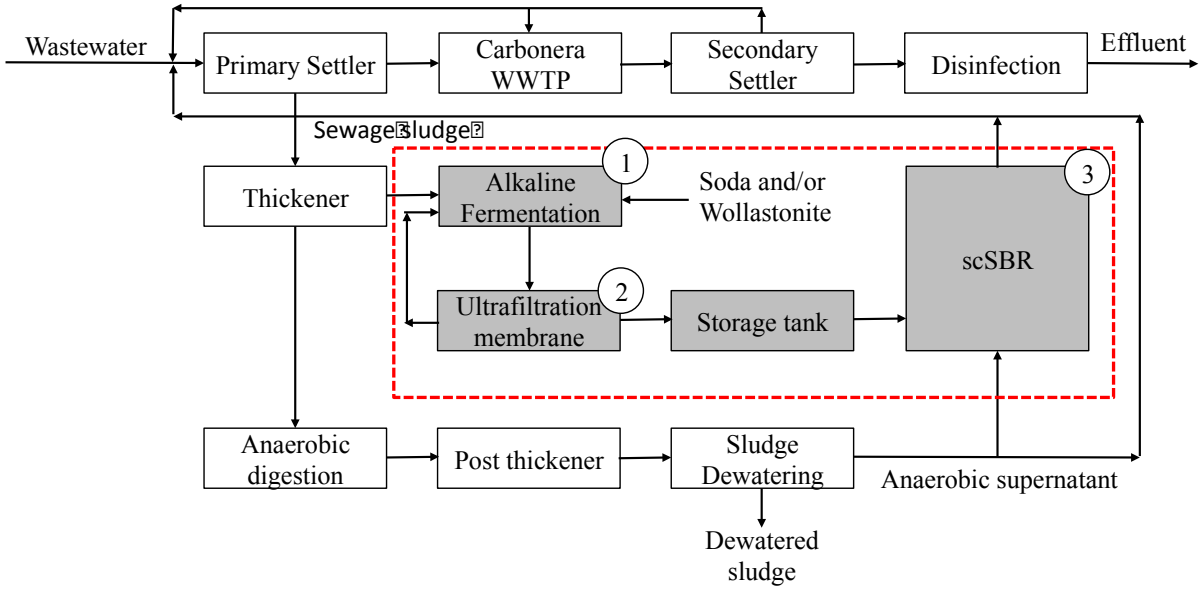

Figure 6.3. The integrated scSBR for the treatment of anaerobic supernatant of sewage sludge.

Characteristics of the anaerobic supernatant from the sewage sludge digestion the operating conditions of the scSBR

The anaerobic supernatant was produced form the anaerobic digestion of the sewage sludge were collected from the full-scale WWTP of Carbonera (Italy). The physicochemical characteristics of both substrate were reported in Table 6.4.

Table 6.4. Characteristics of the anaerobic supernatant and sewage sludge.

Anaerobic Supernatant			Sewage sludge	
Parameter	Unit	Average±St.Dev.	Parameter	Average±St.Dev.

pH	-	7.63 ± 0.4	TS (mg/L)	39796 ± 3319
TSS	mg L^{-1}	323.3 ± 129.5	TVS (mg/L)	33420 ± 3526
TVS	mg L^{-1}	273.0 ± 129.8	TVS (%)	84
COD	mgCOD L^{-1}	363.2 ± 94.0	COD (mg/gTS)	914.2 ± 40.8
SCOD	mgCOD L^{-1}	155.4 ± 86.5	TKN (mg/gTS)	33.9 ± 7.9
NH_4-N	mgN L^{-1}	472.1 ± 100.8	TP (mg/gTS)	13.7 ± 2.1
TN	mgN L^{-1}	546.3 ± 42.2		
PO_4-P	mgP L^{-1}	18.9 ± 7.3		
TP	mgP L^{-1}	25.8 ± 1.9		
Alkalinity pH 5.7	mg$CaCO_3$ L^{-1}	1480 ± 309		
Alkalinity pH 4.3	mg$CaCO_3$ L^{-1}	1955 ± 237		

The cycle of the scSBR reactor was set as follows: 30-35 min (agitated) filling under anaerobic conditions, 180-230 min aerobic reaction, 50-100 min anoxic reaction, 15 min settling and 30-35 min decanting.

However, the optimization of the cycle was carried out in the lab scale reactor, in order to study also the mechanism and the influence of the carbon source on the biological via nitrite phosphorus removal. Two main periods were performed in the lab scale scSBR and the operating conditions are reported in Table 6.6.

Table 6.5. SBR operating conditions.

Parameter	Unit	Period 1	Period 2
		Average ± st.dev.	Average ± st.dev.
$NL_{applied}$	kgN $m^{-3}d^{-1}$	0.45±0.05	0.53±0.09
$PL_{applied}$	gP $m^{-3}d^{-1}$	16.93±2.3	15.43±3.20
OLR	kgCOD $m^{-3}d^{-1}$	1.12±0.09	1.32±0.21
HRT	d	1.16±0.11	1.08±0.16
SRT	d	15	15

Complete accumulation of nitrite was achieved within the first 20 days of SBR operation following the strategy described by Frison et al. 2012. The nitrite oxidizing bacteria (NOB) were completely inhibited due to the high free ammonia (FA) concentration that was maintained in the mixed liquor (>1 mgNH_3-N L^{-1}). After this acclimatization period, the SBR was operated for 118 days to treat up to 24 L d^{-1} of anaerobic supernatant produced from digested sewage sludge and the subsequent solid/liquid separation. The physicochemical characteristics of the anaerobic supernatant are presented in Table 1. As this liquid stream is characterized by a very low

chemical oxygen demand (COD) to nitrogen ratio, an external carbon source must be added to accomplish effective nutrient removal during the anaerobic and anoxic reaction phases.

The operating conditions of the SBR (Table 2) were similar in both periods: the volumetric nitrogen loading rate (vNLR) was 0.45±0.05 and 0.53±0.09 kgN $m^{-3}d^{-1}$ during period 1 (days 1 to 70) and period 2 (days 71 to 118) respectively. The parameter that was altered during periods 1 and 2 was the type of the external carbon source that was applied; pure propionic acid (HPr) was used during period 1 and sludge fermentation liquid (SFL) derived from primary sludge and waste activated sludge was used in period 2. HPr was used as the reference VFA solution since previous findings suggest that propionate may be a more favourable substrate as compared to acetate in terms of enhanced biological phosphorus removal (EBPR) (Li et al., 2011; Wu et al., 2010; Pambrum et al., 2006; Patel et al., 2006). Laboratory scale experiments have shown better EBPR when propionate is used instead of acetic acid during long-term enrichment studies. (Wu et al., 2010; Patel et al., 2006; Zhang et al., 2010; Wang et al., 2004). These studies suggest that propionate may provide a competitive advantage of phosphorus accumulating organisms (PAOs) over glycogen accumulating organisms (GAOs) (Patel et al., 2006). During the first 8 days of period 1, external carbon source was not added. Afterwards, the external carbon source was added during the first 1-3 min of the anoxic phase in order to obtain an initial COD concentration of approximately 330 mgCOD L^{-1} in the reactor as well as during the anaerobic phase to have an initial CODVFA/P ratio of 10.

Batch test to optimize the sewage sludge alkaline fermentation

The optimization of the fermentation process was performed with the aim to maximized the production efficiency and the final composition of the VFA in the sewage sludge fermentation liquid (SFL). The thickened sludge consisted of mixed primary and secondary sludge which was thickened at a total solids of 3.5-4.5%. The impact of the temperature, the sludge total solids (TS) concentration, the type of solution for pH adjustment on sewage sludge fermentation and VFAs accumulation were investigated by operating batch reactors. Each reactors consists which were made of plexiglas and each one had a working volume of 1.0 L. All reactors were stirred at a speed of 100 rpm and the VFA concentration was measured daily.

Temperature: The effect of temperature on the fermentation of sewage sludge was examined by operating two batch reactors at 30±1^{o}C, 55±1^{o}C. The pH was always maintained at 8.5 with the addition of pre-aerated anaerobic supernatant by employing an initial sewage sludge/pre-aerated supernatant volume ratio of 0.4. The total duration of the experiments was 15 d. The TS concentration in the thickened mixed sewage sludge was 4%.

Sludge total solids concentration and use of anaerobic supernatant to control pH: The influence of the initial TS sludge concentration and of the pH control through the addition of anaerobic supernatant on the alkaline fermentation process were examined in six batch reactors that were operated in parallel. Two different solids concentrations were tested; 4.5% TS and 6.5% TS. The experiments were performed with and without pH adjustment. The batch tests that were conducted without any control of the pH were the 'blank' experiments, while in the other batch reactors the pH was controlled with the addition of pre-aerated and non-aerated anaerobic supernatant in order to maintain the pH at 8.5. To produce pre-aerated anaerobic supernatant, samples of 300 mL were introduced into a cylindrical glass tube of 100 mL and were continuously aerated using air diffusers. The pH variation with time was continuously recorded while the temperature was maintained at 25^{o}C±1^{o}C. In the pre-aerated anaerobic supernatant the CO_2 stripping procedure explained above was followed to increase the pH value. In all the batch fermentation reactors the temperature was kept constant at 30^{o}C. After the addition of the sewage sludge in the reactors, the operation time, the pH and VFA concentration were measured every day.

Effect of pH control using caustic soda, : Four batch fermentation reactors were operated in parallel. In each reactor the pH was adjusted daily at of 8, 9, 10 and 11 using NaOH and were monitored for VFAs production for 8 days.

Effect of PH control using calcium silicate (wollastonite): The impact of the concentration of the wollastonite on the efficiency of the fermentation was investigated using four batch reactors experiment (i.e.1, 10, 20, 40 g/L). Wollastonite was added at the beginning of each experiment and the pH was recorded every day. The temperature was maintained at 37 ± 1 °C. The experiments were repeated without any mineral addition to serve as a control experiment.

Finally, the effect of the conditioning reagents (caustic soda and wollastonite) on the sewage sludge fermentation dewaterability was evaluated. Under the optimum operating conditions of

the last two experiments the capillary suction time (CST) with the 340M CST equipment (Triton, UK) and time to filter (TTF) tests were performed according to the methodology of Lo et al., 2001.

Effect of the conditioning sludge on the ultrafiltration process

The effect of caustic soda and wollastonite to buffer the pH, changes SRT on the efficiency of separation UF membrane system were evaluated. Table 6.6 shows the main operating conditions applied in the pilot SF-MS unit. In order to improve the organic loading rate (OLR) of the fermentation, a semi-continuous configuration coupled to UF system was applied by separating and recirculating the solids fraction of the fermented sludge to the fermentation reactor. This way the SRT was maintained significantly higher than HRT. The experimentation was divided in 5 periods. In period 1 (days 1-40) the pH was not controlled, to evaluate the maximum VFAs production in the case of sludge fermentation without any pH buffering. During period 2 (days 41-80) and period 5 (days 151-180) caustic soda was added in order to keep the pH in the range of 9.5-10.5, while in periods 3 (81-120) and 4 (121-150) the use of wollastonite (10 g/L) as buffer was tested in order to avoid the addition of chemicals in the alkaline fermentation process. During the whole experimentation the fermentation process was conducted by applying HRT in the range of 4.6-5.9 days, based on the results of the batch tests. Two different SRT (5 and 14 days) were applied to assess the effect of SRT on the fermentation process. In periods 1-3 the process was conducted at high SRT = 14 days, while during periods 4-5, the HRT was kept very close to SRT (around 5 days).

Table 6.6. Operating condition of the SF-MS pilot unit

Parameter	Unit	Perido 1	Period 2	Period 3	Period 4	Period 5
Days of operation		1-40	41-80	81-120	121-150	151-180
Type of pH conditioning		-	Soda	W	W	Soda
pH	-	5.71±0.45	10.1±0.25	7.15±0.06	7.11±0.02	9.99±0.32
Solids fed	gTVS L^{-1}	7.17±3.19	7.33±0.41	8.54±1.41	9.50±0.51	9.23±0.97
Solids in the reactor	gTVS L^{-1}	16.73±3.95	23.51±3.67	25.18±1.58	14.41±1.12	14.39±0.69
HRT	d	5.42±1.10	5.93±1.05	4.62±0.51	5.13±0.11	5.16±0.23
SRT	d	13.63±0.58	14.17±1.21	13.83±0.32	6.13±0.55	5.21±0.60
Temperature	°C	35±1	35±1	35±1	35±1	35±1

Flow UF module	$m^3 d^{-1}$	-	5.71±0.98	7.18±0.12	-	-
TMP	bar	-	0.68±0.06	0.71±0.056	-	-

6.3 Case study 1: Result and discussion

6.3.1 EBPR with OFMSW FL and nitrite impact

Figure 6.4 shows the variation of sPLR and the specific nitrogen loading rate (sNLR) with the operation time. The sNLR was relatively similar during the two operational periods. However, the volumetric NLR (vNLR) applied during period 1 was variable; specifically, during the first days (27-44) of period 1 vNLR was 0.3 kg $m^{-3}d^{-1}$ and subsequently 0.7 kg $m^{-3}d^{-1}$. The hydraulic retention time of the system was 0.74±0.06 d in the Period 1, while was decreased up to 0.35±0.06 d during the Period 2. The nitrogen and phosphorus loading rates have been calculated taking also into account the contribution by the feeding of the fermentation liquid.

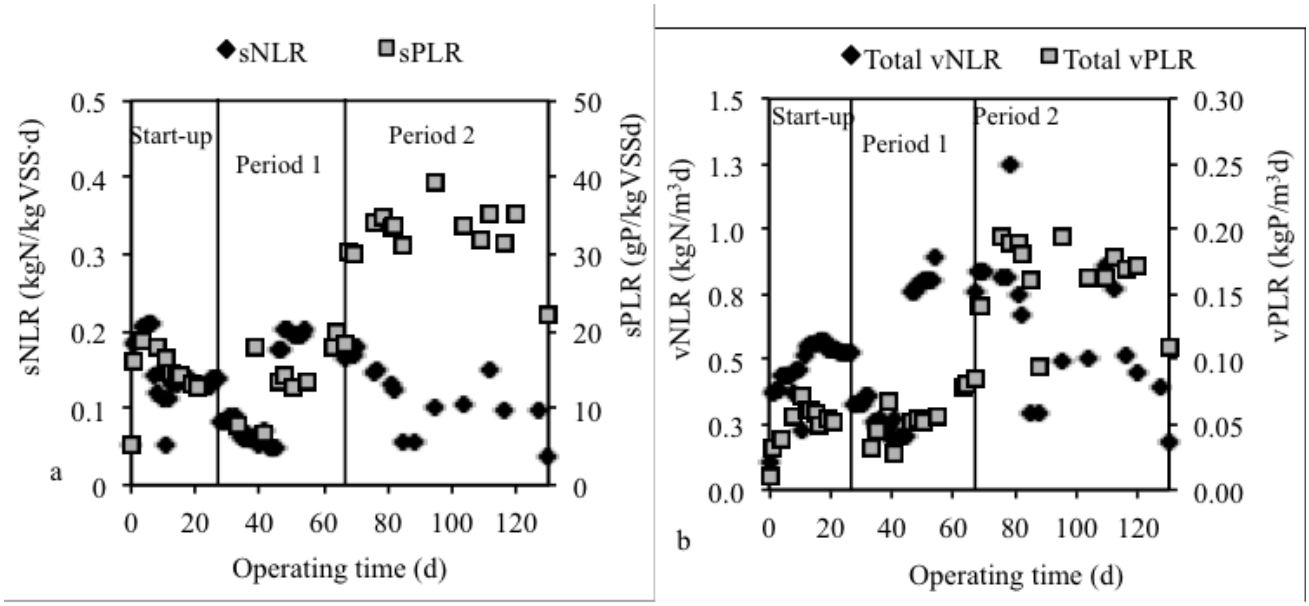

Figure 6.4. Variation of (a) sNLR, sPRL and (b) vNLR, vPLR with the operating time of the scSBR.

Effective nitritation/denitritation was accomplished (NO_2-N/NO_x-N=100%) without any nitrate accumulation. This was achieved by maintaining a high free ammonia (FA) concentration in the reactor (1-2 mgNH_3-N/L). Such FA concentrations are known to inhibit the nitrite oxidizing bacteria (NOB).[2,15] The pH in the SBR was not controlled and fluctuated between 7 and 8. Given

the high ammonium concentration of the supernatant this pH range was adequate to obtain the required FA concentration in the reactor for complete NOB inhibition.

In our SBR the effluent ammonia concentration varied from 25 to 49 mgN L^{-1}, respectively for Period 1 and 2. In both periods the pH in the effluent was 7.8, while the temperature in the mixed liquor was 25 °C. With this parameters, the calculated FA concentration, according with equation reported from Pambrun et al. (2006), varied from 1 to 2 mgN L^{-1}, respectively for Period 1 and 2.

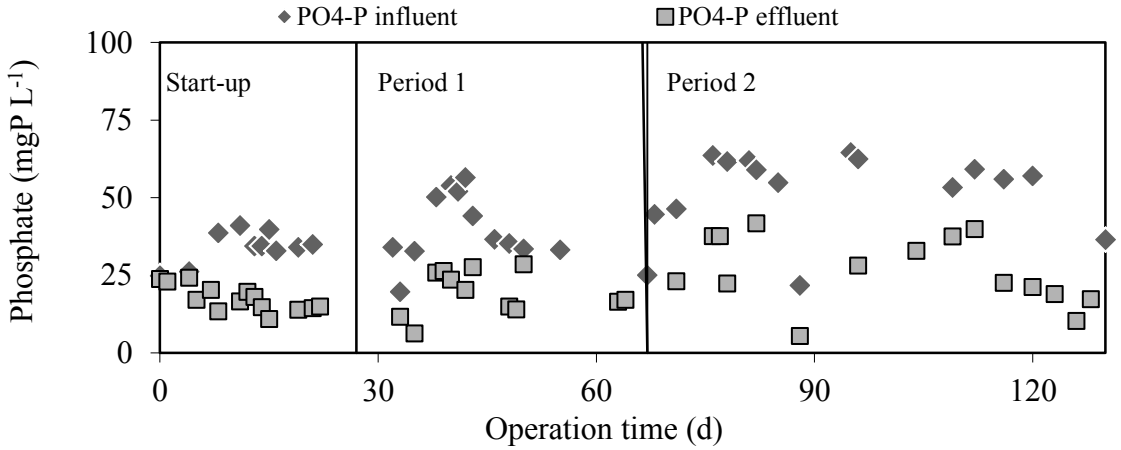

Figure 6.5. Variation of influent and treated effluent phosphate concentration

Table 6.7. Removal efficiency and mass balances for phosphorus (average value ± standard deviation).

Parameter	Units	Start-up	Period 1	Period 2
Inflow phosphorus load	gP $m^{-3}d^{-1}$	58.1	61.1	162.7
Outflow phosphorus load	gP $m^{-3}d^{-1}$	36.2	29.3	91.2
Phosphorus removal (%)	%	38 ± 18	52 ± 18	44 ± 28
Biological Phosphorus Uptake				
-Growth	gP $m^{-3}d^{-1}$ (%)	3.8 (33)	4.6 (22)	5.1 (19)
- P removed by aerobic PAOs	gP $m^{-3}d^{-1}$ (%)	2. 8 (25)	1.8 (8)	6.4 (24)
- P removed by DPAOs	gP $m^{-3}d^{-1}$ (%)	3.5 (31)	13.2 (63)	11.5 (44)
Phosphorus chemically precipitated	gP $m^{-3}d^{-1}$ (%)	1.2 (11)	1.4 (7)	3.4 (13)
Error in P mass balance	%	-	17.6	27.7

Figure 6.5 shows the variation of the phosphate concentration in the influent and treated effluent with operating time. Phosphate removal was variable owing to the fact that complete denitritation was in some cases achieved, resulting in some release of phosphorus due to the prevalence of anaerobic conditions. The average phosphate removal efficiency was 52% in period 1 and 44% in period 2 (
Table 6.7).

Various mechanisms were responsible for the phosphorus removal; these include the EBPR by aerobic PAOs and DPAOs, the growth of normal biomass and chemical precipitation. After the start-up period, the majority of phosphorus uptake (57-63%) was attributed to PAOs and DPAO activity. EBPR was mainly due to DPAOs activity (88% in period 1 and 64% in period 2 of the total EBPR). A significant amount of phosphorus was also removed biologically due to the growth of heterotrophic biomass (19-22%). This was calculated considering the phosphorus content in the conventional activated sludge used as inoculum. The contribution of chemical precipitation to phosphorus removal was low (<7-13%) and thus confirmed the prevalence of biological processes. The $sPUR_{anoxic}$ was similar in periods 1 and 2 (on average 10.7 and 9.2 mgP $gVSS^{-1}h^{-1}$ respectively, Figure 6.6). The increased phosphorus loading that took place during period 2 did not increase the phosphorus removal rate. The standard deviation of $sPUR_{anoxic}$ was significant in both periods, probably owing to the fluctuation in the nitrite concentrations in the mixed liquor. In both periods the $sPUR_{anoxic}$ was much higher than $sPUR_{aerobic}$. The high nitrite content seems to significantly inhibit the aerobic PAOs.

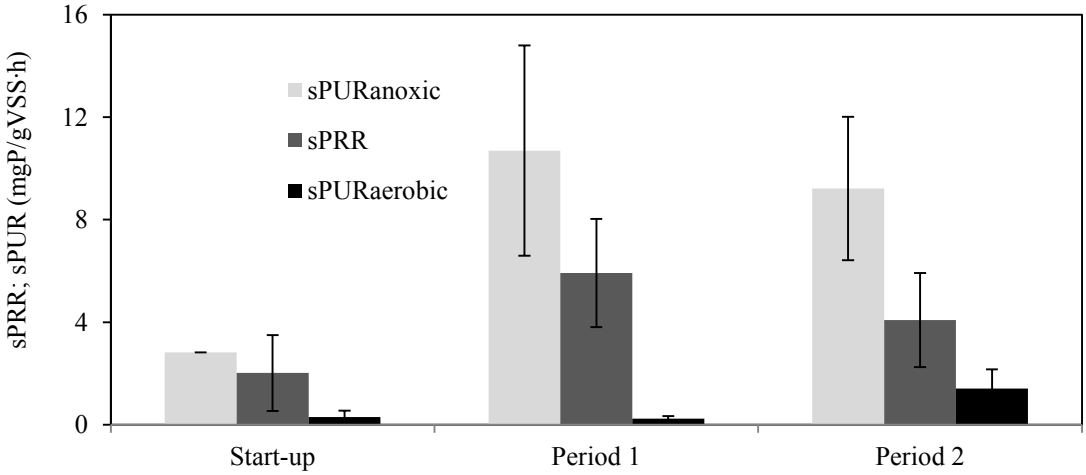

Figure 6.6. $sPUR_{anoxic}$, $sPUR_{aerobic}$ and sPRR for the examined experimental periods

Despite the high nitrite levels of the mixed liquor the average $sPUR_{anoxic}$ was significant showing that DPAOs were less sensitive to nitrite accumulation than PAOs. The specific phosphorus release rate (sPRR) was lower than the $sPUR_{anoxic.}$ In the cases where the nitrite were not depleted at the end of the anoxic phase, some nitrites were also present and the beginning of the anaerobic feeding period, thus shortening the actual duration of actual anaerobic conditions. During the operation of one cycle, phosphorus uptake took place during the aerobic phase and also continued during the anoxic phase, at a much higher rate. Usually, during the first 10 min of operation in the anoxic phase, phosphorus uptake was sluggish, since some PO_4-P release occurred due to the addition of OFMSW FL. Subsequently, phosphate uptake occurred at a high rate and the $sPUR_{anoxic}$ was 9-12 mg $gVSS^{-1}h^{-1}$. Towards the end of the anoxic phase, in several cycles the nitrite was completely depleted and anaerobic conditions prevailed resulting in some phosphorus release. The anaerobic phosphorus release demonstrates that VFAs were not completely consumed despite the fact their dosage was 2.0-2.1 $gCOD/gN_{applied}$. However, in several cycles the nitrite concentration at the end of the anoxic period was >10 $mgNO_2$-N L^{-1} starting from initial nitrite values of 90-140 $mgNO_2$-N L^{-1} at the beginning of the anoxic phase. Despite these high nitrite concentrations, DPAO activity was significant as a result of the high $sPUR_{anoxic}$ that was obtained. The good sludge settling properties (SVI=73±14 mL/g in period 1 and 39±13 mL/g in period 2) is probably related to the polyelectrolyte residual in the anaerobic supernatant. FISH analysis was conducted in both the initial sludge inoculum and in the mixed liquor collected on the 31st day of the scSBR operation. In both samples the presence of GAOs was very low <2%. In the inoculum the presence of PAOs was relatively low (5.2±1.9%). The subsequent scSBR operation resulted in the growth of PAOs since the area of PAO cells increased to 36.4±3.4%. The observations of the micrographs showed the predominance of the coccus morphology versus the rod morphology in *Accumulibacter* indicating the presence of nitrite DPAO versus nitrate DPAO.

6.3.2 Effect of critical parameters on EBPR via-nitrite

The effect of nitrite concentration

The effect of nitrite on the DBPRN was also investigated in ex situ reactors. Different initial NO_2-N concentrations were tested to simulate a wide vNLR applied to the pilot scale scSBR,

while the fermentation liquid was added either simultaneously with nitrite or under anaerobic conditions. In Figure 6.7 the phosphate and nitrite time profiles are shown when the fermentation liquid was added simultaneously with nitrite for different initial PO_4-P/NO_2-N ratios. These conditions may occur at the beginning of the anoxic phase. The initial, steep phosphate increase is due to the addition of OFMSW derived carbon source, which also contained phosphates, thus increasing their content in the mixed liquor. Then, a slow, net increase of phosphate was observed. The phosphate release lasted up to the depletion of VFAs. Other studies have also reported phosphorus release under anoxic conditions (Zhang et al., 2010; Patel et al., 2006) this is probably associated to the high COD concentrations in the solution that may be in excess of the PHB saturation of polyphosphate bacteria (Wang et al., 2004).

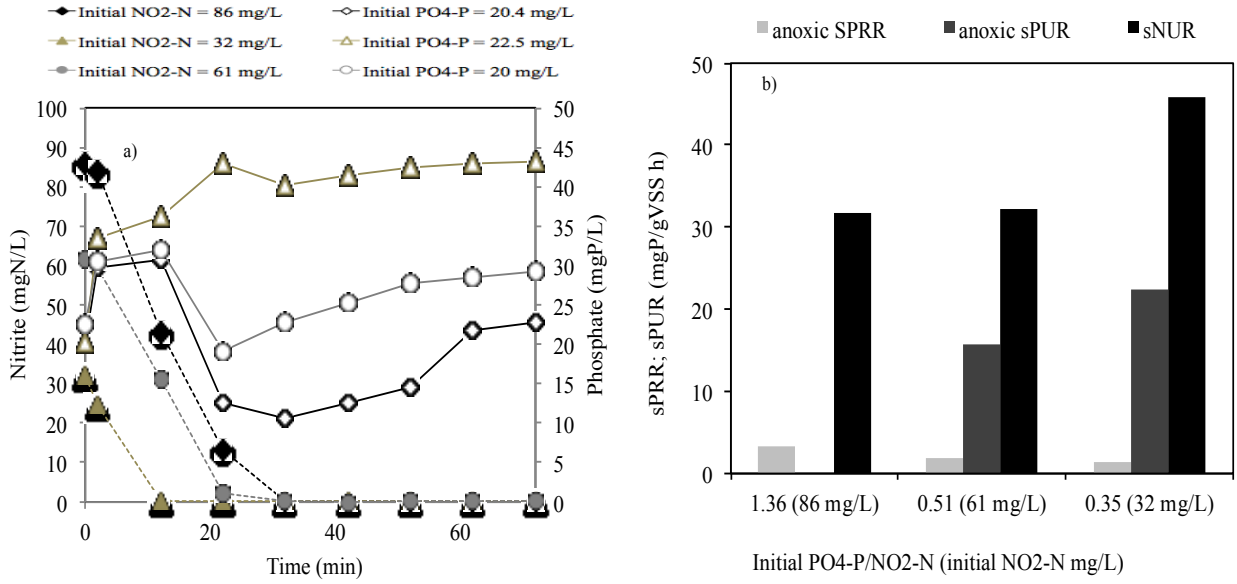

Figure 6.7. (a) Effect of initial PO_4-P/NO_2-N ratio and initial nitrite concentration on (a) phosphate profile and on (b) sPUR and sPRR. In the tests nitrite and fermentation liquid were added simultaneously in the batch reactor (initial COD/PO_4-P = 10).

It has been demonstrated [29] that when both HAc and electron acceptors are present, the oxidation of HAc in the tricarboxylic acid (TCA) cycle, instead of the anaerobic degradation of glycogen, provides the reduction equivalent ($NADH_2$) required for generation of PHB. Energy (ATP) is produced by the oxidation of HAc through the TCA cycle and subsequent oxidative phosphorylation with the $NADH_2$ that are formed. This results in less PHB production and less polyphosphate degradation and thus less phosphorus release. Once, the VFAs were depleted,

phosphate uptake took place under anoxic conditions, until the nitrites were depleted. Then, phosphorus release was observed as strictly anaerobic conditions prevailed. The phosphate release rate observed after the simultaneous spiking of carbon source and nitrite was lower when the biomass was initially spiked with high nitrite concentrations (Figure 6.7b). Although it has been reported that high nitrite levels can inhibit the DNBPR,[9,30] the $sPUR_{anoxic}$ was found to increase with higher initial nitrite concentrations spiked to the biomass. Biological phosphorus uptake was high even at high nitrite concentrations (i.e. 86 mg/L), confirming the in situ biomass activity results obtained from the pilot scSBR. At the highest PO_4-P/NO_2-N ratio that was applied (=1.36), an almost continuous release of phosphorus was observed attributed to the depletion of nitrite within only 12 minutes. In this case, nitrite was not available for P uptake when VFAs were depleted and thus phosphate continued to be released in the mixed liquor.

Addition of the OFMSW FL under anaerobic conditions

The undesirable release of phosphorus under anoxic conditions can be tackled by automatic real time control of the FL dosage. Other options are the addition of the pulsed addition of the carbon source or its addition under strictly anaerobic conditions just before the anoxic phase; the latter option was examined in batch reactors. Figure 6.8 shows the sPUR and sPRR that were obtained when this scheme was followed.

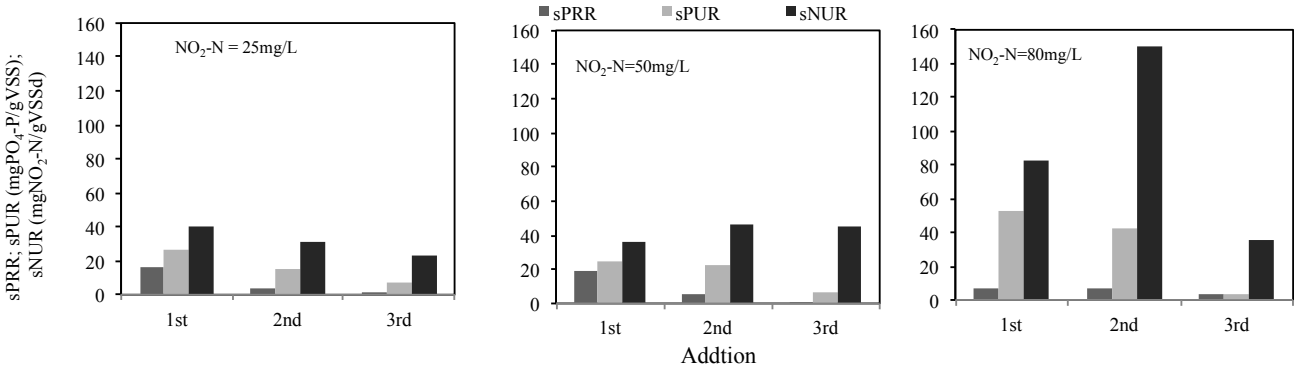

Figure 6.8. Effect of initial nitrite concentration on sPUR and sPRR when the fermentation liquid was added under anaerobic conditions in the batch reactor

Once nitrite was introduced in the reactor, phosphate uptake was immediately observed. More importantly, the DPAOs did not compete with the denitrifiers for the available carbon, since the

DPAOs had already stored PHA using the organic carbon available in anaerobic conditions. In accordance with batch experiments of the previous section, significant phosphorus removal was obtained even at high initial nitrite concentration (i.e. 80 mg/L) due to the availability of nitrite as electron acceptors, which resulted in high phosphate uptake via nitrite (Figure 6.8). This finding demonstrates that DPAOs are much more tolerant to nitrite than aerobic PAOs. The results of this work also show that it is advantageous to introduce a short anaerobic phase prior to the anoxic one in order to avoid the release of phosphorus at the beginning of the anoxic phase. However, the composition of the carbon source is critical since the addition of acetate or sugars to directly to the anaerobic zone may promote the growth of GAOs.[1] The increase of the initial nitrite concentration from 25 to 80 mg L favoured the rate of both nitrogen and phosphorus uptake, since more electron acceptors were available. The difference was more profound when nitrites increased from 50 to 80 mg L. In all cases and irrespective of the initial nitrite concentration, the sPRR and sPUR decreased during the 3rd spiking with nitrite and phosphate, since readily biodegradable organic carbon had been depleted (i.e. approximately 300 min had elapsed from the introduction of the fermentation liquid). During the first two spiking periods sPUR were obtained were high ranging from 15-53 mgP (gVSS h)$^{-1}$.

6.3.3 SBR cycle and real time process control

Figure 6.9 shows a complete SBR cycle in which it is seen that during the anoxic phase the nitrites were depleted very fast (i.e. within the first 25-30 minutes). As a result in the remaining time period, anaerobic conditions prevailed and some phosphorus release occurred. The cycle also shows that the via nitrite phosphorus uptake was much faster than the respective one that occurred under aerobic conditions. From this figure, it is clearly seen that, although uncontrolled anaerobic conditions occurred during the anoxic phase (when nitrite was depleted) and during the sedimentation and discharge phases. Figure 6.10 shows a typical trend of the conductivity during a cycle of the scSBR. The conductivity signal follows very well the nitrogen forms during the aerobic phase. The decrease of conductivity during the aerobic phase coincides with the combined effects of the oxidation of ammonium with the production of the nitrite and the consumption of alkalinity.

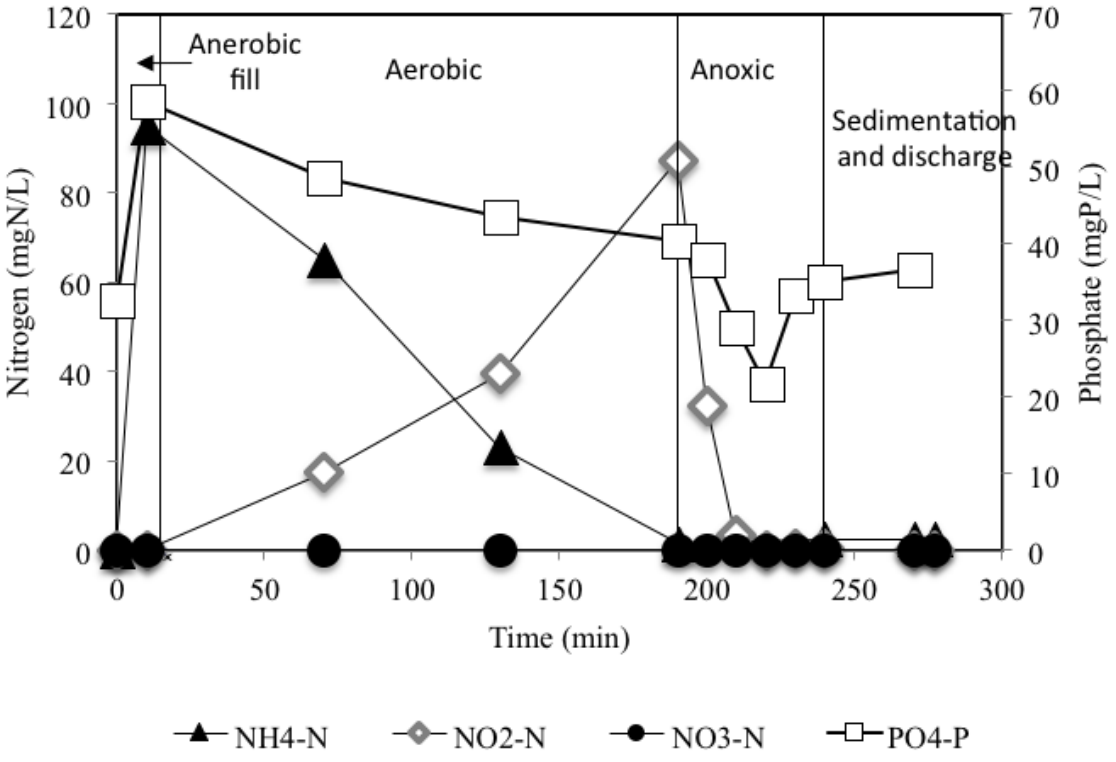

Figure 6.9. Profile of ammonium, nitrite, nitrate and phosphate in a typical cycle of the scSBR

In our case, the decrease in conductivity corresponding to the oxidation of 1 mgNH4-N·L-1 was 9.52 μS/cm. During the anoxic phase, a decrease of conductivity of approximately 1μS/cm for 1 mgNO2-N/L removed was observed, due for the simultaneous processes of denitrification and uptake of phosphorus via nitrite. However, once the nitrite were depleted, a small but steady increase in conductivity was observed which is more clearly seen in the top right of Figure 6.10. The released of 1 mgPO4-P/L corresponded to an increase in the conductivity of 4 μS/cm. Hence, conductivity can be employed as an indirect parameter of real time process control to signal the termination of the anoxic phase so that the undesirable anaerobic phase is avoided.

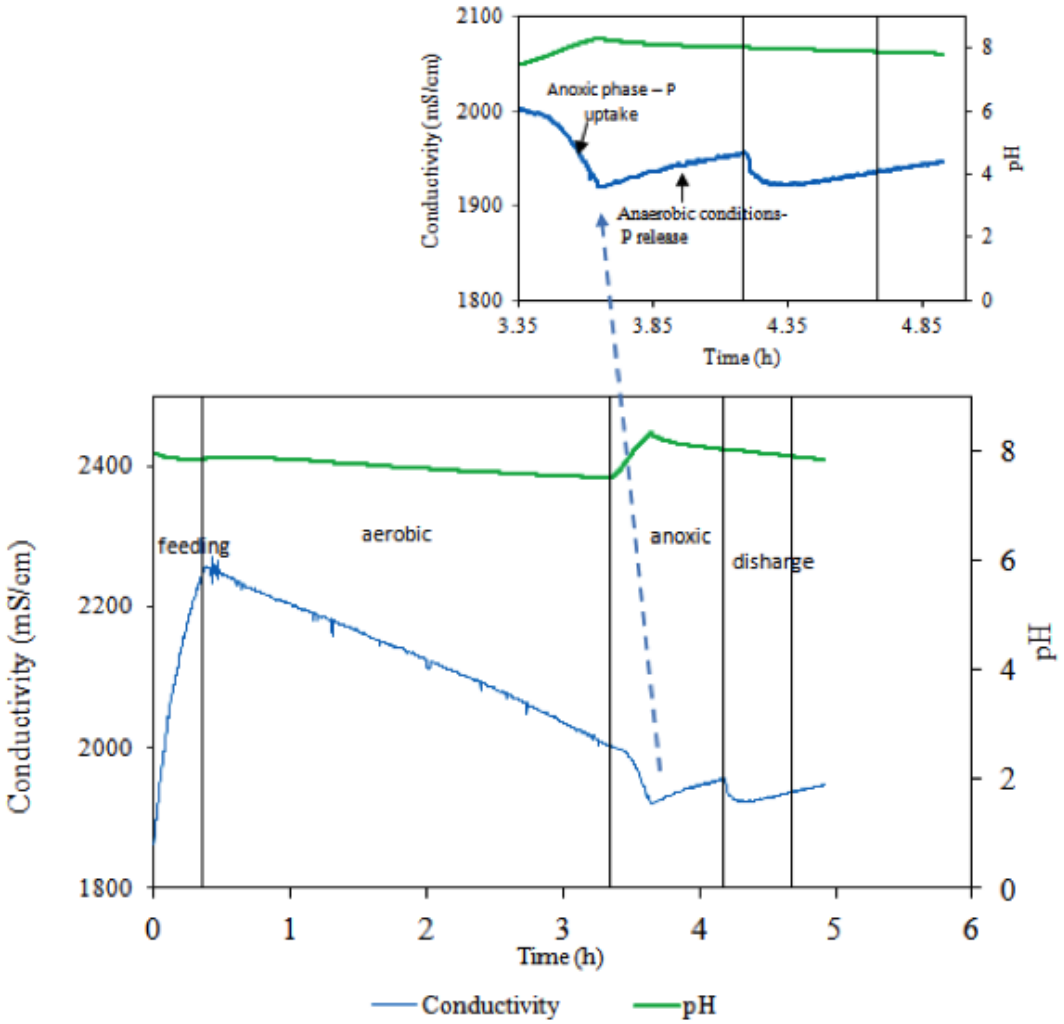

Figure 6.10. Variation of conductivity, pH and DO in the scSBR

6.3.4 Comparison of literature on phosphorus removal via nitrite

Table 6.8 summarizes the works in which the DNBPR has been implemented. It is clear from the literature that the DNBPR has not been examined for anaerobic supernatant effluents. A potential problem when dealing with highly nitrogenous effluents such as the anaerobic supernatant produced from the co-digestion of OFMSW and WAS is the potential inhibition of DPAO activity by high nitrite levels. Several studies have reported that PAO activity in the reactor can be inhibited by significant nitrite levels in the reactor under both anoxic and aerobic conditions, resulting in a decrease of phosphorus removal. (Peng et al., 2011; Yoshida et al., 2009; Sing et a., 2008; Saito et al., 2004; Hu et al., 2003; Ahn et al., 2001; Kuba et al., 1996) On the other hand,

some studies have reported that the DPAOs can acclimatize to nitrite and effectively carry out the via nitrite nitrogen and phosphorus removal (Vargas et al., 2011; Guisasola et al., 2009; Jiang et al., 2006) However, most of these studies have been carried out by adding nitrite in order to investigate the impact of nitrite/free nitrous oxide on PAOs activity (Zeng et al., 2011) The effect of nitrite accumulation in a significant time span of reactor operation effluents has not been examined for strongly nitrogenous effluents.

Table 6.8 Studies that have examined P removal by applying the via nitrite pathway

Type of wastewater	System	Main operating conditions	External carbon	Main finding	Reference
Anaerobic supernatant produced from OFMSW and WAS co-digestion	scSBR	DO control High FA =1-2 mg NH_3-N/L	OFMSW FL	sPUR = 9-11 mg/gVSS·h Significant sPUR achieved even at high nitrite levels Both PAOs and growth are important mechanisms in P uptake	This work
Synthetic	Batch reactors (sludge collected from SBR)	Impact of MLSS concentration	Acetate	P uptake was more efficient in the presence of nitrates compared to nitrites Increase of MLSS resulted in decrease of P uptake (nitrate environment), while the increase of NO_3-N concentration led to increase of P uptake	Ahn et al., 2001
Synthetic (low strength)	(AO) 2 SBR	Nitrate and nitrite environment	Acetate	Complete P removal PUR was higher in the presence of nitrite compared to the presence of nitrate Real time control enhanced the stability and the efficiency of the process	Lee et al., 2001
Synthetic: the nutrients and carbon source solution (HPr)	Lab-scale SBR	nitrite-DPAO enrichment in a anaerobic-aerobic EBPR system	HPr	It was not feasible for the nitrite-DPAO to utilize nitrates	Guisasola et al., 2009
Municipal	SBR	Effect of the	WAS alkaline FL	P removal with sludge FL as	Ji and Chen,

		composition of the applied carbon source	Acetic acid	carbon source was 97.6% while with the use of acetic acid 73.4% Sludge FL resulted in higher DPRN than acetic acid	2010
Synthetic (NH_4-N=114 mg/L)	Batch reactor	Effect of nitrite on PRR, PUR	Acetate, butyric acid, glucose	The increase of NO_2-N levels improved PUR PRR decreased as anaerobic nitrite addition increased. Effective P release and uptake was achieved at nitrites < 2 mg/L. P release with different carbon sources followed the order: acetate > butyric acid> glucose.	Zhang et al., 2010
Municipal	A^2/Os – alkaline WASFermentation – Separation of FL	Improvement of SCFA concentration in the external carbon source	WAS alkaline FL (under controlled pH)	$Ca(OH)_2$ was very effective to adjust the sludge fermentation pH at 10, having the same effects as NaOH, but exhibiting better sludge dewatering, lower cost, and higher FL recovery efficiency	Li et al., 2011
Urban	Autotrophic-SBR Heterotrophic-SBR	pH control		P release: 80 mg/L (anaerobic phase) Half of P was removed in the anoxic phase: Complete P removal under aerobic conditions.	Marcelino et al., 2011
Domestic	SBR	Real-time step feed strategy.	Propionate	The process resulted in >22.3% of PHA saving during P removal Optimization of the process by applying real-time step feeding control strategy. NO_2-N levels of 15 mg/L were not toxic to anoxic phosphorus uptake (batch scale)	Peng et al., 2011
Synthetic	anaerobic/anoxic (An/A) reactors – batch tests	Batch tests	Acetate, propionate acetate/propionate	The increase of carbon source up to a certain and the nitrate concentration enhanced PUR removal	Wang et al., 2011

				ORP can be used as a control parameter for P release	
Domestic(synthetic)	SBR	DO control Control of the aerobic duration	Adjustment of the COD concentration in the wastewater at 200 mg/L (EDTA)	The introduction of pre-anoxic zone and additional carbon source in the treatment scheme resulted in 98% P removal Free nitrous acids levels of 0.002–0.003 mgHNO_2-N/L inhibited P uptake	Zeng et al., 2011

6.4 Case study 2: Result and discussion

6.4.1 Effect of the pH and initial solid concentration on the fermentation efficiency

The anaerobic supernatant of sewage sludge was used as conditioner to adjust the pH in the fermentation process of sewage sludge. The stripping of CO_2 from the supernatant was accomplished through the provision of air by diffusers. The pH increased up to 9.1 after 2h (Figure 6.11). The stripping of CO2 resulted in a reduction of the total alkalinity by approximately 40% and of ammonia concentration by 20%.

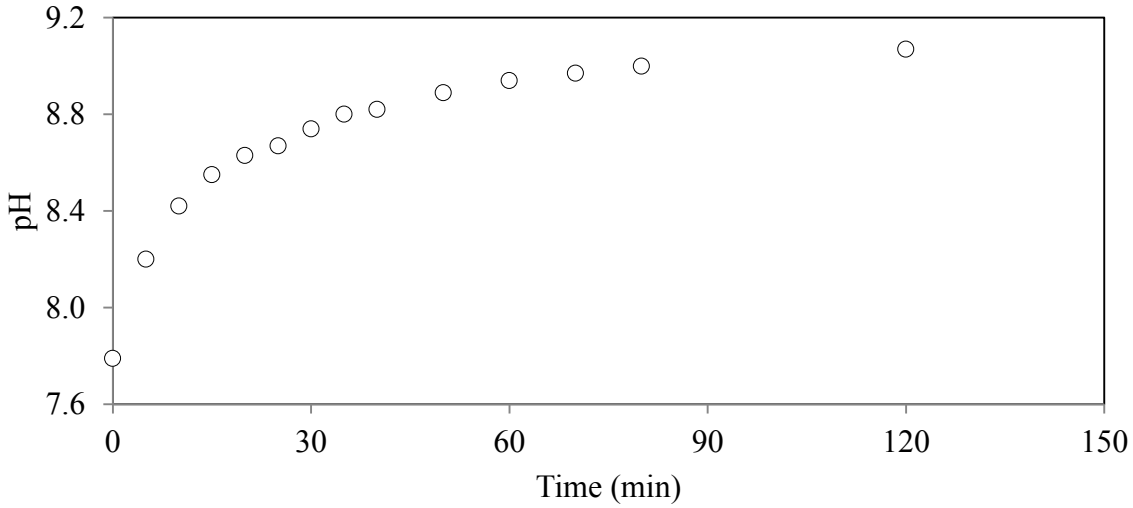

Figure 6.11. Effect of CO_2 stripping on the supernatant pH.

Figure 6.12 shows the effect of the initial TS concentration (4.5% and 6.5%) and the type of anaerobic supernatant used (i.e. pre-aerated, non-aerated) for pH adjustment on the production of VFAs. The highest VFA concentration was achieved 6 days after the initiation of the fermentation process. Higher VFA concentrations (up to 12 gCOD L) were observed at high TS concentrations (i.e. 6.5%). The use of pre-aerated anaerobic supernatant seems to produce a higher VFA content than in the case where non aerated anaerobic supernatant was used. However, the addition of the anaerobic supernatant could not maintain the pH at the desired

levels, thus did not significantly enhance the efficiency of the process.

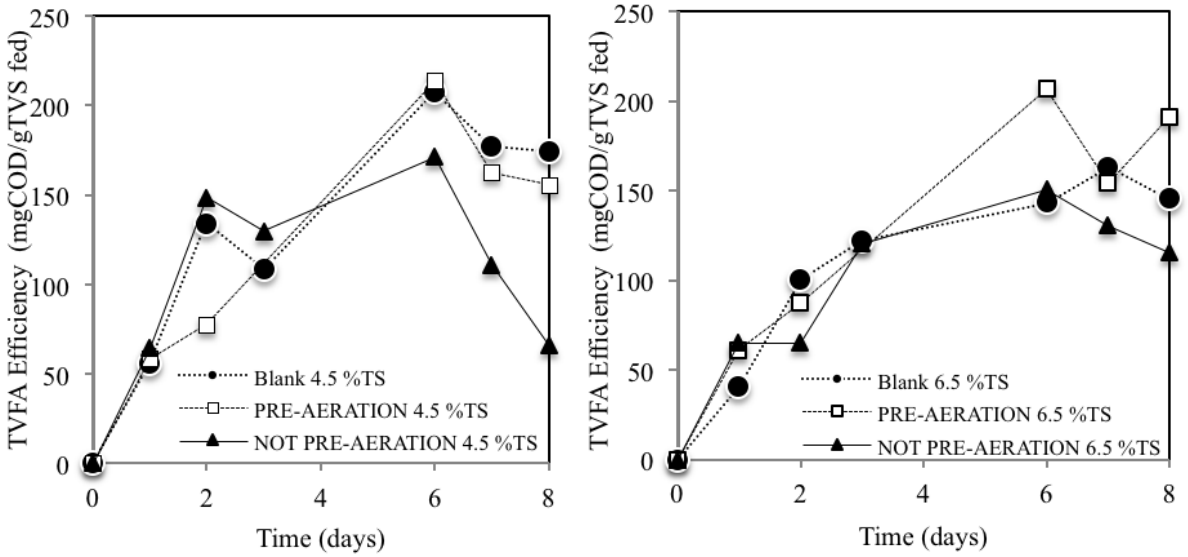

Figure 6.12. Effect of initial solid concentration and solution type on the production of total VFAs in the fermented liquid

The effect of the pH (8-11) on VFA production was better investigated by the daily control of the pH using caustic soda. Figure 6.13 shows the variation in the production of VFAs with time. The maximum VFA production was obtained for a residence time of 6-7 days. The increase in pH from 8 to 9 resulted in enhanced production of VFAs (i.e. from around 200 mgCOD $gTVS^{-1}$ to 300 mgCOD $gTVS^{-1}$), while the further increase of pH (up to 11) did not result in significant differences. The maximum VFA production obtained in the reactor for pH 8 was similar to that obtained with the use of pre-aerated supernatant to initially adjust the pH at 8.5 (i.e. 200mgCOD $gTVS^{-1}$).

Table 6.9 gives the concentrations of VFAs that were contained in the fermented liquid at the sixth day of the fermentation process. The type of anaerobic supernatant used for pH adjustment and the solids concentration of sewage sludge affected the proportions of the VFAs to the total VFAs and the efficiency of the fermentation process. Higher proportion of propionic acid and butyric acid were obtained in the 'blank' and 'without any aeration' anaerobic supernatant. On the other hand, the proportion of propionic acid and butyric acid was higher in the experiments that were performed with higher concentration of solids.

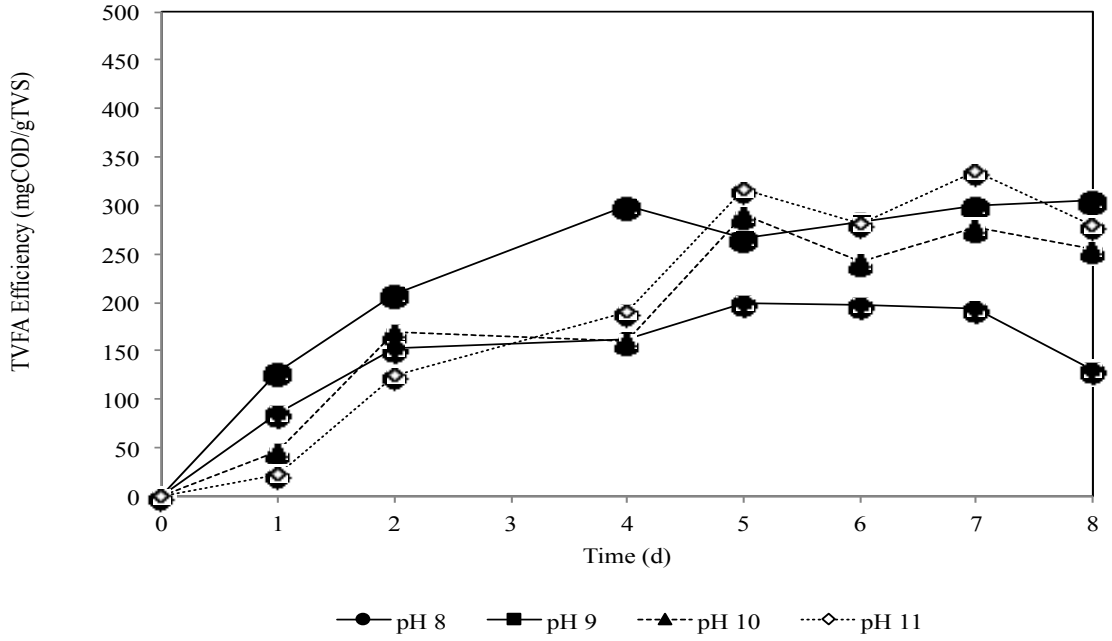

Figure 6.13. Effect of pH on the production of VFAs in the fermentation process

Table 6.9. VFAs concentration in the fermented liquid produced at the 6th day of the fermentation process under different operating conditions.

Acid[a]	TS: 4.5%			TS: 6.5%		
	Blank	Pre-aerated supernatant	Non-aerated supernatant	Blank	Pre-aerated supernatant	Non-aerated supernatant
Hac (mgCOD L)	2595	4011	2143	2989	5291	5291
Hbut (mgCOD L)	1706	1515	1223	2074	2316	1975
Hpr (mgCOD L)	3403	1861	3034	4046	4046	4013
TVFA (mgCOD L)	9547	8597	8084	11409	11945	11219

[a]Hac: acetic acid, Hbut: butyric acid, Hpr: propionic acid

The highest ratio of VFA/NH4-N was obtained in the blank experiment (=17 gCOD/gN). This work demonstrated that high TS concentration (6.5%) and a retention time of 6 days were the most favourable conditions for fermentation.

6.4.2 Effect of temperature on the fermentation process

Figure 2 presents the effect of temperature on the production of VFAs. The fermentation tests were conducted for 15 days in mesophilic and thermophilic conditions (i.e. 30 and 55oC). In order to keep a constant pH (8.3-8.8), during the experimental period some amount of pre-aerated supernatant was added. The maximum concentration of soluble COD was ~350 mgCOD $gTVS^{-1}$ after 12 d of fermentation at 30 °C. It was difficult to maintain stable pH (at 8.5) with the addition of pre-aerated supernatant since continuous dosing was required. Higher temperature improved the hydrolysis of the organic matter, since the concentration of the soluble COD was three times higher compared to the respective one obtained under mesophilic conditions. Furthermore, the time required to reach the maximum VFA concentration was much shorter during the thermophilic temperatures (only 2 days). Moreover, the pH was much more stable; after the first two days of the fermentation process, the pH was stable within 8.5-8.8 and thus pH adjustment with the supernatant was not required.

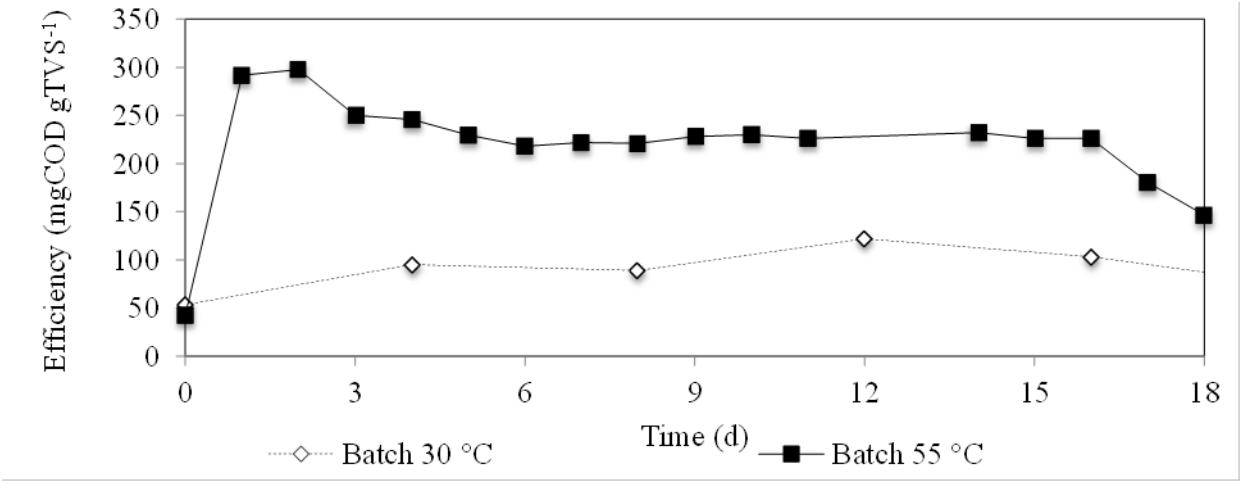

Figure 6.14. Impact of temperature on the efficiency of the fermentation process

On the contrary, under mesophilic conditions continuous adjustment of the pH value was required. The results of other studies have also shown that the production of soluble COD increases with temperature and the sludge hydrolysis is higher at elevated temperatures (Mahmoud et al., 2004) Feng et al. (2009) found that more soluble protein and carbohydrate were produced at higher temperature during WAS fermentation, while the hydrolysis of WAS at pH 10 could be improved by increasing the temperature. Zhang et al. (2010) found that the optimum pH for VFAs production was temperature dependent, while the maximum VFAs yields were obtained in the following order: thermophilic, pH 8 > mesophilic, pH 9 > ambient, pH 10 >

ambient, uncontrolled pH.

6.4.3 Filtration performance and dewatering characteristics

According with the experimental periods, the alkaline fermented sludge was separated through membrane filtration in order to obtain the VFAs within the fermentation liquid. The filtration performance decreased with time due to the deposition of soluble and particulate matter on the membrane surface. The temperature of the SFL was high (35 °C), fact that is expected to favour the filtration process. The filterability of the caustic soda fermentation effluent was evaluated by the step-flux filtration test. Figure 6.15(a) depicts the effect of pH and TMP on permeate flux: the increase in permeate flux resulted in a linear increase of transmembrane pressure (TMP). However, above a certain TMP value the permeate flux decreased. This indicates that there is an optimum operating TMP. Moreover, the increase of pH negatively affected the permeate flux, since at the pH of 10 the obtained permeate flux was lower than that obtained at pH of 8 forgiven TMP (Figure 6.15(a)). Another factor that impacted on the fermented effluent dewatering characteristics is the type of the agent/additive that is used for the fermentation. The sludge dewatering characteristics and the separation process can be adversely affected from the use of caustic soda (Su et al., 2013). To this end, wollastonite was added to increase the pH without chemical use, in order to favour the dewatering characteristics of the sludge and maximize the separation performance. Better dewatering characteristics need to be achieved in order to reduce the energy consumption of the S/L separation process. Sludge fermentation has been found to be temperature-dependent. However, the maximum operating temperature of the membrane module used for the separation process in the pilot scale system is 40°C. This constitutes a limiting factor for the application of the fermentation process at higher temperature level. The average flux measured for soda-fermented sludge was 9.5 LMH. Compared to soda, the flux was increased by 24% (12.5 LMH) in the period where wollastonite fermented sludge was used. The fermented sludge filterability was evaluated through CST and TTF tests. The addition of 10 g L of wollastonite decreased the CST and TTF (by 51% and 59% respectively), resulting in more favourable dewatering characteristics (Figure 6.15(b)).

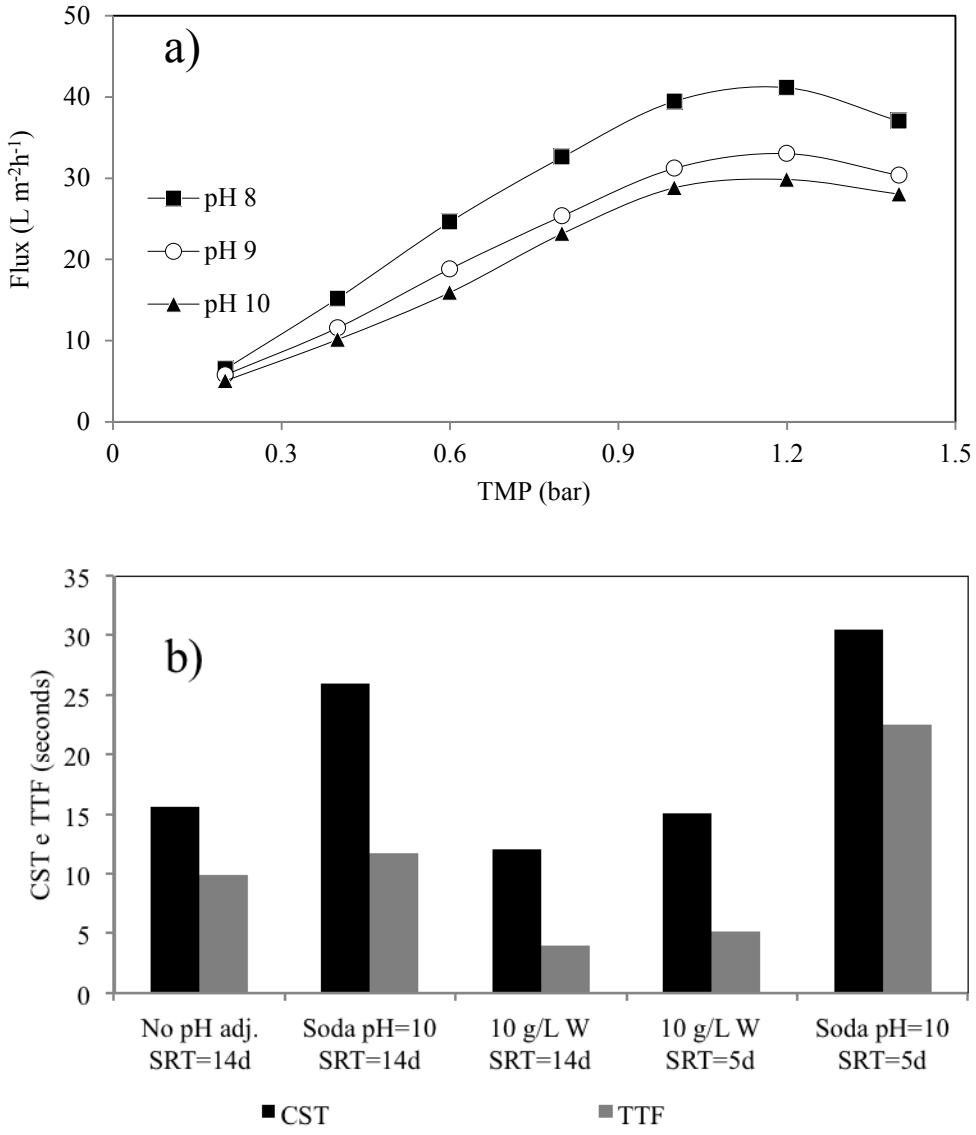

Figure 6.15.(a) Permeate flux versus TMP for different pH values of the soda fermented effluent (20 °C), TS= 14.2 g L; (b) CST and TTF of the fermented effluent for the different experimental periods.

However, during the whole operation of the membrane unit clogging phenomena in the membrane module and in the feed pumps frequently occurred due to the presence of fibrous materials in the sludge fed to the fermentation reactor. When these problems occurred it was necessary to stop the filtration process and manually remove manually such materials from the internal part of the membrane modules. The maintenance of the pumps was performed on weekly basis or when a sudden drop of membrane permeability was recorded. The implementation of an effective pre-treatment step is essential. The use of grinding, upstream of

the fermentation, could eliminate the occurrence of this problem.

6.4.4 Mechanism of nutrients removal treating anaerobic supernatant from sewage sludge digestion

The type and composition of organic carbon source significantly impacted on the sNUR and sPUR. The use of SFL resulted in significantly higher denitritation rates than the ones achieved by HPr (Table 6.10). The increase of the denitritation rate in period 2 was accompanied by an increase in anoxic P uptake.

Table 6.10. Nutrient removal kinetics obtained during the in situ biomass activity tests

Parameter	Unit	Period 1 (HPr)	Period 2 (SFL)
		Average ± st.dev.	Average ± st.dev.
sAUR	mgN $gVSS^{-1}$ h^{-1}	11.47±2.76	9.10±1.28
sNUR	mgN $gVSS^{-1}$ h^{-1}	7.80±1.23	22.39±1.08
$sPUR_{anoxic}$	mgP $gVSS^{-1}$ h^{-1}	1.09±0.12	3.41±1.71
$sPUR_{aerobic}$	mgP $gVSS^{-1}$ h^{-1}	0.15±0.09	0.28±0.12

Previous research works have shown that carbon sources that contain a mixture of SCFAs, such as acetate, propionate and butyrate, can improve denitrifying via nitrite biological phosphorus removal compared to the use of acetate alone (Ji and Chen, 2010; Frison et al., 2013a, 2013b) This study shows that SCFA mixtures are superior to propionate as the sole carbon source for denitritation and P removal. The evaluation of nitrogen removal via nitrite in the SBR was carried out based on mass balances and nutrient removal efficiencies (Table 6.11).

Table 6.11. Nitrogen mass balances (values standardized with the volume of the reactor)

Parameter	Unit	Period 1	Period 2
$NL_{applied}$	(kgN $m^{-3}d^{-1}$)	0.45±0.08	0.53±0.06
$(NH_4\text{-}N)L_{effluent}$	(kgN $m^{-3}d^{-1}$)	0.01±0.001	0.01±0.001
$(NO_x\text{-}N)L_{effluent}$	(kgN $m^{-3}d^{-1}$)	0.11±0.03	0.01±0.003
$NL_{effluent}$	(kgN $m^{-3}d^{-1}$)	0.13±0.02	0.02±0.005
NL_{sludge}	(kgN $m^{-3}d^{-1}$)	0.02	0.03
Nitrogen removal	%	72±11	96±3
Nitritation	%	97±9	98±7
Denitritation	%	67±15	90±9

In the first 10 days of operation, high sAUR values were obtained (up to 24.34 mgN $gVSS^{-1}h^{-1}$), since the growth of autotrophic bacteria was favoured by the complete absence of an external organic carbon source. Once the addition of HPr was implemented, the sAUR stabilized around 11-12 mgN $gVSS^{-1}h^{-1}$, as heterotrophic denitrifying bacteria also developed. In period 2, the average sAUR was slightly lower (9.10 mgN $gVSS^{-1}h^{-1}$), but without compromising the quality of the effluent. In both periods the nitritation efficiency was high (97-98%) and the average ammonium concentration in the effluent was 15.71 and 9.46 mgNH_4-N L^{-1} for periods 1 and 2, respectively. However, in period 1 the denitritation rate was not very high; the average sNUR was 7.80 1.23 mgN $gVSS^{-1}h^{-1}$ and did not allow complete removal of nitrite during the anoxic phase, resulting in a net accumulation. The average nitrite concentration in the treated effluent was 132.9±40.8 mgNO_2-N L^{-1} and the denitritation efficiency was 67%. In period 2, SFL was applied as external carbon source to the reactor, resulting in much higher denitritation activity (sNUR was on average 22.39±1.08mgN $gVSS^{-1}h^{-1}$); nitrite removal was much greater as compared to period 1 when only HPr was applied. The denitritation efficiency was on average 90% and the average nitrite concentration in the treated effluent was only 11.6 mgNO_2-N L^{-1}. The high nitrite level in the effluent at day 113 (135 mgNO_2-N L^{-1}) (Figure 6.16) can be attributed to the higher NH_4-N concentration in the anaerobic supernatant on this day.

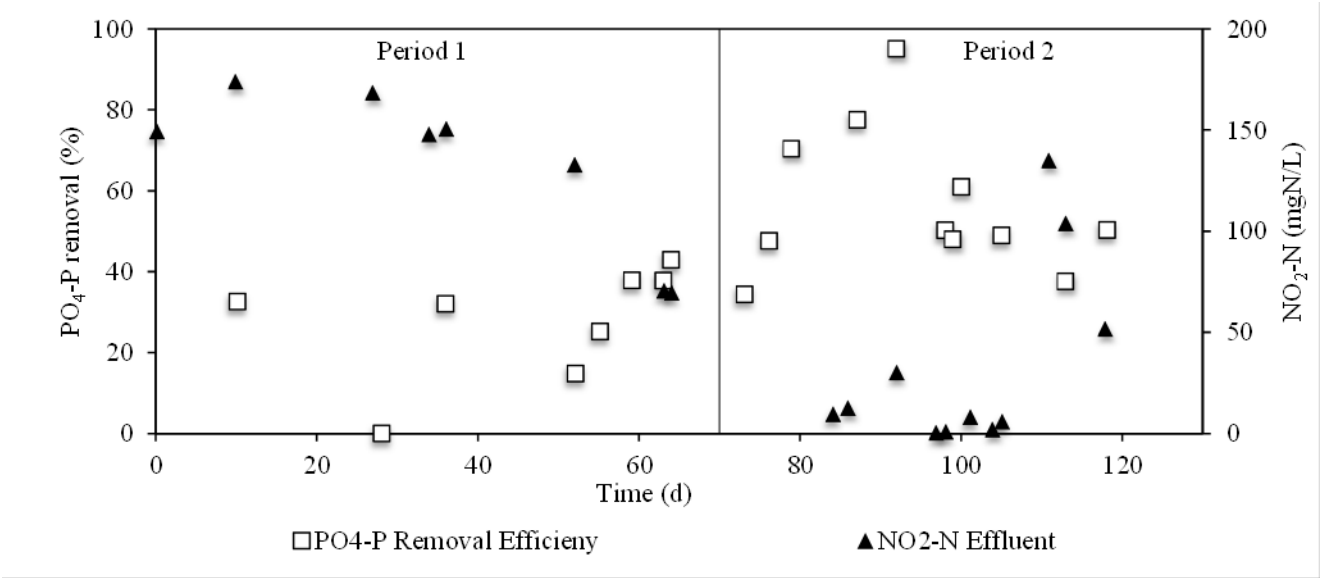

Figure 6.16. Nitrite concentration in the treated effluent of the SBR and phosphate removal efficiency (%) during the operation of the scSBR

Previous works have shown that the exposure of denitrifying phosphorus accumulating organisms (PAOs) to high nitrite concentrations can inhibit their activity (Peng et al., 2011; Saito et al., 2004). The high levels of nitrite during period 1 (Figur 6.16) resulted in low sPUR under anoxic conditions (1.09 mgP $gVSS^{-1}h^{-1}$). In the same period, the average phosphorus removal efficiency was 32%; based on stoichiometric calculations, 89% of phosphorus was removed due to the growth of biomass and only 11% of it was taken up by PAOs (Figure 6.13).

Table 6.12. Phosphorus mass balances (values standardized with the volume of the reactor).

Parameter	Unit	Period 1	Period 2
$PL_{applied}$	gP $m^{-3}d^{-1}$	16.93±2.12	15.43±3.45
$PL_{effluent}$	gP $m^{-3}d^{-1}$	11.48±1.80	6.51±0.97
PL_{sludge}	gP $m^{-3}d^{-1}$	4.73±0.72	8.72±1.46
$P_{removal}$	%	32±14	58±18
PL_{growth}	gP $m^{-3}d^{-1}$	4.20±0.53	5.87±1.01
$PL_{chemical\ precipitation}$	gP $m^{-3}d^{-1}$	<0.1	<0.1
$PL_{enhanced\ biological}$	gP $m^{-3}d^{-1}$	0.53±0.07	2.85±0.42
P removed by growth[a]	%	89±12	67±5

[a]Estimated considering the biomass stoicheometric formula $CH_{1.8}O_{0.5}N_{0.2}P_{0.015}$; [b]Percent is out of the total P removed

The P concentration in the sludge was on average 20±4 gP $kgSS^{-1}$. In period 2 (SFL as carbon source) the anoxic sPUR increased to an average of 3.41±1.71 mgP $gVSS^{-1}h^{-1}$. The use of SFL containing a mixture of VFAs was beneficial for phosphorous removal, as has also been observed by previous studies (Thomas et al., 2003; Chen et al., 2004; Oehmen et al., 2006, 2007) Furthermore, in period 2, a maximum P removal of 95% was obtained (day 92), resulting in P concentration in the treated effluent of 1.1 mgP L^{-1}. In the same period, the high nitrite level in the treated effluent at day 113, resulted in a decrease of phosphate removal to a minimum of 37%. The average phosphorus removal in period 2 was 58% out of which 67% was attributed to the growth of biomass, which is lower than the respective one observed in period 1. The P content in the cell increased to 28±3 mgP $gVSS^{-1}$.

6.5 Conclusion

This chapter focused more on the implementation and optimization of an innovative scheme integrated within two type of WWTP, for the side stream via nitrite phosphorus removal of sludge rejects water.

From the case studies analysed, the following main conclusions can be drawn:

1. In both case studies, the use of fermentation liquid produced from biowaste (i.e. OFMSW) or from sewage sludge (primary and second) seems a sustainable option enabling the reduction of the operating expenses.

2. Examining the two case studies, the phosphorus uptake occurred under anoxic condition even when high concentration of nitrite concentrations up to 140 mg L^{-1} were present.

3. However, when an anaerobic period was not present for the presence of high nitrite concentration, the phosphorus uptake was mainly attributed to heterotrophic biomass growth and partly to PAOs/DPAOs.

4. For the case study 2, the use of sludge fermentation liquid for the via nitrite nutrient removal resulted in much higher nitrogen and phosphorus removal than the use of propionic acid (22.4 mgN (gVSS h)$^{-1}$ and 3.4 mgP (gVSS h)$^{-1}$ compared with 7.8 mgN (gVSS h)$^{-1}$ and 1.09±0.12 mgP (gVSS h)$^{-1}$. The majority of phosphorus removalwas attributed to normal biomass growth rather than PAO activity (89% in period 1 and 67% in period 2).

5. The long-term operation of the pilot sludge fermentation-membrane separation unit suggested that the use of wollastonite could give advantages, maintaining relatively high pH, thus increasing the VFA production, enhancing filterability and limiting the release of ammonium and phosphate in the permeate. The sludge dewatering characteristics and the separation process were adversely affected from the use of soda during the fermentation process.

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7 Chapter. Via-nitrite biopolymers recovery by integrating the existing sewage sludge treatment trains

In this chapter, the recovery of high added value biopolymers from the sewage sludge handling was investigated. The process integrated the via-nitrite processes for the side stream nitrogen removal with the feast and famine alternation with the aim to select PHA storing biomass.

7.1 Introduction

Polyhydroxyalkanoates (PHA) are completely biodegradable polymers that can be produced naturally by many species of bacteria. The family of PHA polymers, including polyhydroxybutyrate (PHB) and PHB-related copolymers, is very versatile and thus, presents significant opportunities for marketability (Salehizadeh H and van Loosdrecht, 2004; Sagastume et al., 2011). The most applied industrial PHA production adopted bacteria from pure cultures using synthetic substrate. However, the PHA market is still limited and not competitive with the conventional plastics from petroleum origin due to cost limitations. Sterile conditions, substrate availability and high energy demand are necessary preconditions to sustain the process. Furthermore, the cost of raw materials plays a significant role for the overall cost of the process and alternative substrates derived from waste streams could potentially increase the cost sustainability of the process (Braunegg et al., 2004). Research studies have demonstrated the feasibility to produce PHA from mixed cultures using renewable organic wastes and/or industrial effluents as a carbon source. Activated sludge from wastewater treatment plants (WWTPs) is a well-known source of PHA-storing organisms that use the stored polymer as carbon and energy source under transient conditions. In the case of open, mixed cultures, the PHA production is accomplished of the following operational units: 1-acidogenic fermentation of biodegradable organic waste for the bioconversion of the waste carbon into VFAs; 2-enrichment in a Sequencing Batch Reactor (SBR) through the selection of PHA-storing biomass from the activated sludge; 3- batch step for the PHA production or accumulation until the complete saturation. The primary and waste activated sludge (PS and WAS) could be a good substrate for the selection of PHA-storing biomass; being always abundant in the WWTPs and very costly for its disposal treatment. On the other hand, PHA production could valorise the wastewater treatment by recycling and channelling carbon towards products and reducing sludge production (Frison et al., 2014). Up to now, the selection of the PHA-storing biomass is carried out under aerobic feast and famine conditions, where bacteria are subjected to alternative high (feast) and low (famine) substrate concentrations. During the feast phase, ammonia and the substrate is taken up and stored as PHA. After, the depletion of the substrate (famine phase), the stored PHAs are consumed along with ammonia. The carbon limitation strategy was found to be

favourable for the enrichment and long-term cultivation of a PHA producing community, while the nitrogen limitation is a successful strategy for reaching high PHA contents during the PHA production step (Katja et al, 2010). The rate of PHB degradation in the famine phase is independent of the electron acceptor present. Under denitrifying conditions in activated sludge cultures, the process of storage and degradation of PHB is the same for anoxic and aerobic environments (Salehizadeh et al., 2004). Some authors have taken advantage of the storage compounds as endogenous carbon source, to satisfy the need of denitrification via-nitrite (Vocks et al., 2005). Ma et al. (2009) demonstrated that compared with the via-nitrate pathway, the via-nitrite may improve up to 20% more the total nitrogen (TN) removal and reduce the aeration costs by 24%. In addition, the via-nitrite processes (i.e. single stage nitritation, nitritation/denitritation and partial nitritation/anammox) became very frequently in the sludge line of the wastewater treatment plant (WWTP) in order to reduce the amount of nitrogen that is recycled in the mainstream with the anaerobic reject water. Although the anaerobic supernatant represents a small percentage of the influent flow (usually around 3%) it increases significantly the nitrogen loading (by 10-30%) to the WWTP (Oleszkiewicz and Barnard, 2006; Cervantes, 2009), and hence the operating cost. The integration of nutrient removal with the PHA production cycle is currently a challenge. Frison et. al. (in press) examined the PHA production in batch reactors under ammonia oxidation via-nitrite. Morgan-Sagastume et al. (2010) evaluated the technical potential for the enrichment of PHA-storing organisms and the PHA-storage capacity of the enriched biomass using HPTH (high pressure thermal hydrolysis) fermented sludge at different organic and nutrient loading rates. The same authors (Morgan-Sagastume et al. 2013), examined a new concept of municipal wastewater treatment plant that enriched biomass with enhanced PHA-storage capacity. However, a post-treatment of the treated effluent was required to improve the nutrient removal, since the pilot-scale SBR removed up to 70–80% of COD, 15–30% of TN, and 30–60% of TP with respect to the incoming filtered wastewater. Meesters (1998) estimated that the energy required for the production of 1 kg of PHAs is 39 MJ/kgPHA employing aerobic feast and famine alternation. Frison et al. (2013), accomplished nutrient removal via-nitrite from real anaerobic supernatant in a pilot-scale nitritation/denitritation reactor using the carbon source from the fermentation of raw organic fraction of municipal solids waste (OFMSW).

In this work, nitrogen removal via-nitrite was integrated with the enrichment of PHA storing biomass for the treatment of anaerobic supernatant in the side stream treatment line. This work

examines a novel scheme that includes: 1) establishment selection phase characterized by carbon limitation, using the mixture of VFAs from the sludge fermentation and the nutrients contained in the anaerobic supernatant, 2) the enrichment phase is carried out under aerobic feast followed by anoxic famine conditions to accomplished the denitritation driven by internally stored PHA as carbon source.

7.2 Material and Methods

7.2.1 Novel configuration for the selection of PHA-storing biomass

The PHA production coupled with the via-nitrite nitrogen removal from the anaerobic supernatant is investigated in a three stage process: 1) enrichment of biomass with high PHA-storage capacity through the aerobic feast and anoxic famine phase for the oxidation of ammonia via-nitrite followed by denitritation driven by PHA consumption. In the 2nd configuration, the nitrites were provided from a separated reactor, which accomplished the oxidation of ammonia via-nitrite; 2) wollastonite primary sludge fermentation (WPSF) coupled with a membrane ultrafiltration (UF) for the production of fermentation liquid rich in VFAs; 3) the aerobic PHA accumulation using wollastonite sludge fermentation liquid (WSFL) using the selected biomass as a inoculum. The scheme is presented in (Figure 7.1).

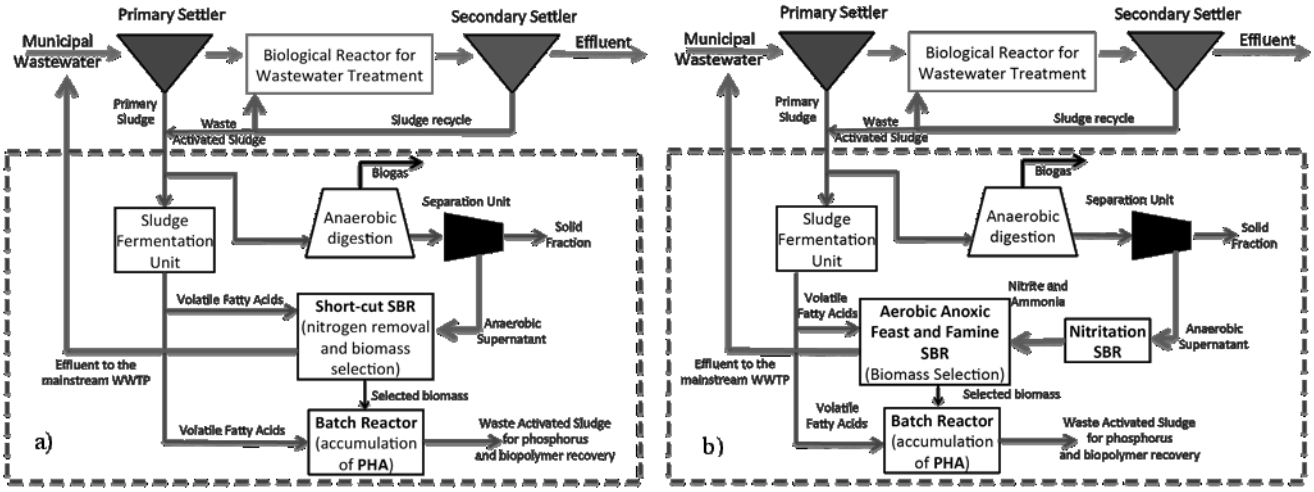

Figure 7.1. Configurations for the selection of PHA storing biomass by applying (a) the feast/famine regime and nitrogen removal via nitrite in a single stage reactor and, (b) two stage process of nitritation (first reactor for the production of electron acceptor) and feast/famine phase – reactor (PHA selection & nitrogen removal).

7.2.2 Short-cut SBR for the enrichment of PHA storing biomass

The lab scale SBR was inoculated with sludge originated from a conventional nitritation and denitritation process which using WSFL as carbon source. The initial specific ammonium uptake rate (sAUR) was 15-20 mgN $(gVSS\ h)^{-1}$, while the initial specific nitrogen utilization rate (sNUR) was up to 45±15% mgN $(gVSS\ h)^{-1}$. The oxygen level during the aerobic conditions was kept higher than 2 mg L^{-1}, while during the aerobic famine conditions, the level of oxygen increased up to the concentration of saturation. The carbon source was added instantaneous at the beginning of the aerobic phase in order to create the feast conditions. The chemical and physical analyses of the anaerobic supernatant fed into the reactor were the same cited in the chapter 6.

7.2.3 Batch test with nitrifying activity

Ex situ activity tests were conducted with the simultaneous addition of SFL and anaerobic supernatant under complete aerobic conditions. 500 mL of biomass was placed in Erlenmeyer flasks under continuous aeration (DO>4 mg L^{-1}), the temperature was maintained at 20±1°C and the pH was controlled at 7.4±0.2. The biomass was spiked with anaerobic supernatant and with SFL in order to reach an initial concentration of ≈100 mg NH_4-N L^{-1} and an initial VFA concentration of either 309±39 mgCOD L^{-1} (F:M=0.07 C-mmol C-$mmol^{-1}$) or 527±18 mgCOD L^{-1} (F:M=0.12 C-mmol C-$mmol^{-1}$) in the mixed liquor. The anaerobic supernatant and the SFL were first analyzed in order to determine the volumes to be added to reach the required concentrations of VFA and ammonium nitrogen in the mixed liquor. The selected initial ammonium concentration used is commonly met in the mixed liquor of SBR treating highly nitrogenous streams. Feast conditions were achieved through the instantaneous addition of the SFL, while famine conditions prevailed after the complete depletion of VFAs, always under aerobic conditions. The total duration of each test was 180-200 minutes. The time profile of ammonium, nitrite, nitrate, phosphate, VFAs, TSS, VSS and PHA was monitored.

7.2.4 Batch test without nitrifying activity

A similar procedure was followed as the one described above, with the difference that 50 mg L^{-1} of allylthiourea were added in the biomass in order to completely inhibit nitritation. In each test the profile of phosphate, VFAs, PHA, TSS, VSS during the time, was monitored. Nitrite and nitrate were also occasionally monitored to demonstrate that there was no nitrification during the tests.

7.2.5 The enrichment of PHA storing biomass

Configuration 1. Two experimental periods were performed in configuration 1, with different level of carbon limitation deg. The COD:NH_4-N ratio applied was adjusted by changing the volumetric nitrogen loading rate (vNLR) and keeping stable the volumetric organic loading rate (vOLR) of the reactor. Hence, in the first period, the COD/NH_4-N ratio was 5.6 by applying a vNLR of 110±27 gN $m^{-3}d^{-1}$, while in the second period the COD:NH_4-N decreased to around 2 gCOD gN^{-1} by increasing the daily volume of the anaerobic supernatant and maintaining a vNLR up to 380±29 gN $m^{-3}d^{-1}$. The SBR cycle consists of the following phases: 5 min of feeding, 330-380 min of reaction phase, 15 min of settling and 5 min of discharge. In order to evaluate the effect of the nitritation efficiency on the PHA biomass enrichment, the percentage of the aerobic versus the total cycle length was varied from 17% to 30% (Period 1, days 0 to 46) and from 30% to 50% (Period 2, days 47 to 108). As a consequence, the aerobic/anoxic ratio was varied from 0.2 to 0.4 (Period 1) and from 0.4 to 1 (Period 2).

Configuration 2. A nitritation SBR (N-SBR) with 14 L of working volume was operated at room temperature (from 20 to 25°C) as a pre-treatment for the anaerobic supernatant, in order to accomplish the ammonia oxidation via-nitrite in a separated reactor from the enrichment SBR. The N-SBR was set-up in order to perform four cycles per day with a vNLR of 0.81 kgN $m^{-3}d^{-1}$. Here, the oxidation of the ammonia via-nitrite took place at high level of nitrogen concentration, thus favouring the ammonium oxidizing bacteria (AOB), while inhibiting the nitrite oxidizing bacteria (NOB). During the cycle, the free ammonia concentration (FA) was kept higher than 1-1.5 mgN L^{-1}, working at high nitrogen concentration and pH in the range of 7.5-8. However, the alkalinity that was consumed during the ammonia oxidation via-nitrite was replaced by dosing

sodium hydroxide (aqueous solution NaOH 30%), according to a ratio of 2.2-2.3 kgNaOH $kgNH_4$-N^{-1} removed (Gustavsson et. al., 2008). During the operation, the specific activity of the ammonium oxidizing bacteria was determined by performing in-situ activity tests followed the procedure described in Frison et al. (2014). The sAUR was 21.3±4.6 mgN $(gVSS\ h)^{-1}$. The HRT was 0.54 days, while the SRT of the SBR was maintained around 15 days. At the end of the aerobic cycle, around 85-90% of the ammonium was oxidized to nitrite, while the production of nitrate was negligible. Therefore, the final ammonium nitrogen concentration in the effluent was in the range of 25 to 67 mg L^{-1}, while the nitrite level was around 350 mgN L^{-1}. The effluent was collected in a tank and it was pumped in the enrichment-SBR during the anoxic phase, according with a volumetric nitrite loading rate of 423±95 gNO_2-N $m^{-3}d^{-1}$. The total nitrogen loading rate was 530±111 gNH_4-N $m^{-3}d^{-1}$. The organic loading rate was kept stable in the range of 730-920 gCOD $m^{-3}d^{-1}$ (2.2±0.1 gCOD gNO_2-N^{-1}), dosing the required amount of WSFL based on the load of nitrite to be denitrified. The cycle operation of the enrichment reactor consisted of 50 minutes of aerobic feast conditions by dosing the carbon source followed by 250 minutes of anoxic famine conditions by feeding the nitrite at the beginning of the anoxic cycle. The HRT of the reactor was 1 day, while the SRT was kept stable at 12-15 days.

7.2.6 Batch reactor for PHA accumulation

The biomass enriched with PHA storing microorganisms was taken under starving conditions at the end of the SBR cycle and used as new inoculum for a fed on demand-batch reactor to examine the PHA accumulation capacity of the biomass. The biomass was concentrated gravimetrically applying 30 min of settling and removing the supernatant in order to reduce the nutrient level from the enrichment reactor. The dewatered biomass was placed in a plexiglass reactor with a working volume of 2 L, equipped with probes to measure the dissolved oxygen, pH and temperature. The on line signals were automatically recorded. The DO level was always maintained above 2 mg L^{-1}. The OUR was determined using the respirometer MARTINA (SPESS, Italy). The reactor was provided with one blower to diffuse air in the mixed liquor. The WSFL was used as a source of VFA during the accumulation. The substrate was divided in fixed volume aliquots in order to dose each time about 1 gCOD L^{-1} of VFAs. The time dosage was decided based on the OUR profile, when consecutive OUR values were 50% less than the previous ones. The accumulation lasted 6-8 h.

7.3 Results and discussion

7.3.1 PHA production under nitrifying conditions

The PHA accumulation was carried out in a batch reactor. The SFL was spiked at the beginning of the experiment and continuous aerobic conditions were maintained throughout the experiment. The 'feast' conditions were defined as the period in which the VFAs were present in the mixed liquor, while the 'famine' period started once the VFAs were depleted. Figure 7.2 presents the nitrite, phosphate and PHA time profile during the aerobic batch tests and Figure 3 the respective VFA concentration. Four cases where examined: a) low F:M (0.07 C-mmol C-mmol^{-1}) without nitritation, b) low F:M with nitritation, c) high F:M (0.12 C-mmol C-mmol^{-1}) without nitritation, d) high F:M with nitritation. The nitritation rate was much higher when the lower F:M was applied (14.6 mgN (gVSS h)$^{-1}$ compared to only 3.3 mgN (gVSS h)$^{-1}$ when the F:M increased). The increased F:M ratio favoured the growth of heterotrophic biomass at the expense of nitrifying bacteria. The uptake rate of the VFAs by bacteria depends on the type of the carbon source that is applied. The use of SFL as carbon source with the application of F:M = 0.07 C-mmol C-mmol^{-1} resulted in a PHA production rate of 0.05 C-mmol (C-mmol h)$^{-1}$ without nitrifying activity; the rate increased to 0.07 C-mmol (C-mmol h)$^{-1}$ when nitritation also took place. The increase of F:M to 0.12 Cmmol (C-mmol h)$^{-1}$ led to PHA production rate of 0.06 C-mmol (C-mmol h)$^{-1}$ (without nitrifying activity) and 0.08 C-mmol (C-mmol h)$^{-1}$ with nitritation. The F:M ratio did not seem to critically affect the PHA production rate, likely since the PHA yield depends on the activity of the PHA storing biomass that was selected. The PHA storing biomass was developed in the SBR process under similar operating conditions for the biomass, and thereafter the high and the low F:M were subsequently applied in the batch reactor. In the batch process, only one spike with SFL was conducted in order to record the PHA production rate (i.e. no feast-famine selection). Since the previous SBR operation was similar, the spiking of carbon source at different F:M was not expected to significantly affect the production rate. The PHA yield was 0.60-0.63 C-mmol (C-mmol VFA h)$^{-1}$ when nitritation and VFA depletion simultaneously occurred and 0.63-0.65 C-mmol of PHA per C-mmol of VFA when nitritation was inhibited. This shows that the nitritation process did not inhibit PHA production, suggesting that the two processes could be achieved simultaneously. Morgan-Sagastume et al. (2010,2014) achieved similar maximum PHA content under aerobic feast and famine with that in the current

work using VFAs derived from the WAS fermentation for PHA accumulation. However, the authors enhanced the quality of the carbon source by removing nitrogen and phosphorus as struvite. The yields of the PHA, PHB and PHV produced in the present work were calculated on the basis of VFAs uptake and are given in Table 5 in comparison with existing literature data. (Morgan Sagastume et a.l., 2010; Jiang et al., 2009; Din et al., 2013; Marang et al., 2013; Moralejo-Garate et al., 2013, Jiang et al., 2011, Menmeng et al., 2009, Valentino et al., 2013, Albuquerque et al., 2010) Figure 3 shows the depletion of VFAs over time for the four examined cases. In all four cases, the uptake rate of VFAs was comparable as it was in the range of 0.08-0.10 C-mmol/C-mmolh. The F:M ratio plays an important role during the enrichment of the bacterial community.21In this study, the uptake rate of the total VFAs derived from the SFL was not significantly affected by the F:M. As it has been previously explained, the operating conditions in the SBR were the ones influencing the PHA yields in the batch reactor, and similar VFA uptake rates are expectable provided that an excess of carbon source is applied during batch operation. In both examined cases (with and without nitritation), the concentration of phosphorus decreased by 7 to 9 mgPO4-P/L during the depletion of VFAs from the initial PO4-P concentration of 15 mg/L; the latter was partly (50%) attributed to the growth of biomass, and partly (50%) to the activity of some organisms with high phosphorus storing capacities. The results demonstrated that under aerobic conditions, PHA production can take place at significant rates under both nitrifying (to nitrite) and non-nitrifying conditions. When nitritation also took place, the PHA production rate even increased by 15-20%. In any case, This article is protected by copyright. All rights reserved there was no adverse effect on PHA production when nitritation also took place. Therefore, the production of PHA can be feasible within the normal operation of the via nitrite SBR, which could facilitate the simultaneous operation of both processes.

Type of carbon Source	F/M or OLR (C-mmol/C-mmol)	sAUR (mgN/gVSSh)	PHA content (C-mmol/C-mmol)100	SCFA uptake rate (C-mmol/C-mmol h)	PHA production rate (C-mmol/C-mmol h)	PHA/SCFA (C-mmol/C-mmol)	PHB/SCFA (C-mmol/C-mmol)	PHV/SCFA (C-mmol/Cmmol)	Reference
SFL	0.07	No nitritation	15	0.08	0.05	0.63	0.60	0.08	Current study
SFL	0.12	No nitritation	14	0.09	0.06	0.65	0.46	0.21	Current study
SFL	0.07	14.6	10	0.09	0.07	0.63	0.54	0.08	Current study
SFL	0.12	3.3	20	0.10	0.08	0.60	0.39	0.22	Current study
Fermented WAS pre-treated via high –pressure thermal hydrolysis	187 C-mmol/L d (as acetic acid)	-	28	-	0.04-0.05	0.4		-	Din et al., 2013
Synthetic municipal wastewater	96 C-mmol/L d (as acetic acid)	-	15	-	0.075	0.24		-	Jing et al., 2009
Butyrate	34.7 C-mmol/L (initial values)	-	57 (wt)	-	6.7	-	0.93	-	Thomas et al., 2003
Acetate/Butyrate	29.3 C-mmol/L, 12 C-mmol/L (initial values)	-	54 (wt)	-	1st feast phase: Butyrate 6.0, Acetate 0.67; 2° feast phase: Butyrate 1.8, Acetate 3.7	-	1st feast phase: 0.90; 2nd feast phase: 0.66.	-	Thomas et al., 2013
Acetate	-	-	52 (wt, Feast)	-	2.2	-	0.67	-	Thomas et al., 2013
Palm oil effluent	-	-	-	-	0.343	0.80	-		
Alkaline SFL	-	-	56.5 HB:HV Ratio 88.1:11.9 (no acclimation)	-	0.23	-	-	-	Ji and Chen, 2010
Mixture 85% Hac, 15%HPr (COD based)	1.55 (F/M)	-	11.6±0.40 (C-mmol/L)	-	0.15 (as PHB)	0.29 (as PHB)	-	-	Valentino et al., 2013
Molasses	-	100/8/1 (for feast/famine); 100/0/0 for PHA accumulation (1.4 mgN/L)	-	-		0.65±0.01	0.11	-	Albuquerque et al., 2010
Glycerol C/N 76 and 770	1.94 and 0 25 C-mmol/C-mmol cycle (F/M)	-	-	-	0.30 (Feast to Famine 0.03); 1.11 (Feast to Famine 0.08)	-	0.19 (Feast to famine 0.03); 0.33 (Feast to Famine 0.08)	-	Moralejo-Gárate et al., 2013

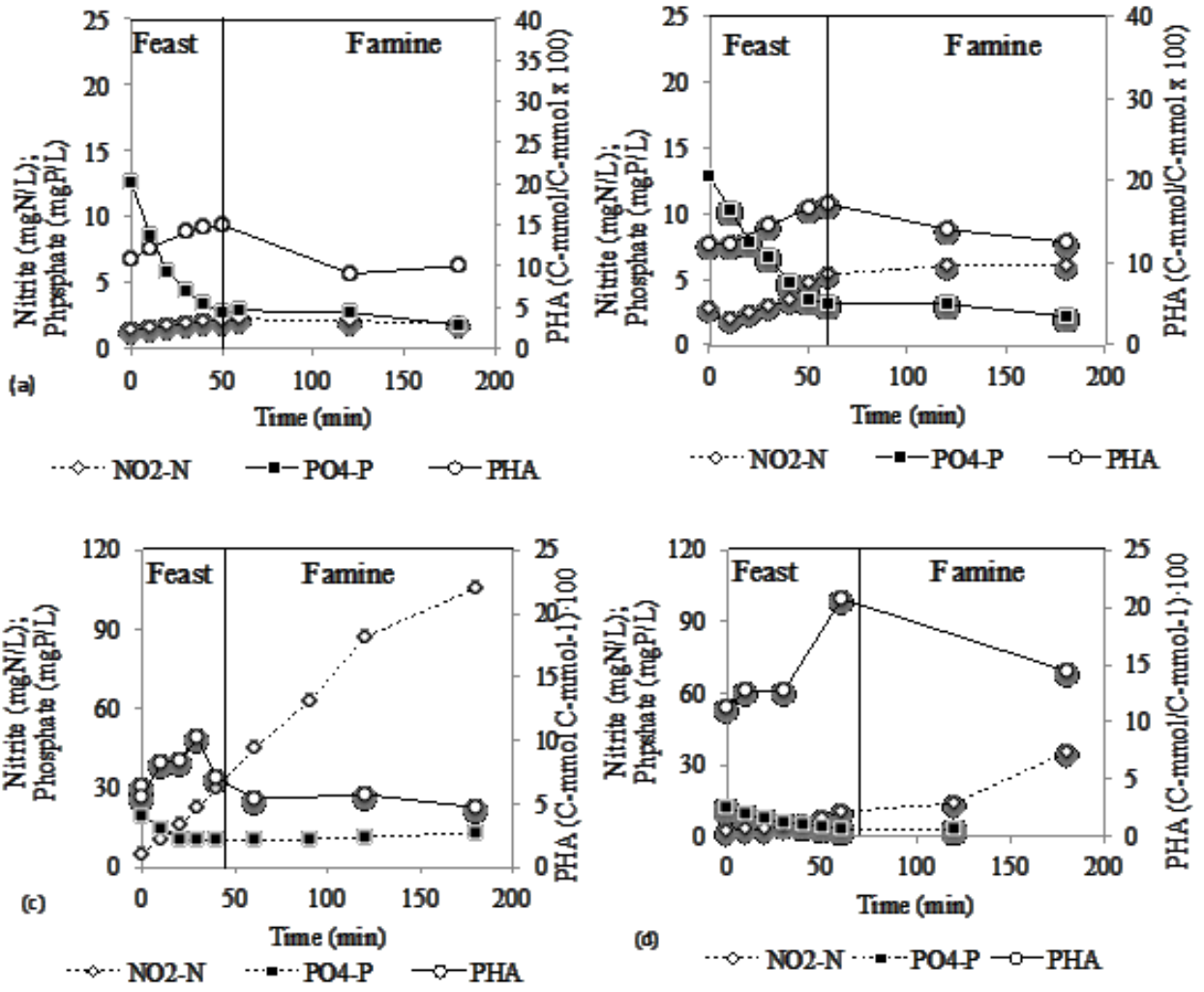

Figure 7.2. Nitrite, phosphate and PHA time profile during the batch tests under aerobic conditions a) F:M=0.07 C-mmol C-mmol^{-1} without nitritation, b) F:M=0.12 C-mmol C-mmol^{-1} without nitritation, c) F:M=0.07 C-mmol C-mmol^{-1} with nitritation, d) F:M=0.12 C-mmol C-mmol^{-1} with nitritation.

7.3.2 Control of VFAs uptake by pH

The pH profile is a reliable control parameter for real time bioprocess monitoring. This study addressed whether it can be used as an indirect parameter to determine the end of the feast and the beginning of the famine conditions. As observed in Figure 7.3, when nitritation did not occur, the complete consumption of VFAs during feast conditions could be identified following the pH profile; the pH rapidly increased as the VFAs were consumed and the concentration of H^+ in the

liquid diminished. Then, the apex in the pH curve indicated the complete depletion of the VFAs, while in famine conditions the pH stabilized at 8.5 or slightly higher due to CO_2 stripping. When both nitritation and VFA depletion took place, the former process resulted in a decrease of the pH and the latter process in the increase of pH. In practice, the net increase or decrease of pH depends on the rate of each process. At the beginning of the feast period, the nitritation rate was very low, since the addition of the external carbon source created favourable conditions for heterotrophic rather than autotrophic biomass.

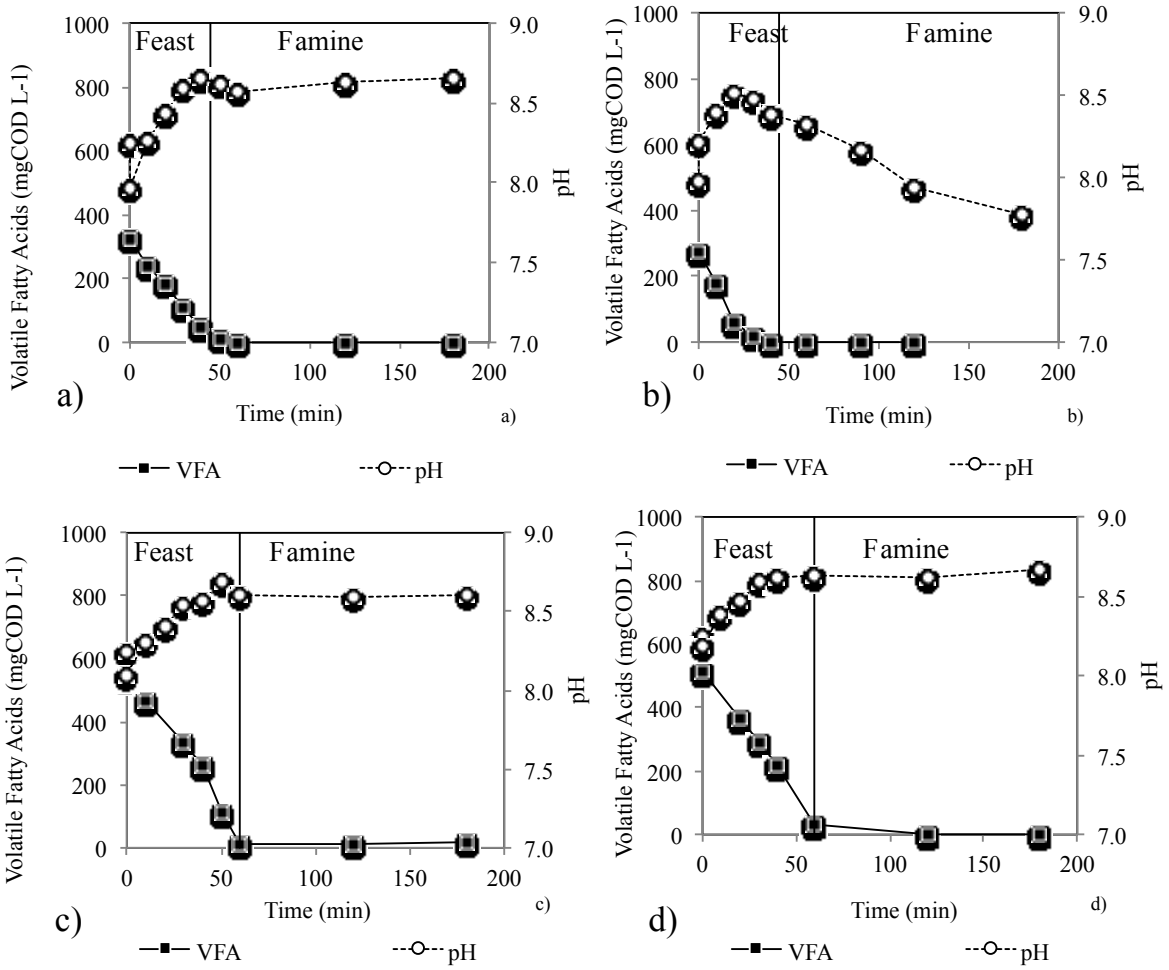

Figure 7.3. Total Volatile Fatty Acids and pH time profile during the batch tests under aerobic conditions a) F:M=0.07 C-mmol C-mmol^{-1} without nitritation, b) F:M=0.07 C-mmol C-mmol^{-1} with nitritation, c) F:M=0.12 C-mmol C-mmol^{-1} without nitritation, d) F:M=0.12 C-mmol C-mmol^{-1} with nitritation.

Thus, the depletion of VFAs was fast. As a result, the pH profile during the first 20 minutes closely resembled the pH profile of the case where nitritation was inhibited. As the time progressed, the nitritation rate increased. At a certain point the pH increase caused by the depletion of VFAs was similar to the pH consumption by nitritation, thus creating the pH apex. After this point, the nitritation resulted in a higher decrease of pH than the pH increase caused by VFAs depletion. This is why the pH dropped. In such a case, the time at which the pH apex occurs depends on the relative rate of VFAs depletion versus ammonia oxidation. Therefore, pH cannot be as readily used as a control parameter to establish the termination of the feast phase. On the contrary, it can be a reliable indirect parameter that signals the end of the feast period when the nitritation process does not take place.

7.3.3 Efficiency of configuration 1

Period 1. The biomass, after the addition of the WSFL, showed the typical feast and famine response. In the first period the length of the aerobic-feast phase was approximately 15.8±0.4% (50 min) of the total cycle.

Figure 7.4 shows representative profiles of nitrogen forms, VFAs and PHA that were obtained during the aerobic-feast and anoxic-famine cycles of the SBR for the first examined configuration. During the aerobic-feast phase, the VFAs were taken up with a constant rate (-qVFA) of 108.5±6.2 mgCOD (gCOD h)$^{-1}$. The biomass PHA concentration increased during the first 50 min, reaching a maximum concentration of 0.06 to 0.07 gCOD per gCOD of biomass. However, during the aerobic-feast phase only 36.4±10.7% of the influent ammonium nitrogen was oxidized to nitrite with a constant rate of 4.18±1.93 mgN (gVSS h)$^{-1}$, producing only 6.5 mgNO_2-N L^{-1}. The ratio between the COD stored as PHA and the nitrite concentration at the beginning of the anoxic-famine phase was around 15.5, showing that the degradation of the PHA was not complete. The denitrification via-nitrite occurred with a constant rate of 3.69±2.60 mgNO_2-N (gVSS h)$^{-1}$; the consumption of the total stored PHA was not more than 41±1%. During the days 15 to 20 of the SBR operation (Figure 7.5) the PHA fraction that was accumulated in the biomass reached a maximal concentration of 0.207 $gCOD_{PHA}$ $gCOD_X^{-1}$. The presence of residual PHA was estimated directly in the reactor, by spiking 100 mgNO_2-N L^{-1} after that the nitrites were denitrified (Figure 7.4, red line). The latter demonstrated that the

nitrite produced during the feast conditions were not enough to oxidize all the PHA produced. During the first period, the biomass lost the VFA uptake capacity and the VFA uptake rate dropped up to 4 mgCOD (gCOD h)-1 (Figure 7.5) The PHA yield decreased, as well, from 0.31 to 0.07 $gCOD_{PHA}$ $gCOD_{VFA}^{-1}$ consumed. In order to enhance the nitrification efficiency, thus the nitrogen removal efficiency, the length of the aerobic phase was extended up to 100 min (days 28 - 50), maintaining constant the vNLR and the vOLR. This strategy resulted in the increase of the amount of nitrite produced in the aerobic phase (up to 26 $mgNO_2$-N L^{-1}), while the nitrogen removal increased up to 70.1%. Furthermore, higher duration of the aerobic phase aided the consumption of the PHA, since there was more availability of the electron acceptors.

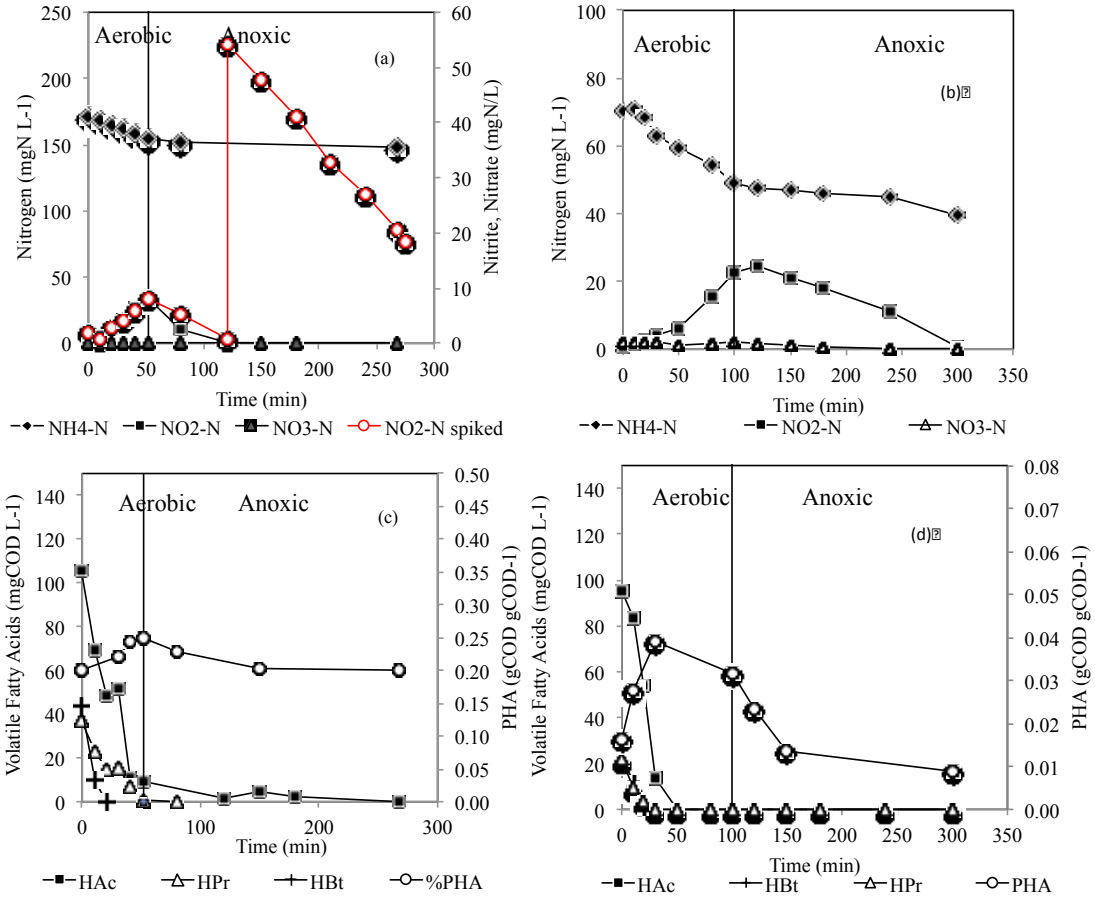

Figure 7.4. a) and b) Typical profiles of nitrogen forms during the aerobic-feast/anoxic-famine in period 1 and period 2 respectively for configuration 1. The red cyrcle shows (a) the nitrite profile after the spiking of nitrite at time 120 min, c) and d) VFAs and PHA profile during the feast and famine phase of the SBR operation .

The VFAs uptake rate increased very fast within the next 10 days of the SBR operation, reaching an average value of 130±34 mgCOD (gCOD h)$^{-1}$. The PHA storage capacity of the biomass was also 'recovered' after the extension of the aerobic conditions. The latter was attributed to the enhanced the consumption of PHA during the famine conditions due to the desirable oxygen level and the higher nitrite concentration during the anoxic phase. After the 'aerobic feast' phase, 31.2% of the PHA that were previously accumulated in the biomass was subsequently consumed during the 'famine aerobic' phase before the initiation of the anoxic-famine period. This resulted enhanced PHA (as COD) storage. The denitration was driven by the residual PHA, with a constant rate of 4.82±0.76 mgNO_2-N (gVSS h)$^{-1}$. As a consequence, the PHA content at the end of the famine conditions decreased till to obtain a constant value of 0.004 - 0.007 gCOD_{PHA} gCOD^{-1} of biomass. Although the denitritation efficiency was always higher than 94%, the average nitrogen removal in that period was low (35.5% as average value). Increasing the length of the aerobic phase (i.e. increasing the efficiency of nitritation) the efficiency of the nitrogen removal increased up to 70.1%.

Period 2. Once the feast and famine cycle and the nitrogen removal efficiency showed a steady state profile, (day 50, Figure 7.4b) and d)) we decided to increase the vNLR to 0.38±0.03 kgN m^{-3}d^{-1} in order to enhance the treatment capacity of the system also in terms of nitrogen removal. The sAUR slightly increased up to 5.5±0.5 mgN (gVSS h)$^{-1}$ (average value) and within 100-150 min of aerobic conditions, the nitrite concentration was around 32 mg L^{-1}. The nitrites produced under aerobic conditions were denitrified almost completely during the famine phase, with an average denitritation rate (sNUR) of 3.74±0.48 mgN (gVSS h)$^{-1}$. The denitritation rate was lower compared to the respective one obtained in period 1. The latter was probably attributed to the lower ratio of COD as PHA stored vs NO_2-N (2.0-2.2 gCOD gNO_2-N^{-1}) available at the beginning of the famine phase. However, the stored carbon was still enough to achieve complete denitritation using PHA as the only carbon source. The nitrogen removal efficiency in that period was 44.2±3.4%. The good capacity of biomass to uptake VFA and to store PHA was confirmed by the stable VFAs uptake rate found of 134.6±32 mgCOD gVSS^{-1}h^{-1} (Figure 7.5). Although this rate was comparable with the respective one obtained in period 1, the yield of PHA production increased from 0.26±0.2 to 0.38±0.03 gCOD_{PHA} gCOD_{VFA} $^{-1}$. After the complete depletion of VFA, the maximal PHA content increased up to 0.02-0.05 gCOD_{PHA}

$gCOD^{-1}$ of biomass, while at the end of the famine phase, it was less than 0.01 $gCOD_{PHA}$ $gCOD^{-1}$ of biomass.

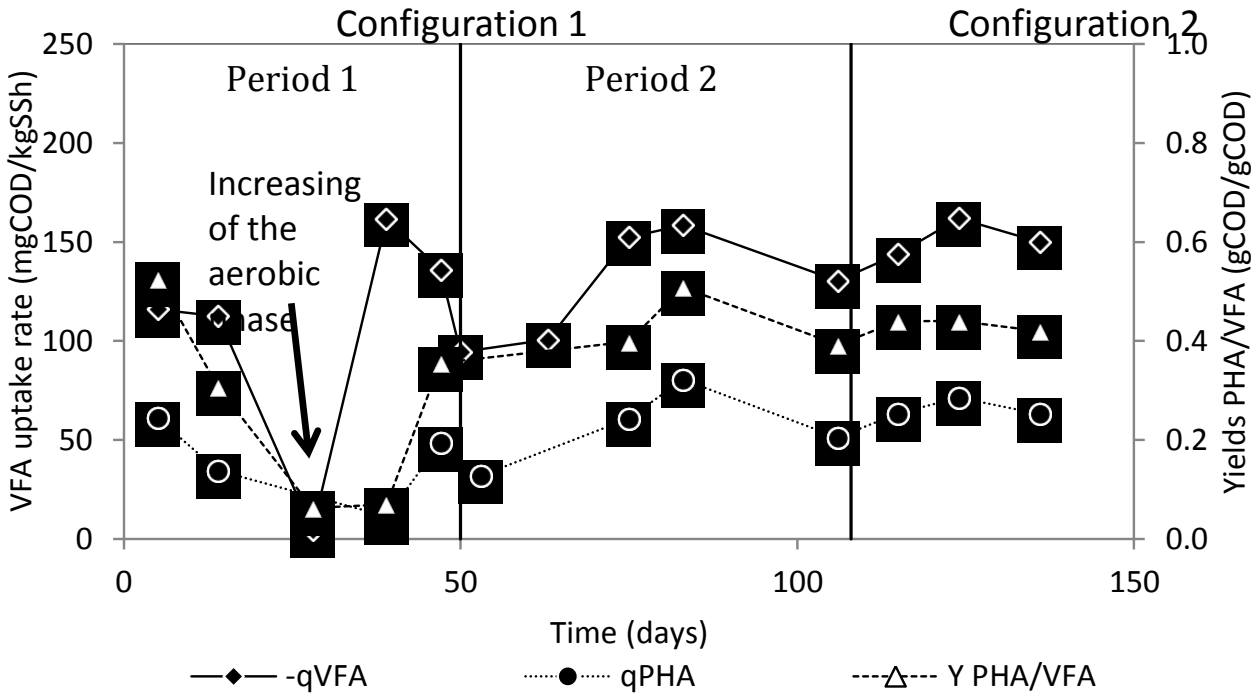

Figure 7.5. VFA uptake rate and PHA yield (gCOD $gCOD^{-1}$) for the configuration 1 (PHA selection - stage).

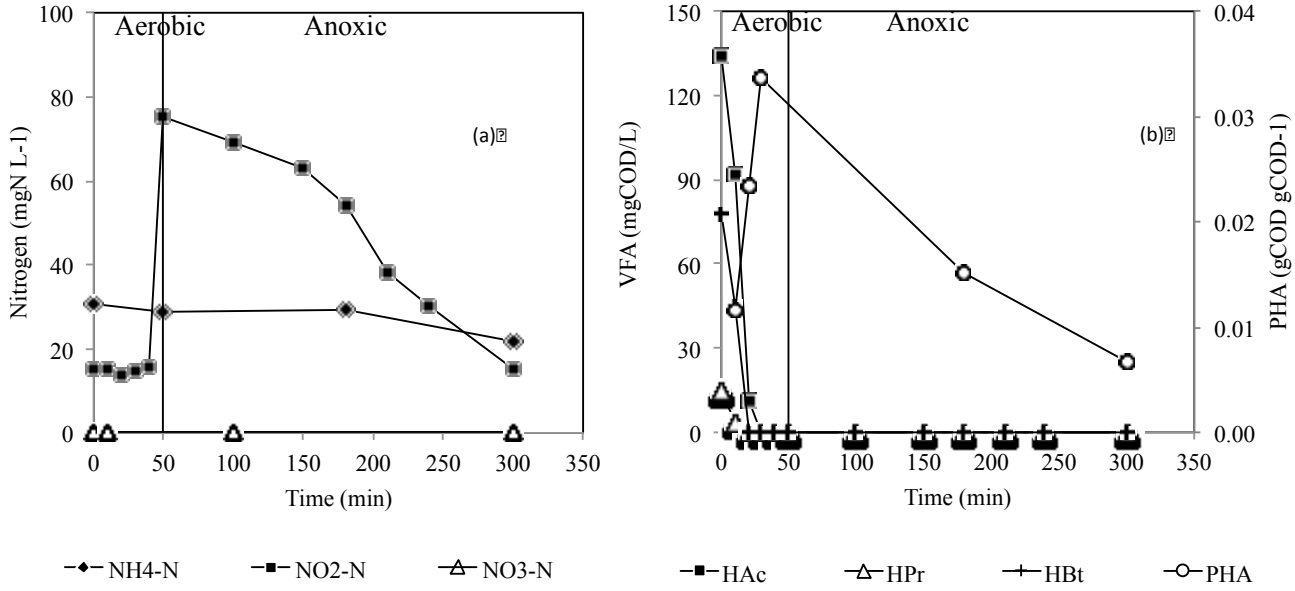

Figure 7.6. Typical profiles of (a) nitrogen forms and (b) VFAs and PHA during the aerobic-feast and anoxic-famine phase in configuration 2.

7.3.4 Efficiency of configuration 2

Figure 7.6 shows the typical profiles of nitrogen, PHA and VFAs observed in the SBR adopting the second configuration (from day 108 to 136; Figure 7.5 for the enrichment of the PHA storing biomass. During the aerobic phase the VFA were completely depleted after 30 min, with a specific uptake rate of 152±9.16mgCOD $(gVSS\ h)^{-1}$. The biomass enriched in the SBR showed a slightly increase of PHA uptake rate (qPHA = 65±4.7mgCOD $(gVSS\ h)^{-1}$) compared with the operation of configuration 1. However, the PHA yield did not change and similar average content (433±10 $mgCOD_{PHA}\ gCOD_{VFA}^{-1}$) was obtained in comparison with configuration 1. The PHA concentration after 30 min of aerobic-feast phase, increased rapidly from 0.02 to 0.033 gCOD of PHA per gCOD of biomass. After 50 min of aerobic phase, the anoxic-famine phase started by switching off of the blower and by feeding the nitrites previously produced in the nitritation SBR. Figure 7.6 (a) shows the increasing of the nitrite content during the anoxic phase up to 75 $mgNO_2\text{-}N\ L^{-1}$. During the famine conditions, the PHA were consumed, reaching a minimum concentration of 0.006 $gCOD_{PHA}\ gCOD^{-1}$ at the end of the anoxic cycle, indicating that almost all the stored PHAs were degraded. Additionally, during the anoxic phase, the ammonium nitrogen decreased from 28 to 21 $mgN\ L^{-1}$; reduction that correlated with the growth of the PHA storing bacteria. Considering the complete cycle, ammonia removal efficiency was around 30%. The nitrite at the end of the cycle decreased up to 15 $mgNO_2\text{-}N\ L^{-1}$. However, additional denitritation occurred (reduction of the nitrite concentration to 5 - 8 $mgNO_2\text{-}N\ L^{-1}$) during the settling of biomass without sludge rising problems. The specific denitritation rate was 5.77 $mgNO_2\text{-}N\ (gVSS\ h)^{-1}$. The application of configuration 2 (nitritation + enrichment SBR) resulted in a nitrogen removal efficiency of 86.8±4.4%, for vNLR of 0.53±0.11 $kgN\ m^{-3}d^{-1}$.

Table 7.1. Performance of configuration 1 & 2 for PHA biomass enrichment; –qVFA:,VFA uptake rate, qPHA, PHA accumulation rate, $Y_{PHA/VFA}$, storage PHA yield based on the VFA uptake.*The efficiency of the nitritation with the enrichment SBR is considered.

Operating conditions	**Configuration 1**		**Configuration 2**
	Period 1	**Period 2**	
vNLR $(gN\ m^{-3}d^{-1})$	0.11±0.03	0.38±0.08	0.53±0.11
VFA:NH_4-N $(gCOD\ gN^{-1})$	5.6±0.06	2.0±0.02	2.2±0.1 (VFA:NH_4-N)
F:M $(gCOD\ gVSS^{-1})$	0.33±0.08	0.22±0.05	0.20±0.07
Aerobic/Anoxic	0.30±0.14	0.73±0.37	0.20

Performance	Configuration 1		Configuration 2
	Period 1	Period 2	
Eff. of nitritation (%)	*35.7±13.1(1.0-70.4)*	*46.2±1.1 (39.9-52.5)*	*89.3±3.1**
Eff. of denitritation (%)	35.5±13.0 (0.9-70.1)	41.1±1.0 (37.9-52.5)	86.8±4.4*
PHA end feast (mgCOD $gCOD^{-1}$)	120±10 (30-200)	27±11	33±4
PHA end cycle (mgCOD $gCOD^{-1}$)	-	18±16	1±0.3
Feast phase fraction (min min^{-1})	0.18	0.14	0.17
-qVFA (mgCOD (gVSS $h)^{-1}$)	106.7±36	134.6±32	152±9.16
qPHA (mgCOD (gVSS $h)^{-1}$)	48.0±9.4	56.1±5.4	65±4.7
$Y_{PHA/VFA}$ (mgCOD $gCOD^{-1}$)	260±20	430±70	433±10

7.3.5 PHA accumulation and properties of the final biopolymer

The maximal capacity of biomass to store PHA was evaluated during the period 2 (from the day 63), when the SBR operation for the biomass enrichment was stable. The harvested biomass from the SBR was taken at the end of the anoxic phase, when the PHA content in the biomass exhibited the minimum concentration. At the end of the accumulation (after 6-8 h), the biomass was able to accumulate 0.16±0.02, 0.19±0.04, and 0.35±0.04 gCOD $gCOD^{-1}$ when primary sludge fermentation liquid (COD:N:P = 100:9.7:2.1, N and P no limited), primary sludge fermentation liquid with wollastonite (COD:N:P = 100:7.8:0.06, P- limited), and mixture of VFA (COD:N:P = 100:0:0, N and P-limited) were respectively applied (Table 7.2).

Table 7.2. Performance of the batch PHA accumulation using different types of carbon source

Parameter	Synthetic mixture of VFA	WSFL	SFL
COD(VFA):NH_4-N:PO_4-P	100:0:0	100:7.8:0.06	100:9.7:2.1
-qVFA (mgCOD (gCOD $h)^{-1}$)	177.4 ± 15.7	133.3 ± 38.7	94.9 ± 22.3
qPHA (mgCOD (gCOD $h)^{-1}$)	81.7 ± 12.6	40.0 ± 4.6	25.3 ± 6.7
PHA (gCOD $gCOD^{-1}$) (6-8 h)	0.35 ± 0.04	0.18 ± 0.04	0.16 ± 0.02
HV (%)	65 (HV+HH)	41	42
$Y_{PHA/VFA}$(gCOD $gCOD^{-1}$)	0.51 ± 0.06	0.35 ± 0.04	0.27 ± 0.04
$Y_{X/VFA}$ (gCOD $gCOD^{-1}$)	0.20 ± 0.02	0.22 ± 0.09	0.28 ± 0.06

Table 7.3. Main properties of the biopolymers obtained with the different carbon sources after the accumulation. (Mw: weight average molar mass, PDI: polydispersity index, Mn: number average molar mass, Td-trans: decomposition temperature (DSC analyses), Tg: glass-transition temperature, Tm: melting temperature, ΔHm: melting enthalpy).

Carbon source	**Mw (g/mol)**	**PDI (Mw/Mn)**	**Tg (°C)**	**T_{m1} (°C)**	**T_{m2} (°C)**	**ΔHm (J/g)**	**$T_{d\text{-}trans}$ (°C)**
Synthetic mixture of VFA	643289	1.35	-1.1	138	147	21	267
SFL	677573	1.30	-0.5	136	144	24	275
WSFL	791949	1.22	-1.6	141	153	27	276

Figure 7.7. Biopolymers extracted after the accumulation stage.

The biopolymer produced had similar characteristics for all the experiments in terms of 3HV and 3HB percentage; the percentage of 3HV was around 40% using the WSFL and SFL, while with the use of synthetic mixture of VFAs as carbon source, the biopolymer was a blend of 65 % of HV and HH, while the remained part was mainly composed of 3HB (Figure 7.7 shown the biopolymer extracted after the accumulation stage.

). Together with the storage activities, the carbon source was also used as substrate to grow new cells. Despite the fact that the applied carbon source presented a favourable ratio of COD:N:P to

limit the growth of new bacteria, the yield of active biomass on the substrate varied between 0.20 and 0.28 gCOD of PHA per $gCOD^{-1}$ of biomass. Nitrogen and phosphorus derived from the liquor of the enrichment reactor and had effect on the metabolic activities of the biomass, that was associated with the volatile fatty acids of the carbon source. Figure 7.7 shown the biopolymer extracted after the accumulation stage.

7.4 Conclusion

In this chapter we reported the feasibility to produce PHA from sewage sludge, based on a new concept for upgrading existing WWTP. In the current work, the via nitrite nitrogen removal was integrated with the selection of PHA storing biomass in the sludge treatment line. The integration of PHA production within a WWTP at full scale is of real added value and was the driving force for the development of our novel treatment scheme. Batch experiments showed that simultaneous nitritation and storage of PHA at significant rates are feasible. The PHA yield was 0.60–0.63 C-mmol PHA C-mmol^{-1} SCFA when nitritation and SCFA depletion simultaneously occurred and 0.63–0.65 C-mmol^{-1} PHA C-mmol^{-1} SCFA when nitritation was inhibited. Finally, it was found that the pH can be used as an indirect parameter for determining the end of the feast period only when nitritation did not occur. The pH profile was a reliable control parameter for real time bioprocess monitoring.

The enrichment of the PHA storing biomass was study in a SBR operating under aerobic-feast and anoxic-famine for nitrogen removal via-nitrite from the anaerobic supernatant. Results showed that the presence of nitrite are fundamental to guarantee complete PHA degradation under famine conditions. The good selection degree was highlight from the VFA uptake rate and the PHA production yields obtained, respectively 152±9.16mgCOD (gVSS h)$^{-1}$ and 433±10 $mgCOD_{PHA}$ $gCOD_{VFA}^{-1}$. After the 8 hours of accumulation in batch reactor, the PHA concentrations were 0.18 gCOD gCOD^{-1} and 0.16 gCOD gCOD^{-1} using respectively the WSFL and SFL, where around 40% was represented by HV. The preliminary analyses of the biopolymer extracted showed similar characteristics of other biopolimery altready present in the market and commercialized.

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8 Chapter. Conclusions

This section includes the final remark and the future trends.

8.1 Final Overview

This thesis described the applications of the via-nitrite nutrients removal processes accomplished in Sequencing Batch Reactor, for the treatment of anaerobic effluents. Compared with the conventional aerobic processes, the anaerobic treatment of municipal wastewater could give several advantages if the energetic balance is considered. Moreover, over the past 20 years, the number of municipal WWTPs in Europe has increased steadily, while their technical standard improved. Furthermore, the anaerobic digestion process became the main core for sewage sludge handling for WWTPs having a population equivalent higher than 40000.

What we are seeing from the last year is a gradual transition between an economy based on fossil fuel to an economy based on green an susistanable practice, which required synergies and scientific innovation, giving effort in the field of the wastewater treatment.

The existing on site sludge handling facilities in municipal WWTPs have significant impact on selecting technologies and eventually sizing the selected ones. Some issues that need to be addressed is whether a side-stream treatment is needed or whether there is a need to oversize the main treatment processes to handle recycle loadings from sludge thickening, digestion, and dewatering.

In addition, if the plant receives landfill leachate or similar hauled or direct waste streams, those loads should be accounted for in the basis of design. Accounting for all these loadings might lead to larger tank sizes, blowers, or chemical storage facilities. The current trend in municipal WWTPs is the installation of ‘swing zones’ equipped with both mixers and aerators; theses zones operate either as anoxic or aerobic bioreactors, depending on the plant’s conditions. However, this practice increases operating expenses and should change in near future.

The results of the current study revealed that the separate treatment of those ‘recycle loads’ before their return in the main biological treatment can be a sustainable option. By applying the envisaged side stream scheme for the removal of nitrogen and phosphorus from anaerobic digested effluents the load in the recycle stream are reduced. The scSBR is operated both under aerobic and anoxic conditions; thus alkalinity lost during nitritation will be partially recovered during denitritation. The side stream will not require neutralization before reintroduction to the mainstream, while the effluent of the SBR contains nitrifiers; the latter is expected to enhance

the nitrifying bacterial population in the main wastewater treatment line. This strategy showed in the previous chapters allows for further reduction of the reactor size, savings in aeration requirements and thus in energy demand and can retrofit/upgrade existing treatments systems or result in the development of new plants with smaller footprints, maximizing nutrients removal capabilities and bioproducts recovery.

Moving towards this direction, the thesis analysed the possible side stream configuration with the aim to integrate the process of biopolymer production with the nitrogen removal from anerobic reject water. The possibility to recovery green products with high added value from wastewater is the current challenge because could enhance the sustainability of the WWTPs. Additional market opportunities could be created by the exploitation of the novel end products, including the bioplastic PHA, the struvite and the nutrient rich compost. Thus, the examined process provides true added value towards the effective treatment of nitrogen in highly contaminated effluents within WWTPs aiming at the same time to maximize resource recovery. Our results revealed that the availability of nitrite is determinant factor to ensure the success of the process. The latter is achieved with the integration of ‘a nitrite supplying stage’ (i.e. nitritation process) that secures the stability of the process and enhances its overall efficiency. Innovative technologies, such as bioaugmentation or partial nitritation are widely applied within the WWTPs.

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Ciclo: 27°

Titolo della tesi : NOVEL BIOLOGICAL SUSTANABLE SOLUTIONS TO OPTIMIZE BIORESOURCE RECOVERY AND ENERGY EFFICIENCY FROM DOWNSTREAM OF ANAEROBIC DIGESTION (S.S.D. ING-IND25/ Impianti Chimici)

Abstract:

The main activities during three years of research, focused on the investigation, optimization as well as the validation beyond bench-scale to pilot reactor, of advanced via-nitrite biological processes for the side stream treatment of anaerobic effluents. The addressing of technical barriers and providing engineering solutions is also fundamental to understand the potential transferability and the commercial interest for large scale applications of such technologies and the strategies for how to exploit it. Therefore, the experimental activities have been carried out using real substrates (e.g. anaerobic supernatant, municipal sewage sludge, OFMSW, municipal domestic wastewater), thus reflecting the real environmental and operating conditions.
The anaerobic processes have gained high attention during the last years, because of the advantages connected with the production of green energy from the biogas utilization. Compared with the consolidated aerobic applications for the treatment of wastewater, the anaerobic processes allow less energy consumption, less sludge production, elimination of the off-gas air pollution and a potential for lower carbon footprint. The upflow anaerobic sludge blanket (UASB), is one af the most upgraded technology for the anaerobic treatment of the municipal wastewater. However, the effluents still contain high amount of nutrients (nitrogen and phosphorus), which can be font of eutrophication for the water bodies. In addition, the effluents derived from the anaerobic digestion or co-digestion of the sewage sludge with organic biowaste (e.g. solid fraction of the municipal solid waste) represent an high strength nitrogenous stream which should be treated before recycled back into the main stream of the wastewater treatment plant (WWTP). Nutrients removal processes via nitrite are recognized to be a sustainable option to treat low and high strength nitrogenous effluents, such as municipal wastewater and the anaerobic supernatant from digested sewage sludge respectively. In this work, the application of innovative bioprocesses that will increase the on-site biological valorization of wastewater and sewage sludge is the major challenge.
The via-nitrite processes were investigated in a sequencing bacth reactor (SBR) for the treatment of anaerobic effluents from a UASB reactor. The municipal anaerobic UASB effluent can be successfully treated through the completely autotrophic nitrogen removal process. The activities of anammox biomass was increased up to 161% compared with the initial inoculum, which correspond with nitrogen removal rate of 2.27±1.31 mgN (gVSS h)-1 at 30 °C. The latter did not change significantly when the volumetric NLR was decreased but lower heterotrophic denitrifying activities was observed and the nitrogen conversion ratios approached to 1.32 NO2-N/NH4-N removed. The Fluorescent In Situ Hybridisation analyses (FISH) confirmed the presence of anammox bacteria and the presence of different filamentous bacteria favoured by the long solid retention time (SRT). Although nitrogen was removed effectively from anammox biomass, phosphorus cannot be eliminated by a complete autotrophic biomass. For this reason, the applicability of the integrated upflow anaerobic sludge blanket (UASB) with a short-cut

nutrient removal was demonstrated feasible by the co-treatment of domestic sewage and biowaste at decentralized level. Occurrence of the simultaneous removal of nitrogen and phosphorus via-nitrite in a robust process was investigated using the best available carbon source produced by the acidogenic fermentation of domestic organic waste (DOW) and vegetable and fruit waste (VFW). Complete nitrite accumulation (NO2-N/NOx-N>97%) was observed operating at low dissolved oxygen (<0.8 mg•L-1) together with high vNLR (= 0.19-0.21 kgN·m-3·d-1). Moreover, the presence of propionic and butyric acid in the carbon source enhanced the denitrification and phosphorus uptake rates up to 6.33±1.92 mgP•(gVSS·h)-1. The specific phosphorus uptake rate (sPUR) via nitrite route was not adversely affected by nitrite concentrations up to 50-70 mgNO2-N·L-1, and was partially inhibited for concentrations of 100-120 mgNO2-N L-1.

Coupling the short-cut SBR with a fermentation unit of biowaste was the innovative side stream scheme investigated and optimized for the treatment of anaerobic digestate. The anaeobic superantant from the co-digestion of secondary sludge and OFMSW was treated in a pilot SBR (2.8 m3 of working volume), accomplishing the via-nitrite biological nutrients removal. Successful start-up operation was achieved in 20 days, which were enough to inhibit the growth of nitrite oxidizing bacteria (NOB) operating at high level of free ammonia (FA) (up to 6 mgN L-1). The change of the bacteria population was observed sequencing the major bands from the DGGE analyses, which indicated mainly the presence of bacterial groups related to β Proteobacteria and Bacteroidetes. Among them, some of the main bands were closely related to the AOB genera, such as Nitrosomonas sp. and Nitrosospira sp. The maximal treatment potential observed was 0.8 kgN m-3d-1. The specific ammonium oxidizing bacteria (sAUR) was observed constant in the range 18-20 mgN (gMLVSS h)−1 during the experimental period. Low temperature of the anaerobic digestor, as well as excess of polyelectrolyte residual in the anaerobic supernatant after dewatering process, were commonly observed during the experimental activities, resulting in wide fluctuations of the volumetric NLR (vNLR) applied and significant fuge of flocculated active biomass from the scSBR. Although the specific ammonium uptake rate (sAUR) was maintained constant, when the vNLR was higher than the maximal biomass nitrifying capacity reduced the nitrogen removal efficiency of the system. From an economic point of view, alternative carbon sources derived from the acidogenic fermentation of biowaste (e.g. organic fraction of mucipal solids waste, cattle manure, maize silage, sewage sludge) could be a good option to replace synthetic originated carbons source (e.g. acetic acid, methanol, ethanol, glycerol). In addtition, the life cycle assessment analyses showed environmental benefits from the fermentation of the biowaste compared with the use of synthetic carbon source (0.28 kg PO4-3 m-3). High denitrifying biological phosphorus removal via-nitrite was observed under anoxic conditions using the fermentation product. The specific phosphorus removal via nitrite was 0.27 kgPO4-P (kgMLVSSd)-1 when the fermentation liquid of OFMSW was dosed, resulting in a hyper-accumulation of phosporus in the activated sludge close to 50 mgP gMLSS-1. During the via-nitrite processes large amount of N2O could be generated which is a strong greenhouse gas and therefore off-set the promising energy and costs saving. In this work, low oxygen and high nitrite concentration in the mix liquor have a negative effect on the production of N2O. Less production the of N2O from the short-cut SBR was observed operating at DO = 1.5 mg L-1 and vNLR = 0.81 kgN m-3d-1 resulted in much lower nitrous oxide emissions (0.24% of influent nitrogen load) compared to the operation at lower DO (0.95 mg L-1) and higher vNLR=1.08 kgN m-3d-1.

When other biowaste are not available in the WWTP, the fermentation of sewage sludge (primary and secondary sludge) is the best candidate to enhance nitrogen and phosphorus removal. The alkaline fermentation of sewage sludge was tested in a pilot sludge fermentation-

membrane separation by the addtion of NaOH. The maximum VFA production increased from 200 mgCOD gTVS-1 to 300 mgCOD gTVS-1.when the pH was increased between 8 to 10. However, it was observed that the addition of NaOH adversely affect the separation by the membrane from the use of soda during the fermentation process. To this end, soda was replaced with wollastonite (Ca2SiO4) to improve the dewaterability of the final fermentation product, which increased the flow by 24% (12.5 LMH). The case studies analysed represented a successfull validation of these technology. The experience point out that the use of sludge fermentation liquid for the via nitrite nutrient removal resulted in high nitrogen and phosphorus removal (22.4 mgN (gVSS h)−1 and 3.4 mgP (gVSS h)−1. High presence of nitrite concentration (> 100 mg L-1) during can interfere with phosphorus uptake mechanism of PAOs, and 65% of the total P removed was attributed to biomass growth. The application of fermentation containing a mixture of SCFAs enhanced the efficiency and the rate of denitrification and P removal.
Finally, the thesis rised the possibility to integrate the side stream nitrogen removal via nitrite from anaerobic supernatant with the enrichment of polyhydroxyalkanoates (PHA) storing biomass. The main goal was to demonstrate the feasibility of the recovery of green added value products from the treatment of the wastewater.After the phase of enrichment, the good capacity of the biomass to store PHA was highlight by the high VFAs uptake rate of 134.6±32 mgCOD gVSS-1h-1 and a PHA production yield which increased during the experimental activities from 0.26±0.2 to 0.38±0.03 gCODPHA gCODVFA -1. Preliminary results of the bioplastics produced showed similar characteristic with conventional biopolymer already present in the market. However, higher effort should be given for the final recovery of the bioplastics, since the extraction of the PHA from the biomass is an important bottleneck, which limited the diffusion of large scale application. Furtrher investigations for suistanable and green methods for PHA extraction are required in order to prevent negative environmental impact due for the use of organic solvents.

Riassunto
Le attività principali durante i tre anni di dottorato, si sono concentrate sulla validazione e ottimizzazione di processi avanzati per la rimozione biologica di azoto e fosforo via-nitrito da effluenti anaerobici in reattore sequenziale (SBR) fino a scala dimostrativa. La necessità di far fronte agli ostacoli tecnici e fornire soluzioni ingegneristiche è di fondamentale importanza per capire da subito il potenziale di trasferibilità e l'interesse commerciale per l'applicazione in piena scala di queste tecnologie innovative. Le attività sperimentali sono state pertanto eseguite utilizzando substrati reali (ad esempio surnatante anaerobica, fanghi di depurazione comunale, FORSU, acque reflue domestiche municipali), riproducendo così le reali condizioni ambientali e operative.
I processi anaerobici hanno ottenuto un elevato interesse negli ultimi anni, dovuto principalmente ai vantaggi connessi alla produzione di "energia verde" tramite l'utilizzazione del biogas. Rispetto ai consolidati trattamenti aerobici, i processi anaerobici permettono minor consumo energetico, minore produzione di fanghi, eliminazione dell'inquinamento dovuto alle emissioni gassose e un impatto ambientale minore. I reattori anaerobici UASB sono tra le tecnologie più evolute per il trattamento anaerobico delle acque reflue municipali. Tuttavia, gli effluenti anaerobici contengono ancora elevate quantità di nutrienti (azoto e fosforo), ed essere fonte di eutrofizzazione per i corpi idrici. Inoltre, gli effluenti derivati dalla digestione o co-digestione anaerobica dei fanghi con rifiuti organici (ad esempio, la frazione organica dei rifiuti solidi urbani, FORSU), rappresentano un flusso concentrato di azoto e fosforo che dovrebbe essere trattato prima di essere ricircolato nella linea principale degli impianti di depurazione. I

processi biologici via-nitrito per la rimozione dei nutrienti sono riconosciuti per essere una scelta sostenibile per il trattamento degli effluenti anaerobici a basso e alto carico poiché consentono aerare il 25% in meno e utilizzano il 40% in meno di fonte di carbonio rispetto ai processi convenzionali. L'applicazione di bioprocessi innovativi per ridurre i costi di trattamento e valorizzare le acque reflue tramite il recupero di risorse è quindi la fida attuale.
Il processo via-nitrito è stata studiato in un reattore sequenziale discontinuo (SBR) per il trattamento di effluenti anaerobici proveniente da un reattore UASB. Le acque reflue domestiche a seguito di un processo anaerobico, sono state trattate prima tramite un processo completamente autotrofo (Anammox). Durante la sperimentazione, l'attività specifica dell'inoculo è stata incrementata del 161%, che corrispondeva ad un tasso di rimozione dell'azoto pari a 2.27±1.31 mgN (gVSS h)-1 a 30 °C. Questo valore è diminuito con il diminuire del carico di azoto volumetrico, portando comunque a ridurre anche l'attività dei batteri eterotrofi denitrificanti, osservando un avvicinamento dei rapporti di conversione tipici della biomassa anammox (1.32 NO2-N / NH4-N rimosso). L'analisi d'ibridazione fluorescente (FISH) ha confermato la presenza di biomassa Anammox e di batteri filamentosi, favoriti dall'elevato tempo di ritenzione dei solidi. Sebbene la biomassa anammox rimuova efficacemente l'azoto, il fosforo non può essere eliminato significativamente da una biomassa completamente autotrofa. Per questo motivo, è stata studiata l'applicazione dello schema UASB-SBR accoppiato al co-trattamento decentralizzato delle acque reflue e dei rifiuti organici domestici, al fine di promuovere la rimozione dell'azoto e del fosforo via-nitrito. La rimozione simultanea di azoto e fosforo via nitrito è stata studiata usando la migliore fonte di carbonio disponibile prodotta dalla fermentazione acidogenica dei rifiuti domestici organici (DOW) e rifiuti di frutta e di verdura (VFW). L'accumulo dei nitriti (NO2-N/NOx-N> 97%) nel reattore SBR è stato osservato operando con basso tenore di ossigeno disciolto (<0.8 mg L-1) e relativamente elevato vNLR (0.19-0.21 kgN m-3d-1). Inoltre, la fonte di carbonio dosata conteneva quantità di acido acetico, propionico e butirrico che ha incrementato le velocità di denitrificazione via nitrito ed il contemporaneo iperaccumulo di fosforo (fino a 6.33±1.92 mgP (gVSS h)-1) da parte della biomassa fosforo accumulante e denitrificante (DNPAOs). Tuttavia, l'attività della biomassa DNPAOs non è stata influenzata negativamente dalle elevate concentrazioni di nitriti nell'ordine di 50-70 mgNO2-N L-1, mentre si notava inibizione da concentrazioni di 100-120 mgNO2-N L-1.
Accoppiando un reattore SBR per la rimozione biologica dei nutrienti via-nitrito con la fermentazione di rifiuti organici di scarto, è stato implementato e ottimizzato un sistema innovativo per il trattamento dei digestati anaerobici. In questa tesi, il surnatante anaerobico proveniente dalla co-digestione dei fanghi secondari e della FORSU è stato trattato in un reattore SBR pilota (con volume utile pari a 2.8 m3), realizzando la rimozione biologica via-nitrito dei nutrienti. Le operazioni di avviamento del reattore sono durate circa 20 giorni, i quali sono stati sufficienti per inibire la crescita dei batteri nitrito ossidanti (NOB) operando ad alte concentrazioni di ammoniaca libera (FA) (fino a 6 mgN L-1) nel reattore. La variazione della popolazione batterica è stata osservata anche tramite il sequenziamento delle principali bande ottenute dall'analisi DGGE, la quale indicava la presenza di gruppi batterici sono connessi a β Proteobacteria e Bacteroidetes. Tra questi, alcune delle bande principali erano strettamente legate ai generi AOB, come Nitrosomonas sp. e Nitrosospira sp. La massima capacità di trattamento osservata è stata di 0.8 kgN m-3d-1. La velocità specifica di ossidazione dell'ammoniaca (sAUR) è stata osservata costante nel tempo, variando in ristretto range compreso tra 18-20 mgN (gMLVSS h)-1 durante il periodo sperimentale. Basse temperature del digestore anaerobico, nonché eccessi di polielettrolita residui nel surnatante anaerobico a seguito del processo di disidratazione, sono stati comunemente osservati durante le attività sperimentali,

causando ampie fluttuazioni della carico volumetrico di azoto applicato e significative fughe di biomassa attiva flocculata dal reattore SBR. Sebbene il tasso di ossidazione dell'ammoniaca sia rimasto costante, quando il vNLR era superiore alla massima capacità di trattamento della biomassa le efficienza di rimozione dell'azoto del sistema diminuivano.
Da un punto di vista economico, le fonti di carbonio alternative derivanti dalla fermentazione dei rifiuti organici (come la frazione organica dei rifiuti solidi urbani, liquami zootecnici, insilati, fanghi di depurazione) potrebbe essere una buona opzione in sostituzione a quelle di origine sintetica (ad esempio acido acetico, metanolo, etanolo, glicerolo). Inoltre, l'analisi del ciclo di vita ha mostrato i benefici ambientali connessi alla fermentazione di rifiuti organici rispetto all'uso di una fonte di carbonio di origine sintetica (0.28 kg PO4-3 m-3). Elevate velocità di denitrificazione via-nitrito con simultaneo accumulo di fosforo è stato osservato in condizioni anossiche, utilizzando il prodotto di fermentazione come fonte di carbonio. Dosando la frazione liquida della FORSU fermentata, la velocità specifica di rimozione del fosforo via nitrito è stata pari a 0.27 kgPO4-P (kgMLVSS d)-1, con il conseguente iper-accumulo di fosforo nei fanghi attivi, ottenendo una concentrazione fino a 50 mgP gMLSS-1. Durante il processo via-nitrito, elevata quantità di gas serra come l'N2O potrebbero essere generate, e quindi venire meno dei vantaggi connessi al risparmio in aerazione. In questa tesi, è stato osservato come il basso tenore di ossigeno accompagnato da un'elevata concentrazione di nitriti nel reattore, incrementano la produzione di N2O. Una riduzione delle emissioni di N2O (0.24% del carico di azoto influente) dal reattore SBR via-nitrito è stata osservata operando a concentrazioni di ossigeno (OD) pari a 1.5 mg L-1 e vNLR pari a 0.81 kgN m-3d-1, rispetto a concentrazioni di OD minori (0.95 mg L-1) e alti carici di azoto applicati (vNLR = 1.08 kgN m-3d-1).
Quando in un impianto di depurazione non sono presenti rifiuti biodegradabili, la fermentazione dei fanghi di depurazione (come i fanghi primari e secondari) è il miglior metodo candidato per migliorare le efficienze di rimozione dell'azoto e del fosforo. La fermentazione alcalina dei fanghi di depurazione (primari e secondari) con l'aggiunta di soda, è stata testata in un fermentatore pilota (del volume utile di 500 L) accoppiato alla separazione della frazione liquida su membrana tubolare. Operando a pH compresi tra 8 e 10 è possibile aumentare le rese di produzione degli acidi organici volatili da 200 fino a 300 mgCOD gTVS-1. Tuttavia, l'aggiunta di soda per l'aumento del pH di fermentazione ne pregiudica anche la disidratabilità, rendendo difficoltosa la separazione. Per questa ragione, l'idrossido di sodio è stato sostituito con wollastonite (Ca2SiO4) che consente di mantenere pH relativamente elevati (pH circa 8.5) e di migliorare la disidratabilità del prodotto di fermentazione finale, aumentandone il flusso di permeato del 24% dalle membrane (12.5 LMH). I casi di studio analizzati e riportati in questa tesi, confermano la validità della tecnologia studiata. Il prodotto di fermentazione derivante dai fanghi primari e secondari consente buone cinetiche di denitrificazione e l'accumulo del fosforo via-nitrito (rispettivamente pari a 22.4 mgN (gVSS h)-1 e 3.4 mgP (gVSS h)-1. Tuttavia, l'elevata concentrazione di nitriti (> 100 mg L-1) può interferire con i meccanismi di rimozione del fosforo da parte della biomassa PAOs: in questo caso il 65% del totale P rimosso è stato attribuito al normale metabolismo di crescita della biomassa. L'applicazione della fermentazione consente pertanto di produrre una miscela ottimale di acidi grassi a catena corta, la quale migliora e incrementa l'efficienza di rimozione di azoto e del fosforo via-nitrito.
In ultima analisi, in questo lavoro di tesi è riportata come è possibile integrare la rimozione dell'azoto da un flusso ad alto carico ammoniacale (come i surnatanti anaerobici) con i processi di selezione di una biomassa con elevate capacità di stoccare poliidrossialcanoati (PHA), precursori delle bioplastiche. L'obiettivo principale era di dimostrare la fattibilità per il recupero di prodotti con valore aggiunto mediante il trattamento di acque reflue. Dopo la fase di arricchimento compiuta in condizioni di "feast and famine", la buona capacità della biomassa a

stoccare PHA è indicata dalle velocità di assorbimento degli acidi organici di 134.6 ± 32 mgCOD gVSS-1h-1, nonché dalle rese di produzione di PHA che sono aumentate nel corso delle attività sperimentali da 0.26±0.2 a 0.38±0.03 gCODPHA gCODVFA-1. I risultati preliminari delle bioplastiche prodotte hanno mostrato caratteristiche simili con biopolimeri convenzionali già presenti sul mercato. Tuttavia, lo sforzo maggiore dev'essere concentrato ai processi di recupero delle bioplastiche poiché ad oggi l'estrazione dei PHA dalla biomassa è un collo di bottiglia che ne sta limitando la diffusione e l'applicazione su vasta scala. Ulteriori ricerche saranno necessariamente focalizzate su metodi più sostenibili e più "green" che consentano di estrarre PHA evitando impatti ambientali negativi a causa dell'utilizzo di dei solventi organici.

Firma dello studente

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