Treatment technologies for recycle liquors: Nutrient removal, mass balances, and potential recovery at wastewater treatment plants

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Declaration

I hereby certify that the work presented in this thesis is my original research work. Due reference is given to literature and any research collaborations where appropriate. No part of this thesis has been submitted previously for a degree at this or any university.

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Abstract

Recycle liquors (RLs) from anaerobic digesters at wastewater treatment plants (WWTPs) can be a challenge to WWTP operations, but also a source of nutrient recovery. For example, Northumbrian Water Ltd.'s Bran Sands WWTP has historically had elevated NH_4-N levels and accumulation of PO_4-P within its works, primarily due to the recycling of nutrient-rich RLs from its sludge treatment process to its activated sludge (AS) units. This has led to chronic scaling problems (as struvite), fluctuations in AS process stability, and increased operational costs (i.e. increased maintenance, chemical and energy costs). In order to tackle these issues, this thesis performed a mass balance on NH_4-N and PO_4-P around the WWTP to understand the material flow across plant operations, which led to various NH_4-N and PO_4-P treatment and recovery studies. Relative to PO_4-P removal and struvite reduction, the addition of $MgCl_2$ (and pH adjustment) were examined to assess their value in removing P and N from the soluble phase (i.e. in RLs) in AD and post-AD units. NH_4-N and PO_4-P removal/recovery increased as Mg^{2+} and pH increased when added to both locations. However, NH_4-N and PO_4-P removal/recovery was most efficient in post-AD applications (i.e. ~49 ± 0.17% and ~83 ± 0.25%, respectively, at pH 8 with 150 mg/L of added Mg^{2+}). A simple cost benefit analysis shows this could potentially offset 83% of the annual cost of dealing with scaling problems on site, and ~£321,000/yr can be generated from the sale of the recovered PO_4-P. The AD experiments also showed increase volatile solids removal and biogas production as Mg^{2+} and pH increased. To further reduce NH_4-N in RLs, the thesis investigated the suitability of aerobic, nitrifying granule bioreactors for NH_4-N removal. Up to 95% NH_4-N removal (mean influent NH_4-N = 300 mg/L) was observed at NH_4-N loading rate of 0.6 $gNH_4\text{-N}\ L^{-1}.d^{-1}$, which was most efficient when nitrite-oxidising bacteria abundances were comparatively higher in the granules relative to ammonia-oxidizing bacteria. Overall, the work shows that a combination of Mg^{2+} addition for PO_4-P removal and aerobic granule bioreactors for NH_4-N removal could reduce nutrient levels in RLs, although work is still needed to translate this potential into actual enhanced NH_4-N and PO_4-P recovery.

Keywords: Anaerobic Digestion, Recycle Liquors, Struvite, Aerobic Granulation

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List of Abbreviations

AAD: Advanced Anaerobic Digestion

AD: Anaerobic Digestion

ADP: Adenosine Diphosphate

AG: Aerobic Granulation

AGT: Aerobic Granulation Technology

Anammox: Autotrophic anaerobic ammonia oxidation

ANG: Anaerobic Granulation

AOB: Ammonia Oxidising Bacteria

AS: Activated Sludge

ATP: Adenosine Triphosphate

BOD: Biological Oxygen Demand

BWB: Berliner Wasserbertriebe

CAS: Conventional Activated Sludge

CSIR: Council for Scientific and Industrial Research

COD: Chemical Oxygen Demand

CT: Crystallactor Technology

DEFRA: Department for Environment, Food and Rural Affairs

DHV: Ingenieursbureau Dwars, Heederik en Verhey

DNA: Deoxyribonucleic acid

DNEBPR: Denitrifying Enhanced Biological Phosphorus Removal

DNPAO: Denitrifying Phosphate Accumulating Organism

DO: Dissolved Oxygen

EA: Environment Agency

EBPR: Enhanced Biological Phosphorus Removal

EC: European Council

EPS: Extracellular polymeric substance

ETW: Effluent Treatment Works

GAO: Glycogen Accumulating Organism

HRT: Hydraulic Retention Time

MBR: Membrane Bioreactors

MBBR: Moving-bed biofilm reactors

NEREDA: is a patented name for aerobic granular sludge technology developed by the RoyalHaskoningDHV engineering consultancy.

NLR: Nitrogen Loading Rate

NOB: Nitrite Oxidising Bacteria

NWL: Northumbrian Water Ltd

OLR: Organic Loading Rate

PAO: Phosphate Accumulating Organism

PCR: Polymerase Chain Reaction

QPCR: Quantitative Polymerase Chain Reaction

REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals

REM-NUT: REMoval of NUTrients

RLs: Recycle Liquors

RNA: Ribonucleic acid

RSTC: Regional Sludge Treatment Centre

SBR: Sequencing Batch Reactors

SHARON: Single reactor system for High activity Ammonia Removal Over Nitrite

SND: Simultaneous Nitrification Denitrification

SRT: Sludge Retention Time

TKN: Total Kjeldahl Nitrogen

TS: Total Solids

UK: United Kingdom

USA: United States of America

UASB: Upflow Anaerobic Sludge Blanket

USEPA: United States Environmental Protection Agency

UWWTD: Urban Wastewater Treatment Directive

VS: Volatile Solids

VS_r: Volatile Solids removed

WWTP: Wastewater Treatment Plant

Chapter 1: Introduction

1.1 Project Background

Wastewater treatment plants (WWTPs) must produce effluents of acceptable quality for discharge or reuse, which has historically been driven by regulations aimed at safeguarding human health and minimizing environmental impact, particularly reducing carbon and microbial releases. However, Lang *et al.* (2013) states that high concentrations of N and P (i.e. NH_4-N and PO_4-P) from WWTP effluents also can cause water quality deterioration in receiving water bodies into which they are discharged. Such deterioration in water quality can cause significant health, environmental and aesthetic problems, and non-compliance with environmental permits that could attract sanctions. Also, the economic implications of this are enormous. For instance, in June 2014, South West Water, UK, was fined a total of £150,000 for three offences under the Environmental Permitting (England and Wales) Regulations 2010. The company was also ordered to pay an additional £3600 in costs to the Environmental Agency for failing to inform the Agency of any failure that may adversely affect effluent quality (EA, 2014). One of the major contributors of high NH_4-N and PO_4-P loads at WWTPs is recycle liquors (RLs) from the dewatering of digested sludge. Hence, in order to attain and maintain acceptable water quality and alleviate environmental pressure, the effective control of NH_4-N and PO_4-P levels in wastewater RLs has become critical.

Nutrient removal (i.e. N and P) from wastewater has often focused on exploiting nutrient cycling reactions to convert reactive forms of nutrients to non-reactive forms. For instance, NH_4-N removal has traditionally been achieved through energy intensive biological nitrification-denitrification (i.e. converting ammonia through nitrite and nitrate to nitrogen gas) which contributes significantly to operational costs in WWTPs (Nancharaiah *et al.,* 2016). Biologically, P removal from wastewater has commonly been implemented through the accumulation of polyphosphate granules by phosphate accumulating organisms (PAOs), which also entails a major cost at WWTPs. Alternatively, P has also been removed by converting it to a non-readily bioavailable form (e.g. the precipitation of P with Iron salts or alum; Wu *et al.,* 2005), which has often made P recovery very difficult. Furthermore, the use of ferric salts in P removal has been reported to cause corrosion in equipment, increase overall sludge production, and has health and safety concerns involved in handling the chemicals (Sharp *et al.,* 2013).

Most WWTPs manage nutrients as components of wastes to be removed, with fewer facilities viewing thiem as a potential resource base (Mehta *et al.,* 2015). N and P have been reported as being critical to intensive agriculture, and there are growing concerns over the cost of extraction and long-term availability of nutrients (especially P whose main source is phosphate rock, a non-renewable resource). More than 90% of global phosphate rock reserves is based in only five nations (i.e. Syria, China, Morocco, Algeria and China; Jasinski, 2012). Uncertainty over the long-term availability of phosphate rock, has led to the recent rise in commercial P prices. With the annual rate of chemical fertilizer production increasing by 1.8% it has been estimated that global demand for P will outweigh supply by 2033 (Cordell *et al.,* 2009; Heffer and Prud'homme, 2014; Ledezma *et al.,* 2015). This could pose significant problems in fertilizer production since it has been reported that ~90% of phosphate rock mined globally is used for fertilizers (Mehta *et al.,* 2015). Furthermore, industrial production of chemical fertiliser is an energy-intensive process requiring the use of non-renewable energy (e.g. natural gas). Also, in fertiliser production N (though a renewable resource) is mostly obtained by converting atmospheric N_2 to NH_4-N through the energy-intensive Haber-Bosch process requiring the use of non-renewable energy (e.g. natural gas) as well (see Figure 1.1).

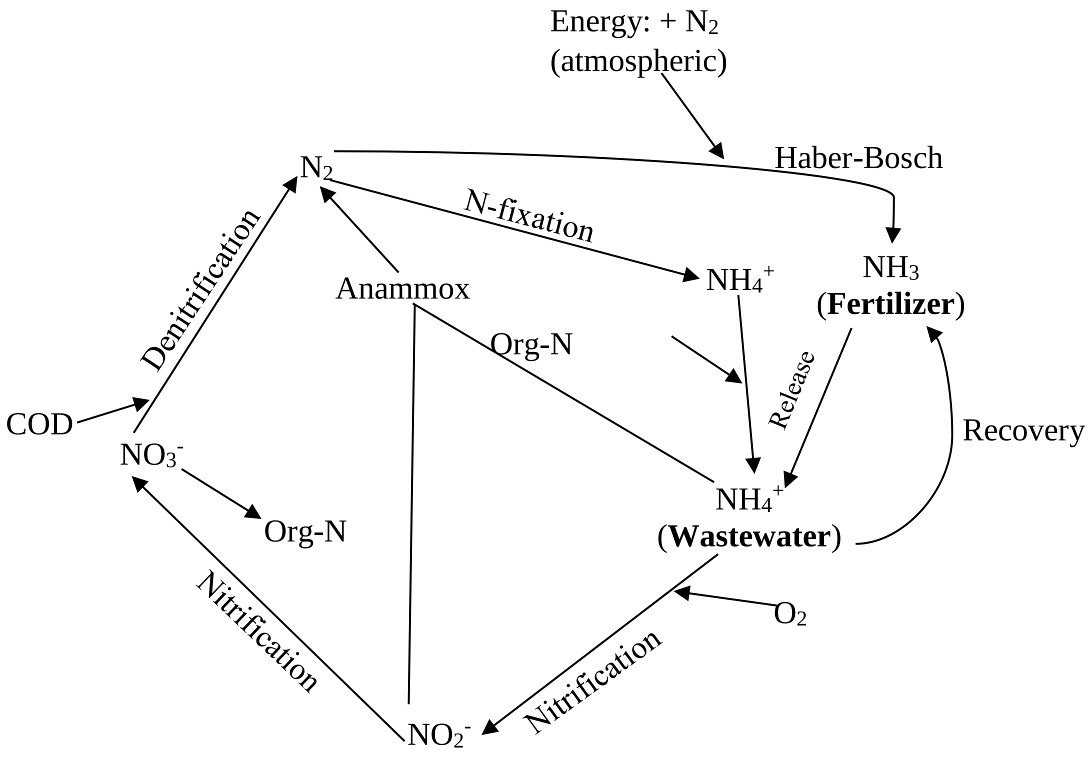

Figure 1.1: Simplified N-cycle showing the main natural and anthropogenic processes (Adapted from Nancharaiah *et al.,* 2016).

Lind *et al.* (2001) and Malamis *et al.* (2014) reported that recycling recovered nutrients (i.e. N and P) from wastewater (especially nutrient-rich RLs), could substitute 35-45% of chemical fertiliser being applied in agriculture. Mehta *et al.* (2015) also estimated that global total P content in human waste (e.g. urine and faeces) can offset ~22% of global P demand. Uludag-Demirer *et al.* (2005) and Uysal *et al.* (2014) highlighted the recent gradual shift from N and P removal from wastewater to recovery being underpinned by the drive towards sustainability in agricultural activities. The recycling of N and P through sustainable methods (rather than their emission or destruction) has gained increasing interest at WWTPs for sustainability as well as economic reasons. Some of the economic benefits of N and P recovery from wastewater (RLs in particular) are as follows: (i) revenue generation from the sale of recovered nutrients; (ii) reduced operating costs at WWTPs due to the reduction in the reagents required for the chemical precipitation of these nutrients; (iii) reduction of sludge production and costs associated with its management; and (iv) reduction in maintenance costs of pipelines and other equipment due to reduced scaling (Liu *et al.*, 2013a; Le Corre *et al.,* 2009; Molinos-Senante *et al.,* 2011).

The chemical precipitation of magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$) (commonly known as struvite) has been extensively studied as an effective method for N and P removal from RLs (Uludag-Demirer *et al.,* 2005). Struvite is a white orthorhombic crystal that forms when magnesium, ammonium and phosphorus react in equimolar amounts as presented in the following chemical reaction (Lee *et al.,* 2003; Barak and Stafford, 2006; Di Iaconi *et al.,* 2010):

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \longrightarrow MgNH_4PO_4.6H_2O_{(s)} \qquad \textbf{Equation 1}$$

Although being generally viewed as a problem due to scaling, controlled struvite formation has recently been viewed as an avenue for P recovery (Doyle and Parsons, 2002; Liu *et al.,* 2013). P recovery (from nutrient-rich wastewater) through the 'controlled' crystallisation of struvite requires a magnesium source (e.g. $Mg(OH)_2$ or $MgCl_2$) and a sufficiently high pH (Hanhoun, 2011), but existing reports reveal several potential markets for struvite if recovered. For example, it has been recommended as a slow release fertilizer, raw material for the phosphate industry, a binding material in cement, and for use in making fire resistant panels (Sarkar, 1999; Schuilling and Andrade, 1999; Demirer *et al.,* 2010).

Most technologies developed to recover P (as struvite) in WWTPs have been applied to the aqueous phase (e.g. digested sludge liquors). There exists very limited information about P-recovery through the controlled crystallization of struvite in the sludge phase; although some recent studies on controlled struvite crystallization in digested sludge have reported the following advantages (Bergmans *et al.* 2014; Geerts *et al.* 2015; Marchi *et al.* 2015): improved dewaterability of digested sludge; better P and N recovery potential; reduced scaling in treatment equipment; and reduced N and P in RLs that are recycled to the head of WWTPs for further treatment. However, much work is still needed to cost-effectively reduce available nutrient loads in post-AD processes (especially N levels in RLs). Further, Laridi *et al.* (2005) reported a meagre NH_4-N removal efficiency of ~17% compared to ~90% PO_4-P removal when magnesium chloride was dosed into the RLs without any addition of PO_4-P, at an optimal pH of 8.5. With reference to this low NH_4-N removal efficiency, this technical option will fail to reduce NH_4-N levels in RLs and combined industrial wastewaters (with 655 ± 108 mg/L NH_4-N levels) at Northumbrian Water Ltd's (NWL) Bran Sands WWTP studied here to below the 250 mg/L NH_4-N consent levels for treated effluent for the NWL site. Hence the need for a cost effective NH_4-N removal technology still exists.

Biological nutrient removal methods have been very successful at NH_4-N removal from RLs. Such NH_4-N removal processes are driven by the following groups of bacteria; denitrifying bacteria, ammonia oxidising bacteria (AOB), nitrite oxidising bacteria (NOB), and anaerobic AOB (anammox) (Caffaz *et al.,* 2008; Cervantes, 2009; Gustavsson, 2010). The basic categories of biological N removal technologies widely used include: (i) conventional nitrification – denitrification (e.g. conventional activated sludge, CAS); (ii) nitritation – denitritation; and (iii) autotrophic anaerobic ammonium oxidation (anammox) (Fux & Siegrist, 2004). These three treatment categories have been reported to achieve 85-95% N-removal from RLs (Fux, 2003). However, the high operating costs of CAS, reduced operational reliability of Anammox, and the high risk of excess N_2O production by nitritation – denitritation technologies (Hedstrom, 2001), have led to need for a more cost-effective, robust and environmentally-friendly technology.

Aerobic granulation technology (AGT) has been extensively used to remove nutrients (N and P) and organic compounds from industrial, toxic and municipal wastewaters, at both high and low organic loading rates (Khan *et al.,* 2013; Szabó *et al.,* 2016). This technology presents the possibility of designing more compact WWTPs due to the fact that the removal of N, P and COD can be achieved simultaneously in a single sequencing batch reactor (SBR) unit (de Kreuk *et al.,* 2005). This simultaneous removal of N, P and COD via AGT is based on a repeatedly fed batch process that relies on the selective growth of microorganisms in granules (through the process of biogranulation). Biogranulation is self-immobilisation of microorganisms (through cell-to-cell interactions governed by biological, physical and chemical phenomena) to form spherical granules. Biological N-removal via AGT has been reported to be driven by the following groups of bacteria; denitrifying bacteria, ammonia oxidising bacteria (AOB), nitrite oxidising bacteria (NOB), and anaerobic AOB (anammox) (Caffaz *et al.,* 2008; Cervantes, 2009; Gustavsson, 2010).

Although little has been done in terms of the application of AGT on simultaneous N- and P-removal from actual wastewater (with complex and highly variable composition; e.g. RLs); and there are few operating full-scale installations like the NEREDA technology in the Netherlands (http://www.royalhaskoningdhv.com/en-gb/nereda). Cassidy and Belia (2005) and Kishida (2009) did report ~97% N- removal when AGT was used to treat wastewater with COD/N = ~5 from an abattoir and animal farms. Cydzik-Kwiatkowska *et al*. (2013) further applied the AGT to assess N-removal from nutrient-rich RLs with high NH_4-N and PO_4-P concentrations, slowly biodegradable organics (BOD/COD =0.4) and a lower COD/N ratio of 1.4 and achieved some N-removal. This supports the findings of Yang *et al.* (2003), which reported that the activity of nitrifying and denitrifying bacteria populations coexisting in the granules, increased with decreasing COD/N ratio in synthetic wastewater. Therefore, there is precedence that AGT could work for RLs, which justifies further developmental work reported herein.

1.2 Problem statement

WWTPs have undergone process innovations over time in order to improve the efficiency of nutrient removal/recovery. The implementation of some Biological Nutrient Removal processes (especially Enhanced Biological Phosphorus Removal; EBPR) in secondary wastewater treatment, has resulted in greater accumulation of N and P in sludge, which is released during anaerobic digestion (AD) processes. This has caused diverse nutrient-related problems in WWTPs. AD of sewage sludge is a very popular technology for biosolids reduction, energy recovery from waste and for the potential reduction of greenhouse gas emissions (compared to aerobic treatment processes) in line with the Kyoto protocol (UNFCCC, 1997; USEPA, 2007). However, this technology has come under some scrutiny, especially associated with nutrient-rich effluent streams that require further treatment prior to disposal (Obaja *et al.,* 2003; Marti *et al.,* 2008; Khalid *et al.,* 2011; Xavier *et al.,* 2014). As such, RLs (also known as digester liquors) that result from the dewatering of AD sludge have become a major concern in the wastewater industry (Malamis *et al.*, 2014). Specifically, RLs can have exceedingly high levels of P, Chemical Oxygen Demand (COD), and ammonium-N typically >100 mg/L PO_4-P, >1230 mg/L, and >1000 mg/L NH_4-N, respectively (USEPA, 1979; Arnorld *et al.*, 2000; Wu *et al.*, 2001; Jenicek *et al.*, 2004), which are not readily treatable by single waste treatment technologies.

RLs have historically been recycled directly (without pre-treatment) back to secondary treatment units in WWTPs for additional treatment and this has often resulted in significant operational and financial challenges (Teichgräber and Stein, 1994; Janus *et al*., 1997; Wett *et al*., 1998; Pitman, 1999; Jaffer et al., 2002; Fux and Siegrist, 2004; Solley, 2006; Gustavsson, 2010). Bran Sands WWTP (operated by NWL) has been experiencing elevated NH_4-N levels and a build-up of PO_4-P in its treatment processes due to the recycling of nutrient-rich RLs from its Advanced Anaerobic Digestion (AAD) process to its activated sludge units due to a lack of NH_4-N and PO_4-P removal/recovery measures. This has led to scaling problems, fluctuations in process stability, and increased operational costs (i.e. increased maintenance, chemical and energy costs). In addition, despite the relatively lenient consent for NH_4-N (i.e. 250 mg/L) and PO_4-P (no consent) levels in treated effluent for the site, the current technology for NH_4-N removal from RLs and combined industrial wastewaters to attain consent levels on site is very expensive. Hence, the need for alternative cost-effective technologies exist. Also, one of the biggest hurdles to tackling these nutrient-related problems at Bran Sands WWTP is a limited understanding of the interdependence of individual treatment units especially associated with the mass flow of nutrients.

1.3 Project Aim and Specific Objectives

The overall aim of the thesis was to assess and determine the effect of ‘controlled’ precipitation of struvite in sludge (through Mg addition and pH adjustment) and the application of aerobic granular sludge technology on ‘free’ NH_4-N and PO_4-P levels in RLs to mitigate scaling problems at Bran Sands WWTP. In general terms, tha aim was to compare options for any WWTPs with an AD unit, which require sustainable treatment of RLs including potential nutrient recovery.

1.3.1 Specific Objectives

The specific objectives of the thesis were as follows:

- To review literature on existing treatment technologies for NH_4-N and PO_4-P removal/recovery from wastewater (especially high-strength wastewaters).

- To establish a mass balance for NH_4-N and PO_4-P around the Bran Sands WWTP with emphasis on the AAD processes and RLs.
- To assess the effect of Magnesium addition and pH adjustments on potential NH_4-N and PO_4-P recovery in AD and post-AD units.
- To compare the benefits of potentially recovering NH_4-N and PO_4-P in AD units, and post-AD systems.
- To grow aerobic granules from activated sludge.
- To investigate the feasibility of aerobic granulation technology in NH_4-N removal from RLs.
- To investigate the effect of NH_4-N loading rates (NLRs) and wastewater composition on nitrification activity and nitrifying communities in aerobic granulation systems.

1.4 Thesis Outline

The thesis outline is as follows:

Chapter 1: Introduction - This chapter presents the thesis background, research problem statement, aims and specific objectives.

Chapter 2: An overview of existing nutrient removal and recovery technologies from recycle liquors – This chapter covers the first objective of the thesis. It commences with an overview of potential NH_4-N and PO_4-P related challenges posed by RLs at WWTPs. It also identifies the main drivers of NH_4-N and PO_4-P removal/recovery as being legal/compliance-related, operational and financial challenges, environmental concerns (e.g. eutrophication and offsetting phosphate rock mining), and potential economic gains (through the sales of recovered NH_4-N and PO_4-P, and reduced maintenance cost). Existing treatment technologies (biological and physicochemical) for N and P removal/recovery from wastewater (RLs in particular) are also reviewed. Research knowledge gap that necessitated the planning and execution of this project are also highlighted.

Chapter 3: Mass Balance of N and P around the AD at Bran Sands WWTP - This chapter tackles the second objective. It presents mass balances of NH_4-N and PO_4-P around the Bran Sands WWTP with emphasis on the AAD processes and RLs. It also assesses the possible impact that the reduction in incoming NH_4-N and PO_4-P loads (between 2011 and 2013; e.g. as a result of the ban of P in detergents in 2012) has had on the transfer and exchange of nutrients throughout the treatment process, based on a comparison mass balance completed in 2011 around when the AAD was installed. This mass balance then guided the choice and experimental design of possible remediation actions, which are tested experimentally by work reported in Chapter Four.

Chapter 4: Nutrient recovery and struvite control associated with AD processes - Work here investigates the feasibility of recovering PO_4-P (as struvite) around ADs via the addition of Mg^{2+} (as $MgCl_2$) and pH adjustment, either directly to AD unit or in post-AD systems. Experiments involving Mg^{2+} addition and pH adjustment in post-AD sludge (i.e. digested sludge) were conducted to compare NH_4-N and PO_4-P removal rates with that of recovery experiments in the AD units. The study also quantified the effect of Mg^{2+} addition and pH adjustment on the performance of the AD units (in terms of methane production and VS removal). Cost benefits analysis are performed for NH_4-N and PO_4-P recovery in AD and post-AD units.

Chapter 5: The application of aerobic granular technology (AGT) in n-removal from recycle liquors – The chapter investigates the feasibility of using AGT to treat NH_4-N in RLs from Bran Sands WWTP. The AG biomass used in the study was cultivated from nitrifying-activated sludge collected from Bran Sands WWTP. The study also quantified the effect of varying feed conditions, NH_4-N loading rates (NLRs) and COD/NH_4-N ratio on NH_4-N removal via AGT.

Chapter 6: Effects of feeding conditions and Nitrogen loading rates on nitrifying communities in aerobic granular systems – This chapter provides microbial descriptions (e.g. about nitrifying bacteria community) to better understand NH_4-N removal data presented in Chapter 5. Here, qPCR analysis were performed on granular sludge collected from the reactors throughout their operation. Data from the analysis facilitated the determination of abundances for “Total bacteria”, AOB and NOB during the different feeding conditions and NLRs. Statistical analysis were performed to test for significant differences (between the abundances) and correlation with chemical analysis data presented in Chapter 5.

Chapter 7: Conclusion and Recommendations – The main results of the different chapters are summarised, recommendations made and future work discussed.

Chapter 2: An overview of existing nutrient removal and recovery technologies from recycle liquors

2.1 Drivers of nutrient removal and recovery from recycle liquors

Effective nutrient removal or recovery is central to the water industry's sustainability goal and is fundamental to maintaining nutrient balances within the natural water cycle (Bloech, 2005). Therefore, the removal of N and P from wastewater is one of the key objectives of the European Commission's Urban Wastewater Treatment Directive (98/15/EC; regulating effluent quality), the Water Framework Directive (2000/60/EC) and the Nitrates Directive (91/676/EEC) (Blöch, 2005; DEFRA, 2014). However, meeting these objectives is not achieved by most wastewater treatment technologies; especially when treating high strength wastewaters, such as RLs from dewatered digested sludge.

Historically, these liquors have been recycled directly (without pre-treatment) back to secondary treatment units (especially with activated sludge units) in WWTPs for additional treatment prior to ultimate disposal (Teichgräber and Stein, 1994; Janus *et al.*, 1997; Pitman, 1999). However, although RLs only account for about 1% of the volumetric flow, they can contribute 20-50% and 10 – 30% of total P and N loads, respectively, at most WWTPs, making them a significant and costly component in the secondary treatment influents (Wett *et al.*, 1998; Jaffer *et al., 2002;* Fux and Siegrist, 2004; Solley, 2006; Gustavsson, 2010). The large nutrient load and the composition of RLs, alongside other factors (e.g., operational challenges, resource depletion, economic and environmental benefits of nutrient recovery), have made their efficient handling and treatment one of the most important areas in the water industry for the identification of new solutions, which is the central goal and theme of this thesis.

2.1.1 Nutrient-related Challenges facing wastewater treatment plants

WWTPs have experienced numerous nutrient-related problems over time that have necessitated continuous retrofitting and/or substitution of treatment technologies, which constitute an additional cost. One of the biggest hurdles to tackling these nutrient-related problems at WWTPs is a limited understanding of the interdependence of individual treatment units especially associated with the mass flow of nutrients (e.g. N and P) (see Figure 2.1). Establishing a comprehensive mass balance model for N and P around WWTPs is very important in identifying and quantifying issues of great concern and also locating critical points of occurrence within the WWTP. A mass balance also helps in assessing the performance of the treatment units. In addition, a good N and P mass balance does not only identify problematic areas of a WWTP, but also highlights potential points for effective nutrient recovery. However, a lack of data due to less complete monitoring regimes and the complex nature of most WWTPs (e.g. the recycling of liquors from sludge dewatering processes) has made developing mass balance models at most WWTPs very difficult.

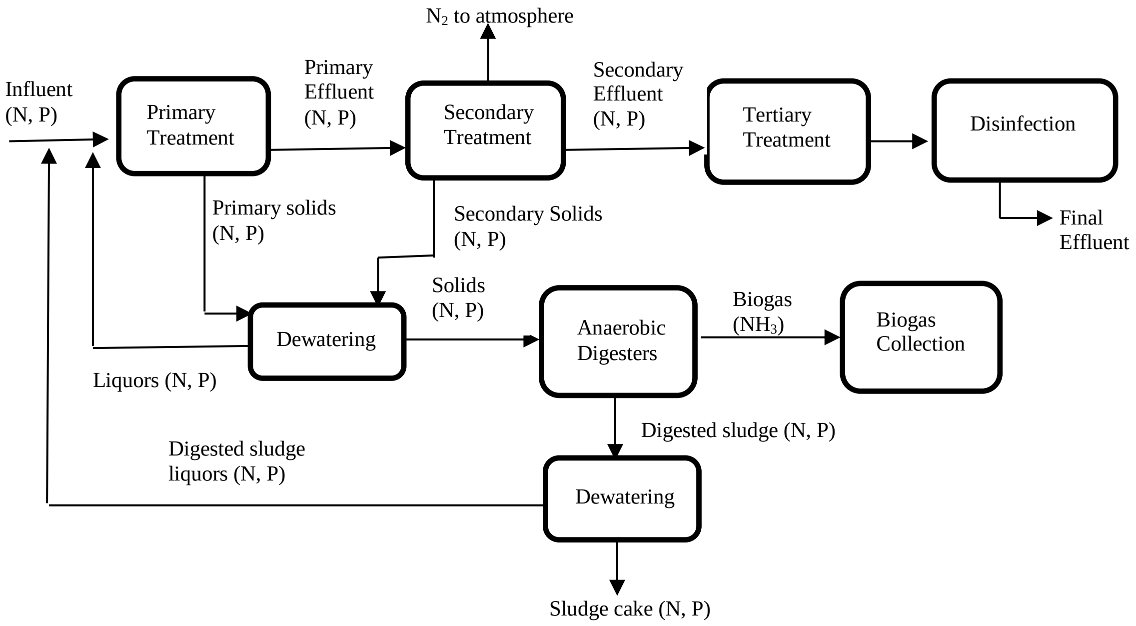

Figure 2.1: Schematic layout of a typical WWTP showing the major mass flow of N and P

The conventional practice of recycling digester liquors to the secondary treatment units at WWTPs poses a plethora of operational and financial challenges. For example, this practice can increase loading rates to secondary treatment units at WWTPs, which causes the plants to be operated beyond their designed loading capacities. Operation instability, poor performance, poor effluent quality and non-compliance with regulatory standards can result (Arnold *et al.*, 2000). Further, the composition of digester liquors can be highly variable (compared to influent wastewater), depending on variations in sludge dewatering or digestion processes (Thorndahl, 1993), although they are usually very high in suspended solids, NH_4-N, and P concentrations. They also have higher pH, pathogen levels, and less biodegradable organic matter (Laurich and Gunner, 2003; De la Rubia *et al.,* 2010; Andreottola *et al.,* 2012; Malamis *et al.*, 2014). Thus, direct recycling of RLs to the biological stage of WWTPs can unbalance COD/N/P ratios in influents to secondary units and destabilise the biological communities (Caffaz *et al.*, 2008; Malamis *et al.*, 2014). This especially results in reduced performance of N removal processes and potentially leads to additional costs, such as the provision of nitrification/denitrification systems within WWTPs (Siegrist, 1996; Hellinga *et al.*, 1998; OFWAT, 2006).

Furthermore, the complex chemical composition of RLs can lead to other problems, such as the formation and precipitation of magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$; i.e., struvite) in digested sludge processing lines and equipment (Momberg and Oellermann, 1992). Struvite is a white orthorhombic biogenic crystalline mineral of low solubility (Barak and Stafford, 2006), which forms and precipitates when concentrations of soluble magnesium, ammonium and phosphate exceed saturation levels (i.e. when the ionic product of struvite component ions exceed the solubility product, K_{sp}, for struvite), typically between pH 6 and 10 (Doyle and Parsons 2002; Sharp *et al.,* 2013; Mudragada *et al.,* 2014). The 'uncontrolled' formation of struvite is one of the major challenges facing most WWTPs as this leads to significant operational problems (e.g. pipeline blockages, fouling of pumps, interrupted plant operations) and financial costs (i.e. increased maintenance costs for equipment repairs and replacements, and increased capital investments to cater for control/remediation measures) (Neethling and Benisch 2004; Barat *et al.,* 2005; Marti *et al.,* 2008).

For instance, Benisch *et al.* (2000) reported that the operational costs directly pertaining to uncontrolled struvite formation at a medium-sized WWTP with a design flow of ~95,000m^3/day can easily exceed $100,000/year. Struvite component ions commonly get resolubilised during the AD process making them available for struvite formation (Mamais 1994; Parsons and Smith 2008; Marchi *et al.,* 2015), which is accompanied by a rise in pH (due to carbon dioxide stripping); hence the prevalence of 'scaling' problems in processes downstream of the AD (Wu *et al.,* 2005). Most of the struvite scaling problems have been observed in areas of high turbulence (e.g. pumps, elbows, propellers, valves, etc.) where there is increased CO_2 stripping resulting in locally increased pH (Ohlinger *et al.,* 1999). Also the suspended solids in digester liquors have been observed to facilitate the formation of nuclei for the formation and growth of struvite crystals (Neethling and Benisch 2004). Table 2.1 presents some examples of publications describing struvite-related problems in WWTPs.

Despite the challenges being posed by RLs on WWTPs, a few studies have identified potential opportunities related to "smarter" handling of RLs. Wett and Alex (2003) observed that high ammonium concentrations and temperatures of RLs could be harnessed to promote bacterial growth in activated sludge treatment units during winter. Also, struvite can be potentially used as a slow release fertilizer, which has boosted interests in more efficient digester liquor processing (Demirer *et al.,* 2010). Furthermore, Benisch *et al.* (2000) and

Barnard (2009) showed that the phosphate content of human wastes represents more than 10% of phosphate rock production, and suggested the ‘controlled’ precipitation of struvite from wastewater (RLs in particular) is a potentially viable method for supplementing the phosphorus cycle to offset the impact of human dependence on the limited global phosphate rock reserve.

Table 2.1: Some of struvite-related problems in WWTPs

Publications	Location of scaling problem	Severity of scaling problem	Case-study
Rawn *et al.* (1937)	Walls of the pipeline conveying Digester liquors	Accumulating to about 2.5 cm thick	WWTP in USA
Borgerding (1972)	The walls of the suction side of the pump drawing digested sludge for dewatering	Reduction of the diameter of the suction side from 310 mm to 150 mm	Hyperion WWTP in USA
Ohlinger *et al.* (1998)	Accumulation on the walls of pipelines transporting liquors from sludge basins supernatant systems	Scaling problems in about 5.6 km length of pipeline	WWTP in USA
Williams (1999)	The walls of pipeline carrying digested sludge from sludge holding tanks to the centrifuges for dewatering	Reduced the internal diameter of the pipeline from 100 mm to 50 mm	WWTP in UK
Doyle *et al.* (2000)	Walls of pipeline carrying digester liquors	Reduced the internal diameter of the pipeline from 150 mm to 60 mm within 12 weeks	Sludge Treatment plant in UK
Battistoni *et al.* (2004)	Walls of pipeline carrying digester liquors	Reduction in the internal diameter of the pipeline	Pilot trials for a fluidised bed reactor plant at a WWTP in Italy
Neethling and Benisch (2004)	Flowmeters on streaming pipes regulating polymer dosing.	Accumulation in a 3 inches rubber lined 90^o elbow over a period of 2 months	WWTP in USA

2.1.2 Depletion of Phosphate Rock Reserves

Phosphate is essential to life. It plays a vital role in intercellular energy transfer (during the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) (see Figure 2.2) and is a central building block in DNA, teeth and bones. The conversion of phosphate constitutes part of the P-cycle and entails two major pathways: (i) organic short-term cycles, constituting the uptake of phosphates by plants and algae from soil and water; which are then consumed by animals, and returned to water and soil via animal faeces/urine or upon death; and (ii) inorganic long-term cycles, which involve the continuous transport of phosphate from land towards inland or coastal waters via mechanical and chemical erosion of the earth's crust (see Figure 2.3). After tens of millions of years, the phosphate in the soils and sediments in water has undergone mountain formation processes and become exposed as 'phosphate rock' (Bergmans *et al.,* 2014). However, natural phosphate cycles have become disrupted by anthropogenic activities, diminishing phosphate rock reserves too quickly and possibly making our society P-limited (Cordell *et al.,* 2009).

Desertification and intensification of agriculture have tremendously accelerated the natural transportation of phosphates to coastal water bodies, and the subsequent depletion of phosphate reserves in farmlands. Increased urbanisation also has led to 'unbalanced' transport of phosphates towards urban centres while causing the gradual depletion of phosphate reserves in other areas. Furthermore, the high dependence in developed countries on phosphate fertilisers to sustain high crop yields has further disrupted the global phosphate balance due to the extensive transportation from the few nations with natural phosphate rock reserves (see Table 2.2) to those with less significant P-reserves. Global food transport (which carries phosphates as well) also has been observed to flow away from sustainability over time. Moreover, the complex mechanism of phosphate mining, transportation and processing leads to further significant phosphate losses.

Overall, our global phosphate reserves are diminishing in quality and quantity. For example, out of the estimated 12,000 million tonnes phosphate rock that can be mined at low cost (i.e. <$40 per tonne), about 6,370 million tonnes has been used (CRU, 2003; von Horn and Sartorius, 2009). Barnard (2009) revealed that about 80% of the phosphates mined was being lost during fertiliser production, field application and food processing; never reaching food that is actually consumed. Their report further suggested that known phosphate reserves will be depleted within 200 years if nothing is done to better recover and recycle 'used' phosphate.

This problem is exacerbated because our remaining phosphate rock reserve is often contaminated by heavy metals, such as cadmium (von Horn and Sartorius, 2009), which is also a common problem with P-recovery from industrial processes, and generally when dosing metal salts in municipal wastewater.

In order to make an attempt at tackling this growing phosphate crisis, the recovery of phosphates from nutrient-rich digester liquors is becoming increasingly desirable. Rahaman *et al.* (2014) highlighted this by stating that recovery of phosphates from wastewater ‘is no longer a possibility, but rather an obvious reality’. Phosphate in water exists as orthophosphates (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}), polyphosphate, and organic phosphate (Metcalf & Eddy, 2004). Current estimates suggest that >90% of phosphates in digester liquors could be recovered via struvite crystallization, achieving about 50-80% total P recovery (Ueno and Fujii, 2001; von Munich and Barr, 2001; Wu and Bishop, 2004; Battistoni *et al.,* 2005; Suzuki *et al.,* 2005; Suzuki *et al.,* 2007; Westerman *et al.,* 2009). Recovered struvite then can be used as a slow release fertiliser in high rate applications (due to its low solubility), and is recommended for forest or grassland environments where high solubility fertilisers are less desirable (Ohlinger *et al.*, 1998; De-Bashan and Bashan, 2004). Recovering and/or recycling phosphates will not only help reduce extensive mining of natural phosphate rock, but may also assist in providing greater balance in the natural P-cycle.

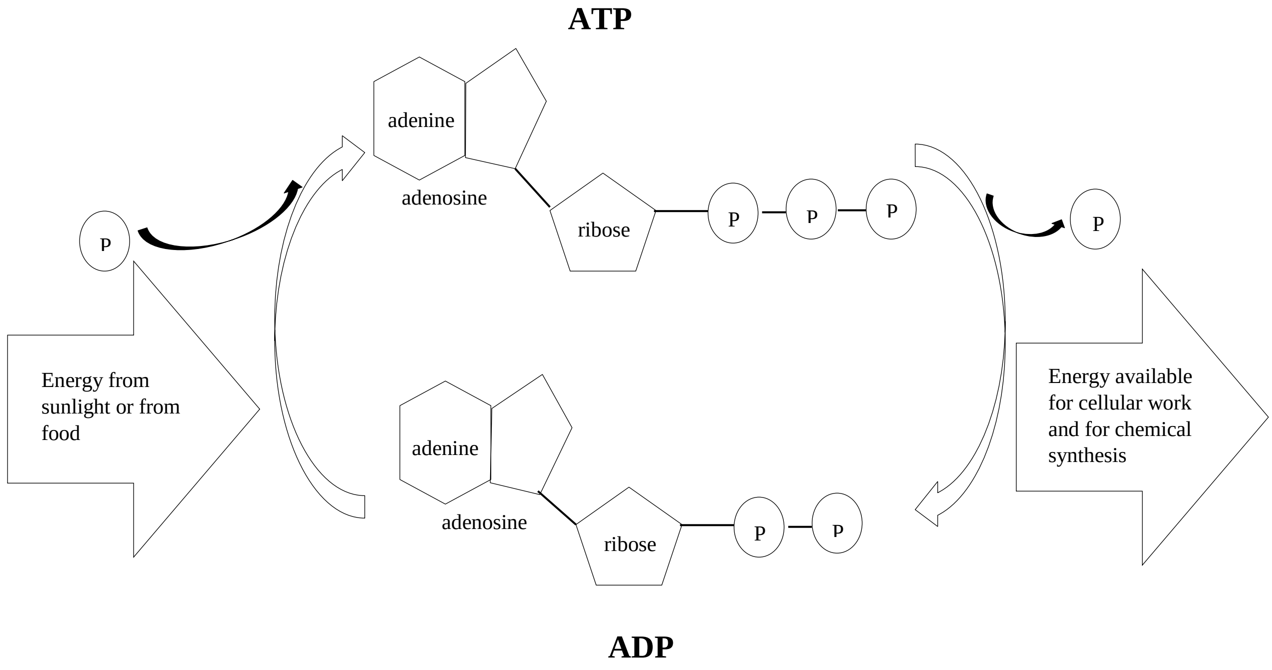

Figure 2.2: The role of phosphates in the conversion of ADP to ATP (Alberts *et al.*, 2004)

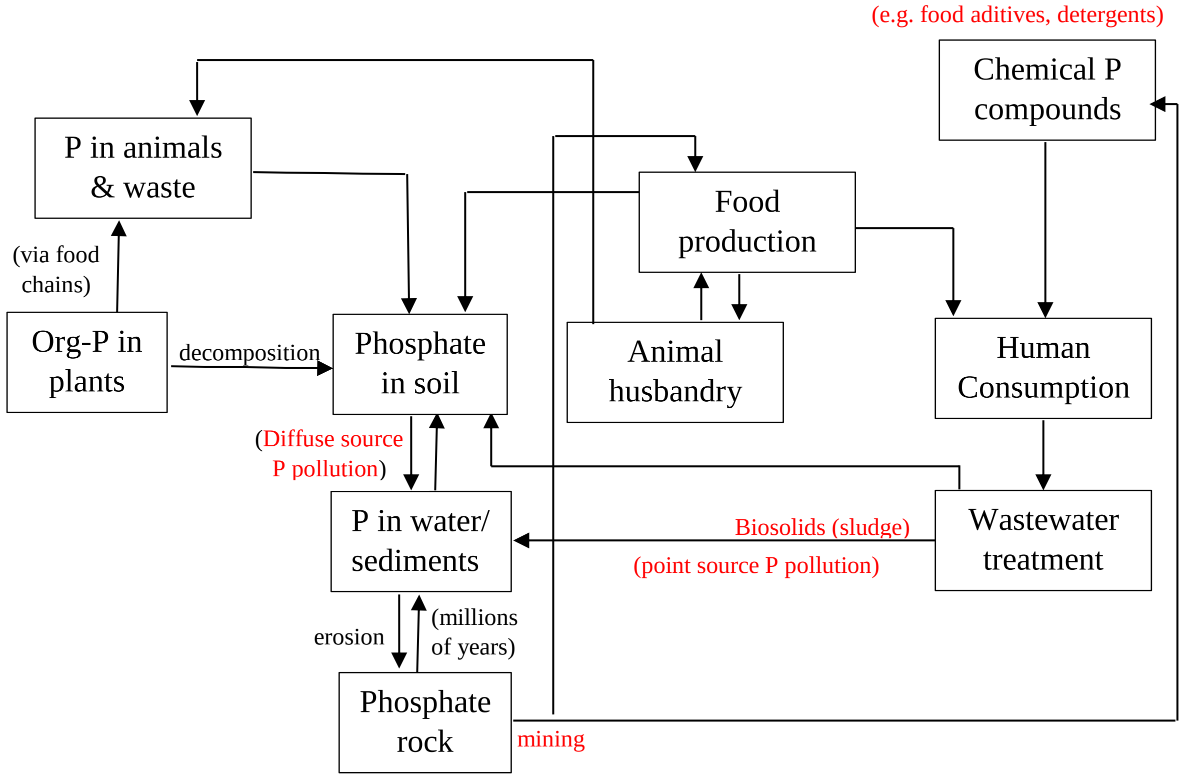

Figure 2.3: The P-Cycle (Adapted from Withers *et al.*, 2015)

Table 2.2: Global phosphate rock reserves (von Horn and Sartorius, 2009)

Nation	**Reserves (1000 tonnes)**
Morocco	5,700,000
Jordan	900,000
China	500,000
Israel	180,000
Russia	150,000
Senegal	150,000
South Africa	100,000
USA	100,000
Togo	30,000
Other Countries	1,200,000
World Total	**12,000,000**

2.1.3 Economic benefits of nutrient removal and recovery from RLs

Recent research has shown that the recovery of N and P from wastewater '*is no longer a possibility but rather an obvious reality*' (Rahaman *et al.,* 2014). The overall benefits of N and P recovery from RLs are as follows: (i) revenue generation from the sale of recovered nutrients; (ii) reduced operating costs at WWTPs due to the reduction in the reagents required for the chemical precipitation of these nutrients; (iii) reduction of sludge production and costs associated with its management; and (iv) reduction in maintenance costs of pipelines and other equipment due to reduced scaling (Molinos-Senante *et al.,* 2011).

Lind *et al.* (2001) reported that recycling recovered nutrients from wastewater, could substitute 35-45% of chemical fertiliser being applied in agriculture. The N in RLs can be precipitated out as ammonium sulphate through ammonia stripping and sold as a fertiliser or as an industrial chemical (Thorndahl, 1992). However, the recovery of N and P as struvite has been shown to be cheaper than other recovery technologies (e.g. membrane separation, algae systems, ammonia stripping, Recovery of gasified N as solids using polymers) and the end-product is relatively cleaner (Moerman *et al.*, 2012; Muster *et al.*, 2013; Egle, 2014). Dockhorn (2009) observed savings of €2 to €3 per kg of P recovered while comparing the operational cost of struvite formation technologies and other conventional removal technologies. The struvite recovery technology is becoming increasingly desirable as recent studies have revealed alternative Magnesium ions sources which could further enhance the financial viability of the struvite formation technology (Lahav *et al.,* 2013; Kruk *et al.,* 2014).

Van Dijk and Braakensiek (1984) and Münch and Barr (2001) estimated that about 1.0 kg struvite can be recovered from 100m^3 of typical municipal wastewater. Stoichiometrically, this can be achieved in wastewaters with >10 mg P/L. Hence, municipal wastewaters with total-P levels typically 7 – 8 mg/L, will require industrial wastewater inputs as total-P concentrations in raw municipal wastewaters with minor industrial wastewater contributions are typically between 15 and 25 mg/L (Henze, 2008). Comparatively, total-P in sewage sludge is typically 40 – 300 mg/L (Henze *et al.,* 2001), which promotes the growing interest in P-recovery in sludge. For example, Tables A.6 and 3.3 show PO_4-P levels of 27 ± 4.4 mg/L and 127 ± 4.4 mg/L in combined influent industrial wastewater and raw sewage sludge at NWL's Bran Sands WWTP. Ueno and Fujii (2001) indicated that struvite is recovered in Japan from digester liquors and being sold to fertiliser companies at €227 per tonne. In Australia, the market value for struvite recovered from digester liquors is estimated at €171 - €285 per tonne by Münch and Barr (2001) and more recently at €421 per tonne (Shu *et al.,* 2006). Based on comparative analysis with the market value for commercial fertilisers, Dockhorn (2009) later estimated the value of struvite at €692 per tonne.

The ‘controlled’ precipitation of struvite from RLs has also been seen to reduce the amount of NH_4-N and PO_4 being recycled to the secondary stage of WWTPs (for further treatment), which can reduce overall operating costs at WWTPs. This also reduces the amount of sludge to be treated and disposed to landfills; hence a reduction in related transportation costs. There is a further reduction in maintenance costs associated with the reduced potential for ‘uncontrolled’ struvite scaling in WWTP processes (Malamis *et al.*, 2014).

2.1.4 Environmental Impacts of the excess nutrients in digester RLs

Following recycling of nutrient-rich RLs to the secondary stage of WWTPs, ‘untreated’ N and P in the effluent can lead to reduced water quality in ‘receiving’ water bodies (e.g. coastal water bodies) due to oxygen depletion and eutrophication (Sharpley and Menzel, 1987). Furthermore, Chambers *et al.* (1994) noted that the loading of large amounts of nutrients in surface water bodies via the effluent from WWTPs, significantly alter the chemistry of riverbed sediments. Finally, the release of excess ammonia into aquatic environments can be toxic to the fish and other aquatic life (Dhakal, 2008), and can result in odour problems (Jaffer *et al.,* 2002). For instance, in 1995, there was an incidence of nutrient-related pollution in 56 miles of Missouri streams, which resulted in the death of over 302,000 organisms (fish inclusive) (Auckley, 2000).

In an effort to resolve negative environmental consequences from excess nutrients released in wastewater (RLs in particular), their efficient removal or better, potential recovery has become vital. More stringent regulations (e.g. the UWWTD (98/15/EC)) to reduce these problems have been enacted throughout Europe that have triggered interests in the development of innovative technologies for tackling nutrients in wastewater (RLs in particular). These regulations particularly target releases to water bodies sensitive to eutrophication. Some of these technologies have been tested and found beneficial both economically and environmentally (Hernández *et al.*, 2006), but more work is needed, especially appropriate nutrient removal options for RLs.

2.2 Technologies for nutrient removal from RLs

The separate treatment of digester liquors has been suggested as an ideal solution for meeting more stringent WWTP effluent standards regarding nutrient removal (Wett & Alex, 2003; Fux

& Siegrist, 2004; Gustavsson, 2010). It is considered potentially cost-efficient and, if appropriately designed, could reduce the N and P loads fed back to the secondary stage of the WWTP; and thereby safeguarding the robustness of the treatment system (Janus and van der Roest, 1996). Two principal categories of treatment technologies have been considered for nutrient removal; i.e. physico-chemical and biological treatment technologies (see Figure 2.4). Some of the physico-chemical treatment technologies identified include: (i) for P removal: crystallisation as struvite (e.g. via the OSTARA process) or as hydroxyapatite (via DHV (Ingenieursbureau Dwars, Heederik en Verhey), CSIR (Council for Scientific and Industrial Research) or Kurita technologies); and (ii) for N removal: crystallisation as struvite, and ammonia stripping (i.e. via steam or air stripping). Some of the biological treatment technologies identified include: Nitrification – Denitrification (e.g. conventional activated sludge systems), Nitritation – Denitritation (e.g. SHARON), and Anammox technologies for N removal; and Enhanced Biological P removal (EBPR), and denitrifying P removal to tackle P in RLs. The efficiency of these technologies depends on pH, and other physical, chemical and biological parameters of RLs.

Some early studies dismissed the use of physico-chemical techniques for N removal as being more expensive than biological N removal. However, factors such as the low BOD/P ratio of RLs and high sludge age of digested sludge (reported to inhibit biological P-removal processes; Neethling *et al.,* 2005), have made physico-chemical processes more appealing for P-removal from RLs. In addition, most biological P removal technologies tend to have long start-up periods (Barnard, 2006). Further, some physico-chemical techniques (e.g. struvite crystallisation) can be particularly applicable in recovering N and/or P from RLs as commercial products (e.g. struvite (magnesium ammonium phosphate-$NH_4MgPO_4.6H_2O$) (Siegrist, 1996; Fux & Siegrist, 2004; Gustavsson, 2010). This technology was chosen and assessed experimentally within this thesis as the primary P removal technology due its ability to simultaneously recover N and P. The technology also has been reported to be cheaper than other physico-chemical techniques, easier to operate, produces a relatively clean commercial product, and also can be applied to the ‘sludge’ phase (Geerts *et al.* 2015; Marchi *et al.* 2015). While this technology can potentially remove ~90 % P from digested sludge and even more from RLs, the N load and N/P ratio in RL are so high that it is often not economically viable to recover more than 50 % via this technology (or other physico-chemical technologies). As a result, biological N removal options (which have been very successful in >90 % N-removal from RLs) may be considered as a ‘polishing’ step. Metcalf and Eddy (2003), Gao *et al.* (2003), and Zhang *et al.* (2016), reported that conventional activated sludge (CAS) systems have been the most successful biological N-removal technology. However, its high operating cost, high potential for nitrous oxide emissions from nitritation-denitritation and low industrial applicability of anammox technologies, have led interests towards more innovative cost-effective technologies, such as aerobic granular sludge technology. This technology was studied in this thesis as a biological N-removal option for RLs. The technology was chosen due its operational advantages highlighted in existing reports (e.g. De Kreuk and van Loosdrecht, 2006; Pronk *et al.*, 2014), including: its low space requirement, low oxygen demand, low operating cost, and its low susceptibility to adverse changes in influent wastewater compared to CAS. Detailed descriptions of possible nutrient treatment options are summarised in the following sections.

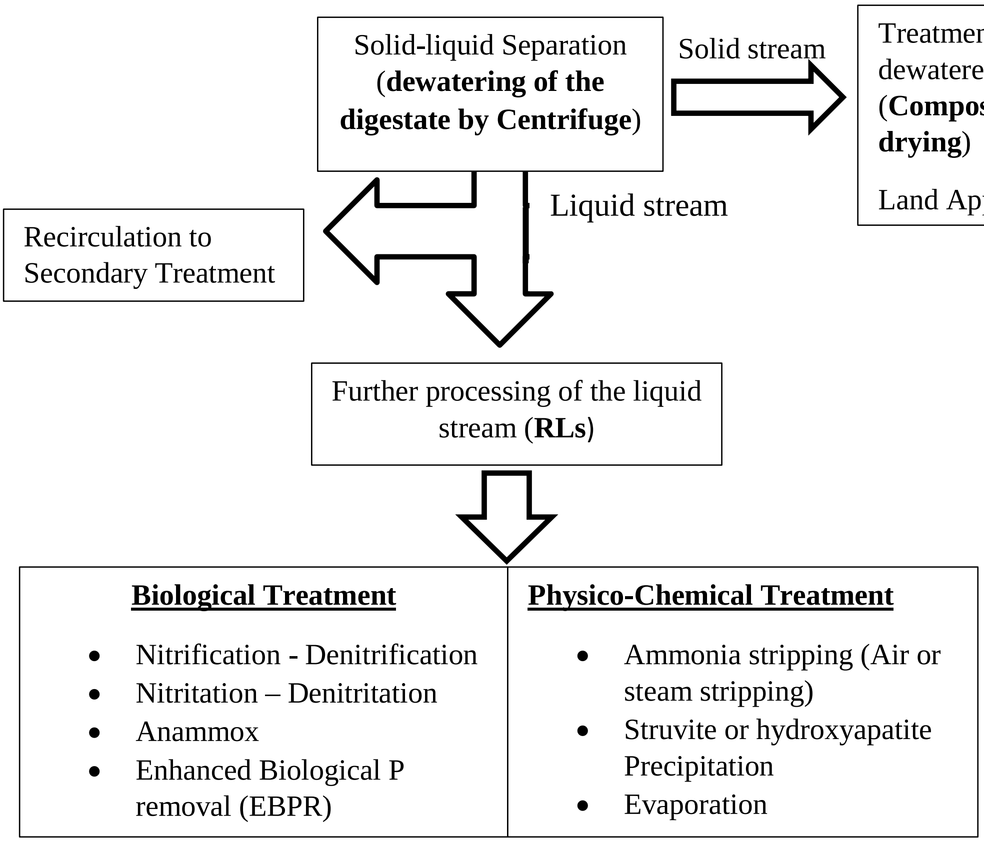

Figure 2.4: Some existing treatment paths for digester liquors and digestate (Adopted from Wäger-Bauman, 2011; Malamis *et al.*, 2014)

2.3 Physico-chemical techniques for P removal from RLs

2.3.1 Crystallisation processes (e.g. DHV Crystalactor Technology & Ostara Pearl Process)

The Crystalactor Technology (CT) was initially designed for municipal wastewater with total P- concentrations between 9 mg/L and 23 mg/L. The process entails the crystallisation of phosphate on sand grains as calcium phosphate in a fluidised-bed reactor (as crystalliser) and can achieve an effluent quality of 0.5 mg P/L. Alternative phosphate recovery products (e.g. struvite, magnesium phosphate, potassium magnesium phosphate) yield effluent quality of 5-10 mg P/L, which implies CT can achieve lower P levels. The final products (pellets) of CT are of reasonable quality/purity with the potential of being used as fertilisers or raw materials for the production of phosphate fertilisers. However, readily available lime is needed to raise pH as well as the injection of seeding grains (i.e. sand), since sand levels become depleted as part of the final product (Giesen, 2009). The CT process has no hydrocarbon-fuel requirement (hence lower carbon-footprint) and can be used to reduce turbidity, BOD content and heavy metals in the RLs, but it also requires the application of chemicals and high operation skills. In the UK, a pilot plant was installed at Chelmsford WWTP. The technology has also been commercialised globally for water softening. For example, the technology has been used for P-recovery in Westerbork (12,000 population equivalent; p.e.), Heemstede (35,000 p.e.) and Geesmerambacht WWTPs (230,000p.e.) in the Netherlands; and also from dairy wastewater at Alto Dairy (Procorp Inc.) in the USA (Environment Agency, 2012).

In contrast, the Ostara Pearl process is based on the controlled chemical precipitation of phosphate compounds in a "*fluidised bed reactor that recovers struvite as highly pure crystalline pellets".* The final product has been commercialised as a fertiliser known as Crystal Green (which has been endorsed by the Environment Agency and UK Trading Standards Service to be sold in the UK as a premium-quality fertiliser). The Ostara process has the potential to remove ~90% of P from nutrient-rich RLs with 60-160 mg/L PO_4-P. RLs are mixed with magnesium chloride (and a dose of sodium hydroxide, if necessary) and then fed into the Ostara Pearl reactor where struvite 'seeds' form (Britton, 2009) (see Figure 2.5). The seeds then expand in size via a mechanism driven by the hydrodynamics of the system. This process also requires high operation skills and chemicals inputs, but purity of the fertilizer product can be high and also controlled.

In the UK, pilot-scale Ostara plants have been operating at Severn Trent Water's Derby and Thames Water's Slough WWTPs since 2009 and 2010, respectively (Environment Agency, 2012). Full-scale installations also are operating in; Edmonton, Canada (since 2007); Portland, Oregon, USA (since 2008); Nansemond, Suffolk and York WWTPs, Virginia, USA (since 2010), Rock Creek Advanced WWTP, Hillsboro, Oregon, USA (since 2010) and Nine Springs WWTP, Madison, Wisconsin, USA (since 2011) ((Ostara, 2011).

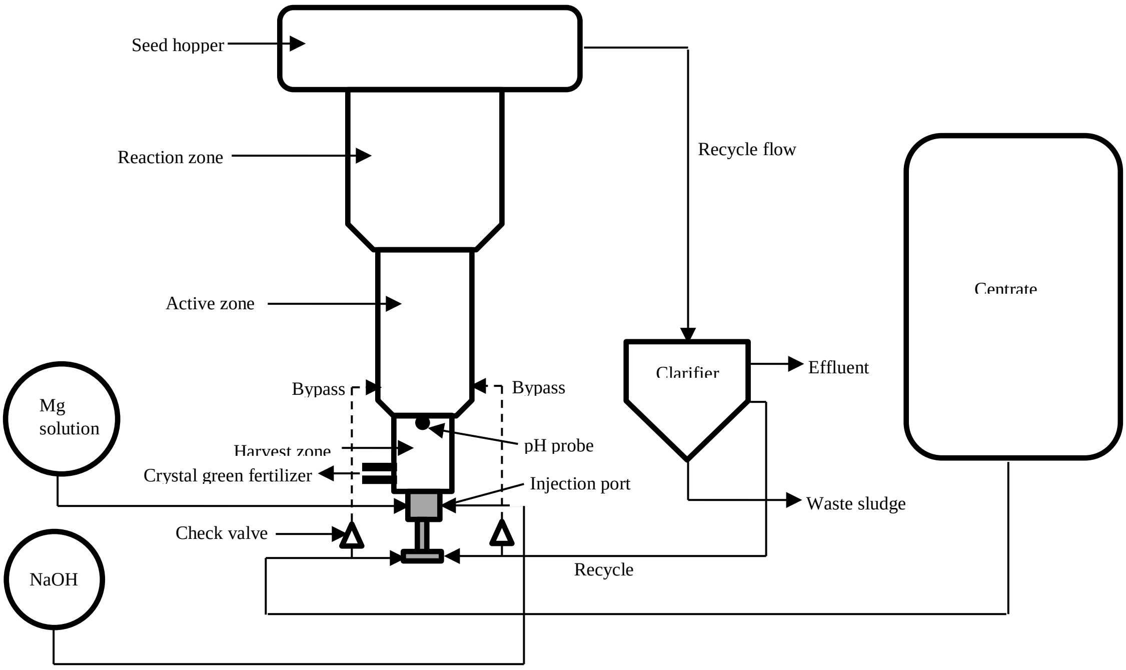

Figure 2.5: A schematic of the Ostara Pearl Process (Britton, 2009)

2.3.2 CSIR technology

This technology is similar to CT and was designed as a fluidised-bed crystallisation column. Its efficiency relies on the composition of the RLs and pH conditions; i.e. P-concentration ranging between 30-80mg P/L and pH of ~9.5 are usually required for effective crystallisation. The final product can be recovered as hydroxyapatite (HAP) or struvite. However, this technology has only been tested at lab-scale in South Africa (Environment Agency, 2012).

2.3.3 Kurita Process

This is a fixed-bed crystallisation process developed for phosphate removal from secondary treatment effluents (hence assumes low total P-input; ~1 mg P/L) with the use of phosphate rock particles as ‘seed’ material. The final product can be recovered as hydroxyapatite (HAP) or struvite (Cecchi *et al.*, 2003; Environment Agency, 2012). In 1998, a pilot scale plant was installed in Osaka-Minami ACE centre in Japan (Mishina *et al.*, 1999).

2.3.4 REM-NUT (i.e. REMoval of NUTrients) Technology

The technology was developed in the 1970s for struvite recovery from municipal wastewater using ion exchange and chemical precipitation by the Water Research Institute of Italy’s National Research Council. The process constitutes a natural zeolite, two ion exchangers, and a strong base anion resin that simultaneously remove potassium, ammonium and phosphate ions, respectively from biologically oxidised wastewaters. These ions are then recovered by precipitation from the exchangers in the form of ammonium and/or potassium struvite (Liberti *et al.*, 1979; Petruzelli *et al.*, 2004). This technology has only been applied at lab- and pilot-scale at Massafra WWTP in Southeast Italy. However, the technology never reached full-scale application because of various limitations: (i) unbalanced NH_4^+/HPO_4^{2-} molar ratio (commonly 10/1 influents at WWTPs), (ii) difficulty in finding sorbents which are strictly phosphate-selective, and (iii) potential fouling of the exchangers (Petruzzelli *et al.*, 2004).

2.3.5 AirPrex Technology

This technology was developed by Berliner Wasserbertriebe (BWB) in Germany to prevent ‘uncontrolled’ struvite precipitation after AD of sludge at an EBPR WWTP. It is usually installed directly after digesters and applied to digested sludge (prior to dewatering), in order to facilitate the crystallization of struvite in a controlled process environment. In the AirPrex process, digested sludge is pumped into an aerated precipitation tank where the pH increases through intensive aeration that strips CO_2. Magnesium (in the form of $MgCl_2$) is then added to form struvite in the precipitation tank, followed by sedimentation and collection at the bottom. This is followed by the washing and gentle drying of the formed struvite to remove any organic and inorganic impurities (see Figure 2.6). The final product (e.g. ‘Berliner Pflanze’) is registered and approved by ‘REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals)’ as a fertiliser. AirPrex technology is currently marketed by cnp-Technology Water and Biosolids GmbH. There are full-scale installations in Wassmannsdorf WWTP in Berlin - Germany, MG-Neuwerk WWTP in Niersverband – Germany, Echten WWTP in Reest and Wieden, Netherlands, and Amsterdam-West WWTP in Waternet, Netherlands. AirPrex technology has been observed to: potentially remove 90 – 95% P from sludge, improve the dewaterability of digested sludge and minimise scaling in treatment units downstream of the AD. However, the supply of air to help elevate the pH of the process constitutes an additional energy cost (Forstner, 2015).

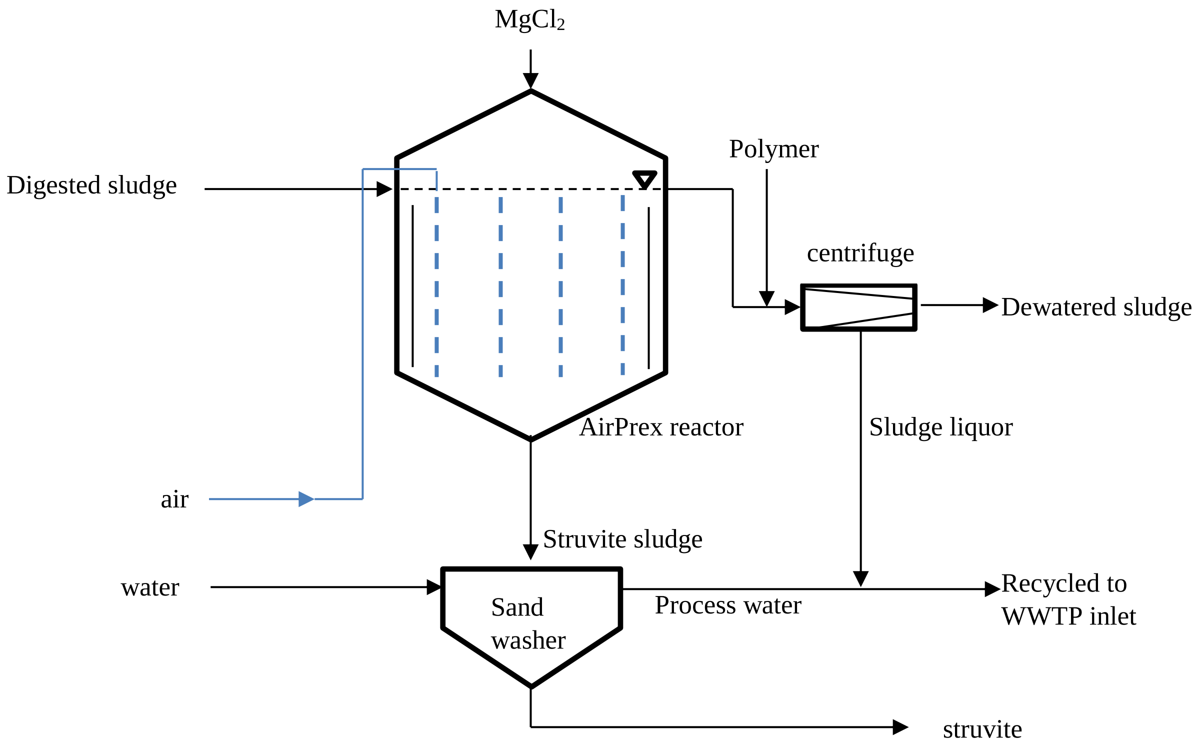

Figure 2.6: Schematic of the AirPrex process (Forstner, 2015)

2.4 Enhanced Biological P removal (EBPR) and Denitrifying EBPR (DNEBPR)

EBPR refers to the process of removing P from wastewater by accumulating it within bacterial cells as poly-P (polyphosphate) in excess of levels commonly needed for growth and metabolism (Seviour *et al.*, 2003). It is achieved by alternating aerobic and anaerobic conditions to foster the proliferation of poly-P-accumulating organisms (PAOs). The principle behind this technology is that, during the anaerobic phase, there is the uptake of organic matter and the release of P, while PAOs take up P in the subsequent aerobic phase (see Figure 2.7). P is typically stored in sludge as high energy poly-P and released during the anaerobic phase as ortho-P (orthophosphate) from the cell. During this phase, organic matter (such as VFAs) is taken up and stored intracellularly as poly-β-hydroxyalkanoates (PHAs) (Marias *et al.*, 1983; Mino *et al.*, 1988; Satoh *et al.*, 1992). The formation of PHAs is facilitated by the glycolysis of glycogen and polyphosphate (stored in cells) and the tricarboxylic acid (TCA) cycle under anaerobic conditions. During the aerobic phase, the released ortho-P is taken up and stored in the cell as Poly-P while the stored PHA is used for energy production through oxidative phosphorylation. During this phase glycogen is also seen to be replenished in the cell structures (see Figure 2.7) (Sathasivan, 2008). Net P removal is achieved by sedimentation and removal of phosphate-rich sludge.

Fuhs and Chen (1975), first reported *Acinetobacter spp.* as the predominant species in the EBPR process; however further studies (Jenkins and Tandoi, 1991; Kampfer *et al.*, 1996; van Loosdrecht *et al.*, 1997; Mino *et al.*, 1998) revealed that *Acinetobacter spp.* showed no major P-uptake characteristics. Early studies aimed at understanding microbial communities associated with EBPR were hampered by limited techniques to isolate and identify PAOs. However, recent developments in microbial molecular techniques, have facilitated research in PAOs, with *Candidatus* '*Accumulibacter phosphatis*' (commonly called *Accumlibacter*) nowreported as the predominant species in EBPR sludge (Hesselmann, *et al.*, 1999). The work of Oehmen *et al.* (2007) and Mao *et al.* (2015) also showed a correlation between the abundance of *Accumlibacter* in many different full scale EBPR and the P content of the sludge. For instance, in some cases, *Accumulibacter* abundance as high as 90% was reported in a sludge with excellent EBPR performance.

EBPR has been widely applied to treat various types of wastewaters. For example, Frison *et al.* (2012a) demonstrated the applicability of EBPR for treating RLs. P-removal efficiency via biological systems depend on the P-content (e.g. BOD/P ratio >10/1) and the efficiency of the sedimentation/ clarification process. Although EBPR has been observed to be cost-effective in most cases, its P-removal efficiency fluctuates and remains inconsistent unless the conditions for its effective operation are well understood and properly managed (Oehmen *et al.*, 2007; Mao *et al.*, 2015). Furthermore, under certain operating conditions (e.g. at pH <7), glycogen accumulating organisms (GAOs) outcompete PAOs for available substrates and this leads to EBPR failures.

Earlier reports emphasized the importance of preventing the entry of nitrates into the anaerobic phase of stable EBPR systems because elevated nitrates may provide the opportunity for denitrifying bacteria to deprive PAOs of their competitive advantage fpr available substrates (Kern-Jespersen and Henze, 1993; Kuba *et al.*, 1993; Kuba *et al.*, 1996; Jorgensen and Pauli, 1995; Barker and Dold, 1996). However, other reviews have shown the occurrence of EBPR in the presence of nitrate during the anaerobic phase in the absence of exogenous carbon (C) sources. This was presumed to have happened as a result of organisms utilising stored PHAs as energy and C sources to take up P and store as poly-P (as PAOs do), while using nitrate (not oxygen) as terminal electron acceptor (Mino *et al.*, 1995; Mino *et al.*, 1998; Seviour *et al.*, 2003). Hence the name denitrifying EBPR (DNEBPR). This general biological mechanism has also been used to treat RLs under anoxic conditions associated with denitrification via nitrite (i.e. denitritation) (Peng *et al.*, 2011). This latter case observed that some denitrifying bacteria have the ability to accumulate large amounts of poly-P because they possess similar metabolism to many PAOs found in conventional EBPR (Meinhold *et al.,* 1999; Nielsen and Nielsen, 2002; Seviour *et al.,* 2003); hence earning them the name denitrifying PAOs (DNPAOs). As an example, Frison *et al.* (2012a; 2013) reported significant P removal in association with nitrite-processing during denitrification. They showed that these phenomena can be achieved in a single reactor under anoxic conditions. Denitrifying P removal via nitrite has been observed to be relatively cheap as there is a low external carbon and energy requirement since nitrite is being used as an electron acceptor (Malamis *et al.*, 2014). However, other studies have shown that high nitrite concentrations and its derivatives in the mixed liquors could inhibit the growth of PAOs (Saito *et al.,* 2004; Peng *et al.,* 2011).

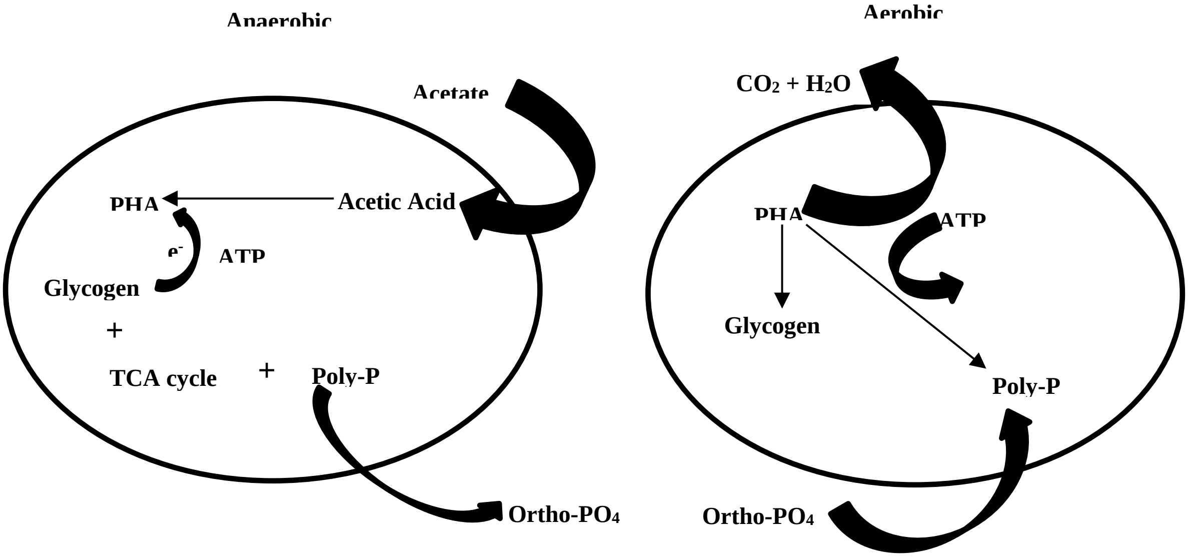

Figure 2.7: Schematic of the main biochemical features of EBPR (Seviour *et al.*, 2003; Forbes *et al.*, 2009)

2.5 Physico-chemical techniques for N removal from RLs

2.5.1 Air stripping

This involves the stripping of N from wastewater as ammonia (USEPA, 2000). The majority of N in RLs is present as NH_4-N, and therefore requires pH adjustment to basic conditions to convert the NH_4-N to ammonia gas for air stripping (Janus and van der Roest, 1997; Lei *et al.,* 2007; Walker *et al.,* 2011). Lime or caustic soda is usually used to achieve a target pH of 10 - 11.5. This shifts the equilibrium such that the NH_4-N moves from the aqueous phase into the gaseous phase as ammonia (Thorndahl, 1993). The stripping process is carried out in a cross-flow or counter current stripping tower. In the cross-flow tunnel, air is introduced throughout the column and it flows through the packing material (in the tunnel) as the RLs flow downwards. Whereas, in the counter current system, air is drawn through pores at the bottom of the tunnel as the liquor is introduced from the top. Free ammonia is then stripped from falling water droplets into the air stream and is either released into the atmosphere, combusted or absorbed with an acid scrubber (e.g. sulphuric acid) for commercial purposes (e.g. ammonium sulphate which can be used as a liquid fertiliser) (Thorndahl, 1992; Mertooetomo *et al.,* 1993; Little and Marinas, 1997).

The ammonia removal efficiency of the air stripping technique is highly dependent on the air temperature, and the air and/or water ratios where treatment performance decreases significantly with decreases in air temperature (Nirmalakhandan *et al.,* 1997). About 90 to 95% ammonia removal efficiency was reported at air temperatures of 20°C (68°F) and a corresponding 75% drop in removal efficiency was observed as the temperature fell to 10°C (50°F) (USEPA, 2000).

Advantages of air stripping include: it doesn't create any backwash; its performance is not affected by the presence of toxic chemicals in RLs as opposed to biological methods; and its operation is relatively simple assuming the temperature and pH of the liquors remain stable. On the other hand, some of the drawbacks of the technology include: higher maintenance and power requirements associated with the constant re-pumping of water up the stripping tower; it cannot be operated in freezing conditions due to the effect of temperature on its performance; the discharge of ammonia and other gases (e.g. sulphur dioxide) into the atmosphere may not comply with air quality regulations; it doesn't remove nitrites and nitrates; operation and maintenance concerns may arise from the addition of lime for pH adjustment; possible noise problems; and the susceptibility of packing material (wood) in the stripping tower to high pH (Lamarre and Sherouse, 1994; USEPA, 2000; Huang and Shang, 2006). Also, air stripping is only suitable for wastewater with 10-100mg/L of ammonia. For wastewaters with higher ammonia levels (i.e. >100mg/L), alternative removal technologies (e.g. biological or steam stripping methods) are recommended (USEPA, 2000).

2.5.2 Steam stripping

The operating principle of this technique is similar to air stripping except for the final discharge of the stripped ammonia. Unlike the air stripping system whereby the ammonia is either acid scrubbed or combusted, the steam stripping method produces aqueous ammonia that can be concentrated in a subsequent distillation step (Janus and van der Roest, 1997). This process is very energy intensive and hence more expensive than air stripping. The aqueous ammonia product is economically usable (containing about 20% ammonia). Its purity allows for its easy reuse (e.g. Denox units) (USEPA, 2000).

2.5.3 Struvite precipitation

Struvite has been reported to form spontaneously in some biological media. For example, Omar *et al.* (1994) reported its formation in rotting organic material (e.g. animal manure and guano), through the biochemical reaction of NH_4^+ (from bacterial metabolism) with available PO_4^{3-} and Mg^{2+} ions. The main physico-chemical properties of struvite are presented on Table 2.3 below:

Table 2.3: Physico-chemical properties of struvite (Adapted from Le Corre *et al.*, 2009)

Chemical name	Magnesium ammonium phosphate hexahydrate
Formula	$MgNH_4PO_4.6H_2O$
Molecular weight	245.43 g/mol
Nature	Mineral salt
Aspect	White glowing crystal (Bassett and Bedwell, 1933)
Structure	Orthombic: regular PO_4^{3-} octahedral, distorted $Mg(H_2O)_6^{2+}$ octahedral, and NH_4^+ groups all linked together by hydrogen bonds (Abbona and Boistelle, 1979)
Specific gravity	1.711 (ρ = 1.711 g/cm^3) (Borgerding, 1972)
Solubility	Low solubility in water: 0.018 g/100ml at 25˚C in water High in acids: 0.033 g/100ml and 0.178 g/ml at 25˚C in 0.001 N HCl and 0.01 N HCl, respectively (Bridger *et al.,* 1961)
Solubility constant	$10^{-13.26}$ (Ohlinger *et al.*, 1998)

Struvite precipitates in the presence of excess NH_4^+, PO_4^{3-} and Mg^{2+} ions according to the chemical reaction below (see Equation (1)) when the pH is sufficiently high and the thermodynamic solubility product (K_{sp}) of struvite is exceeded. Struvite precipitates in an equimolar ratio (i.e. a NH_4^+:PO_4^{3-}:Mg^{2+} ratio of 1:1:1). (Celen and Turker, 2001; Doyle & Parsons 2002; Ali *et al.*, 2004; Ali, 2007; Sharp *et al.*, 2013; Mudragada *et al.*, 2014):

$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \Leftrightarrow MgNH_4PO_4.6H_2O$ **Equation 2**

From Equation 2, the thermodynamic solubility of struvite (K_{sp}) is:

$K_{sp} = [Mg^{2+}]\,[NH_4^+]\,[PO_4^{3-}]$ **Equation 3**

Struvite precipitation occurs when supersaturation is attained and Equation 3 becomes:

$K_{sp} < [Mg^{2+}]\,[NH_4^+]\,[PO_4^{3-}]$ **Equation 4**

The thermodynamic equilibria of the different complexes involved in struvite formation are presented in Equations 5 to 12.

Ammonia systems:

$NH_4^+ \Leftrightarrow NH_{3\,(aq)} + H^+ \quad K_n = \frac{[NH_{3\,(aq)}].[H^+]}{[NH_4^+]}$ **Equation 5**

Phosphate systems:

$H_3PO_4 \Leftrightarrow H_2PO_4^- + H^+ \quad K_{p1} = \frac{[H_2PO_4^-].[H^+]}{H_3PO_4}$ **Equation 6**

$H_2PO_4^- \Leftrightarrow HPO_4^{2-} + H^+ \quad K_{p2} = \frac{[HPO_4^{2-}].[H^+]}{H_2PO_4^-}$ **Equation 7**

$HPO_4^{2-} \Leftrightarrow PO_4^{3-} + H^+ \quad K_{p3} = \frac{[PO_4^{3-}].[H^+]}{HPO_4^{2-}}$ **Equation 8**

Magnesium systems:

$MgOH^+ \Leftrightarrow Mg^{2+} + OH^- \quad K_{M1} = \frac{[Mg^{2+}].[OH^-]}{MgOH^+}$ **Equation 9**

$MgH_2PO_4^+ \Leftrightarrow Mg^{2+} + H_2PO_4^- \quad K_{M3} = \frac{[Mg^{2+}].[H_2PO_4^{2-}]}{MgH_2PO_4^+}$ **Equation 10**

$MgHPO_4 \Leftrightarrow Mg^{2+} + HPO_4^{2-} \quad K_{M3} = \frac{[Mg^{2+}].[HPO_4^{2-}]}{MgHPO_4}$ **Equation 11**

$MgPO_4^- \Leftrightarrow Mg^{2+} + PO_4^{2-} \quad K_{M4} = \frac{[Mg^{2+}].[PO_4^{2-}]}{MgPO_4^{2-}}$ **Equation 12**

The total concentration of NH_4^+, PO_4^{3-} and Mg^{2+} (i.e. $C_{T,\,NH4}$, $C_{T,\,PO4}$, and $C_{T,\,Mg}$, respectively) in the ‘reaction mix’ is simply a sum of the individual complexes as shown Equations 13 to 15.

$C_{T,\,NH4} = [NH_{3\,(aq)}] + [NH_4^+]$ **Equation 13**

$C_{T,\,PO4} = [H_3PO_4]+[H_2PO_4^-]+[HPO_4^{2-}]+[PO_4^{3-}]+[MgH_2PO_4^+]+[MgHPO_4]+[MgPO_4^-]$

Equation 14

$C_{T,\,Mg} = [Mg^{2+}] + [MgOH^+] + [MgH_2PO_4^+] + [MgHPO_4] + [MgPO_4^-]$ **Equation 15**

Its formation and precipitation occurs in two stages: nucleation (i.e. crystal birth or the formation of struvite nuclei/embryo) and crystal growth (i.e. the development of struvite crystals) (Jones, 2002; Le Corre *et al.*, 2009). Predicting and possibly controlling these processes is complex as they are driven by a combination of factors such as: the thermodynamics of the liquid-solid equilibrium; kinetics of the struvite formation reaction; the crystal state of the initial compounds, the principle of material transfer between the liquid and solid phases; and various physico-chemical factors (e.g. pH of the wastewater or sludge, supersaturation, mixing energy, presence of other ions, and temperature) (Mullin, 1992; Aage *et al.,* 1997; Ohlinger *et al.,* 1999; Bouropoulos and Koutsoukos, 2000; Doyle *et al.,* 2002; Jones, 2002).

The pH and saturation of struvite component ions in solution, have significant effect on most of the aforementioned factors, and the overall crystal formation and development process. For crystallisation to occur, supersaturation needs to be attained. Bouropoulos and Koutsoukos (2000) reported that supersaturation ratio (**Ω**; see Equation 16) was directly proportional to crystal growth rate and inversely proportional to induction time (i.e. the period between blending solutions containing the reactants for struvite precipitation and the first measurable indication of precipitation (Ohlinger *et al.*, 1999)). Bouropoulos and Koutsoukos (2000) further reported that at a fixed pH of 8.5, increasing **Ω** from 1.13 to 3.33, reduced the induction time by a factor of 20 and the rate of crystal growth increased >55 times. The pH influences the rate of crystallization as it is closely linked to struvite solubility and supersaturation. For instance, Neethling and Benisch (2004) reported that struvite scaling problems at WWTPs was partly due to increasing pH resulting from the stripping of CO_2. In addition, Borgerding (1972) reported that increasing the pH from 5 to 7.5, decreased struvite solubility from 3000 mg/L to <100 mg/L, while Buchanan *et al.* (1994), observed minimum struvite solubility at pH 9. This was consistent with observations of Bouropoulos and Koutsoukos (2000) and Ali (2007), which reported that the optimum rate of struvite precipitation was observed between pH 8.5 and 9.5. Ohlinger *et al.* (1999) also reported that an increase in pH caused an increase in supersaturation with a corresponding increase in crystal growth rate. It was also reported that mean struvite crystal sizes decreased by a factor 5 from 96.5 µm as pH rose from 9 to 11 (Matynia *et al.*, 2006).

$$\Omega = \frac{\{Mg^{2+}\}\{NH_4^+\}\{PO_4^{3-}\}}{K_{sp}} \qquad \textbf{Equation 16}$$

Temperature has also been observed to have a direct relationship with the solubility product (K_{sp}) of struvite. For example, Ksp values were reported to increase from 0.5×10^{-14} at 10˚C to 4.0×10^{-14} at 60˚C (Burns and Finlayson, 1982; Aage *et al.*, 1997). It was also reported that since K_{sp} is linked to supersaturation such that, it is more difficult to achieve struvite precipitation at higher temperatures (>35ºC); hence the reason why most studies on struvite precipitation from RLs and digested sludge have been carried out between 25ºC to 35ºC (Le Corre *et al.*, 2009). Temperature also affects the morphology of struvite. For instance, Babic-Ivancic *et al.* (2002) reported that at high Mg^{2+} concentrations, high temperatures caused struvite to transform into newberyite ($MgHPO_4.3H_2O$) faster than at lower temperatures.

The chemical precipitation of struvite is considered to be an effective method for N removal from RLs (Uludag-Demirer *et al.*, 2005). In a pilot study wherein phosphoric acid and magnesium oxide were dosed into RLs (to adjust the Mg:NH_4-N:PO_4-P ratio to 1.3:1:1 to promote struvite precipitation), about 70% NH_4-N removal was observed when the pH was increased by CO_2 stripping only. When the pH was further adjusted to 9.0 with NaOH, NH_4-N removal rose to about 85 – 90%. With the high pH, NH_4-N removal was linked to both struvite formation and NH_3 stripping. Based on Jiang *et al.* (2010), NH_3 stripping could have accounted for about 50% of the NH_4-N removed. It was reported that excess Mg was needed to lower the equilibrium concentration of NH_4-N and prevent the recycling of PO_4-P (originating from excess phosphoric) to the secondary treatment stage (Celen and Turker, 2001). Laridi *et al.* (2005) later reported an NH_4-N removal efficiency of about 17%, whereby magnesium chloride was dosed into the digester liquors without any addition of PO_4-P, at an optimal pH of 8.5 and 30minutes retention time. The precipitate can then be dewatered (with a centrifuge) to 50% dry solids which can then be commercialised based on its level of purity.

2.6 Biological N removal from digester liquors

Biological nutrient removal methods have been very successful at N removal from RLs and in the subsequent reduction of eutrophication potential in aquatic environments. Both attached and suspended growth processes have been applied in treating RLs, including membrane bioreactors (MBRs), sequencing-batch reactors (SBRs), conventional activated sludge (CAS) technology, and moving-bed biofilm reactors (MBBRs). In some instances, two or more of these technologies have been jointly applied to treat RLs (Malamis *et al.,* 2014). Biological N-removal comprises known reactions within the N-cycle. With respect to these reactions, there is a high potential for the emission of nitrous oxide (N_2O; which is a greenhouse gas) (Hedstrom, 2001). Hence, there is need for caution when choosing a treatment technique. The major treatment processes either involve reactions oxidising ammonia and/or nitrate removal.

Biological N-removal processes are driven by the following groups of bacteria; denitrifying bacteria, ammonia-oxidising bacteria (AOB), nitrite-oxidising bacteria (NOB), and anaerobic AOB (anammox) (Caffaz *et al.,* 2008; Cervantes, 2009; Gustavsson, 2010). The basic categories of biological nitrogen removal have been identified as: (i) conventional nitrification - denitrification; (ii) nitritation – denitritation; and (iii) autotrophic anaerobic ammonium oxidation (anammox) (Fux & Siegrist, 2004). The difference in facilitating the three processes is in terms of their costs, start-time and reliability. These three treatment options can achieve 85-95% N-removal from RLs (Fux, 2003) and all have potential advantages, but they also have drawbacks.

For example, autotrophic nitrification-heterotrophic denitrification processes are well established treatment technologies (which are straightforward to design and operate within engineering operations), but they have high operating costs (in terms of. high aeration energy requirements to oxidise NH_4-N) and require additional carbon for heterotrophic denitrification. On the other hand, autotrophic anaerobic ammonium oxidation (Anammox) is somewhat less expensive, but tends to be less operationally reliable based on current methods (Hedstrom, 2001; Malamis *et al.,* 2014). Nitritation – denitritation is a promising technology due to its low aeration energy and additional carbon requirement (see Figure 2.8), but it poses the risk of excess N_2O production when treating mixed liquors with elevated nitrite levels (Joss *et al,* 2009; Kampshreur *et al,* 2009; Weissenbacher *et al,* 2010; Gustavsson and la Cour Jansen, 2010). Therefore, low-cost innovative technologies for nutrient removal/recovery from RLs are still needed. A brief summary of each technology is provided below.

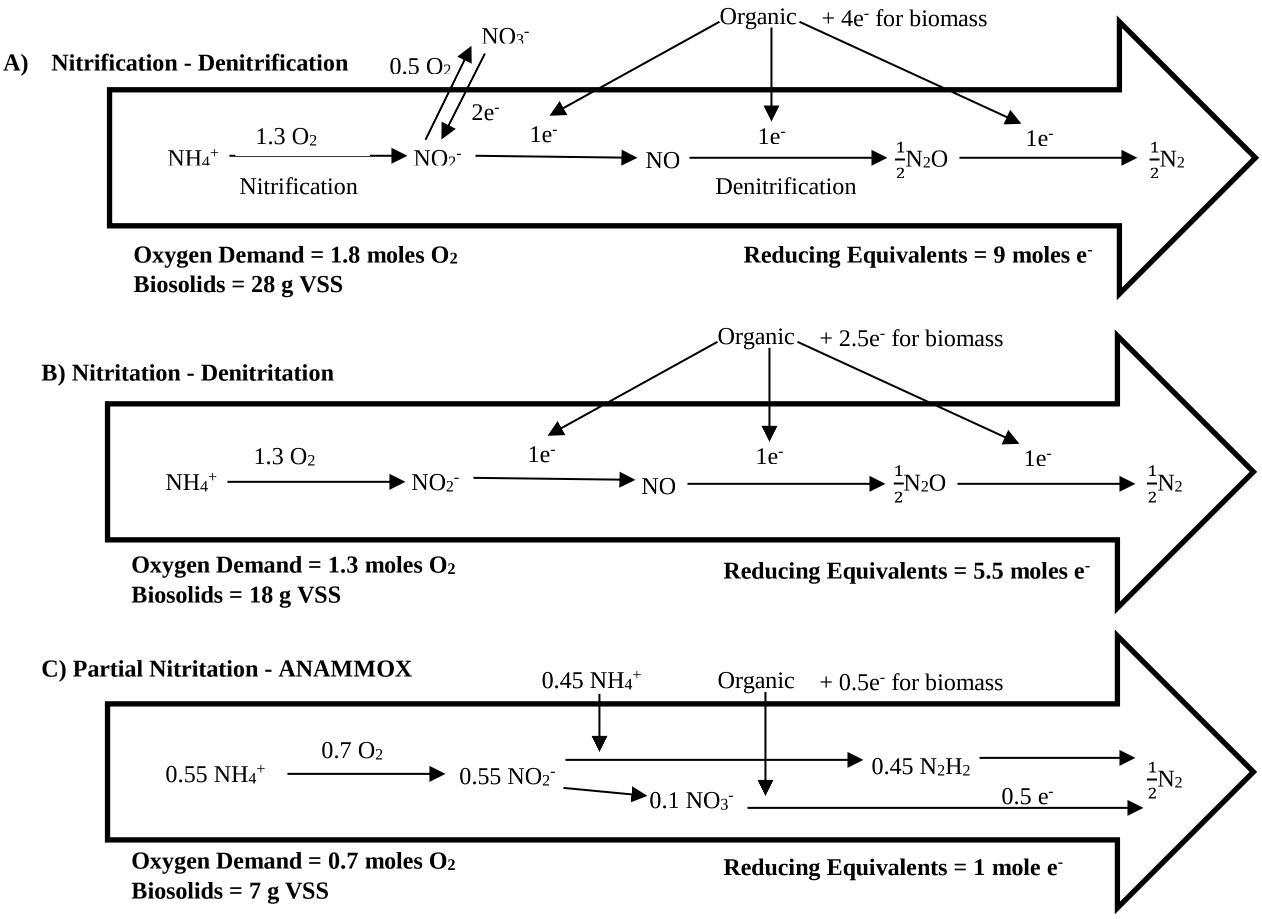

Figure 2.8: Comparison of A) nitrification – denitrification, B) nitritation – denitritation, and C) Partial nitritation - anammox processes (Gao *et al.*, 2014)

2.6.1 Conventional Autotrophic Nitrification - Heterotrophic Denitrification

At an operational scale, this involves nitrification (i.e. ammonium and nitrite oxidation) and then denitrification (i.e. NO_3^- and NO_2^- reduction by denitrifying bacteria to N_2 gas) processes (see Figure 2.8A) in separate reactors or in one larger reactor under aerobic conditions; a configuration known as simultaneous nitrification-denitrification (SND) (Hedstrom, 2001). Digester liquors usually contain significant amounts of organic matter that is not readily biodegradable under anaerobic conditions, which must be treated via associated aerobic processes (Mayer *et al.*, 2009). However, the BOD_5/TKN ratios of RLs tend to be unfavourable for denitrification (Andreotta *et al.*, 2012) and can only allow for the nitrification of about 50-60% ammonia due to alkalinity issues (i.e. two moles of bicarbonate is consumed for each mole of ammonium nitrified and 3.57 mg $CaCO_3$/L of alkalinity is recovered for each milligram of NO_3-N denitrified). Hence complete nitrification and denitrification are only achieved with RLs with additional alkalinity (e.g. lime, soda) and carbon provision (Fux & Siegrist, 2004). Furthermore, Zanetti *et al.* (2012) observed that additional external carbon (C) could potentially increase the operational cost of the system by 40-50%; thereby making this technology less desirable in terms of cost. This among other reasons has led to research in more cost-effective technologies with less external C and energy requirement such as nitritation-denitritation and anammox processes.

2.6.2 Nitritation-Denitritation

This process involves the short-cut oxidation of ammonium to nitrite and its subsequent reduction to nitrogen gas (see Figures 2.8B). Higher temperatures (25ºC-35ºC) typical of RLs favour the dominance of the AOBs over NOBs, since the former grows faster than the latter when temperatures exceed 20ºC (Hellinga *et al.,* 1998; Malpei *et al.,* 2008). This subsequently promotes partial nitrification (i.e. nitritation). The nitritation-denitritation process has been observed to consume only 75% of oxygen and 60% of additional carbon required by the conventional nitrification-denitrification process. The stoichiometric COD:N required mass ratio for denitritation was estimated at 1.72 as opposed to 2.86 for denitrification (Mulder *et al.,* 2006). In addition, Gustavsson (2010) also found that the nitritation-denitritation process potentially reduces sludge production and CO_2 emission by 30% and 20% respectively, compared to the nitrification-denitrification process. This process has been observed to achieve between 90-95% N-removal (Mayer *et al.,* 2009; Fatone *et al.,* 2011; Frison *et al.,* 2012b). A wide range of factors, such as alkaline pH >7.5, high temperatures, high free NH_3, inhibition of free nitrous acid, low dissolved O_2 concentration, sludge retention time (SRT) are suspected to suppress NOBs and favour nitrite accumulation, but potential conditions need further examination to apply them at larger scales (Yang & Alleman, 1992; Hellinga *et al.,* 1998; Zanetti *et al.,* 2012). However, Hawkins *et al.* (2010) reported that AOB activity and pH fluctuations are the most important factors. Furthermore, recent studies have shown that nitrite oxidation can be permanently suppressed in Sequencing Batch Reactor (SBR) configurations (at higher temperatures and controlled sludge age) or continuous-flow reactor configurations (e.g. patented SHARON) without sludge retention (Fux *et al,* 2003). These two configurations of nitritation-denitritation are described as follows:

- **Single reactor system for High activity Ammonia Removal Over Nitrite (SHARON):** This system operates as a chemostat with hydraulic retention time (HRT) being equal to SRT. The system achieves nitrite accumulation by making use of the difference in growth rates between NOBs and AOBs. The N-removal efficiencies depend on the influent concentration (Hellinga *et al.,* 1998). The operating HRT is chosen to suppress NOB without AOB washing out. The HRT for 100% nitrite accumulation at lab-scale and full-scale operations was first set at 1 day (at 35ºC) and 1.3-1.8 days, respectively. The recommended HRT for the anoxic phase has been set at 0.5-0.75 day, with volumetric N-removal rates estimated at 0.2-0.8 kg-N/m^{3}d for influent concentrations 500-1500 mg NH_4^+-N/L (Mulder *et al.,* 2006). The SHARON process can also be set-up in two separate tanks (one for nitritation and the other for denitritation with possible recirculation), but this entails a large investment cost (in construction), although it can have lower operational costs (due to lower aeration requirements) (van Loosdrecht, 2008). Mulder *et al.* (2006) reported that there exist ~12 SHARON systems in operation around the world.

- **Sequencing Batch Reactor (SBR):** SBR with floc-type suspended biomass is the most common nitritation-denitritation configuration applied in the full-scale treatment of RLs (Jardin *et al.,* 2006), and was developed and implemented prior to the SHARON process (Fux *et al.,* 2006; Gustavsson *et al.,* 2010). The SBR cycle commonly consists of the rapid filling and reaction phases (although continuous feeding was seen to improve process stability and speed-up reaction rates; Fux *et al.,* 2006), the settling, and then withdrawal phases. Internal hydrolysis and generally long SRTs of SBRs leads to higher bacteria decay and a corresponding increase in the internal available carbon for denitritation (hence lower demand for external carbon) (Gustavsson *et al.,* 2010). The nitritation-denitritation process depends on high ammonium concentrations, pH, inhibition of free nitrous acid and hydroxylamine, low dissolved oxygen, carbon source, nitric oxide concentrations and intermittent aeration. However, among these factors, the most critical ones for full-scale operations are not yet known. SBR design and size (relatively half the size of a corresponding SHARON system) depends on N-load, reaction rates and settling characteristics. Nitritation rates are commonly estimated between 1.2 kg-N/m^{3}d and 1.4 kg-N/m^{3}d, while the full-scale denitritation rates normally range from 1.4 kg-N/m^{3}d to 2.2 kg-N/m^{3}d, with volumetric nitrogen removal rates at 0.5 kg-N/m^{3}d to 0.8 kg-N/m^{3}d. The currently recommended HRT adequate for settling and withdrawal is 0.16 days (Fux *et al,* 2003; Vadivelu *et al.,* 2007; Gustavsson *et al,* 2008; Gustavsson *et al.,* 2010).

Both the SHARON and SBR processes have been observed to have foaming problems. However, more recent reactor designs have incorporated sprinklers with anti-foaming agents reduce foaming-related issues. However, these modifications inhibit effective oxygen transfer (Fux *et al,* 2003; Mulder *et al.,* 2006; Gustavsson *et al.,* 2010). Additionally, there is also the growing concern of excess N_2O production associated with nitritation-denitration processes when treating RLs with elevated nitrite levels (Joss *et al.*, 2009; Kampshreur *et al.*, 2009; Weissenbacher *et al.*, 2010; Gustavsson and la Cour Jansen, 2010).

Other observations are as follows. Mayer *et al.* (2009) observed that despite the fact the inorganic nitrogen content in the treated effluent from nitritation-denitration processes was quite low, there was still a significantly high amount of recalcitrant organic matter. Björlenius (1994) found that 33% of the N-removed in the SBR system was emitted as N_2O in a nitritation-denitritation process operated at lower dissolved oxygen levels; i.e. <1.0mg/l. This further suggests possible concerns with increased N_2O emissions from 1.5 to 4.5% from WWTPs. It was also revealed that 1.7% and 3.8% of the total N removed during 50% and 90% ammonia oxidation to nitrite, respectively, was emitted as N_2O. However, this reduced to 0.4% and 0.6% when oxidised to nitrate (Joss et al, 2009; Kampshreur et al, 2009; Weissenbacher *et al.*, 2010; Gustavsson and la Cour Jansen, 2010).

2.6.3 Anammox

This process was discovered more than two decades ago and was observed to be facilitated by Planctomycete-like bacteria (‘anammox’ bacteria) characterised by extremely slow growth rate with an average doubling time of 11days (Strous *et al.*, 1999a). These bacteria use nitrite as electron acceptor to oxidise ammonium, yielding low biomass according to the following equation (van de Graaf *et al.*, 1990; Strous *et al*, 1998; Van Hulle *et al.*, 2010):

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

Equation 17

This technology theoretically eliminates ~89% of the N-load in digester liquors as dinitrogen gas (Hedstrom, 2001). The alkalinity of the digester liquors is usually adequate to oxidise 50% of the ammonium to nitrite to provide a suitable NH_4^+:NO_2^- molar ratio (Fux & Siegrist, 2004). The main drawbacks with Anammox systems include slow growth rate of ‘anammox’ bacteria; i.e. doubling time of ~11 days at 32-33ºC, and process start-up time can take several months (Strous *et al.*, 1999a; Furukawa *et al.*, 2009; Gistavsson, 2010; Malamis *et al.*, 2014). The required SRT is always long enough to allow for the growth of ‘anammox’ bacteria and for increases in internal carbon (COD) and ‘anammox’ bacteria often face competition from NOBs over available carbon. However, control strategies such as low dissolved oxygen concentration and ammonia inhibition, can be applied to reduce NOB competition. ‘Anammox’ bacteria are generally sensitive to exposure to significantly higher NO_2^-

concentrations and oxygen (Strous *et al*, 1999). The NO_2^- toxicity levels depend on acclimation periods and the size of biomass aggregates (Wett *et al*, 2010). However, when anammox is paired up with other technologies the advantages become quite significant (e.g. Nitritation-Anammox (see Figures 2.8).

Nitritation-Anammox configurations (also known as deammonification): This can either be a 'one' or two reactor system. For theoretical 100% N removal, these systems use 57% less oxygen (see Figure 2.8C) and 86% less carbon than commonly used in conventional nitrification-denitrification. However, the actual N-removal is usually lower than in the nitritation-denitritation process; usually between 70 and 86% (Gustavsson, 2010). The nitritation- anammox process is generally operated as a granular sludge and biofilm system (moving-bed biofilm reactors; MBBRs), where nitrifiers (i.e. aerobic AOBs) in the outer layers of flocs or films are supplied with oxygen and the 'anammox' bacteria based in the relatively anoxic inner layer (depending on the oxygen diffusion), both oxidise ammonia and denitrify via nitrite (van Dongen *et al*, 2001; Wett *et al*, 2010). Anammox bacteria are usually grown from smaller granules which are denser than sludge flocs" (Trigo *et al.*, 2006). Some key applications of the Nitritation-anammox process include:

- **DeAmmon:** This was the first full-scale nitritation-anammox process operated as a one-reactor cascaded MBBR system. This was marketed by Purac and installed in 2001 at the Hattingen WWTP, Germany for 53,000p.e. Initially designed for 120 kg-N/day and a flow of 200 m^3/day, it achieved 80% N-removal from digester liquors after 1.5 years of its operation (Rosenwinkel *et al*, 2005). There are effectively three DeAmmon processes in operation globally, with the second one installed in 2007 in the Himmerfjärden WWTP, Sweden for 260,000 p.e, 670 kg-N/day and the third one in Dalian, China for 2200 kg-N/day. These processes usually require long start-up periods; typically 6-7months (Ling, 2009).
- **SHARON-ANAMMOX:** This process was first installed in 2002 in Dokhaven WWTP, Rotterdam, Netherlands for 620,400 p.e (van Dongen *et al*, 2001). This is a two-reactor system, developed in collaboration with Paques and Grontmij companies and Delft University of Technology. The first reactor (SHARON) was used for nitritation with aerobic HRT of 1.4 days, while the second reactor (Anammox) receives the partially aerated liquors to obtain biomass retention via a three-phase separator. The design load was set at 500 kg-N/day, but this was only attained after 3.7 years of operation due to technical problems with the prototype during scale-up from lab testing (van der Star *et al*, 2007). Paques later developed and installed a one-

reactor system (with a design capacity of 600 m^3 and 1200 kg-N/day) in Olburgen WWTP, Netherlands using anammox granules from Dokhaven WWTP as inoculum to treat digester liquors and pre-treated sewage from a potato processing factory. The actual start-up time to reach the actual working load of 700 kg-N/day was reduced to just five months (Abma *et al,* 2007). The system is operated with continuous aeration and can be adjusted based on online monitoring of ammonium and nitrite concentrations in the effluent so as to achieve the desired treatment outcomes. There are presently eight full-scale plants installed around the world by Paques; three of which are domestic WWTPs (Gustavsson, 2010).

- **SBR-Anammox (DEMON process):** In 2004, the first full-scale one-reactor system with floc-type suspended biomass in SBR was installed at the Strass WWTP, Austria (200, 000 p.e.) (Wett, 2006). Actual full-scale (500 m^3 working volume) start-up time of six months was preceded by two years of enrichment with four litres inoculum from the EAWAG pilot plant in Zurich. The process is operated with low dissolved oxygen (0.3 mg/L) and pH-controlled aeration, set to operate within a tight pH range of ±0.01. Nitritation was thought to reduce the pH, while anammox reactions tend to increase the alkalinity, but increases in pH were primarily linked to the continuous loading with digester liquors. The low dissolved oxygen limit was set to inhibit rapid nitrite accumulation while constantly suppressing further nitrite oxidation by NOBs. The DEMON process, which consists of a patented hydrocyclone is marketed by Grontmij and Cyklar-Stulz companies (Wett *et al.*, 2010).

The high costs of conventional nitrification-denitrification, the unreliability of the anammox technology, especially its long start-up period and low biomass yields, and also the potential for increased nitrous oxide from nitritation-denitritation processes have prompted further research into alternative, innovative low cost treatment technologies for RLs. This is key knowledge gap and underpins research within this Thesis, especially the treatment of RLs.

2.7 Aerobic Granulation Processes

Aerobic granulation technology (AGT) is growing in the wastewater treatment industry and has been applied in both industrial and domestic wastewater treatment applications; although there is still limited data on full-scale operation (de Bruin *et al.,* 2004; de kreuk *et al.,* 2005; Coma *et al.,* 2012; Show *et al.,* 2012; Morales *et al.,* 2013; Pronk *et al.,* 2015). This technology presents the possibility of designing more compact WWTPs due to the fact that the removal of N, P and COD can be achieved simultaneously in a single SBR unit (de Kreuk *et al.*, 2005). Simultaneous removal of N, P and COD via AGT is based on a repeatedly-fed batch process that relies on the selective growth of microorganisms in granules through the process of biogranulation. Biogranulation is self-immobilisation of microorganisms through cell-to-cell interactions governed by biological, physical and chemical phenomena to form spherical granules. It is also referred to as the formation of biofilms without supporting carrier material, which was initially conceived as a wastewater treatment option in the early 80s (Liu & Tay, 2002; Yang *et. al.*, 2004a; Jing, 2008; Bindhu and Madhu, 2013). However, recent successes of these biofilm-based technologies have suggested them as potential substitutes for conventional wastewater treatment because of their intrinsically small footprint (De Kreuk and van Loosdrecht, 2006; Pronk *et. al.*, 2013).

There are two categories of biogranulation technologies; anaerobic and aerobic granulation technologies. Anaerobic granulation (ANG) technology has been extensively applied in the wastewater industry and studied in Upflow Anaerobic Sludge Blanket (USAB) systems. However, there are drawbacks to ANG systems including long start-up periods, and the need for relatively high operating temperatures, which have triggered increased interest in aerobic granulation (AG) technologies (Schmidt & Ahring, 1996; Morgenroth *et. al.*, 1997; Jing, 2008; Bindhu and Madhu, 2013), which have potential for RL treatment.

The development of aerobic granules; i.e., with average diameter 2 - 8mm, was first reported by Mishima and Nakamura (1991) in a continuous aerobic upflow sludge blanket reactor. AGT has since been studied in sequencing batch reactors (SBRs) and has been utilised in treating both high and low strength wastewaters (with organics, phosphorus, nitrogen and toxic substances) (De Kreuk *et. al.*, 2005a; Buitron and Moreno-Andrade, 2011). The technology has been observed to be a potential replacement for conventional activated sludge systems based on some of the advantages of the former presented in Table 2.4.

Table 2.4: Differences between Conventional Activated sludge Technology and AGT

Conventional Activated sludge Technology	AGT
Traditionally, weak 'flocs' with low settling velocities (< 9m/h) applied (De Kreuk *et. al.*, 2005a; Wang *et. al.*, 2007; Bindhu and Madhu, 2013).	'Flocs' are substituted by strong, compact and fast settling spherical granules (with velocity of 12 – 157m/h) with low sludge volume index (SVI) (Etter and Wilderer, 2001; De Bruin *et. al.*, 2004; Wang *et. al.*, 2007; Bindhu and Madhu, 2013).
Large settling tanks are required to separate clean effluent from the biomass, with little biomass retention resulting in lower biomass concentrations (3 – 5g/l) (van Loosdrecht and De Kreuk, 2004; De Bruin *et. al.*, 2007)	Granules promote biomass separation in the treatment reactor, thereby retaining higher biomass concentrations (15 – 20g/l) and minimising plant size. Hence low footprint (De Kreuk and De Bruin, 2004; van Loosdrecht and De Kreuk, 2004; De Kreuk, 2006)
The need for separate tanks to accommodate different treatment processes (COD, N- and P-removal) with large recycle flows and high hydraulic retention times (Metcalf & Eddy, 2003).	Due to diffusion gradients in the granules, various redox conditions commonly accommodated in various tanks in conventional treatment processes, are now accommodated inside the AG sludge and thus effectively only a single tank is required for several parallel biological treatment processes (P-removal, nitrification and denitrification) without the need for large recycle flows (van Loosdrecht and De Kreuk, 2004; De Bruin *et. al.*, 2007).
Surplus sludge requires various dewatering stages (thickening and filter pressing) prior to it being processed ((De Kreuk *et. al.*, 2005a)	Less surplus sludge volume
	AG uses only 20% of the surface area required by conventional activated sludge processes (De Bruin *et. al*, 2004). AG systems could lower the CAPEX and OPEX (reducing energy consumption by ~30%) at WWTPs than conventional activated sludge systems (van Loosdrecht and De Kreuk, 2004).

2.8 Factors determining the formation of aerobic granular sludge

2.8.1 Substrate composition and Organic Loading rate (OLR)

A wide variety of wastewaters have been used to grow aerobic granules and a strong correlation has been observed between the microstructure and morphology of these granules, and the composition of the wastewater in which they are cultivated. For example, Schwarzenbeck *et al.* (2005), reported instabilities in aerobic granules during the treatment of dairy wastewater due to the proliferation of filamentous bacteria. It was also observed that flocculation during the treatment of wastewaters containing carbohydrates and low dissolved oxygen levels tended to be caused by the growth of favoured filamentous bacteria (Gaval and Pernell, 2003; Martins *et al.,* 2003). Aerobic granules grown in glucose showed a filamentous structure; while those grown in acetate were non-filamentous and compact with predominantly rod-like species (Tay *et al.,* 2001).

It has also been reported that the potential for aerobic biogranulation generally decreases with increasing microbial growth rates in some growth media. For instance, more compact granules were more readily grown in methanol versus acetate due to relatively lower microbial growth rates on methanol than acetate. However, there are exceptions. Glucose-grown aerobic granules can exhibit better formation of compact granular sludge despite the generally high microbial growth rate in glucose. This is because microbial growth rate in glucose is typically lower in biofilm and granules (de Kreuk *et al.,* 2005). Further, aerobic nitrifying granules with excellent nitrification activity have been observed to grow across a wide range of OLR; i.e. 0.4 to 15 kg COD/m^{3}d (Morgenroth *et al.,* 1997; Moy *et al.,* 2002; Tsuneda *et al.,* 2003; Liu and Tay, 2004; Tsuneda *et al.,* 2006). The morphology and kinetic behaviour of aerobic granules was observed to be directly related to the substrate loading rate; i.e. as the OLR increased from 3 to 9 kg COD/m^{3}d, the granular mean sizes increased from 1.6 to 1.9 mm (Moy *et al.,* 2002; Liu and Tay, 2004; Zheng *et al.,* 2006). The OLR was also observed to affect the stability of the granules. It was reported that it was possible to cultivate bacteria-dominated aerobic granules of about 1 mm mean diameter at a high OLR of 6 kg COD/m^{3}d in SBR within a 30 days' period. However, these granules were unstable and easily dominated by filamentous strains due to poor mass transfer and the presence of anaerobes in the large-sized aerobic granules (Zheng *et al.,* 2006).

2.8.2 Shear force and aeration intensity

A high hydrodynamic shear force and aeration rate are both vital for the stable operation of aerobic granular processes (Liu and Tay, 2004). It was observed that relatively high shear forces due to aeration up-flow velocities of 2.4 to 3.2 cm/s generated compact and stable granules that exhibited relatively better treatment performance. Whereas, lower shear forces from aeration up-flow velocities of 0.8 to 1.6 cm/s produced large-sized filamentous granules characterised by their loose structure and irregular shapes with subsequent operational instability and poor performance (Chen *et al.,* 2007). It was also seen that the production of extracellular polysaccharides (EPS) was directly proportional to shear force and the stability of granules; i.e. higher shear forces stimulated the secretion of EPS by the back (Adav *et al.*, 2008).

2.8.3 Reactor configuration

Reactor configuration affects the flow pattern in the reactor (Beun *et al.*, 1999; Liu and Tay, 2002). All reactors used for AG has been designed to include some form of agitation. Most recently, controlling superficial air velocity (especially in SBR) is being incorporated in designs to influence AG via improved oxygen supply and hydrodynamic shear stress conditions. The recommended design for up-flow column-type SBRs should be such that the height to diameter ratio is high enough to ensure a longer circular flow trajectory, and a subsequent high hydraulic shear stress for effective granulation. However, if the height to diameter ratio of the reactor is too high, it may lower the shear stress on the aerobic granules forming towards the top of the reactor, which may result in the formation of filamentous-type granules (de Kreuk *et al.,* 2005). Beun *et al.* (2000) reported that shear stress triggered by aeration rate depends on the reactor configuration.

2.8.4 Cycle Time

In SBRs, a single cycle involves, a feeding phase, an aeration/reaction phase, a settling phase and a withdrawal phase. The volumetric exchange ratio and settling time at the end of each cycle constitutes the screening step to decant non-granular biomass (i.e. the treated effluent). Shorter cycle times imply shorter HRT, which is used as a selective pressure (due to the washing out of slow settling biomass) (Pan *et al.,* 2004). Cycle time has also been seen to influence the kinetic behaviour of aerobic granules. It was reported that as the cycle time increased from 1.5 to 8 hrs, the specific biomass growth rate and biomass growth yield of aerobic granular sludge fell from 0.266 to 0.031 day^{-1} and 0.316 to 0.063 gVSS/g COD, respectively (Liu *et al.,* 2007). The length of the settling phase was reported as an important selective pressure on the microbial community within SBRs. With shorter settling times, there is preferential selection for rapidly settling communities while poor settling biomass is washed out. Furthermore, shorter settling times can preferentially select aerobic granules, whereas in anaerobic-aerobic alternating systems with PAOs, settling times seem to be less significant because PAOs apparently possess an inherent tendency to aggregate (de Kreuk *et al.,* 2005).

2.8.5 Feeding pattern

Unlike a continuous-flow activated sludge reactor, SBRs have a unique cycle of operation that incorporates a periodic ‘starvation/famine’ phase that has been observed to be critical to effective AG formation (Tay *et al.,* 2001). Sanin *et al.* (2003) further reported a correlation between the hydrophobicity of the granular sludge and carbon-starvation that further facilitates the agglomeration of the sludge into more compact and stable granules. Yang *et al.* (2005) also reported that a shorter starvation phase resulted in faster AG, whereas Li *et al.* (2006) showed AG was initiated by starvation and further facilitated by anaerobic metabolism and shear force effects. McSwain *et al.* (2004) also recommended intermittent (or pulse) feeding in SBRs as an effective means of enhancing the formation of compact aerobic granules.

2.8.6 Temperature

De Kreuk *et al.* (2005) demonstrated that temperature has a significant influence of the performance of the AG treatment process. They found that, when the start-up temperature of the aerobic granular reactors was relatively low (e.g. wintery temperatures), the stability of granules deteriorated and most of the biomass was washed out. However, their work also reported that when the reactors were started-up at relatively higher temperatures (e.g., summer temperatures), aerobic granules were more stable and it was possible to operate the reactors at lower temperatures without significant effect on treatment efficiency or the general stability of the granular sludge.

2.8.7 pH

Although the influence pH on microbial growth rate has been widely reported, information on its influence on species selection and AG is still quite limited. A study on the effect of pH and alkalinity on AG revealed that at lower pH (e.g. pH ~3), and alkalinity (e.g. ~28.7 mg $CaCO_3$/L), fungi-dominating granules were rapidly formed within one week; whereas at relatively higher pH (e.g. pH ~8.1) and alkalinity (e.g. ~301 mg$CaCO_3$/L), bacteria-dominating granules were obtained within four weeks of operation (Yang *et al.,* 2007).

2.8.8 Dissolved Oxygen (DO) levels

Most aerobic granulation processes have been successfully achieved at DO levels above 2.0 mg/L (Yang *et al.,* 2005; Adav *et al.,* 2008); although small aerobic granules with 0.3 to 0.5 mm diameter have been observed to agglomerate into larger ‘flocs’ during the settling phase in SBRs at 1.0 mg/L of DO (Peng *et al.,* 1999). Furthermore, it was revealed that dropping the oxygen saturation to 40% resulted in a decrease in the density, deterioration and subsequent disintegration of the aerobic granules (Mosquera-Corral *et al.,* 2005).

2.9 Applying AGT in the Treatment of AD Supernatant

Based on the review on the aforementioned determinants, it is apparent that AG is a complex process, but can be successfully achieved through a wide range of potentially controllable interactions among process variables. Hence it is becoming increasingly possible to cultivate aerobic granules for the treatment of complex wastewaters, including wastes as strong as RLs. In fact, AGT has been successfully applied to study N-removal during the treatment of synthetic wastewater with both high (Tsuneda *et al.*, 2003; Kim and Seo, 2006; Cydzik-Kwiatkowska and Wojnowska-Baryla, 2011) and low N-concentrations (Wang *et. al.*, 2008; Yuan and Gao, 2010).

However, little has been done in terms of the application of AGT on simultaneous N- and P-removal from actual wastewater with complex and highly variable composition; and there are few operating full-scale installations like NEREDA technology in the Netherlands. Cassidy and Belia (2005) and Kishida (2009) did report ~97% N- removal when AGT was used to treat wastewater with COD/N = ~5 from an abattoir and animal farms. Cydzik-Kwiatkowska *et al*. (2013) further applied the AGT to assess N-removal from nutrient-rich RLs with high N- and P- concentrations, less biodegradable organics (i.e. BOD/COD =0.4) and a low COD/N ratio of 1.4, and achieved some N-removal. This supports the findings of Yang *et al.* (2003), which reported that the activity of nitrifying and denitrifying bacteria populations coexisting in the granules, increased with decreasing COD/N ratio. Therefore, there is precedence that AGT could work for RLs, which justifies further developmental work reported herein.

2.10 Understanding the struvite problem in post-AD processes

Struvite is composed of magnesium, ammonium and phosphate in an equimolar ratio; i.e. 1:1:1 (Lee *et al.*, 2003; Di Iaconi *et al.*, 2010). It usually precipitates in areas of high turbulence (which causes CO_2 stripping and a consequent local increase in the pH of wastewater), and has been observed (Ohlinger *et al.*, 1998) to cause clogging in pipes, and fouling of aerators, pumps, screens and other equipment. Struvite is readily soluble in acidic medium and highly insoluble in neutral and alkaline media (Münch and Barr, 2001). However, the operational and financial implications of struvite accumulation are serious to water companies including significant reduction in the stability/reliability of treatment processes as well as significant increases in operational and maintenance costs.

Marchi *et al.* (2015) reported that the component ions of struvite (i.e. Mg^{2+}, NH_4^+ and PO_4^{3-}) are generally released from the solid phase to the liquid phase during the anaerobic digestion of sewage sludge due to the breakdown of organic matter. As these ions exceed supersaturation levels, struvite rapidly precipitates upon release (Doyle and Parsons, 2002). Bhuiyan *et al.* (2007) reported other factors that affect struvite precipitation, such as pH, temperature (see Figure 2.11), mixing rate and the presence of other ions (e.g. Ca^{2+}). Struvite formation potential was found to be directly proportional to pH (i.e. it increases with increasing pH (between pH 6 and 10); with the maximum struvite precipitation observed at pH 9(see Figure 2.10)) (Mudragada *et al.*, 2014). Hence the relatively high struvite precipitation incidence in post-AD processes could be linked to the pH of the digestate which is usually between 7.2 and 8.5. Furthermore, Borgerding, (1972) identified the following physical attributes of the post-AD treatment processes which also promote struvite formation:

- Pipeline roughness, especially at fittings and joints.
- The relatively high surface area to volume ratio from the digester to the pipelines conveying the digestate, thereby promoting struvite precipitation by providing a large area for crystal growth.
- Increased energy in pipelines resulting from the vibration of the sludge screens which could potentially cause low pressure zones and temperature drops.

Struvite crystallization in treatment processes has been observed to occur in two chemical stages; i.e. nucleation and crystal growth (Momberg and Oellermann, 1992; Jones, 2002). These two stages are driven by factors such as; the interrelationship of some physico-chemical parameters (e.g. molar ratios, pH, supersaturation and mixing energy), thermodynamics of liquid-solid equilibrium, kinetics of chemical reactions, and mass transfer between liquid and solid phases (Ohlinger, 1999; Jones, 2002). Nucleation has been described as the generation of crystal nuclei from solution, whereas crystal growth refers to the transportation and orientation of struvite component ions to the surface of the nuclei to form the crystal lattice (Momberg and Oellermann, 1992).

In order to control struvite precipitation in treatment processes, it is very important to understand the underlying factors responsible for its formation. Table 2.5 shows some existing struvite control measures alongside their advantages and disadvantages.

Table 2.5: Potential and Existing struvite control measures

CONTROL MEASURES	ADVANTAGES	DISADVANTAGES
A) Non-chemical Control		
B)		
Dilution with fermenter overflow (2:1 dilution) (Neethling and Benisch, 2004)	Simultaneously reduces the concentrations of all 3 struvite component ions. Thereby minimising the deposition of struvite and/or other precipitates. It is a relatively cheaper option	It effectiveness varies and depends on site-specific wastewater chemistry
Minimise the number fittings (except for taps and isolation valves to operate cleaning loops for routine scale removal) on digested sludge and centrate/filtrate conveying lines straight (Williams, 1998)	This minimises turbulence and the subsequent loss of CO_2, hence preventing pH rise Minimises the build-up of pipe scaling/clogging Minimises operation costs	
Use smooth pipe materials or PVDF to line pipes on the centrate pump suction side (Ohlinger, 1998).	Minimises struvite precipitation and subsequent build-up in pipelines and other equipment downstream of the AD PVDF lining is cheap compared to other fluoropolymers and is also commercially available PVDF lining is known for its high purity, flexibility, high resistance to chemical corrosion and heat, low smoke generation during a fire event, low weight and low thermal conductivity. Low operational cost	Additional capital cost to purchase PVDF liners
Minimise the length (<250ft) of digestate transfer and centrate/filtrate conveying lines and keep the pipelines straight (Giesen, 1999)	Minimises reaction time for reactants to form struvite Minimises initial capital cost Minimises operation cost as well	
Allow for easy accessibility to pipes and equipment conveying digestate and centrate, so as to facilitate regular inspection (Neethling and Benisch, 2004)	Reduces time and personnel needed for cleaning up. Minimises/eliminates struvite build-up Reduces operational cost	

…Continued

Table 2.5 Continued

Provide sufficient positive suction pressure head to avoid negative pressure upstream of pump (Neethling and Benisch, 2004)	Minimises reaction and settling time for the possible formation and deposition of struvite	More energy cost incurred in supplying sufficient pressure upstream of the pump
Avoid free discharge of dewatering centrate (Evans, 2007; Bergmans, 2011).	Prevents the loss of CO_2 involved in splashing and a subsequent pH rise	
Avoid surface turbulence in centrate storage tanks and if possible eliminate the storage of untreated centrate (Williams, 1998)	Prevents struvite deposition in the sumps due to the loss of CO_2.	
C) Chemical Control Measures		
Using Ferrous/Ferric Chloride and Alum (Mamais *et al.*, 1994; Thaler, 2011)	Reduces PO_4^{-3}; hence minimises struvite formation and deposition	Expensive large chemical dose is required for effectiveness Increased inorganics in biosolids Increased risk of other deposits (e.g. Ferrous phosphate(vivianite)) P-recovery from ferric or aluminium phosphate is almost impossible
Using acid to Lower the pH (Bergmans, 2011)	Increases struvite solubility; thereby preventing precipitation	Large dose required for any significant change Increased risk of corrosion Handling of hazardous material is required
Injection of CO_2 (Bergmans, 2011)	Lowers pH, thereby eliminating conditions favourable for struvite precipitation	
Airprex System (This is a bioreactor that can be installed downstream of the AD but upstream of the centrifuge. The reactor utilises the dosing of $MgCl_2$ and CO_2 stripping to recover P as struvite) (Langereis & Geraats, 2013)	Lower (~20% reduction) polymer consumption prior to the dewatering of the digestate Increase in dry solids content of dewatered sludge Elimination of scaling in systems conveying digestate and centrate 80 – 90% recovery of P Product currently sold in German markets for €60-80 per ton Full-scale applications in Mönchen-Gladbach WWTP; Waternet, Amsterdam-West WWTP	Struvite product is not completely pure Results in the precipitation of other compounds in the digested cake. High capital cost involved in the purchase of $MgCl_2$ and the installation of the Airprex system The economics depends on local costs of dewatering and sludge disposal

…Continued

Table 2.5 Continued

Using nanofiltration (NF) to separate Mg(II) from seawater for struvite recovery from centrate (Lahav *et al.*, 2013)	>50% cheaper Mg-source compared to commercial Mg-based chemicals Struvite produced is of high purity Lower addition of other ions into the liquors than commercial Mg-based chemicals >90% P- recovery	Further research is required on the effect of anti-scalants (used to protect NF membranes) on the morphology of the struvite Further research is also needed to identity NF membranes with more selective Mg(II) separation properties
Dosing of $Mg(OH)_2$ in the AD and in centrate for controlled struvite recovery (Wu *et al.*, 2001)	Improves reduction in TSS, VSS, COD (total and soluble) Reduces the PO_4 and NH_3 load in centrate Improves maintains dewaterability of post-AD sludge; thereby reducing polymer consumption Improves biogas production Dissolution of $Mg(OH)_2$ can neutralise VFAs produced by the acidogens resulting in better pH conditions for the methanogens ~93% P-removal from centrate Eliminates CO_2 stripping stage since the $Mg(OH)_2$	Additional costs in purchasing $Mg(OH)_2$ Extra care is needed as the digesters are very delicate (because any damage could take a longer time to restore or could even be irreversible)

2.11 Summary and knowledge gaps

Establishing a comprehensive mass balance model for N and P around WWTPs constitutes the first step in considering treatment options for N and P. Mass balance models are very important in identifying and quantifying issues of greatest concern and also identifying critical points within WWTPs. They also help in assessing the performance of individual WWTP unit operations. Further, a good N and P mass balance does not only identify problematic areas within WWTPs, but also highlights potential points of interest for cost-effective nutrient recovery. However, the lack of adequate data due to limited monitoring routines and the complex nature of most WWTPs (e.g. the recycling of liquors from sludge dewatering processes), have often restricted the development of meaningful mass balances at most WWTPs. Hence, as part this thesis, a mass balance for struvite component ions around the AD unit at Bran Sands WWTP (operated by Northumbrian Water Ltd) was performed, in order to identify critical areas and facilitate decision-making on treatment options. This site was chosen due to challenges in treating its nutrient-rich RLs and serious incidences of scaling in some treatment units.

Early reports about struvite formation in WWTPs, described it as a natural scaling problem. However, this view is fast changing as it is now viewed as potential means of P and N recovery. Controlled struvite crystallisation through the addition of $MgCl_2$ or $Mg(OH)_2$ has been recommended as one of the most effective methods of controlling struvite scaling problems in post-AD processes. This control measure has been widely studied on synthetic wastewater and digester liquors. This has proven to be quite effective at N and P recovery; but its ability to reduce scaling problems is only limited to processes downstream of digested sludge dewatering units. Most struvite scaling problems being reported at WWTPs have been seen in digested sludge processing equipment (e.g. most common pipelines conveying the sludge, pumps and dewatering equipment). There are also reports of scaling problems in anaerobic digesters themselves.

In this thesis, the feasibility of struvite control through pre-precipitation of P and N from sludge was assessed. Controlled struvite crystallization experiments in bench-scale anaerobic digesters (AD) through the addition of $MgCl_2$ (and pH adjustment) was performed in order to assess the suitability of pre-precipitating P and N in the AD in reducing the potential for struvite scaling in post-AD processes. The impact of controlled struvite crystallization (in the AD) on the performance of the AD (in terms of methane production and volatile solids breakdown) was also assessed. Struvite crystallization experiments were also performed on digested sludge through the addition of $MgCl_2$ (and pH adjustment). Comparative studies were then performed on both struvite control methods (i.e. between struvite control in AD and post-AD) based on the differences in their P and N recovery efficiencies. Cost benefit analysis were also performed on both methods.

With relatively high levels of N still observed in digester liquors (following struvite crystallization), there is growing interest in tackling excess N to comply with regulatory consents. Current attempts at resolving N-related water quality problems have transcended the capabilities of conventional aerobic treatment technologies (which are usually energy intensive and expensive); therefore, the development of alternate cost-effective treatment technologies are needed. SBRs have been widely studied due to their relatively small footprint compared to other existing biological treatment technologies. SBRs are also generally easily adaptable to regulatory changes in effluent quality pertaining to nutrient removal, however more robust and compact systems other than conventional SBRs are required for effective nutrient removal from wastewaters with high nutrient load (e.g. digester liquors).

Aerobic granulation in SBRs is fast gaining interest in the wastewater treatment industry as an efficient alternative to existing biological treatment technologies, due to its relatively low sludge production and the compactness of the granular sludge involved in the treatment process. Unlike conventional activated sludge systems, these systems allow the self-immobilization of microorganisms of interest to form and accumulate large amounts of active biomass (in the form of granules), with a dense microbial structure and excellent settleability. It is as a result of these characteristics that aerobic granular sludge technology is being proposed as a reliable substitute in order to minimise space and increase loading rates in WWTPs. However, there is limited information on the applicability of aerobic granulation technology in treating complex wastewaters like digester liquors with high nutrient (N and P) loads and high levels of recalcitrant organic carbon (less biodegradable).

In this thesis, comparative studies were conducted to investigate the feasibility and efficiency of aerobic granulation in treating synthetic wastewater and then actual digester RLs. A settling time reduction technique was first applied to three identical aerobic granular reactors in order to select fast settling granules over slower ones. A comparative study also was done to assess the relationship between N loading rates and the value of nitrification/denitrification processes within granular reactors. Finally, the effect of the feed type and loading rates on the microbial community structure in the aerobic granules was also be examined. The technology was also tested for phosphorus removal during the simultaneous nitrification/denitrification.

Chapter 3: Mass balance of N and P around the AD at Bran Sands WWTP

3.1 Introduction

There is growing interest in the recovery of N and P from wastewater for commercial purposes vis-à-vis complying with regulatory standards (e.g. WWT-UK, 2012) for treated effluent from WWTPs. The depletion of P-reserves; the growing population stress on limited natural resources; and increasing energy and fertiliser costs have triggered interest in nutrient recovery. Worrell *et al.* (2000) reported that the production of N-fertiliser accounts for about one percent of global energy use. Further, Doyle and Parsons (2002) and von Horn and Sartorius (2009) reported that global phosphate reserves may last only two-hundred years more if the population growth and P-use rates remain the same. These concerns and more (e.g. regulatory and environmental concerns such as eutrophication problems) have necessitated the recovery of N and P from nutrient rich wastewaters (especially RLs).

Overtime, WWTPs have undergone process innovations in order to improve the efficiency of nutrient recovery. When considering optimal nutrient (i.e. N and P) recovery options from WWTPs, the first requirement is to better understand and quantify how nutrients are distributed in the various treatment process units (Starman, 2009). As such, to facilitate the design, operation and optimisation of N and P recovery processes, it is essential to establish their relative mass flow through the WWTP. A WWTP mass balance model is, therefore, essential in ensuring that N and P in all the individual treatment units are included and that the interconnection between operating units is clearly known (Wild and Siegrist, 1999).

Mass balance models for WWTPs are generally expressed as a function of wet or dry matter (de Araújo *et al.*, 2008). One of major benefit of a mass balance analysis is that it can be used to assess the overall performance of WWTPs as well as each of the individual unit operations (Pognani *et al.*, 2011). It also highlights the interdependence among individual treatment units at WWTPs, including how the operation/optimisation of each unit might affect the economics and performance of other upstream and downstream units (Wild and Siegrist, 1999). For instance, the recycling of digester liquors to the secondary treatment stage (e.g. conventional activated sludge systems) of a WWTP can significantly affect performance and stability of not only the secondary treatment unit, but also the entire WWTP (Pitman *et al.*, 1991; Janus and Van der Roest, 1997). Ekama *et al.* (2006) highlighted other potential benefits of a mass balance as follows:

- It facilitates the tracking of compounds of interest through the WWTP.
- It aids the identification of the biological, physical and chemical characteristics of streams from one treatment unit to the next.
- It helps in assessing the effect of recycling digester liquors and sludge thickening from downstream units on upstream units.
- It facilitates the identification of critical points and overloaded treatment units that affect the overall performance of the WWTP.
- It aids the design, operation and optimisation of key treatment units.
- It provides an opportunity to assess the impact of mitigation techniques; e.g. controlled N and P precipitation as struvite from RLs.
- The easy identification of analytical and operational data that do not comply with the principles of mass balance and continuity.

Nutrients in wastewater are usually partitioned into two major treatment trains; i.e. solids and liquids. Therefore, it is important to consider both treatment trains when establishing a comprehensive mass balance of N and P throughout a WWTP. The total N content in most WWTPs primarily consists of ammonia/ammonium-nitrogen (NH_4-N), organic nitrogen (i.e. particulate, dissolved and colloidal), and nitrate/nitrite (NO_x-N) (Mȩlkinia *et al.,* 2009). These forms of N represent the products of various N-transformation pathways occurring in the individual treatment units (at WWTPs) and within the wastes. This is consistent with reports of strong correlations between the design and operation conditions of wastewater treatment processes, with the biodegradable and non-biodegradable fractions of dissolved organic N in treated effluent (Czerwionka *et al.,* 2008). Furthermore, during AD of sewage sludge (i.e. the solids treatment train), some of the total N is converted to NH_3 (i.e. the gaseous phase), and further resulting in high NH_4-N concentrations in RLs (in the aqueous phase) (Hobson and Wheatley, 1993). It also has been observed that as the SRT in AD increases, the concentration of NH_3 increases while that of organic N decreases. Other factors such as temperature and pH have also been seen to influence the concentration of reduced forms of N such as NH_3 in the gaseous phase or NH_4-N in the aqueous phase; with higher concentrations of gaseous NH_3 seen at high pH ranges and higher concentrations NH_4-N seen with decreasing pH (Cacho, 2005).

At WWTPs, significant loads of P arrive daily from diverse sources (e.g. livestock, industrial, domestic wastes) (Rahman *et al.,* 2011, 2014). Like N, P is also being mass transferred through the different treatment processes in WWTPs via the solid (i.e. when precipitated in sludge or when incorporated in PAOs) and liquid phases (e.g. as free PO_4^{3-} ions). In addition, following AD of sewage sludge, significant amounts of P are released from the solid phase to the liquid phase. This causes high concentrations of P (especially orthophosphates) in RLs. If these liquors are recycled to the secondary treatment units, scaling problems can ensue, and deterioration in process stability, treatment performance, and poor effluent quality can result (Caffaz *et al.,* 2008; Malamis *et al.*, 2014).

The Bran Sands WWTP (operated by NWL) has been experiencing a continuous build-up of N and P in its treatment processes due to the recycling of nutrient-rich RLs from its Advanced Anaerobic Digestion (AAD) process to its activated sludge units due to a lack of N and P removal/recovery measures. This has led to scaling problems, fluctuations in process stability, and increased operational costs (i.e. increased maintenance, chemical and energy costs). In order to tackle these issues, it was important to establish a mass balance for N and P around the site, in order to identify critical points for possible intervention and to characterise the problem. Therefore, this chapter presents mass balances of P and N around the Bran Sands WWTP with emphasis on the AAD processes and RLs. It also assesses the possible impact that reducing incoming N and P loads has hamight have on the transfer and exchange of nutrients throughout the treatment process, based on a comparison mass balance completed in 2011 around when the AAD was first commissioned. This mass balance was then used to guide the choice and experimental designs of possible remediation actions, which were tested experimentally by work reported in Chapters Four and Five.

3.2 Description of the main treatment units at Bran Sands

3.2.1 The Liquid Treatment Train or Effluent Treatment Works (ETW)

Bran Sands WWTP is located in the Teesport industrial area in Northeast England. It was constructed on a former landfill site with its pipelines located over-ground due to an array of underground issues related to the closed landfill. The WWTP has a design capacity of 1.2 million p.e. and a wet weather flow of 171,000m^3/d. Treated effluent from the WWTP is discharged via a combined sewer outlet to the Dabholm Gut, a tributary of the River Tees Estuary. The Environment Agency’s consent to discharge (Consent Number 254/1920) for the site stipulates that treated effluent shall contain: <250 mg/L of suspended solids, <250 mg/L BOD, 5 < pH <9, and <40 mg/L NH_4-N. The facility receives wastes from a very complex sewage collection network, including both municipal and industrial wastewaters a volumetric ratio of ~1:2 at the time of writing. Based on COD equivalence, the municipal wastewater contributes ~500,000 p.e., whereas the industrial wastewaters account for ~1,100,000 p.e. The municipal wastewaters come from Portrack, Eton and Middlesbrough. The sources of known industrial wastewaters are summarised in Table 3.1:

Table 3.1: Sources of industrial wastewater treated at Bran Sands WWTP.

Type of industry	Name of Industry
Steel processing	South Bank coke Ovens
Oil refining	Conoco Phillips
Pure terephtalic acid (PTA) for polyethylene terephthalate (PET) the production of bottles	Lottie
Differentiated chemicals production	Huntsmann
Food processing	Unknown
Landfill	Unknown

Due to the complex nature of waste inputs to Bran Sands, the liquid treatment train is divided into three sub trains as presented on Figure 3.1. These trains largely flow in parallel due to possible toxicity issues from the industrial sources, although the sludge from the trains are jointly treated in the same AAD units.

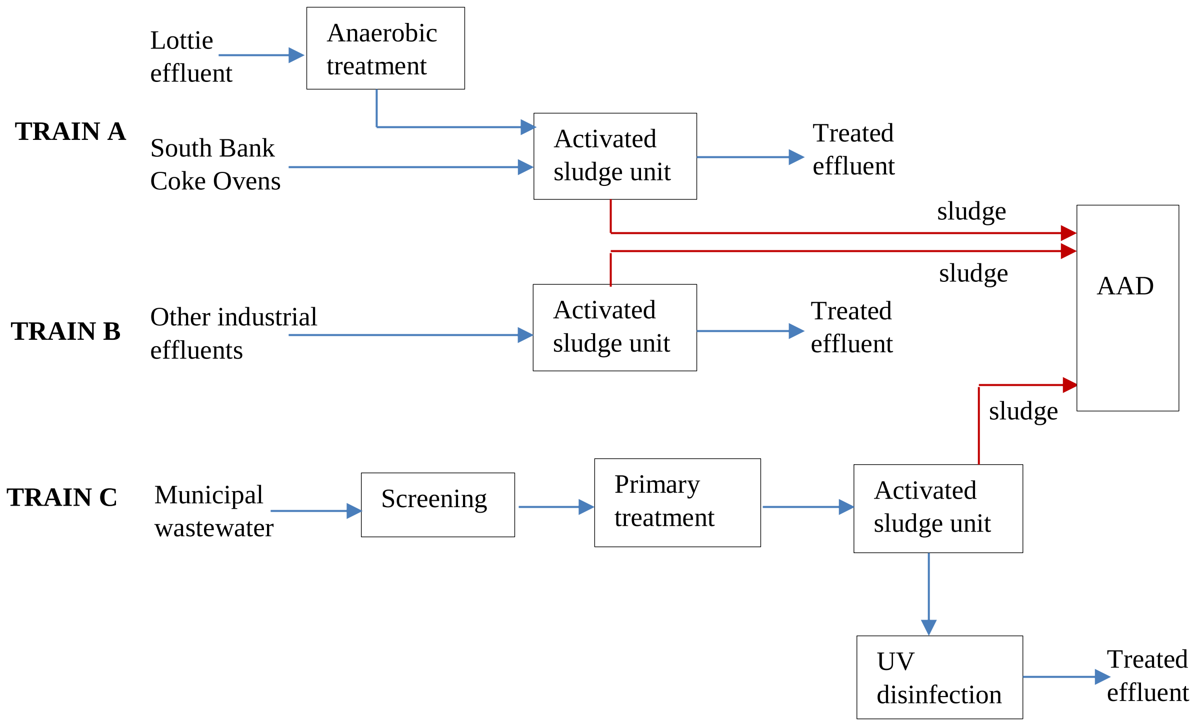

Figure 3.1: The liquid treatment train or ETW at Bran Sands WWTP

As seen on Figure 3.1, Trains A and B are dedicated to the treatment of the industrial wastewaters while Train C receives and treats municipal wastewaters that arrive at the WWTP. Based on special arrangements between NWL and Lottie, PTA effluents that arrive at Bran Sands are treated by anaerobic treatment units prior to treatment by the conventional activated sludge treatment units. Municipal wastewater with its relatively high suspended solids content first undergoes primary treatment before secondary treatment, and subsequently undergoes tertiary treatment in the UV disinfection chamber. It is worth noting that the ETW at Bran Sands WWTP primarily aims to remove C and N; i.e. P-removal is not designed into any of the treatment processes.

3.2.2 The Biosolids Treatment Train

Bran Sands WWTP also acts as a regional sludge treatment centre (RSTC) treating both indigenous sludge (produced on-site) and imported sludge from neighbouring WWTPs in approximately volumetric ratio of 1:1. The processes involved in the treatment of sludge on site are shown in Figure 3.2 and detailed as follows:

- **Conditioning of the different incoming sludge:** Both indigenous and imported sludge cakes (~25% w/w dry solids) are first mixed, diluted to 17% w/w dry solids and then to 16% w/w dry solids as required by the CAMBI process. Part of the conditioning process involves dewatering by centrifugation, which produces ‘Return Liquor 1’ (see Figure 3.2). These liquors are returned to the primary treatment units of the ETW.
- **Thermal hydrolysis through the CAMBI process:** This process also known as the Thermal Hydrolysis Plant (THP) constitutes two banks of four reactors with an associated pulper, flash tank and foul gas skid. During hydrolysis, the conditioned sludge is held at 165°C and 60 bars for 30mins, resulting in ~14.3% w/w dry solids, after which the sludge is further diluted with treated water to 10% w/w dry solids. The hydrolysed sludge is then transported to three parallel AAD units on-site via air-cooled heat exchangers that reduce the temperature to just below 40°C, as required by the digesters.
- **Anaerobic Digestion (mesophilic):** This is achieved in three parallel concrete digesters, each with a capacity of 6700m^3. Each digester operates at up to 5.5 kg VS/m^3/d (with an SRT of 25days). Mixing in the digesters is provided by recirculation (by pump). The biogas produced is transferred to membrane gasholders at a design

rate of 44,000Nm^3/d. The digestate is then pumped into storage tanks on-site, which have been retrofitted with air mixing systems to reduce odour issues.

- **Dewatering of the digestate by centrifuge:** The stored digested sludge is pumped to the designated centrifuge on-site to be dewatered to ~35% w/w dry solids. This is then transferred to the cake store where it is held for up to 7 days to optimise the potential for the further release of liquid from the cake. The liquors/centrate from the dewatering process is recycled directly to Train A in the ETW for further treatment, whereas the dewatered sludge is available for sale/spreading to land.
- **Combined Heat and Power (CHP) Plant:** The biogas produced during the AD process is utilised in both the CHP plant and boiler plant. The CHP plant constitutes four Jenbacher gas engines with waste heat recovery modules, and capable of generating up to 4.7MWe of power. This recovered waste heat is used on-site and offsets approximately 40% of the annual energy required for the CAMBI sludge pre-treatment process.

Locating the liquid treatment train adjacent to the sludge treatment train has many benefits such as; the opportunity to re-use a large number of existing assets on-site, and the potential to utilise the power generated by the sludge treatment process to offset the power demand of the liquid treatment train. However, the production of nutrient-RLs by the sludge treatment train and their recirculation to the liquid treatment train has had repercussions including; fouling of pipes and pumps, and the occasional reduction in the performance of some of the treatment processes.

3.3 Sampling and Analysis

3.3.1 Sampling

To develop the mass balances, a sampling campaign was performed over ten months (from 7th February to 5th December 2013). Over 200 samples were collected from eight different points around the sludge treatment line and three other sampling points; i.e. combined industrial effluent (CIE), settled activated sludge (RAS) and final effluent from Train A (Figure 3.2). Specifically, grab samples of settled activated sludge (SAS), final effluent (FE), strained raw sludge (SRS), Return Liquor 1 (RL1), Dewatered Raw sludge (DRS), Feed sludge to the THP (FST), Digester feed sludge (DFS), digested sludge (DS), Return Liquor 2 (RL2), and sludge cake (SC) were collected fortnightly on Thursdays (at 1pm). The sampling time was chosen for consistency and convenience as it was easier and more likely to get assistance from site operators immediately after their lunch. Samples collected were mainly fixed spot samples despite the overall preference for composite samples as being a ‘true’ representation of variations in composition during the course of a day. Grab samples were used due to limited availability of autosamples to install on all sampling locations to get composite samples. However, statistical tests (i.e. t-test) were performed on NH_4-N data between collected fixed spot RLs samples and composite samples collected from the technical team at Bran Sands WWTP to if they were statistically similar.

Sampling most centred on the sludge treatment lines due to observations of scaling problems in this section of the WWTP, especially downstream of the AD units. In addition, a simple mass balance for Total Kjeldahl Nitrogen (TKN), NH_4-N, PO_4-P, and Mg^{2+} around the sludge treatment line at Bran Sands WWTP established in 2011 by an earlier study, highlighted this section of the plant as being most likely to show high available N, P, and Mg loads (See Figures 3.4, 3.7, and 3.10). Volumetric flow data were collected by the supervisory control and data acquisition (SCADA) system installed at Bran Sands WWTP. Flow is measured on site with the help of flow meters.

Sterile wide-mouth 1 L polypropylene bottles were used to collect the sludge samples, while 250 mL polyethylene teraphtahlate (PET) bottles were used to collect the liquid samples.

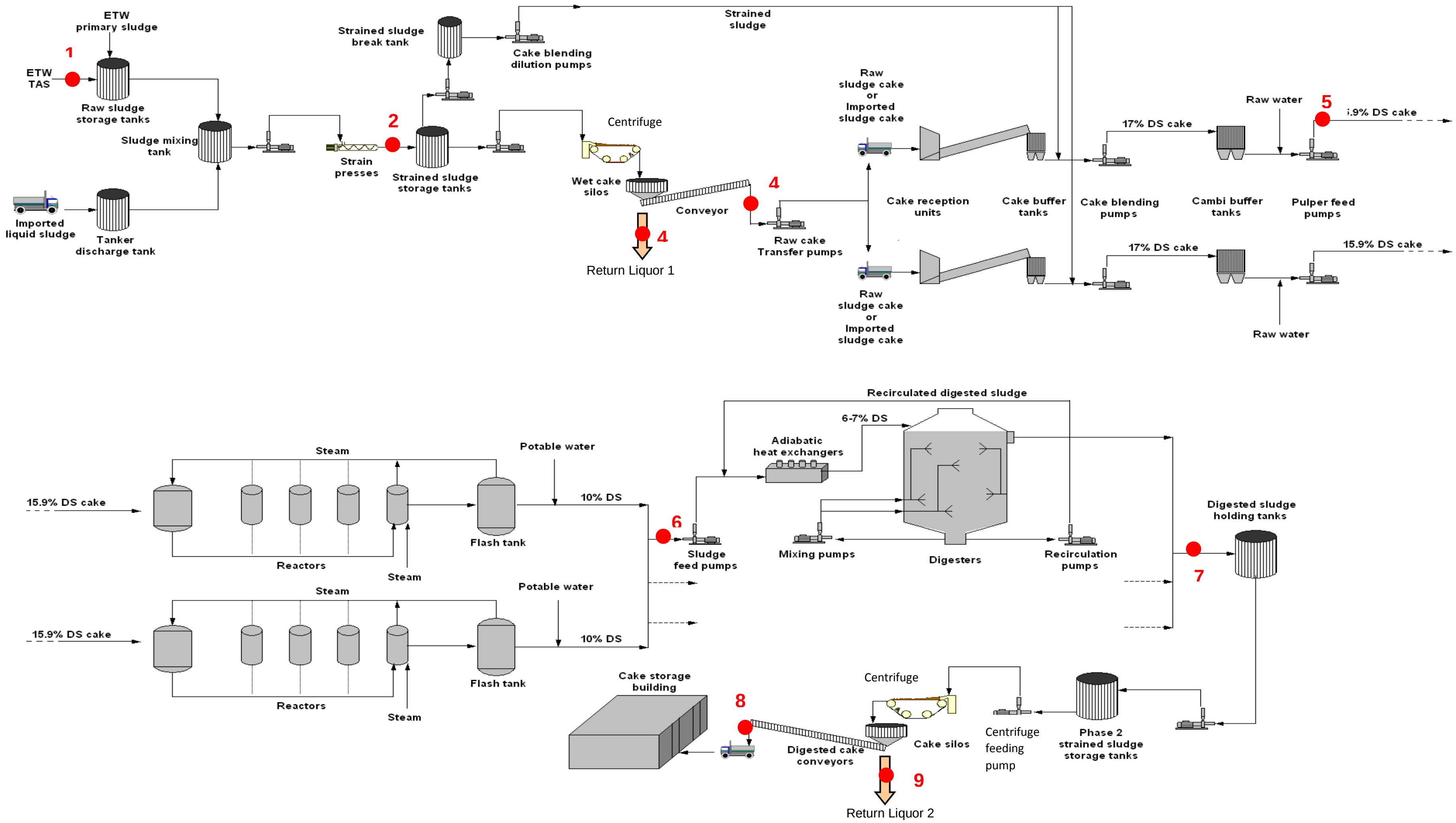

Figure 3.2: Schematic of sludge treatment at Bran Sands WWTP. Red dots indicate sample points

1 = SAS; 2 = SRS; 3 = DRS; 4 = RL1; 5= FST; 6 = DFS; 7 = DS; 8 = SC; 9 = RL2

3.3.1 Chemical analysis of samples

The pH of the samples was measured during sampling using a HACH portable pH meter. The pH of each sample was measured three times and the average taken. Grab samples were analysed at Newcastle University's Environmental Engineering Research Lab for TS, VS, NH_4-N, COD, TKN, PO_4, and Mg^{2+}. While RL2 data collected from the technical team at Bran Sands WWTP was obtained from composite RL2 samples.

Solids

Total solids (TS) and volatile solids (VS) were analysed following Standard Methods for the Examination of Water and Wastewater (Methods 2540B and E, respectively) (APHA, 2005) and measured within 24 hrs of sampling. Each analysis was done in triplicate and the average taken. Each test was carried out on 20 ml of each sample. This volume was chosen due to the available holding capacity of the crucibles used in the analysis. In addition, the volume is within the range used in other publications (Rao et al., 1999; Kobelev et al., 2006; Daphne et al., 2011). VS removed (VS_r) was calculated as the difference between the VS concentrations influent and effluent from treatment units.

Nitrogen and COD

The NH_4-N and COD concentrations (both total and soluble) in the samples were measured within 24 hrs of sampling using Merck Spectroquant test kits (i.e., HC604710 2018/07/31 for 100 tests; test range: 2.0-150 mg/L NH_4-N and HC603159 2019/09/30 for 25 tests; test range: 25 – 1500 mg/L COD; respectively) according the manufacturer's specifications (www.analytical-test-kits.com). Samples for NH_4-N and soluble COD measurement were first centrifuged in 50 ml sterile centrifuge tubes with conical bottom using a Sigma 3-16 centrifuge for 10 mins at 4200rpm (3392 x g) and then filtered through sterile syringe filters with 0.45 µm pore sizes. The samples were stored at -18°C until further analysis was carried out. Each analysis was done in triplicate and averages taken. All samples except treated effluent samples were diluted with dionised water prior to NH_4-N analysis. The dilution factor for samples from treatment processes upstream of the AD was 1:10, while for samples from post-AD units it was 1:50. These dilution factors were chosen to comply with the measurable test range for the NH_4-N test kits used. On the other hand, NH_4-N analysis on composite RL2 samples was carried out daily by the technical team at Bran Sands WWTP using Hach Lange ammonium cuvette test kits, LCK 303 for 25 tests (test range: 2.0 – 47.0 mg/L NH_4-N).

Total Kjeldahl Nitrogen (TKN) the grab samples was measured using the Digestion Tube and Distillation apparatus (VADOPEST method). Procedural details were obtained from Standard Methods for the Examination of Water and Wastewater (Method 4500 B; APHA, 2005).

Phosphates and Magnesium

The PO_4 levels in the samples were measured using Ion Chromatography (IC) according to Standard Methods for the Examination of Water and Wastewater 4110B (APHA 2005); whereas Mg^{2+} concentrations were measured using inductively coupled plasma mass spectroscopy (ICP-MS). Liquid samples (i.e. CIE, SAS, FE, RL1, and RL2), 12 mL each, were digested in 1.2 mL concentrated nitric acid, whereas sludge samples (i.e. SRS, PF, FST, DFS, DS, and SC) were digested with 5 mL nitric acid and 0.5 mL hydrogen peroxide. After digestion, these samples were centrifuged in 50 ml sterile centrifuge tubes with conical bottom using a Sigma 3-16 centrifuge for 10 mins at 4200rpm (3392 x g) and filtered through sterile syringe filters with 0.2 µm pore sizes (Description: MinisartCA; 0.2µm; 28mm) The PO_4 data obtained was then converted to PO_4-P using Equation 18 below. Prior to PO_4 analysis, the filtered samples for were diluted in deionised water as follows: dilution ratios of sample to deionised water of 1:2 for samples from the chosen ETW units, and 1:10 for all samples from the test sludge treatment line processes. These dilution factors were chosen to comply with the maximum PO_4 concentration of 40 mg/L in the 'standard' used in the IC analysis. Diluted samples with dilution ratios of sample to deionised water of 1:10 were also used for all Mg^{2+} analysis.

$$PO_4\text{-P Concentration (in mg/L)} = \frac{\boldsymbol{Measured\ concentration\ of\ PO_4^{3-}\ X\ \ Atomic\ mass\ of\ P}}{\boldsymbol{Molecular\ weight\ of\ PO_4^{3-}}}$$

Equation 18

Daily NH_4-N, TKN, PO_4-P and Mg^{2+} Loads

The daily NH_4-N, TKN, PO_4-P and Mg^{2+} loads for the different treatment units used in the mass balances at Bran Sands WWTP were calculated by multiplying their mean concentrations by the mean volumetric flow within the treatment units. The calculations were done as follows:

$$NH_4\text{-N load (in tonne } NH_4\text{-N/d)} = \frac{\boldsymbol{mean}\ NH_4{-}N\ concentration\ \left(\frac{mg}{L}\right)X\ \ mean\ Volumetric\ flow\ (\frac{m^3}{d})}{1000000}$$

Equation 19

$$\text{TKN load (in tonne TKN/d)} = \frac{\boldsymbol{mean}\ TKN\ concentration\ \left(\frac{mg}{L}\right)X\ \ mean\ Volumetric\ flow\ (\frac{m^3}{d})}{1000000}$$

Equation 20

$$\text{PO}_4\text{-P load (in tonne PO}_4\text{-P/d)} = \frac{\boldsymbol{mean}\ PO_4{-}P\ concentration\ \left(\frac{mg}{L}\right)\ X\ \ mean\ Volumetric\ flow\ (\frac{m^3}{d})}{1000000}$$

Equation 21

$$\text{Mg}^{2+}\text{ load (in tonne Mg}^{2+}\text{/d)} = \frac{\boldsymbol{mean}\ Mg^{2+}\ concentration\ \left(\frac{mg}{L}\right)\ X\ \ mean\ Volumetric\ flow\ (\frac{m^3}{d})}{1000000}$$

Equation 22

3.4 Data Analysis

Data obtained from laboratory analyses were first tested for normality using SPSS prior to being subjected to analysis of variance (ANOVA) to determine if samples collected through out the sampling campaign were statistically similar. Mass flows of the individual constituents were calculated by multiplying the measured concentration in a given stream by the average daily flow of that stream (Yoshida et al., 2015). Uncertainty ranges in this study were presented as standard error of sample means. Mass balances for TKN, NH_4-N, PO_4-P, and Mg^{2+} around key units of interest at Bran Sands WWTP were then established using the calculated mass flows (see Figures 3.4, 3.5, 3.7, 3.8, 3.9, 3.10 and 3.11). Outlying data and flow data on days when flow was zero or SCADA was interrupted were ignored during analysis.

3.5 Results and Discussions

3.5.1 Hydraulic and solid fluxes within the sludge treatment train at Bran Sands WWTP

Hydraulic flow data for the various streams (presented in Figure 3.3) represent the mean and median daily flow values for sludge and liquid transport streams respectively, obtained from SCADA throughout the sampling campaign. Mean and standard error values were used for the sludge transport streams because normality tests (i.e. Shapiro-Wilk test using SPSS) on their respective ‘raw’ flow data (presented in Table A.8) showed that the data was normal ($P>0.05$ at 95% confidence interval limit). In contrast, median and interquartile range values were used for the liquid streams as the Shapiro-Wilk tests on their respective ‘raw’ flow data (presented on Tables A.6, A.7 and A.8) showed these data were not normal ($P<0.05$ at 95% confidence interval limit). Hydraulic and solid flow data shown in Figure 3.3 start from primary effluent (of train B) due to the lack of flow data for the influent into the plant. RLs from the sludge treatment process were recycled back to the ETW (Figure 3.3).

RLs from the dewatering of digested sludge at Bran Sands WWTP contribute ~1% (i.e. 1216 ± 62 m^3/d) of the total hydraulic flow (111,175 ± 5514 m^3/d) entering the activated sludge treatment unit (including 108,422 ± 64 m^3/d from the primary settling units). This is consistent with studies (such as; Wett *et al.*, 1998; Jaffer *et al., 2002;* Fux and Siegrist, 2004; Solley, 2006; Gustavsson, 2010) that report RLs contribute 1% of the volumetric flow into WWTPs. The figure also shows that ~66.4% of the daily hydraulic flow entering the RSTC gets recycled to the ETW as RL1. Out of the 1420 ± 61 m^3/d that goes through the remaining RSTC units, ~7.1% reduction was observed post-CAMBI, which may be linked to possible errors in flow readings as some the flow meters were reported flooded during the sampling campaign. In addition, a further 31.7% reduction was seen post-AD which can also be linked to possible flowmeter errors. Finally, post-AD hydraulic flow losses can be partially linked to the biodegradation of organic matter to form biogas (CH_4, NH_3, CO_2) and water loss as water vapour to biogas collection systems. However, statistical analysis (t-test using SPSS) revealed these differences in hydraulic flow across the RSTC units were not statistically significant ($P>0.05$ at 95% confidence interval limit).

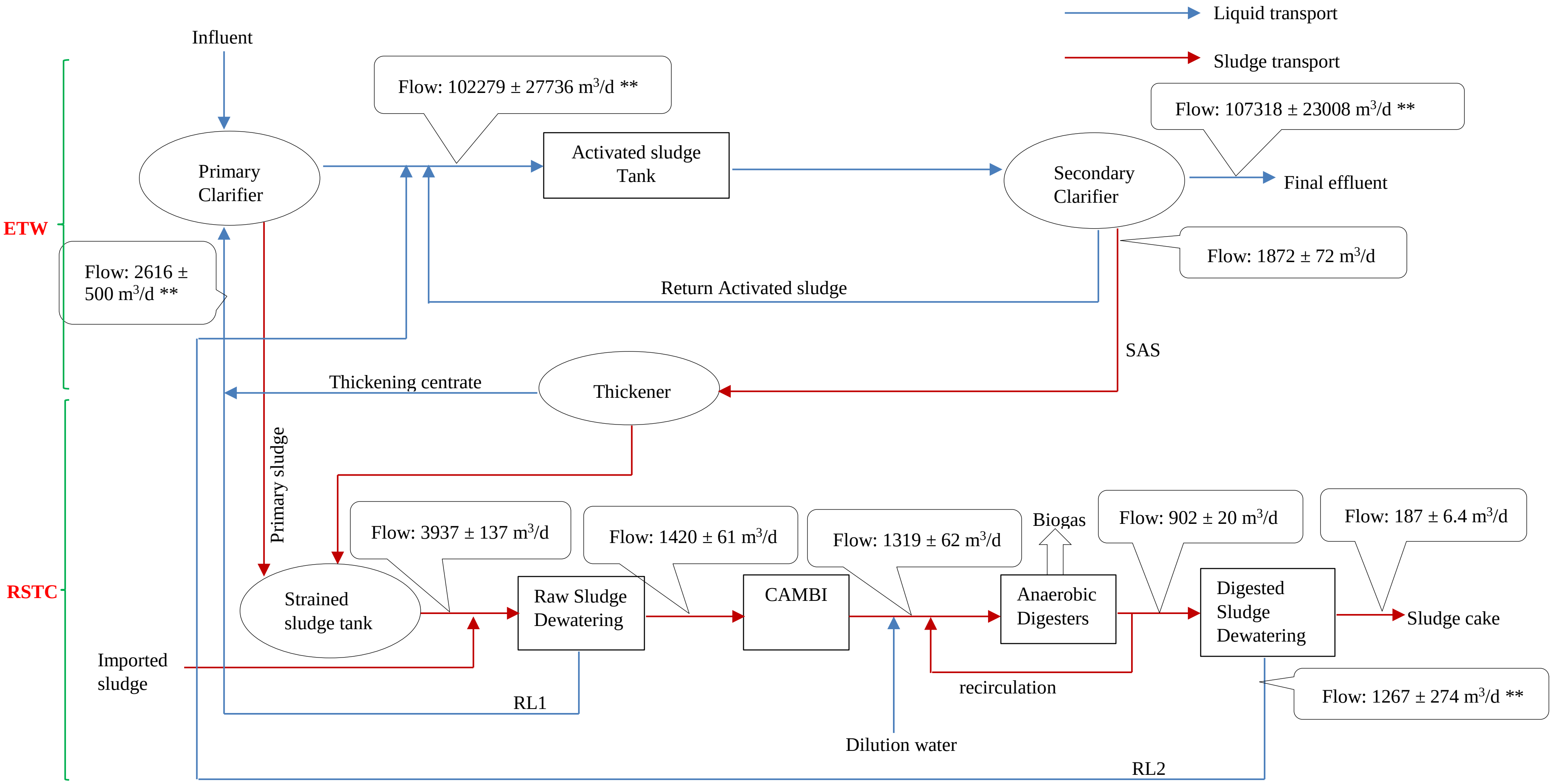

Figure 3.3 : A schematic of the hydraulic and solid flows at Bran Sands WWTP. **Uncertainty ranges presented as interquartile range. Uncertainty ranges presented as standard error of sample means**

3.5.2 Nitrogen Mass Balance

Due to the complex nature of the ETW and scaling problems at the RSTC (owing to the historically high N loads going through the various treatment units), the N mass balance here primarily focused on sludge treatment units. Moreover, Figure 3.4 which presents a mass balance for N around the key units at RSTC at Bran Sands established in 2011, further highlighted sludge treatment units on-site is critical points in terms of nutrient recovery. The N load data presented on Figures 3.4 and 3.5 are calculated means from the daily of NH_4-N and TKN load data presented on Tables A.9 and A.10, respectively. Mean and standard error values were used as the Shapiro-Wilk test for normality showed the data were normal.

Comparing mass balances of NH_4-N and TKN in 2011 (see Figure 3.4) with that of 2013 (see Figure 3.5), ~47.5% and ~15.5% decreases in the NH_4-N and TKN load entering the sludge treatment line were seen between 2011 to 2013, respectively (see Figure 3.6); i.e. from 1.6 tonne NH_4-N/d to 0.84 tonne NH_4-N/d and from 8.4 tonne TKN/day to 7.1 tonne TKN/d, respectively. This can be linked to increased operator care with respect to organic loading rates following increased incidences of scaling (i.e. with higher ‘available’ N, Mg and P loads being transferred through the treatment units). In addition, reductions in daily NH_4-N and TKN in influent sludge in 2013 resulted in overall reductions in associated loads in downstream processes; with the highest percentage reductions in TKN (i.e. 57.1%) seen in RL1 and the least being 3.1% seen in the post-mixing and dilution sludge (see Figure 3.6).

Both Figures 3.4 and 3.5, show that in 2011 and 2013 N mass balances, >75% NH_4-N loads in influent raw sludge at the RSTC in Bran Sands WWTP went through the sludge treatment process following raw sludge dewatering (see Table 3.2). Figure 3.4 shows a 0.5 tonne TKN/d loss following raw sludge dewatering probably due to errors in sampling, sampling time, and data analysis as data collected from NWL had been transformed to two significant figures. This loss is consistent with the ‘unaccounted’ 6% loss in TKN on Table 3.2 after adding up the 8.3% and 85.7% TKN content in RL1 and dewatered raw sludge, respectively in 2011. As expected, Figures 3.4 and 3.5 both show NH_4-N loads in 2011 and 2013 increased following thermal hydrolysis of sludge in CAMBI (compared to loads in pre-CAMBI sludge), while the TKN load remained relatively consstant, probably due to the breakdown of organic N component in the sludge to form the additional NH_4-N/d and gaseous N (NH_3 due to high temperatures in CAMBI). Specifically, post-CAMBI NH_4-N load increased by ~0.1 tonne/d and ~0.41 tonne/d in 2011 and 2013, respectively. This is consistent with the observations of Jaffer *et al.* (2002) of a significant increase in NH_4-N loads and a ~0.5% increase in TKN loads following AD of sewage sludge at Slough WWTP. A two-sample t-test analysis on pre- and post-CAMBI sludge NH_4-N loads showed their means were statistically significantly different ($P>0.05$ at 95% confidence interval limit; df = 40).

In addition, Figures 3.4 and 3.5 show further increase in NH_4-N loads, respectively, in the AD. For instance, in 2011, Table 3.2 shows that %NH_4-N loads in post-AD sludge increased to ~175% from 81.3% in post-CAMBI sludge. The high post-AD NH_4-N load in the N-mass balances, could have been as a result of the digestion of more proteinaceous organic matter prior to sampling, chemical reduction of N due to anaerobic conditions, and periodic disintegration of struvite crystals in the AD units at the relatively high temperatures (>35°C). This is consistent with reports (Marchi *et al.*, 2015) that show increased NH_4-N loads in AD units were associated to the anaerobic biodegradation of organic-N and the potential resolubilization of struvite component ions. The alkaline pH conditions (~7.8) and high temperatures (38 – 40°C) post-AD could also have led to the volatilization of ammonia. However, in the context of this mass balance, pH-related ammonia volatilization is suspected to be minimal based on other reports (Liao et al., 1995; Bonmati & Flotats, 2003; Ruiz et al., 2003; Jiang et al., 2010; Guskin & Marinsek-Logar, 2011) which suggest effective ammonia volatilization/stripping (i.e. >20%) requires pH > 8.5.

Over 43% of the original NH_4-N load in the influent sludge ended up in the digested sludge cake in both the 2011 and 2013 N-mass balances (Table 3.2). Additionally, Figure 3.8 also showed that RLs account for ~30%; i.e. 1.9 tonne/d of total NH_4-N load i.e. 6.7 tonne/d entering the aeration tank (i.e. the activated sludge tank) in the ETW for treatment. This is consistent with existing reports that RLs contribute 10-30% of N load entering WWTPs (Wett *et al.*, 1998; Jaffer *et al.*, 2002; Fux and Siegrist, 2004; Solley, 2006; Gustavsson, 2010). Further, it can be seen in Table 3.2 that ~35% of the raw sludge TKN load in both 2011 and 2013 was recycled to the ETW as RLs. Finally, Table A.2 shows the concentration of NH_4-N also went up during the CAMBI process probably because of the thermal hydrolysis of cell walls and release in the sludge.

The above preliminary information on the historical and current mass transport of N throughout these key treatment units at Bran Sands has necessitated the process of developing adequate nutrient recovery trials that have been presented in Chapter 4. To further guide experimental design for nutrient recovery systems (especially control of the scaling problems on-site), a mass balance for P and Mg also were performed and reported in subsequent sections below.

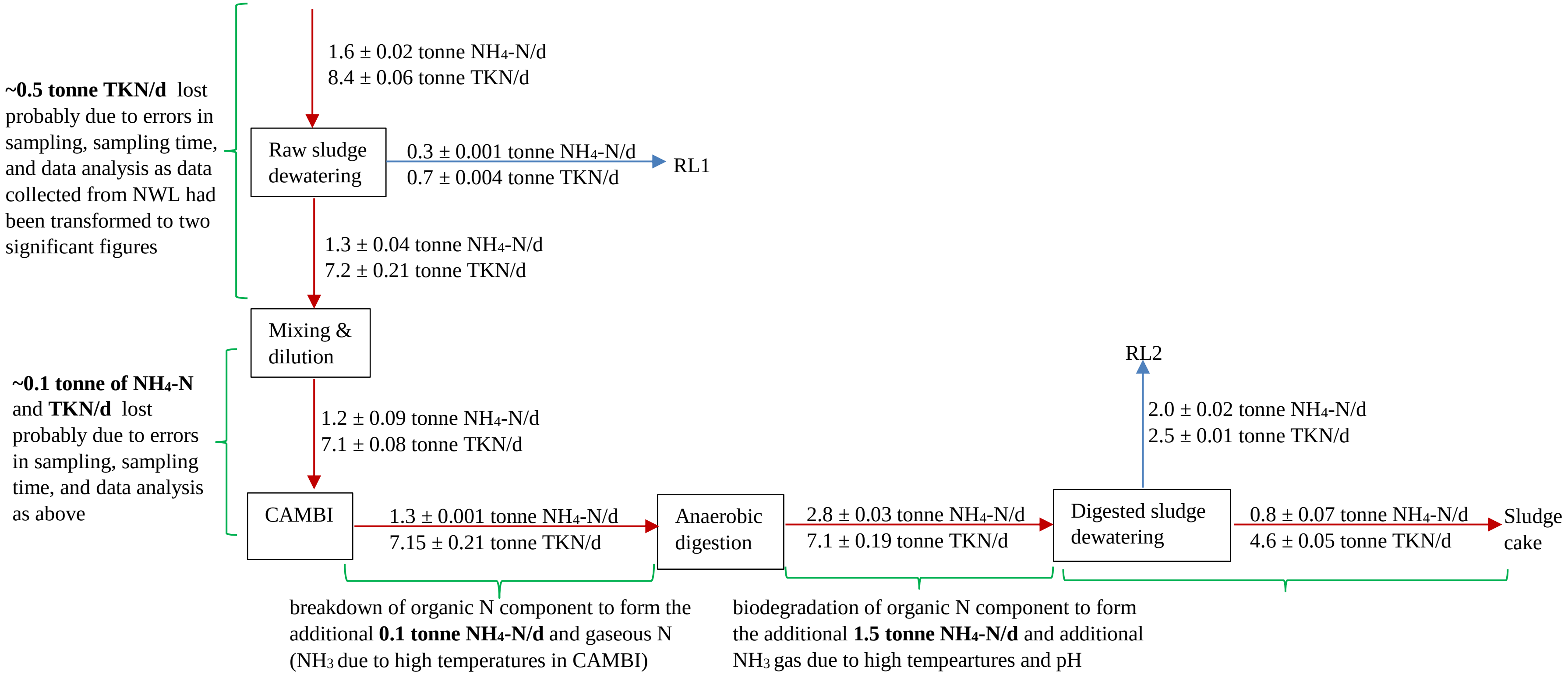

Figure 3.4: Mass flow of NH_4-N and TKN in sludge treatment processes at Bran Sands WWTP in 2011. Uncertainty ranges in this study were presented as standard error of sample means.

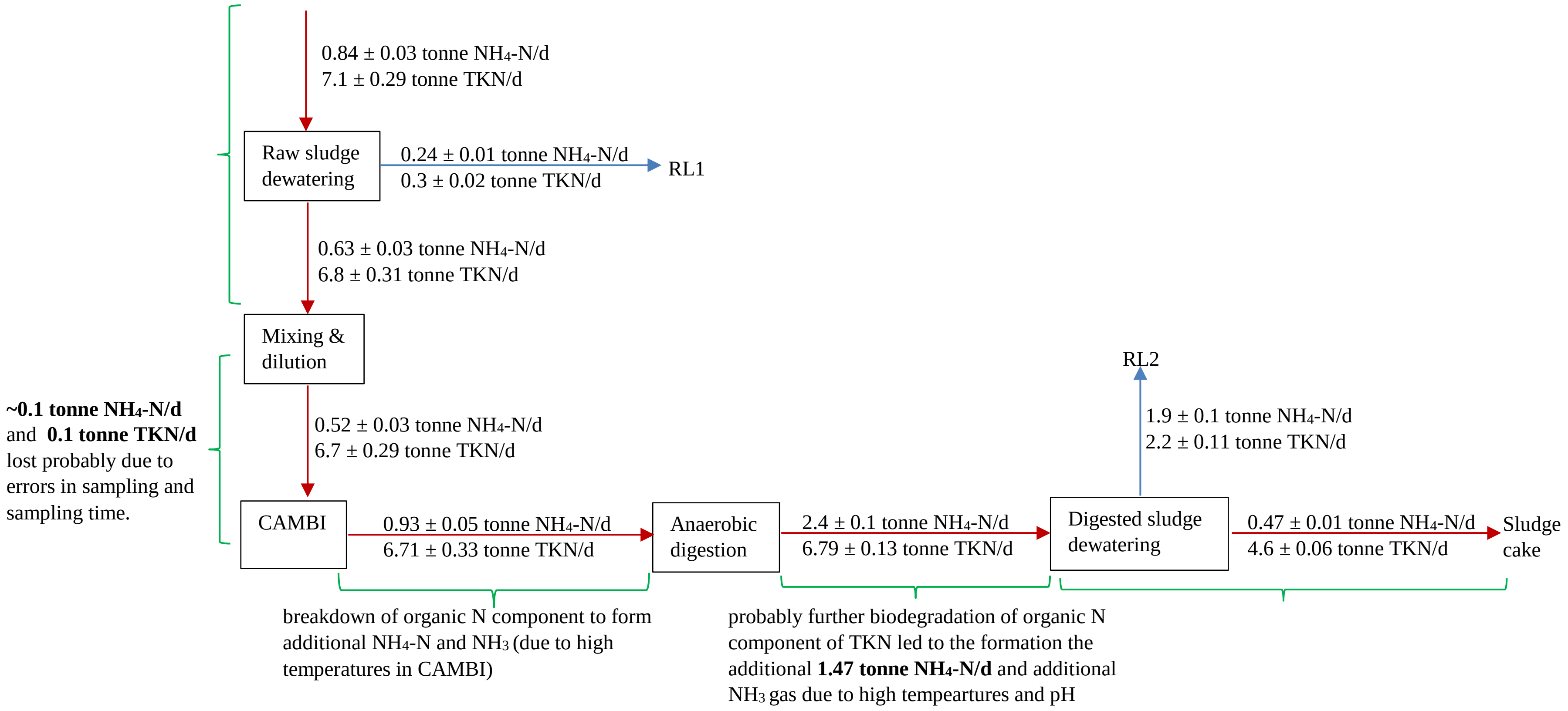

Figure 3.5: Mass flow of NH_4-N and TKN around sludge treatment processes at Bran Sands WWTP in 2013. Uncertainty ranges in this study were presented as standard error of sample means.

Table 3.2: Percentage of influent sludge NH_4-N load in samples in 2011 and 2013

Sample Type	% NH_4-N transfer -2011	% NH_4-N transfer-2013	% TKN transfer-2011	% TKN transfer-2013
Raw sludge (RS)	100	100	100	100
RL1	18.8 ± 0.06*	28.6 ± 1.2	8.3 ± 0.005	4.2 ± 0.3
Dewatered RS	81.3 ± 2.5	75.0 ± 3.6	85.7 ± 2.5	95.8 ± 4.4
Post-mixing and dilution	75 ± 5.6	61.9 ± 3.6	84.5 ± 0.9	94.4 ± 4.1
Sludge from CAMBI	81.3 ± 0.06	110.7 ± 6.0	85.1 ± 3.9	94.5 ± 4.6
Digested sludge - DS	175 ± 1.9	285.7 ± 11.9	84.5 ± 2.5	95.6 ± 3.3
RL2	125 ± 1.3	226.2 ± 11.9	29.8 ± 0.1	31.0 ± 1.5
DS cake	43.8 ± 4.4	47.6 ± 1.2	54.8 ± 0.6	64.8 ± 0.8

The data presented represent the ratios (as a percentage) between the mean NH_4-N and TKN loads at the different sampling points and the respective raw sludge loads in 2011 and 2013.
**Uncertainty ranges in this study were presented as standard error of sample means.*

Table 3.3: Physico-chemical characteristics of sludge and RLs around sludge treatment processes at Bran Sands WWTP

Samples	Parameters					
	TKN (mg/L)	NH_4-N (mg/L)	PO_4-P (mg/L)	Mg^{2+} (mg/L)	Temp (°C)	pH
Influent Raw sludge (RS)	1823 ± 57*	215 ± 6.8	127 ± 4.3	50 ± 2.3	18	6.13
RS Liquors (RL1)	125 ± 3.9	98 ± 3.7	46 ± 1.6	20 ± 0.9	18	6.39
Dewatered RS	4807 ± 152	447 ± 14.1	269 ± 9.06	122 ± 5.5	18	5.52
Post-mixing and dilution sludge	4632 ± 144	364 ± 14.8	256 ± 8.6	111 ± 5	18	5.61
Post-CAMBI sludge	5214 ± 140	705 ± 19.3	297 ± 10	124 ± 5.5	66	5.41
Digested sludge (DS)	7508 ± 115	2775 ± 110	439 ± 14.8	102 ± 4.6	35	7.89
DS Liquors (RL2)	2015 ± 58	1546 ± 61.2	194 ± 6.6	9 ± 0.42	28	8.15
DS Cake	24554 ± 532	1920 ± 76	791 ± 26.7	85 ± 3.9	33	8.38

**Uncertainty ranges in this study were presented as standard error of sample means.*

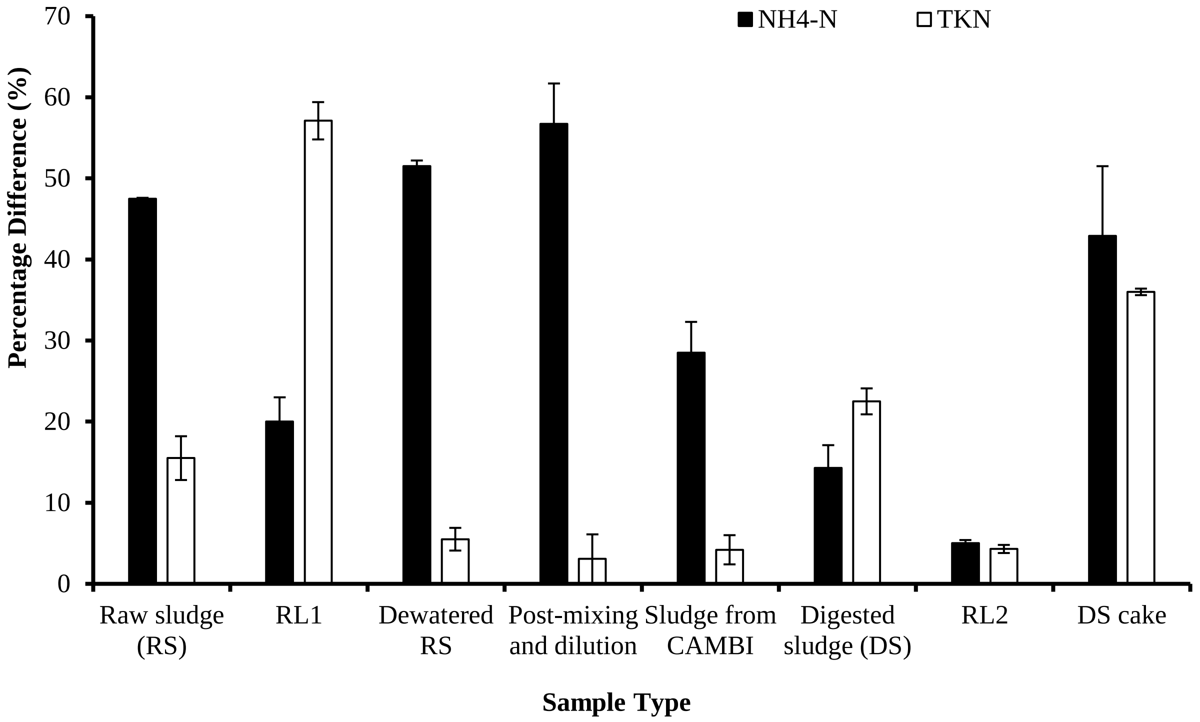

Figure 3.6: Percentage reduction in NH_4-N and TKN loads around sludge treatment processes at Bran Sands WWTP between 2011 and 2013. Uncertainty ranges in this study were presented as standard error of sample means.

3.5.3 Mass Balance for P

At Bran Sands WWTP, there is no P-removal or recovery technology in place. Therefore, similar to the N-mass balance, a detailed P-mass balance was performed around the sludge treatment line. A comparison between a P-mass balance for 2011 (Figure 3.7) and that of 2013 (Figure 3.8) around the sludge treatment line was also performed. A two-sample t-testing showed statistically significant differences between the mean PO_4-P load in the raw sludge entering the sludge treatment line in 2011 and 2013 (p-value < 0.05 at 95% confidence interval limit, df = 40). Specifically, Figures 3.7 and 3.8, and Table 3.4 show that PO_4-P load in the raw sludge dropped by ~20.6% between 2011 and 2013 (i.e. from 0.63 tonne/d in 2011 to 0.5 tonne/d in 2013). This difference probably reflects first attempts at minimising the scaling problems on-site by reducing P-loads entering the sludge treatment line. This could also have been a consequence of the EU-wide ban on P in household detergents in 2012 (EU Regulation No. 259/2012).

Like the N-balances, Table 3.4, also shows that reducing influent sludge PO_4-P loads between 2011 and 2013 resulted in reduced PO_4-P loads in downstream processes processes. For example, the P-balance shows a 25% reduction in PO_4-P loads recycled to the ETW via RLs;

i.e. 0.48 tonne/day in 2011 to 0.36 tonne/d in 2013. In addition, the two mass balances show that ~40 % (2011) and 48% (2013), respectively, of the PO_4-P load in the raw sludge ended-up in the RLs (see Table 3.4). These proportions are similar, although, the slightly higher proportion in 2013 might reflect greater resolubilization of precipitated struvite after as the problem got worse.

Like NH_4-N, PO_4-P concentrations and load increased in the AD system from 2011 to 2013 (see Figures 3.7 and 3.8, and Table 3.3). For instance, Figure 3.8 shows a ~2% increase; i.e. from 0.38 tonne PO_4-P /day in post-Cambi sludge to 0.39 tonne PO_4-P/day in digested sludge, which is similar to the ~1.6% increase reported by Jaffer *et al.* (2002) and Kleeman (2015) in the P-mass balance at the Slough WWTP. Although not significant this may be associated with the increased release of PO_4-P from PAOs under anaerobic conditions (Satoh *et al.*, 1992) and/or the potential resolubilization of struvite related ions in the AD units (Marchi *et al.*, 2015). This increase in PO_4-P resulted in higher PO_4-P loads in the RLs from the digested sludge dewatering process, with ~60 % of the PO_4-P load in digested sludge ending up in the RLs following dewatering. It was observed that RLs from digested sludge accounted ~43% (i.e. 0.24 tonne/day) of the PO_4-P load entering the activated sludge unit in 2013 in the ETW (see Figure 3.9). This is consistent with reports that digested sludge liquors contribute 20-50 % of the total P load entering WWTPs (Wett *et al.*, 1998; Jaffer *et al.*, 2002*;* Fux and Siegrist, 2004; Solley, 2006; Gustavsson, 2010). Data in Figures 3.7 and 3.8 also show the PO_4-P load in RL2 in 2011 and 2013 were quite similar (i.e. ~4% less; see Table 3.4). This may be associated to the relatively longer ‘holding’ period of digested sludge in 2013 prior to dewatering, which would have potentially resulted in the further release of PO_4-P from the digested sludge solids into the liquid phase.

Furthermore, it was observed that operating conditions (i.e. pH of 7.8 – 8.38, high available PO_4-P concentrations; see Table 3.3) in post-AD processes resulted in major scaling problems. These conditions favour the formation and precipitation of struvite. The mass balance indicates about 40% (i.e. 0.12 tonne/d in digested sludge cake and 0.04 tonne/d probably precipitated out on walls of pipes and centrifuge) of the 0.4 tonne/d PO_4-P load in digested sludge in the 2013 P-balance was precipitated out of the aqueous phase into the solid phase (see Figure 3.8). X-ray diffraction (XRD) analysis of the precipitate showed that 95% of scale was struvite. Moreover, assuming 0.04 tonne/d (i.e. 10% of PO_4-P load in digested sludge) ‘unaccounted for P’ in the 2013 P-balance was precipitated out as struvite on the walls of pipes and dewatering equipment conveying digested sludge, it is possible that it must have built up in the pipes and equipment ‘unnoticed’ over time and only discovered when treatment processes start failing; i.e. clogged pipes and pumps impede the effective flow of sludge, and difficulties in effective heat transfer due to clogged heat exchangers. This makes struvite control measures on site highly imperative.

In addition, attempts at transforming the problem into economic gains could even be more justified, due to the fact that Figure 3.9 shows that almost all the PO_4-P load that gets recycled to the ETW ends up in the final effluent (i.e. 89% PO_4-P load) due to the ‘relaxed’ P consent for the site and the absence of P-removal technologies on site. Hence, struvite control through potential P-recovery, targeting processes downstream of the AD will be of paramount importance given their natural operating conditions that favour effective nutrient recovery at relatively low costs.

In summary, unlike in the N-mass balance where there was NH_4-N removal of ~70 % (i.e. 3.3 tonne/d in the activated sludge unit at the ETW, ~89 % (i.e. 0.5 tonne/d) of the PO_4-P entering the secondary treatment unit was disposed via the final effluent while only ~11% was recycled through the various treatment units at WWTP. An overall summary is provided in Figure 3.9. However, before conclusions are made, a similar mass balance for Mg is needed because of its significant role in struvite formation around the RSTC at Bran Sands WWTP.

Table 3.4: Percentage of influent sludge PO_4-P load in samples in 2011 and 2013, and percentage reduction in PO_4-P load in samples between 2011 and 2013

Sample Type	% of influent sludge PO_4-P load in samples in 2011	% of influent sludge PO_4-P load in samples in 2013	% reduction in PO_4-P load in samples between 2011 and 2013
Raw sludge (RS)	100	100	20.6 ± 3.2
RL1	36.5 ± 4.7*	24 ± 2.0	47.8 ± 8.7
Dewatered RS	63.5 ± 11.1	76 ± 4.0	5 ± 1.3
Mixed and diluted sludge	60.3 ± 4.8	74 ± 4.0	2.6 ± 0.2
Sludge from CAMBI	73 ± 15.9	78 ± 4.0	15.2 ± 1.7
Digested sludge-DS	84.1 ± 7.9	80 ± 4.0	24.5 ± 5.7
RL2	39.7 ± 4.8	48 ± 2.0	4 ± 0.8
DS cake	36.5 ± 15.9	30 ± 14.0	17.5± 1.3

**Uncertainty ranges in this study were presented as standard error of sample means.*

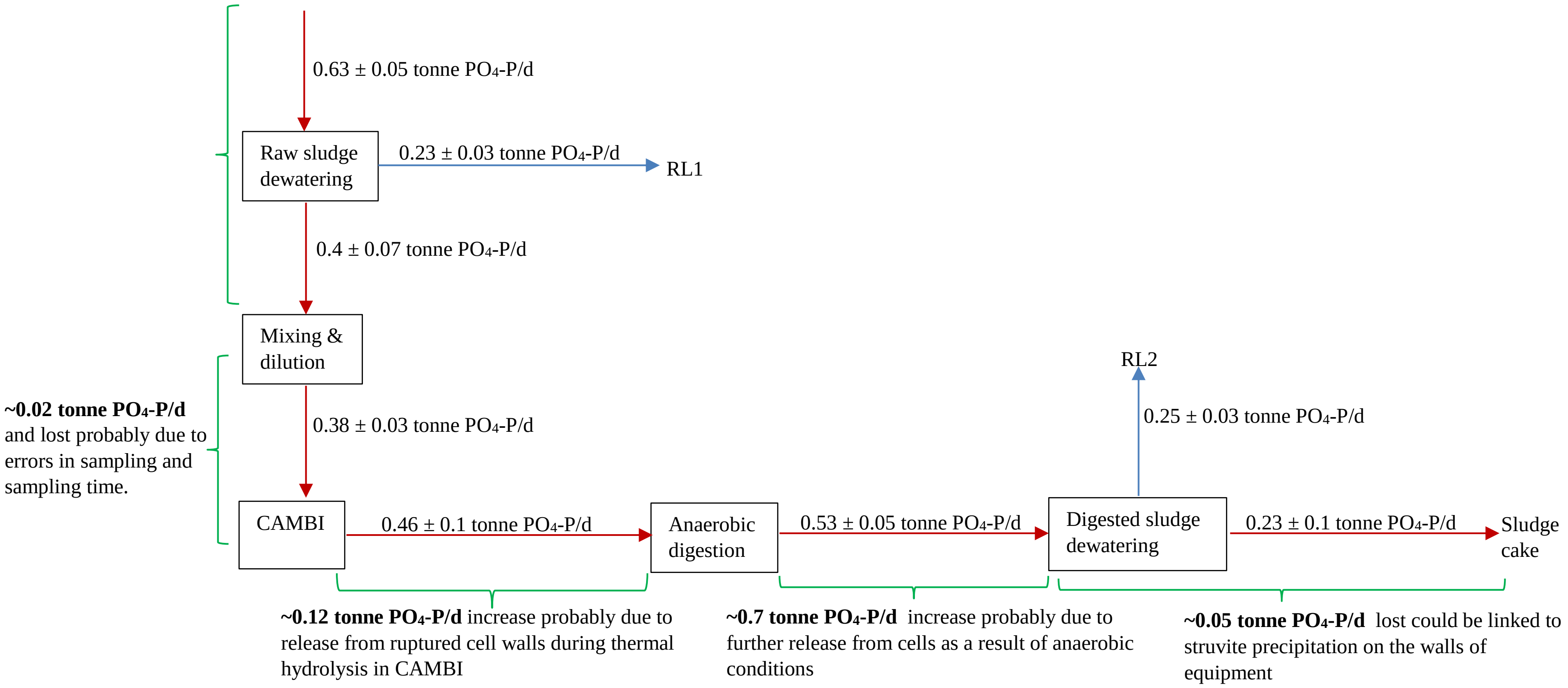

Figure 3.7: Mass flow of PO_4-P in sludge treatment processes at Bran Sands WWTP in 2011. Uncertainty ranges in this study were presented as standard error of sample means.

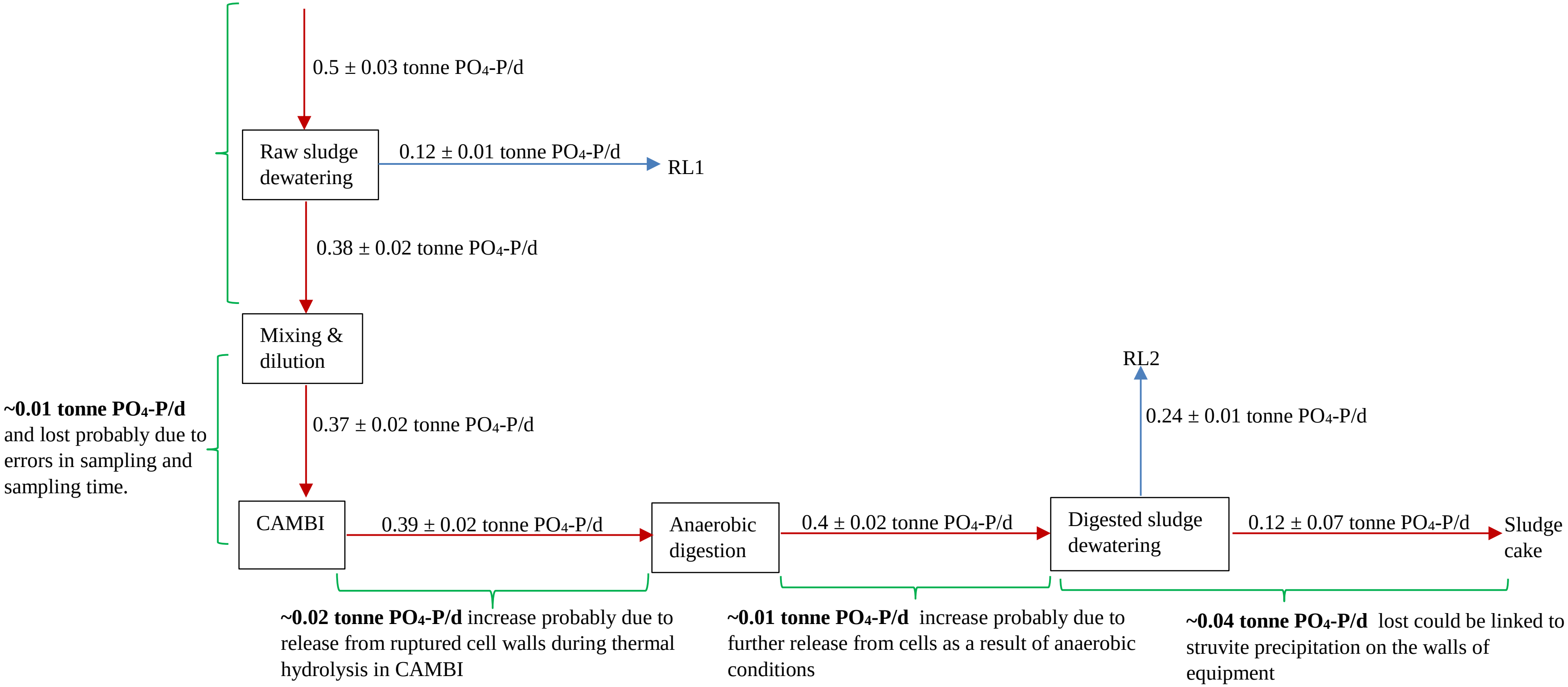

Figure 3.8: Mass flow of PO_4-P around sludge treatment processes at Bran Sands WWTP in 2013. Uncertainty ranges in this study were presented as standard error of sample means.

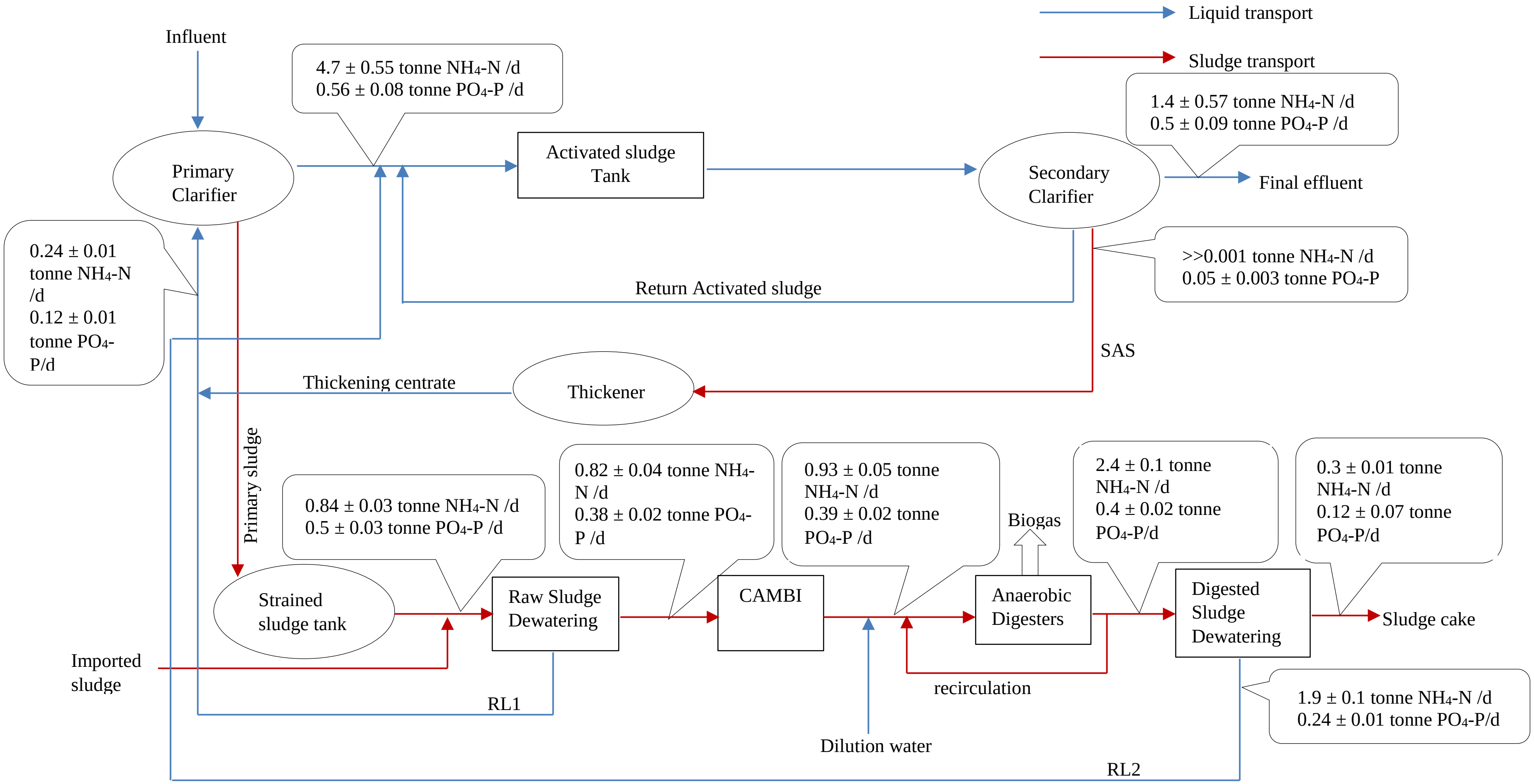

Figure 3.9: Mass flow of NH_4-N and PO_4-P in the ETW and contributions from the RSTC via RLs at Bran Sands WWTP in 2013. Uncertainty ranges in this study were presented as standard error of sample means.

3.5.4 Mg-mass Balance

Similar to N and P, Mg-mass balances around the RSTC at Bran Sands were performed for 2011 (Figure 3.10) and 2013 (Figure 3.11). A two-sample t-test showed a statistically significant difference between Mg^{2+} load in the raw sludge entering the sludge treatment line in the 2011 compared with 2013 (p-value < 0.05 at 95% confidence interval limit; df = 40). Specifically, Figures 3.10 and 3.11, and Table 3.5 show that Mg^{2+} load in the raw sludge dropped by ~63% (from 0.54 tonne/d in the 2011 to 0.2 tonne/d in 2013). This drop is consistent with the drop in sludge loading rates in 2013 that led to the drop in NH_4-N and PO_4-P loads entering the sludge treatment line. In the 2011 Mg-balance, ~32.2% of the Mg^{2+} load in the raw sludge ended-up in the RLs, whereas ~30.5% of the Mg^{2+} load in the raw sludge in the 2013 Mg-balance ended-up in the RLs (see Table 3.5).

Like NH_4-N and PO_4-P, reduced transport of Mg^{2+} (~88% of the 0.09 tonne/day Mg^{2+} load in digested sludge; see Figures 3.11) due to crystallization was apparent from the aqueous phase in post-AD processes to the solid phase (i.e. scaling in pipes and in the digested sludge cake). This confirms AD and post-AD locations are potentially suitable for P-recovery via struvite precipitation. Nelson *et al.* (2003) reported that a Mg:PO_4 molar ratio of 1.2:1 is required for the effective P-recovery of struvite from AD effluents vis-à-vis minimising scaling problems on site. However, the Mg:PO_4 molar ratio in digested sludge from Bran Sands WWTP is 0.3 : 1 and 0.06 : 1 in RLs from digested sludge dewatering (see Figure 3.12). These lower Mg:PO_4 molar ratios suggest that Mg level is probably the 'limiting' nutrient in the struvite formation reaction, thereby potentially making it practically difficult to recover P via 'controlled' struvite formation. These data suggest the need for additional Mg to facilitate P-recovery via struvite formation at Bran Sands. Upstream Mg addition was concluded to be potentially 'cost-effective' option for controlling scaling problems in the post-AD RSTC equipment, and also for reducing N and P loads being recycled to the ETW via RLs.

Table 3.5: Percentage of influent sludge Mg^{2+} load in samples in 2011 and 2013, and percentage reduction in Mg^{2+} load in samples between 2011 and 2013

Sample Type	% of influent sludge Mg^{2+} load in samples in 2011	% of influent sludge Mg^{2+} load in samples in 2013	% reduction in Mg^{2+} load in samples between 2011 and 2013
Raw sludge (RS)	100	100	63 ± 13
RL1	29.6 ± 5.6*	25 ± 1.5	68.8 ± 16.7
Dewatered RS	75.9 ± 3.7	85 ± 6.0	58.5 ± 4.0
Mixed and diluted sludge	77.8 ± 1.8	80 ± 5.5	61.9 ± 2.3
Sludge from CAMBI	77.8 ± 9.2	80 ± 6.0	61.9 ± 19
Digested sludge (DS)	63 ± 3.7	45 ± 2.5	73.5 ± 4.4
RL2	2.6 ± 0.4	5.5 ± 0.5	21.4 ± 0.7
DS cake	53.7 ± 0.6	8 ± 0.5	94.5 ± 7.1

**Uncertainty ranges in this study were presented as standard error of sample means.*

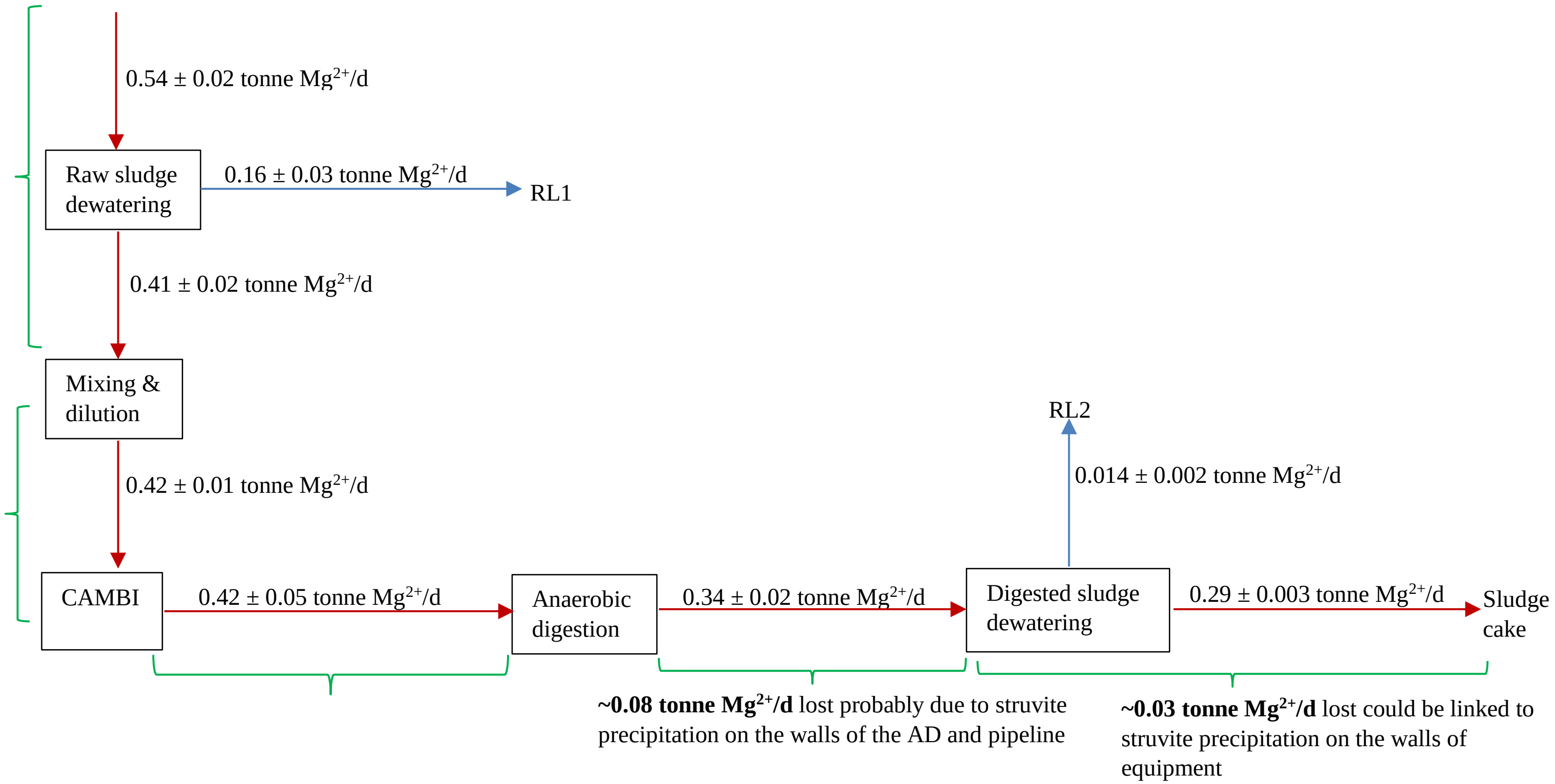

Figure 3.10: Mass flow of Mg^{2+} around sludge treatment processes at Bran Sands WWTP in 2011. Uncertainty ranges in this study were presented as standard error of sample means.

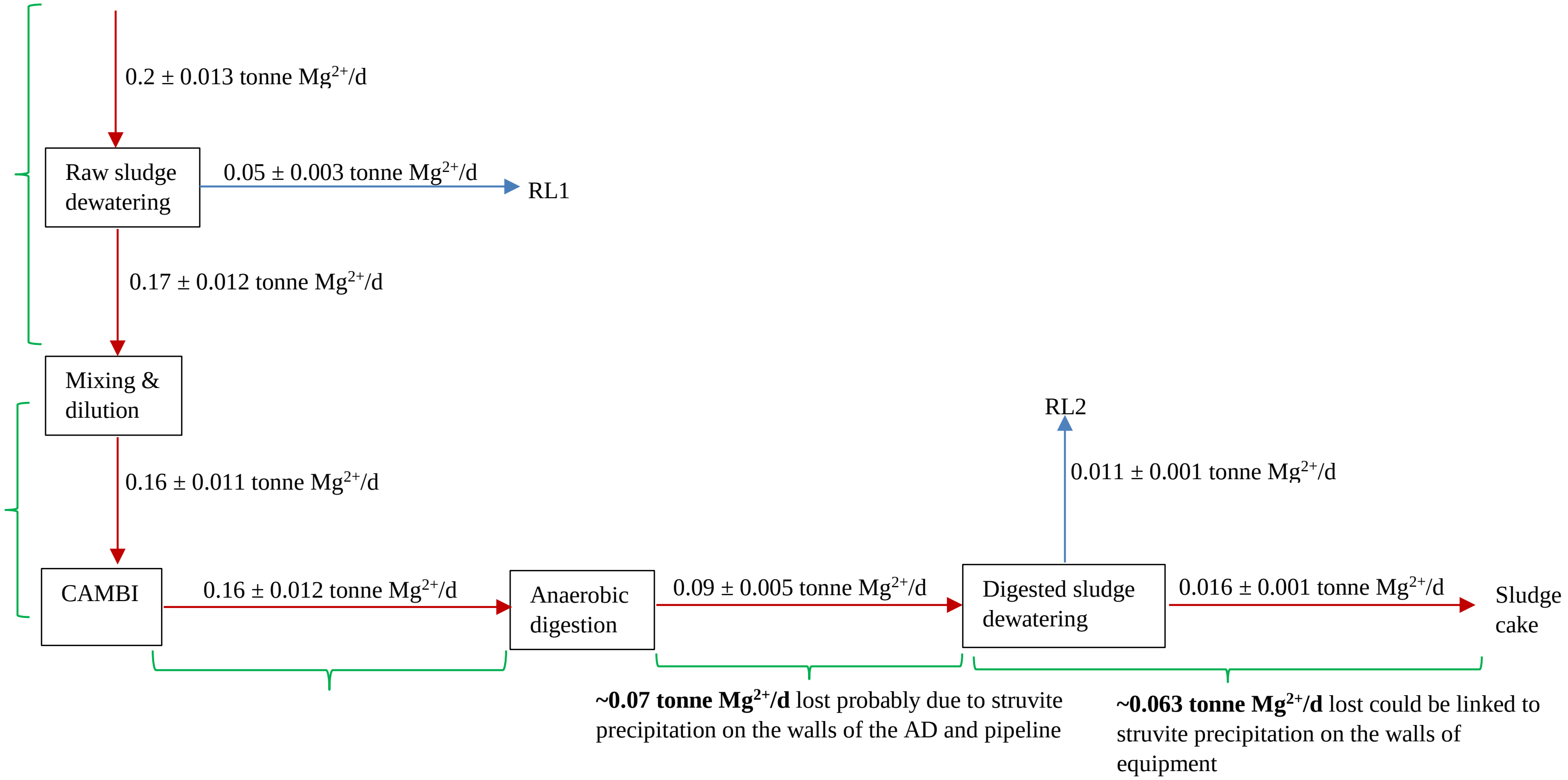

Figure 3.11: Mass flow of Mg^{2+} around sludge treatment processes at Bran Sands WWTP in 2013. Uncertainty ranges in this study were presented as standard error of sample means.

3.6 Struvite precipitation potential around the RSTC at Bran Sands WWTP

Theoretical supersaturation conditions that favour struvite formation (see Equation 4 in Chapter 2) were used to assess struvite formation and its reduction at Bran Sands. To apply this concept, Stumm and Morgan (1970) and Snoeyink and Jenkins (1980) proposed the thermodynamic solubility product constant (K_{sp}) that takes into account total molar concentration of struvite component ions in solution. Sharp *et al.* (2013) and Mudragada *et al.* (2014) reported that when a solution containing struvite constituent ions is in equilibrium with the solid phase, K_{sp} is equal to the ionic activity product (IAP) (see Equation 3, in Chapter 2). However, supersaturation occurs when $K_{sp} <$ IAP (see Equation 4). Solubilility product constant (K_{sp}) has been estimated to be $\sim 10^{-13}$ over the pH range of 6.61 to 8.72 (Bhuiyan *et al.*, 2007).

IAP and Mg^{2+}: PO_4^{3-} molar ratio values are shown in Figure 3.12 for sludge and liquor samples collected from treatment units around the RSTC at Bran Sands WWTP. The IAP values are mean values of the product of the molar concentrations of Mg^{2+}, NH_4^+, and PO_4^{3-} ions in samples from the various treatment units presented on Table A.13. Using $K_{sp} = 10^{-13}$, Figure 3.12 shows that IAP for struvite component ions was greater than K_{sp} in all RSTC units sampled for the mass balances by a factor of $>10^4$. In addition, t-test analyses showed that mean IAP for each of the sampled treatment units was statistically significantly different from K_{sp} (p-value < 0.05 at 95% confidence interval limit). The high IAP values in the RSTC units could have been as a result of the significantly high concentrations of the struvite component ions resulting from a potential build-up within the systems and potentially higher loading rates. As expected, IAP values were even higher after CAMBI and there were further increases in the AD system. Differences in mean IAP in pre- and post-CAMBI sludge, post-CAMBI and post-AD sludge were statistically significant (p-value < 0.05 at 95% confidence interval limit). In contrast, the mean IAP in the digested sludge and digested sludge cake following dewatering on-site were similar; probably due to relatively higher struvite constituent ion concentrations. The decrease in IAP in RL2 could have resulted from the drop in constituent ion levels relative to the digested sludge (probably due to struvite precipitation).

Mg^{2+}:PO_4^{3-} molar ratio were generally higher in treatment units upstream of the AD units in contrast to those downstream of the AD system (Figure 3.12). For example, a mean Mg^{2+}:PO_4^{3} molar ratio of < 0.5 was seen in post-AD sludge and lower following the dewatering of digested sludge (e.g., mean Mg^{2+}: PO_4^{3-} molar ratio of 0.06 was seen in RL2). This decrease in post-AD Mg^{2+}: PO_4^{3-} molar ratios could be linked to the higher pH in processes downstream of the AD, which could have resulted in the reduction of ‘free’ Mg^{2+}

ions through precipitation. Despite the higher Mg^{2+}: PO_4^{3} molar ratios in post-AD processes (compared to those downstream of the AD), the potential for struvite precipitation is lower in the former due to the lower pH (i.e. pH <7). This is consistent with the observations of Jaffer *et al.* (2002) who reported that struvite precipitation potential was higher in digested sludge, digester sludge centrate and dewatered digested sludge with pH between 7.3 -7.9 compared to sludge in pre-AD units with pH < 7. This explains the struvite-related scaling problems in post-AD processes at Bran Sands WWTP.

IAP data presented on Figure 3.12 alongside the Mg^{2+}, NH_4-N and PO_4-P loads and the pH in the various RSTC treatment units, led to performance of P-recovery trials as a function of Mg dosing (in AD and post-AD) which are presented in Chapter 4. The high IAP, suitable pH and stoichiometrically favourable Mg^{2+}:PO_4-P molar ratio in AD units and in digested sludge further suggested 'controlled' struvite precipitation might be cost-effective N and P recovery as the conditions were amenable to struvite formation. This provides the added value by strategically targeting N and P recovery at points that would reduce scaling problems.

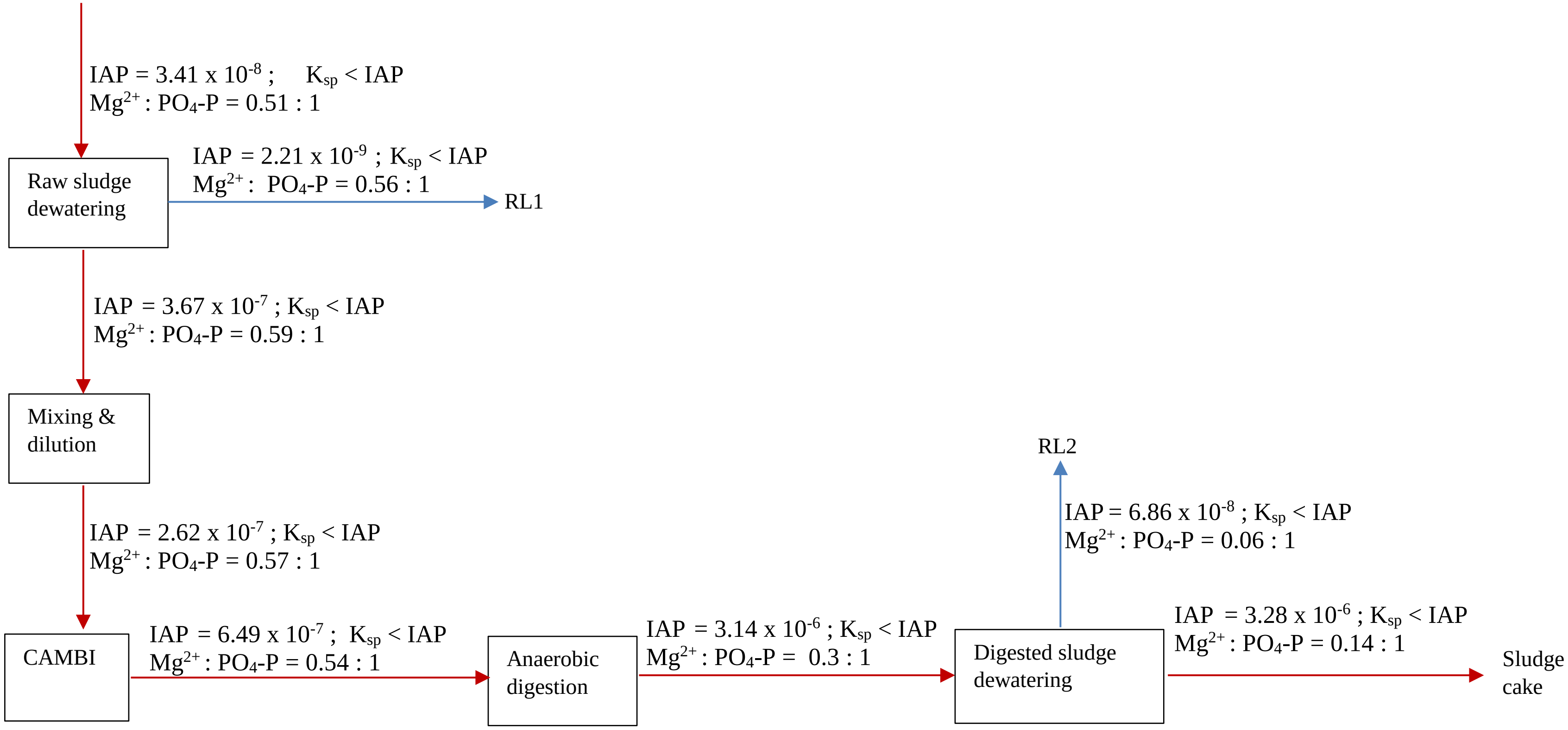

Figure 3.12: Comparison between ion activity product (IAP) and struvite solubility product constant (K_{sp}) around sludge treatment processes at Bran Sands WWTP in 2013

3.7 Conclusion and Recommendations

Bran Sands WWTP includes a liquid treatment train (or ETW) that is roughly in parallel with a sludge treatment train (or RSTC). The RSTC provides operational benefits, including: the opportunity to re-use waste-associated assets/resources on-site and the potential to harvest/utilise energy from the waste via the sludge treatment process to offset power demands from the ETW. However, the production of nutrient-rich RLs in the RSTC and their recirculation back to the ETW has had various repercussions. They include scaling in pipes and pumps, and occasional reductions in performance of some of the treatment processes (e.g. the activated sludge treatment units due to high N-content).

In order to better define these issues, a mass balance for N and P around the site was performed, to identify critical locations of concern and identify potential points for intervention. The mass balances showed there were increases in NH_4-N and PO_4-P in CAMBI following thermal hydrolysis and in the AD units. Both increases increased potential for resolubilisation of struvite constituent ions that promoted actual struvite formation and re-entry into the ETW. Specifically, NH_4-N and PO_4-P loads of 2.23 tonne/d and 0.36 tonne/d respectively, are returned to the ETW via post-AD RLs. Based on 2013 data, about 13% of NH_4-N, 40% of PO_4-P and 88% of Mg^{2+} in digested sludge precipitated out of solution (in post-AD processes) into the solid phase, which caused scaling in equipment and precipitates in digested sludge cake (due to the elevated nutrient levels and favourable conditions for struvite formation). Further, NH_4-N recycled back to the ETW accounts for ~ £224,260/yr in additional energy costs to reduce NH_4-N to consent levels (250 mg/L) at Bran Sands WWTP (see calculation in Appendix A). In addition, scaling problems linked to the ‘uncontrolled’ struvite crystallization in equipment reported on site occurred between the AD and the digested sludge centrifuge.

Although elevated nutrients and evident struvite formation are problems, this highlights these locations as a suitable place for N and P recovery as struvite. However, other mass balances show these areas are relatively deficient in Mg^{2+} (i.e. in digested sludge), which suggests that Mg^{2+} levels are rate-limiting relative to struvite formation reactions or new processes to be considered. Hence, it was decided to perform experiments to determine the feasibility of Mg^{2+} addition (in AD and post-AD processes) in conjunction with pH adjustment to promote N and P removal, both to reduce struvite problems, but also to reduce N and P loadings in RLs returned to the ETW. These experiments and their results are presented in Chapter 4.

Chapter 4: Nutrient recovery and struvite control associated with AD processes

4.1 Introduction

The high cost of treating nutrient-rich wastewaters (especially RLs), coupled with the stringent regulations limiting the discharge of N and P into aquatic environments, have stimulated research on in-stream process options capable of reducing P and N levels while minimising net system cost (Capdevielle *et al.,* 2013). For example, about 60 - 70% of sewage sludge in the UK is currently treated via mesophilic anaerobic digestion (AD) (Roberts, 2015). Most of these AD processes produce liquors (after digested sludge dewatering) with elevated pH, and high soluble P and N concentrations. Wastes with such traits are often difficult to treat, and also favour struvite formation (i.e. Magnesium ammonium phosphate hexahydrate) in sludge processing equipment, resulting in significant financial (i.e. increased maintenance costs) and operational problems (e.g. pipeline blockages, fouling of pumps, interrupted plant operations).

Unmanaged formation and precipitation of struvite in WWTPs leads to significant increases in maintenance costs for the replacement of plugged pipelines and operational problems, such as the instability of the treatment processes (Neethling and Benisch, 2004; Barat *et al.,* 2005; Marti *et al.* 2008; Mudragada *et al.,* 2014). The situation is worsened by the P accumulation across the whole system which can result from recycling of digester liquors back to the 'head' of the WWTP requiring re-treatment. Further, Mudragada *et al.,* (2014) reported struvite also can accumulate inside AD units, which can result in reductions in the available digester volume. Finally, high ammonia concentrations inside AD units, have been observed to impair methanogenic activities and, at extremely high concentrations (i.e. ~6000 mg NH_4-N/L), can completely inhibit entire methanogenesis (Sawayama *et al.,* 2004). Hence there is need for better removal/recovery of N and P in AD processes, to boost performance, reduce secondary costs, and minimise struvite-related problems in WWTP systems.

Preventing struvite accumulation in treatment processes is a potentially costly task. Therefore, in order to control struvite-related problems, it is important to understand the underlying factors responsible for its formation in each case. Parsons *et al.* (2007) proposed pH to be a very important factor in struvite control. Some existing struvite control measures include: the installation of water 'softening' devices before and after the AD (Williams, 1999); the dilution of digested sludge with secondary effluent (Borgerding, 1972; Stratful *et al.,* 2001);

and in some critical cases, central treatment processes have been redesigned (Williams, 1999; Stratful *et al.*, 2001). Other studies suggest the use of metal salts (e.g. ferric chloride, magnesium chloride, magnesium hydroxide, magnesium oxide, alum etc.) for the control of struvite formation (Mamais *et al.*, 1994; Bergmans *et al.,* 2014) by precipitating some and/or all free struvite component ions. However, Wu *et al.* (2005) discouraged the use of ferric salts and alum in controlling struvite formation because P recovery from the compounds formed (e.g. ferric phosphate) is very difficult. In addition, the use of ferric salts in P removal has been reported to cause corrosion in equipment, increase overall sludge production, and has health and safety concerns involved in handling the chemicals (Sharp *et al.*, 2013).

An alternative method to prevent struvite accumulation in treatment processes is to encourage its formation in a 'dedicated' reactor (Stratful *et al.*, 2001) by 'controlled' Mg addition. Such controlled formation of struvite is a potential avenue for P recovery (Doyle and Parsons, 2002; Liu *et al.,* 2013). P recovery (from nutrient-rich wastewater) through crystallisation of struvite requires a Mg source (e.g. $Mg(OH)_2$ or $MgCl_2$) and a sufficiently high pH (Hanhoun, 2011), and several potential markets for resultant struvite exist. For example, it has been recommended as a slow release fertilizer, raw material for the phosphate industry, a binding material in cement, and for use in making fire resistant panels (Sarkar, 1999; Schuilling and Andrade, 1999; Demirer *et al.,* 2010).

Most technologies developed to recover P (as struvite) in WWTPs have been applied to the aqueous phase (e.g. digested sludge liquors). However, limited information about P-recovery through the controlled crystallization of struvite in the sludge phase exist; although recent studies (Bergmans *et al.* 2014; Geerts *et al.* 2015; Marchi *et al.* 2015) on controlled struvite crystallization in digested sludge have reported advantages, such as: improved dewaterability of digested sludge; better P and N recovery potential; reduced scaling in treatment equipment; and reduced N and P in digester liquors recycled to the head of WWTPs for further treatment. However, much work is still needed to cost-effectively minimise available P and N levels in processes downstream of anaerobic digesters (ADs).

The NWL Bran Sands WWTP near Middlesbrough (equipped with a sludge treatment facility that uses mesophilic AD to treat sewage sludge) has been experiencing significant struvite deposition problems (like the clogging of pipes and pumps downstream of the AD). This is coupled with production of nutrient-rich RLs that account for ~22% of the WWTP's annual energy consumption when returned to the head of the works for treatment) (and up to ~38% in December 2012). This poses financial, mechanical and operational problems for the company; hence the dire need for remedial action.

Within this context, this chapter investigated the feasibility of the potential for P recovery (as struvite) from sludge around AD processes through pH adjustment and addition of Mg^{2+} (as $MgCl_2$) either directly to AD units or in post-AD systems, based on recommendations from the N, P, and Mg mass balances. Experiments involving Mg^{2+} addition and pH adjustment were performed in eight bench scale AD units and in eight post-AD effluents (i.e. digested sludge), and comparisons on their P- and N-removal rates were made. The study also sought to evaluate the effect of Mg^{2+} addition and pH adjustment on the performance of the AD process (in terms of methane production and VS removal).

4.2 Methodology

After establishing a basic mass balance for P, N and Mg around process units of concern at Bran Sands WWTP (presented in Chapter Three), bench experiments were designed and conducted to assess the efficiency of PO_4-P and NH_4-N removal from sludge train by Mg^{2+} addition and pH adjustment (both pre- and post-AD). Magnesium Chloride ($MgCl_2$) was used as Mg^{2+} source due to its high solubility in water and the comparative ease by which Mg^{2+} is released for reaction with PO_4^{3-} and NH_4^+ (compared to $Mg(OH)_2$ as the Mg^{2+} source; Parsons *et al.* 2007).

4.2.1 pH adjustment experiments – Jar Tests

Prelimnary jar test experiments were performed on digested sludge samples from Bran Sands WWTP to assess the effect of varying pH (only) on the removal of NH_4-N and PO_4-P from digested sludge. Assays were conducted in 500ml beakers (each containing 250ml of digested sludge) in duplicate at adjusted pH of 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. The pH of the sludge (which was originally 7.8) was modified to each test pH using either 5N NaOH or 5N HCl as required.

The tests were performed at ambient temperature of 25ºC and stirring speed of 100rpm for 10 minutes followed by a settling period 3 hours. The chosen mixing time was based on recommendations of Li *et al.,* (2012) and was based on optimal times for the recovery of N and P through stable struvite formation. After the reaction, aliquot of the supernatant was collected centrifuged, filtered and analysed for Mg^{2+}, NH_4-N and PO_4-P as described in Chapter 3. Samples were then collected from the remaining reaction ‘mix’ and centrifuged in 50 ml sterile centrifuge tubes with conical bottom using a Sigma 3-16 centrifuge for 10 mins at 4200rpm and 3392 x g; after which crystal-like particles were picked out and dried in an incubator at 35ºC for 1 week. The dried particles were collected, weighed and analysed by X-ray Diffraction (XRD) to characterise product crystalline structures and ascertain the purity of struvite formed.

4.2.2 X-ray Diffraction (XRD)

Prior to XRD analysis, dried crystal-like particles were crushed manually using a mortar and pestle. The powdered were then exposed to Cu-K_α X-ray radiation (with a wavelength, λ=1.5418 Å). The PANalytical X'Pert Pro MPD, powered by a Philips PW3040/60 X-ray generator and fitted with an X'Celerator* detector was used for the XRD analysis. The X-ray radiation were generated from a copper (Cu) anode supplied with 40 kV and a current of 40 mA. Diffraction data were then collected over a range of 5 to 80.00017 °2Θ with a step size of 0.0668451 °2Θ and nominal time per step of 50.165s, using the scanning X'Celerator detector (hence the counting time per step). The X'Celerator is an ultrafast X-ray detector that uses Real Time Multiple Strip (RTMS) technology to operate as an array of a hundred channels that can simultaneously count X-rays diffracted from a sample over the range of 2Θ angles specified during a scan. It can therefore produce high quality diffraction data in a shorter period of time than the 'older style' diffractometers. Fixed divergence and anti-scatter slits of 0.38 mm were used together with a beam mask of 10 mm and all scans were carried out in 'continuous' mode.

Phase identification was by means of the X'Pert accompanying software programme PANalytical High Score Plus in conjunction with the ICDD Powder Diffraction File 2 Database (2004), ICDD Powder Diffraction File 4 – Minerals (2014), the American Mineralogist Crystal Structure Database (March 2010) and the Crystallography Open Database (May 2013; www.crystallography.net).

4.2.3 Bench-scale Post-AD Mg^{2+} dosing Jar Test Experiments

Further jar tests were performed on digested sludge samples from Bran Sands WWTP to assess the removal rates of NH_4-N and PO_4 as a direct consequence of dosing Mg^{2+} and adjusting the pH in post-AD sludge.

These were comparative tests to ascertain which Mg^{2+} feed location and what doses (pre-AD Mg^{2+} dosing or post-AD Mg^{2+} dosing) were best for controlled struvite precipitation, and also assess the effect of pH on NH_4-N and PO_4 levels under conditions closer to real operations.

Specifically, these experiments involved adjusting the pH of Bran Sands sludge in the beakers to pH 7 and 8 using 5N HCl and 5N NaOH, respectively, and then adding the following Mg^{2+} doses 0, 50, 100 and 150 Mg^{2+}mg/L (in duplicate per dose) as $MgCl_2$. In each test experiment, mixing after dosing was for 10 mins at 100 rpm, followed by a settling period of 3hours. The NH_4-N and PO_4 levels in the supernatant were then determined as described above.

4.2.4 Bench-Scale AD Reactor Experiments

Two sets of eight 1.0 L flat-bottomed cylindrical glass culture vessels were used as bench-scale AD simulation reactors. The units were seeded with digested sludge (DS) samples from Bran Sands WWTP and fed daily with post-CAMBI sludge (see constituents in Table 4.2). The units were operated in duplicate under eight different pH/Mg^{2+} conditions as presented on Table 4.1. The pH 7 and 8 regimes were chosen because previous studies had shown that pH values outside this range often reduced the dewaterability of digested sludge (Bergmans *et. al.*, 2014). The 0 – 150 mg/L Mg^{2+} range was chosen due to results from previous post-AD Mg^{2+} dosing experiments. In addition, the Mg doses; 0, 50, 100 and 150 mg/L were used to give the test Mg:PO_4 and Mg:NH_4:PO_4 molar ratios in the sludge (presented in Table 4.1). The Mg:PO_4 molar ratio range was chosen based on observations from previous studies that showed that P-recovery as stable struvite crystals was best at Mg:PO_4 molar ratio of 1.2:1 (Rahaman *et al.* 2014).

One set of eight AD units were adjusted to pH 7 using 5N HCl, while 5N NaOH was used to adjust the pH in the second set of 8 AD units to pH 8 (which was closest to the operating pH conditions (pH ~7.8) of the full-scale ADs at the aforementioned WWTP). The pH of the AD units was adjusted once a day; i.e. during feeding and the various Mg^{2+} doses (as $MgCl_2$) were added alongside the feed. The AD units were maintained at temperatures between 38-40˚C and solid retention time (SRT) of 18 days to approximate operating conditions of the full-scale ADs at Bran Sands WWTP. Mixing (at 90 rpm) in the AD units was provided by magnetic stirrers.

The units were operated for 102 days to investigate the effect of varying pH and Mg-doses on PO_4, NH_4-N and VS removal rates. The effect on methane(CH_4) production also was assessed. Gas sampling bags (2L, ALTEF) were connected to each bench AD, into which biogas was collected and the volume measured daily. CH_4 concentration in the biogas was measured using a Carlo Erba Gas Chromatograph coupled to a flame ionisation detector (GC-FID). Prior to the measurement of methane concentrations in the biogas samples the GC-FID was calibrated using a standard; n3.5 Methane (99.95%) pure gas (Cylinder 112DA) supplied by StG. The calibration process involved using a gas tight syringe (Part 005279/100R-V-GT-100ul Syringe) supplied by VWR to inject 20, 40, 60, 80 and 100 µm of the standard gas into the GC-FID in replicates of 3, after which the methane concentrations were then measured.

The measured methane concentrations in the biogas samples were then used to estimate daily methane volumes from the measured biogas volumes.

Table 4.1: Characteristics of Post-C and digested sludge from Bran Sands WWTP used for N and P recovery tests

Mg^{2+} Dose (mg/L)	Nº of Reactor replicates		Mg : PO_4 molar ratio	Mg : NH_4 : PO_4 molar ratio
	pH 7	pH 8		
0.0	2	2	0.5 : 1	1 : 7.6 : 1.9
50.0	2	2	0.8 : 1	1 : 5.4 : 1.3
100.0	2	2	1 : 1	1 : 4.2 : 1
150.0	2	2	1.2 : 1	1 : 3.4 : 0.8

Table 4.2: Characteristics of Post-CAMBI and digested sludge from Bran Sands WWTP used for N and P recovery tests

Parameter	Post-CAMBI sludge	Digested sludge	Unit
pH	5.41	7.89	-
Temperature	39 - 41	25	°C
NH_4-N	705 ± 19.3*	2775 ± 17	mg/L
PO_4-P	297 ± 10	439 ± 14.8	mg/L
Mg^{2+}	124 ± 5.6	102 ± 4.6	mg/L
COD (soluble)	46125 ± 3270	7300 ± 636	mg/L
COD (Total)	111420 ± 7540	64260 ± 2800	mg/L
Total Solids (TS)	213900 ± 8240	54000 ± 2450	mg/L
Volatile Solids (VS)	128300 ± 6780	33000 ± 1750	mg/L

**Uncertainty ranges in this study were presented as standard error of sample means*

Samples of the feed and effluent from the AD units were analysed three times per week for pH, volatile solids (VS), COD, NH_4-N and PO_4. The NH_4-N and COD concentrations were measured using Merck Spectroquant test kits (www.analytical-test-kits.com) supplied by VWR (see Chapter 3 for details); while the VS and PO_4 levels were determined by standard methods (APHA, 2005) (see Chapter 3). The VS removal rate and the cumulative CH_4 volume were used to assess the effect of varying pH and Mg doses on AD performance.

4.2.5 Data Analysis

Data obtained from laboratory analyses were first tested for normality before being subjected to statistical analysis (e.g. t-test and Regression analysis) using SPSS to determine whether there were statistical significance between N and P removal with pH, varying Mg doses, and N and P removal between pre- and post-AD Mg dosing tests. Uncertainty ranges in this study were presented as standard error of sample means. Mean and standard error of mean values were used in presenting data from the Jar test and AD experiments because normality test (i.e. Shapiro-Wilk test using SPSS) analysis performed on their respective 'raw' NH_4-N, PO_4-P, Mg^{2+}, VS, and CH_4 data, revealed that the data were normal ($P>0.05$ at 95% confidence interval limit).

4.3 Results and Discussions

4.3.1 Effects of pH on N and P recovery in digested sludge by simple precipitation in Jar test experiments

It was found that pH level and residual NH_4-N levels in digested sludge after the pH tests had an inverse relationship (as shown by the negative gradient in Figure 4.1). A strong negative correlation (Pearson Correlation, $R^2 = -0.976$) was observed between pH and NH_4-N removal. Prior to pH adjustments, the pH of the sludge was 7.8 and the NH_4-N concentration was ~2775 ± 110 mg/L (see Table 4.2). Figure 4.1 and Table 4.3 show that as the pH was increased to 9, liquid NH_4-N levels fell by ~25.41 ± 1.4%. On the other hand, when the pH was adjusted down to 6.5, NH_4-N levels increased by ~14.27 ± 0.5%. This increase in NH_4-N levels with falling pH is probably linked with a shift in equilibrium of reduced-N concentrations from the gaseous phase (NH_3) to the aqueous phase (NH_4^+). The increase in NH_4-N levels was also likely linked to the resolubilization of NH_4^+ ions following associated with increasing struvite solubility (with falling pH) and NH_3 (or NH_4^+) potential release from organic-N. While the decrease in NH_4-N levels as the pH increased can be partially linked to the conversion of NH_4^+ to NH_3 (i.e. potential volatilization of NH_3). For instance, the ≥20% NH_4-N removal observed at pH ≥8.5 is consistent with reports (e.g. Liao *et al.*, 1995; Bonmati & Flotats, 2003; Ruiz *et al.*, 2003; Jiang *et al.*, 2010; Guskin & Marinsek-Logar, 2011) that pH > 8.5 is needed for reasonable NH_3 stripping (i.e. >20%). Ammonium-N removal with increasing pH was also linked to possible precipitation of available NH_4^+ ions (as struvite) and falling struvite solubility with increasing pH. This is consistent with the observations of Borgerding (1972) who reported that increasing pH from 5 to 7.5, decreased struvite solubility from 3000 mg/L to <100 mg/L and confirmed by Buchanan *et al.* (1994) who also reported that minimum struvite solubility was observed at pH 9. Simple linear regression analysis showed a significant effect of pH on the concentration of NH_4-N in digested sludge (p-value = 0.001 at 95% confidence interval limit, DF = 30).

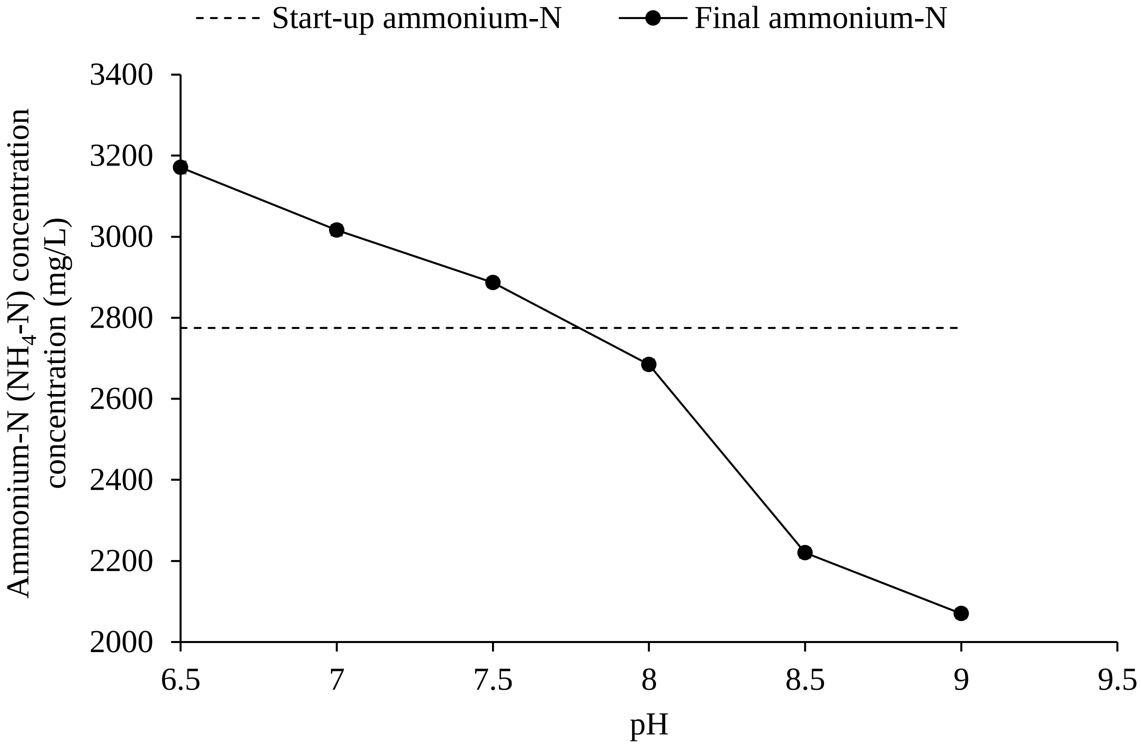

Figure 4.1: The effect of pH on NH_4-N concentration in digested sludge in Jar Test experiments. Results show mean 'soluble' NH_4-N concentrations in effluent from Jar test experiments carried out on digested sludge samples (in separate beakers) from Bran Sands WWTP, with mean start-up NH_4-N concentration and pH of 2775 mg/L and 7.8, respectively. Error bars in this study were presented as standard error of sample means

As with NH_4-N levels in digested sludge, a strong correlation (Pearson Correlation, R^2 = 0.961) was also seen between pH and residual PO_4-P levels in the sludge. As the pH increased, PO_4-P levels in digested sludge decreased. At the start of pH tests, initial PO_4-P concentration in the sludge was ~439 ± 14.8 (see Table 4.2 and Figure 4.2); however, as pH was increased from 7.8 to 9.0, PO_4-P levels fell by ~35.03 ± 1.33%. On the other hand, when the pH was adjusted down to 6.5, and PO_4-P levels increased by ~13.43 ± 1.3% (see Table 4.3). Increase in PO_4-P levels with falling pH was linked to the resolubilization of PO_4^{3-} ions (with falling pH) as seen with the increase in NH_4-N above. This is consistent with the observations of Mamais *et al.* (1994), Ali (2007), Parsons and Smith (2008), and Marchi *et al.* (2015), which reported the resolubilization of struvite component ions as pH decreased (see Subsection 2.5.3 in Chapter 2).

While the decrease in PO_4-P levels as the pH increased was also linked to the potential precipitation of PO_4^{3-} ions (as struvite). Figures 4.1 and 4.2 showed that NH_4-N and PO_4-P concentrations in digested sludge declined to a lesser extent between pH 8.5 and 9 indicating that the maximum rate of N and P recovery as struvite probably occurred around pH 9. Hence adjusting the pH to values >9 should not be necessary to achieve higher N and P removal/recovery. This was consistent with observations of Bouropoulos and Koutsoukos (2000) and Ali (2007), who reported that the optimum rate of struvite precipitation was observed between pH 8.5 and 9.5 (see Subsection 2.5.3 in Chapter 2). A simple linear regression analysis showed a statistical significant effect (p-value = 0.001 at 95% confidence interval limit, DF = 30) of pH on the concentration of PO_4-P in digested sludge.

Two important parameters to consider during N and P recovery as struvite include; the pH and supersaturation ratio of struvite component ions in the feed. Evaluating the performance of a system for N and P removal/recovery, using pH as controlling factor, is relatively simple and useful since it clearly controls supersaturation in the reactor. However, using pH as a stand-alone controlling parameter is inadequate in fully understanding the driving mechanism behind struvite crystallization process, as it does not take into account $Mg:NH_4:PO_4$ molar ratios and absolute concentrations. Hence the need for Mg-dosing experiments presented in the following sections.

Table 4.3: Effects of pH on % removal of liquid NH_4-N and PO_4-P in digested sludge

pH	**% NH_4-N Removal**	**% PO_4-P Removal**
6.5	-14.27 ± 0.5*	-13.43 ± 1.3
7.0	-8.68 ± 0.27	-6.03 ± 1.24
7.5	-4.04 ± 0.2	-1.39 ± 0.18
8.0	3.24 ± 0.001	14.58 ± 0.9
8.5	20.00 ± 2.3	29.20 ± 1.9
9.0	25.41 ± 1.4	35.03 ± 1.33

**Uncertainty ranges in this study were presented as standard error of sample means*

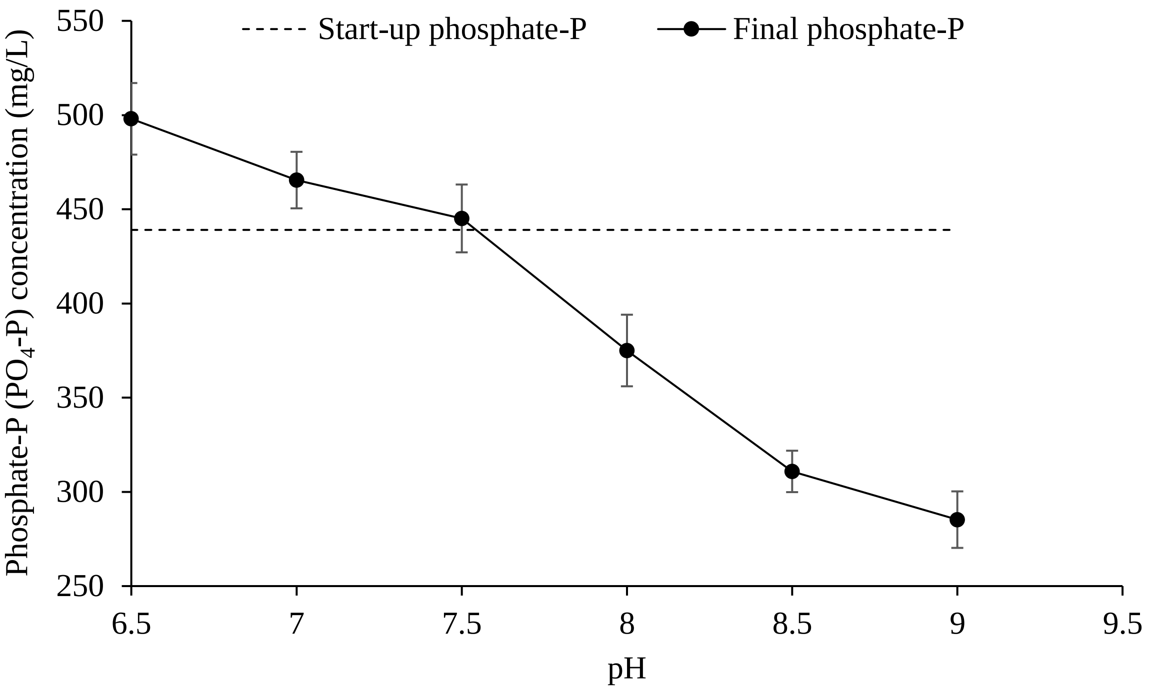

Figure 4.2: The effect of pH on PO_4-P concentration in digested sludge. Results show mean 'soluble' PO_4-P concentrations in effluent from Jar test experiments carried out on digested sludge samples (in separate beakers) from Bran Sands WWTP, with mean start-up PO_4-P concentration and pH of 439 mg/L and 7.8, respectively.Error bars in this study were presented as standard error of sample means.

4.3.2 Effects of pH and Mg^{2+} on N and P in digested sludge in Jar Test experiments

Addition of Mg^{2+} to post-AD sludge also showed strong correlations between added Mg^{2+} doses and reduction in NH_4-N (R^2 = -0. 93, at both pH 7 and 8) and PO_4-P (R^2 = -0.97 at pH 7 and R^2 = -0. 93 at pH 8) levels (see Figures 4.3 and Figure 4.4, respectively). It was observed that varying the pH and Mg^{2+} levels in digested sludge had a statistically significant effect on the rate of precipitation of NH_4-N (P value = 0.002 at 95% confidence interval limit, DF = 35) and PO_4 (P value = 0.001 at 95% confidence interval limit, DF = 35) in the sludge. Figures 4.3, 4.4 and 4.5 showed that NH_4-N and PO_4-P removal efficiencies in digested sludge increased with increasing Mg^{2+}. For instance, removal efficiencies of up to 18 ± 0.16% and 46 ± 3.4% for NH_4-N and PO_4-P, respectively, were observed when 150 mg/L Mg^{2+} was added into digested sludge at pH 7. However, higher removal efficiencies were observed at pH 8 with up to ~46 ± 0.7% and ~89 ± 1.1% NH_4-N and PO_4-P removal respectively (see Figures 4.5). The relatively high NH_4-N removal at pH 8 could also have partially been linked to possible ammonia stripping although to a lesser extent compared to potential struvite precipitation. This is based on reports (e.g. Liao *et al.*, 1995; Bonmati & Flotats, 2003; Ruiz *et al.*, 2003; Jiang *et al.*, 2010; Guskin & Marinsek-Logar, 2011) that pH > 8.5 is needed for reasonable NH_3 stripping (i.e. >20%). Therefore, assuming that at pH 8, ~20% of the NH_4-N removed was due to ammonia stripping, estimating the molar concentration of NH_4-N in the remaining 26% of NH_4-N removed would yield a Mg:NH_4:PO_4 molar ratio of 1:1:1 assumed to be in the precipitated struvite product. In addition, the relatively improved Mg:NH_4:PO_4 molar ratio following the addition of 150 mg/L of Mg (see Table 4.1) which is comparatively closest to the recommended Mg:NH_4:PO_4 and Mg:PO_4 molar ratios of 1:1:1 and 1.2:1 (Celen and Turker, 2001; Doyle & Parsons 2002; Ali *et al.*, 2004; Ali, 2007; Sharp *et al.*, 2013; Mudragada *et al.*, 2014) for effective N and P recovery via struvite precipitation, and suitable pH (i.e. pH 8) would most probably have been main reason for the high N and P removal.

Obviously, Mg^{2+} addition clearly reduces N and P levels, but optimal doses must be considered. The slopes between 100 mg/L and 150 mg/L of added Mg^{2+} shown in Figures 4.4 and 4.5 indicate PO_4-P removal does not change drastically above Mg^{2+} dose 100 mg/L at pH 8. Analysis showed no statistical significance (P value = 0.536 at 95% confidence interval limit) between PO_4-P concentrations in digested sludge between 100 mg/L and 150 mg/L of added Mg^{2+}. This implies further addition of Mg^{2+} doses >100 mg/L in digested sludge at pH 8 does not yield significantly better PO_4-P removal.

In contrast, simple extrapolation of linear regression on theoretical PO_4-P data showed that up to ~300 mg/L of added Mg^{2+} might achieve 100% PO_4-P removal at pH 7 and ~255 mg/L of added Mg^{2+} might be required to achieve 100% PO_4-P removal at pH 8 (see Figures B.1 and B.2, respectively, in Appendix B).This was because the 300 mg/L and 255 mg/L of added Mg^{2+} doses potentially raised the 'effective' Mg^{2+}:PO_4-P molar ratio in the digested sludge samples from 0.5 and 0.3 at pH 7 and 8 respectively, to 1.2, which was the Mg^{2+}:PO_4-P molar ratio necessary for effective precipitation of soluble P as struvite. This is consistent with existing research that shows P recovery as struvite crystals increases as Mg^{2+}:PO_4-P molar ratio approaches 1.0 (Rahaman *et al.*, 2014; Bergmans *et al.*, 2014). Barat *et al.* (2005) also reported that increasing the available Mg^{2+} concentration, increases their competitiveness over Ca^{2+} ions during ionic exchange reactions to form phosphate compounds. Although such high Mg^{2+} doses may be advantageous in achieving slightly greater nutrient recovery and completely preventing 'scaling' in treatment equipment, applying such doses could be relatively uneconomical.

To characterise precipitates after Mg^{2+} addition, XRD analysis was performed on precipitates from the post-AD reactors. XRD showed that ~99% of the "new" crystalline matter obtained was struvite (see Figure 4.6). This confirmed that N and P removal observed in the sludge was due to struvite precipitation, and could theoretically be recovered and processed to potentially improve on its commercial value. Any potentially ‘controlled’ struvite precipitation within a specific reactor might minimise scaling problems in downstream equipment and also potentially reduce N and P loads being recycled to the ‘head’ of the treatment works via RLs.

‘Controlled’ N and P recovery through Mg^{2+} addition was also performed in ADs in order to compare nutrient recovery in AD and post-AD reactors, which is presented in the next section.

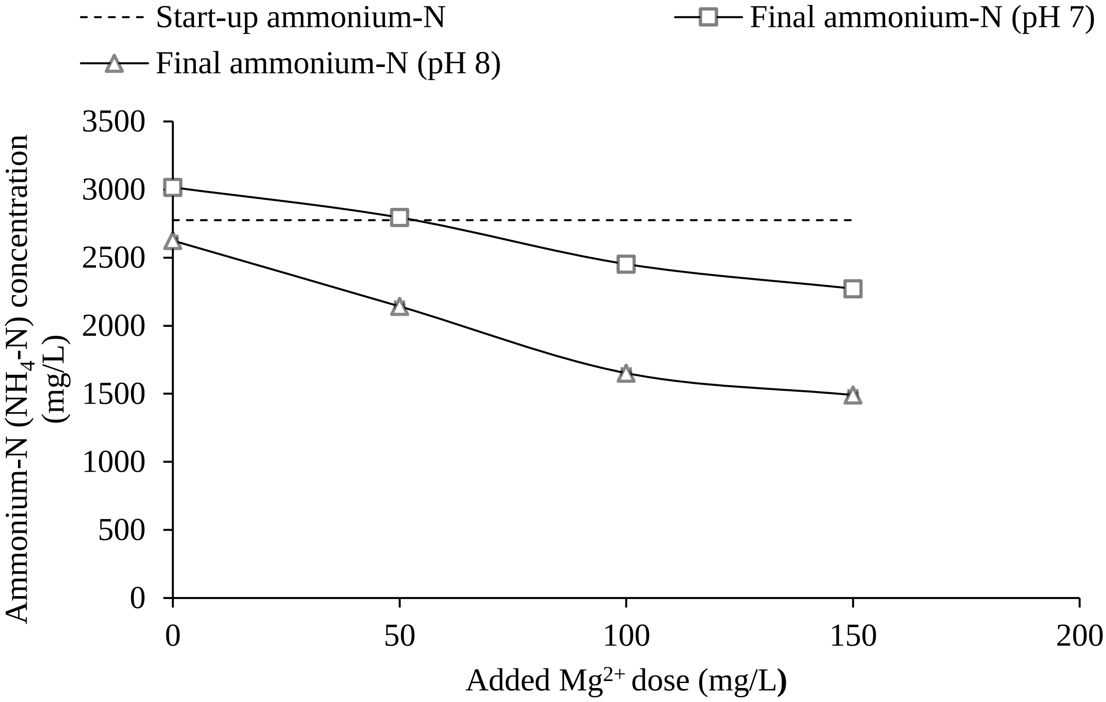

Figure 4.3: Effect of pH and Mg^{2+} addition on NH_4-N concentration in sludge from post-AD units. Results show mean ‘soluble’ NH_4-N concentrations in effluent from Jar test experiments carried out on digested sludge samples (in separate beakers) from Bran Sands WWTP, with mean start-up NH_4-N concentration and pH of 2775 mg/L and 7.8, respectively. Error bars in this study were presented as standard error of sample means.

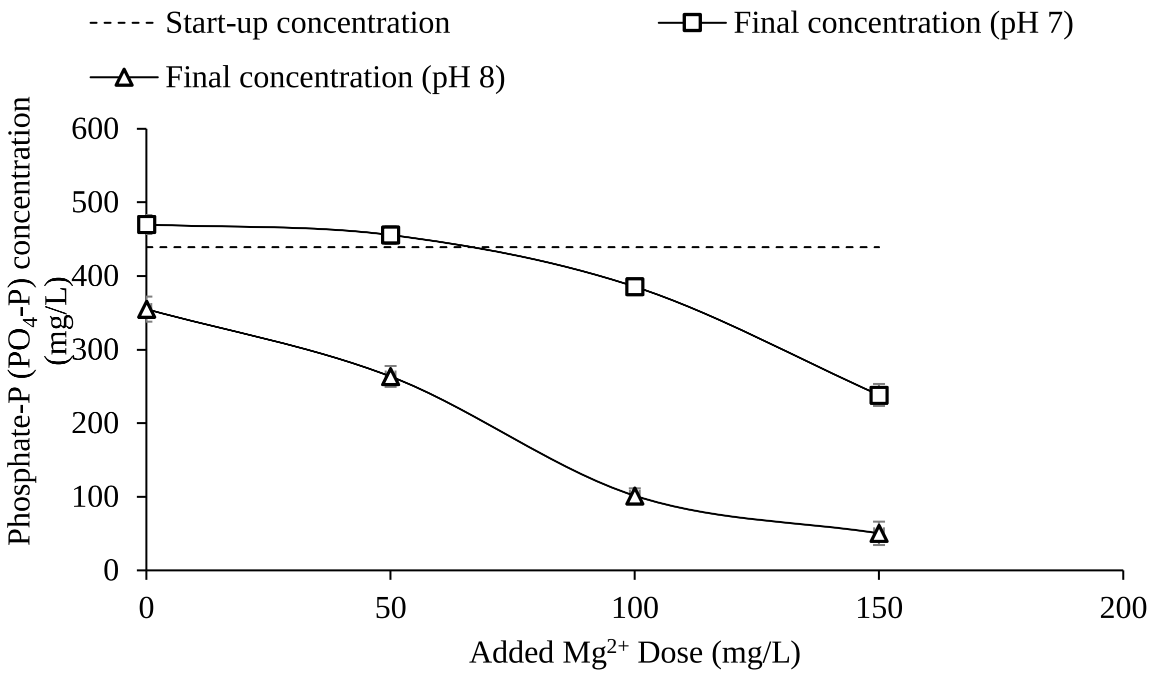

Figure 4.4: Effect of pH and Mg^{2+} addition on PO_4-P concentration in post-AD units. Results show mean 'soluble' PO_4-P concentrations in effluent from Jar test experiments carried out on digested sludge samples (in separate beakers) from Bran Sands WWTP, with mean start-up PO_4-P concentration and pH of 439 ± 14.8 mg/L and 7.8, respectively. Error bars in this study were presented as standard error of sample means.

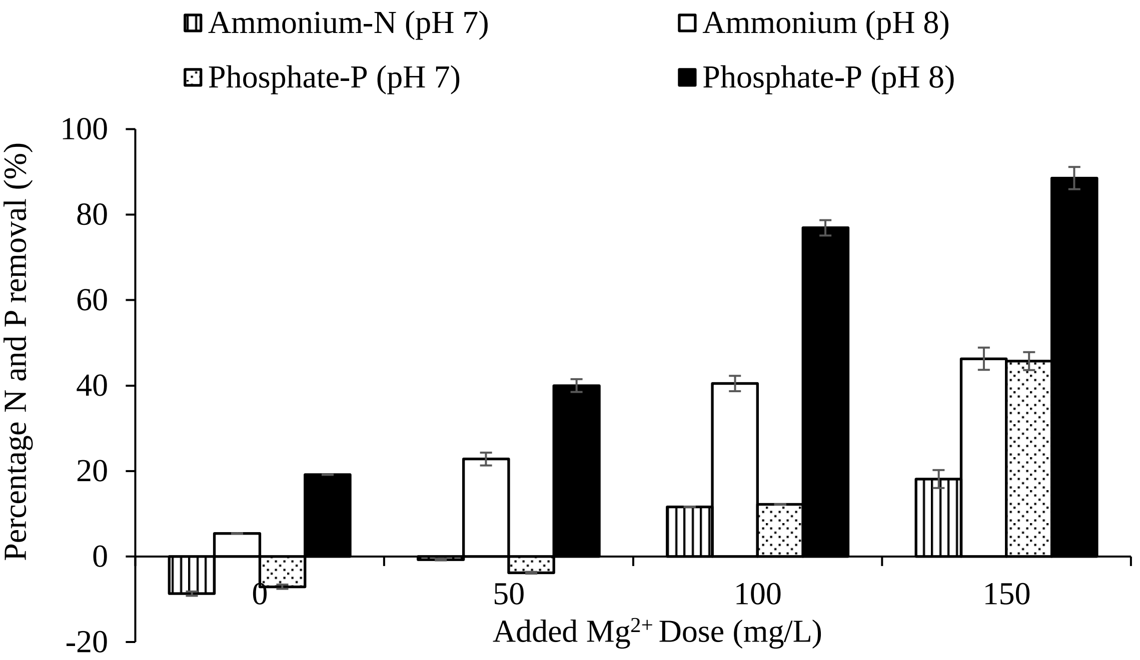

Figure 4.5: Effect of added Mg^{2+} dose on Percentage NH_4-N and PO_4-P removal from digested sludge in Jar test experiments at pH 7.0 and 8.0. Error bars refer to standard errors.

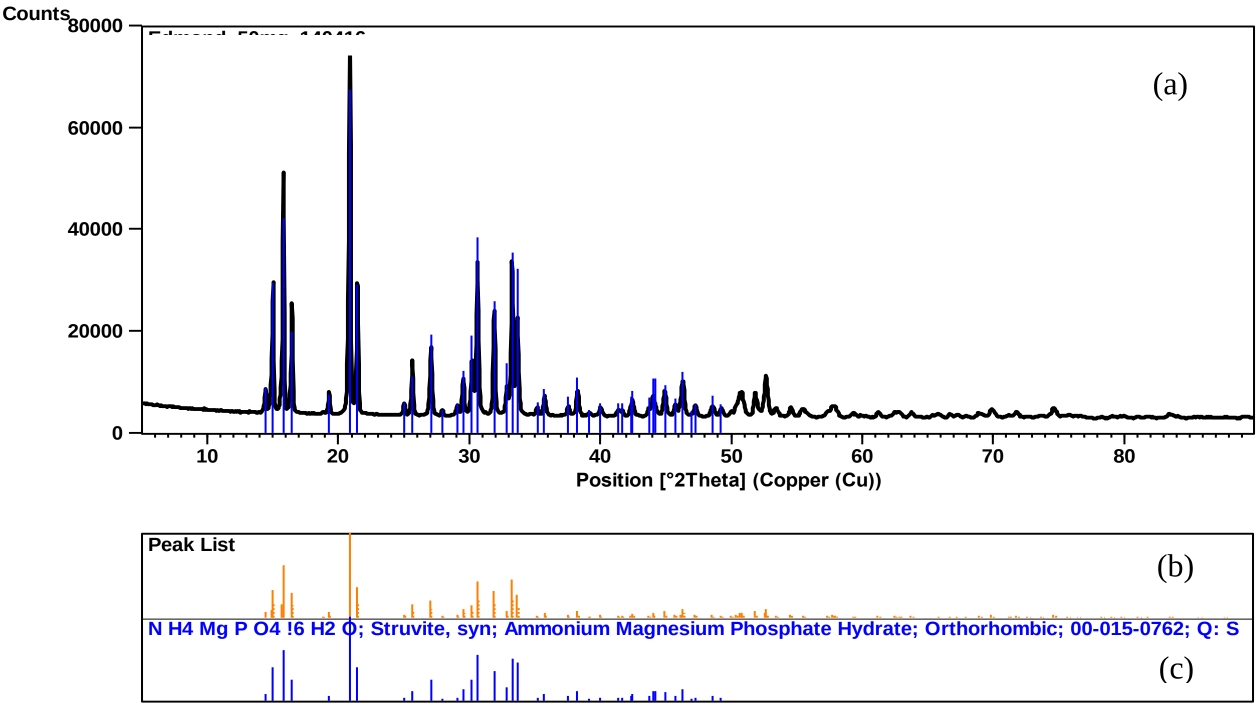

Figure 4.6: Diffractogram of XRD analysis showing how patterns in crystalline precipitate samples (following the dosing of 100mg/L Mg^{2+} in digested sludge) conform to standard struvite spectra. Where, (a): Sample spectrum; (b): Library spectrum; (c): Standard struvite spectrum

4.3.3 Effects of pH and Mg^{2+} on N and P recovery in bench-scale AD reactors

AD experiments with and without Mg^{2+} addition also showed a strong correlation between Mg^{2+} dose, and NH$_4$-N and PO$_4$-P concentrations in sludge within AD units (see Figure 4.7 and 4.8). NH$_4$-N and PO$_4$-P concentrations in AD effluents clearly decreased with increasing pH and Mg^{2+} dose. This is consistent with the observed N and P removal from digester liquors following the Mg addition and pH adjustment >7.5 reported by Laridi *et al.* (2005) and Uludag-Demirer *et al.* (2005). Furthermore, varying the pH and Mg^{2+} levels in AD units had a significant effect on the NH$_4$-N levels (P value = 0.014 at 95% confidence interval limit, DF = 32) and PO$_4$-P (P value = 0.004 at 95% confidence interval limit, DF = 32) in the sludge. Figure 4.9 shows that the removal efficiencies of NH$_4$-N and PO$_4$-P increased with increasing pH and Mg^{2+} dose. For instance, NH$_4$-N removal efficiency rose from ~8.1 ± 0.93% (pH = 7.0 and 150 mg/L Mg^{2+} addition) to 23.4 ± 0.94% (pH = 8.0 and 150mg/l Mg^{2+} was added). Assuming upto ~20% of NH$_4$-N removal at pH 8 was attributed to ammonia stripping (as reported by Liao et al., 1995; Bonmati & Flotats, 2003; Ruiz et al., 2003; Jiang et al., 2010; Guskin & Marinsek-Logar, 2011; for treating N-rich wastewaters), the remaining 3.4% NH$_4$-N removed was assumed to be as a result of struvite formation. Using the 6.2% NH$_4$-N removal efficiency at pH 8, a Mg:NH_4:PO_4 molar ratio of 1:1:1 was estimated to be in the precipitated struvite product (assuming all the PO_4-P and Mg^{2+} removed at pH 8 went into the formed struvite). PO_4-P removal efficiency also rose from ~36 ± 3.3% (pH = 7.0 and 150mg/L Mg^{2+} addition) to 69 ± 2.4% (pH = 8.0 and 150 mg/l Mg^{2+} was added). These increases potentially resulted from precipitation of N and P as struvite due to the increased pH and Mg:PO_4-P molar ratios in AD units. This is consistent with improved PO_4-P removal efficiency observed at Mg:PO_4 molar ratio of 1.2:1 in the post-AD Jar test experiments and in studies on digester liquors and digested sludge reported by Doyle & Parsons (2002), Ali *et al.* (2004), Sharp *et al.* (2013) and Mudragada *et al.* (2014). This was confirmed by XRD analysis on AD residuals, which showed that ~93% of the precipitated crystalline matter was struvite (see Figure 4.10). This confirmed that N and P removal observed in the AD was precipitated out of the aqueous phase as struvite, which can be potentially recovered and processed for possible commercial sale, and also reduce post-AD struvite precipitation and minimise scaling problems in downstream equipment.

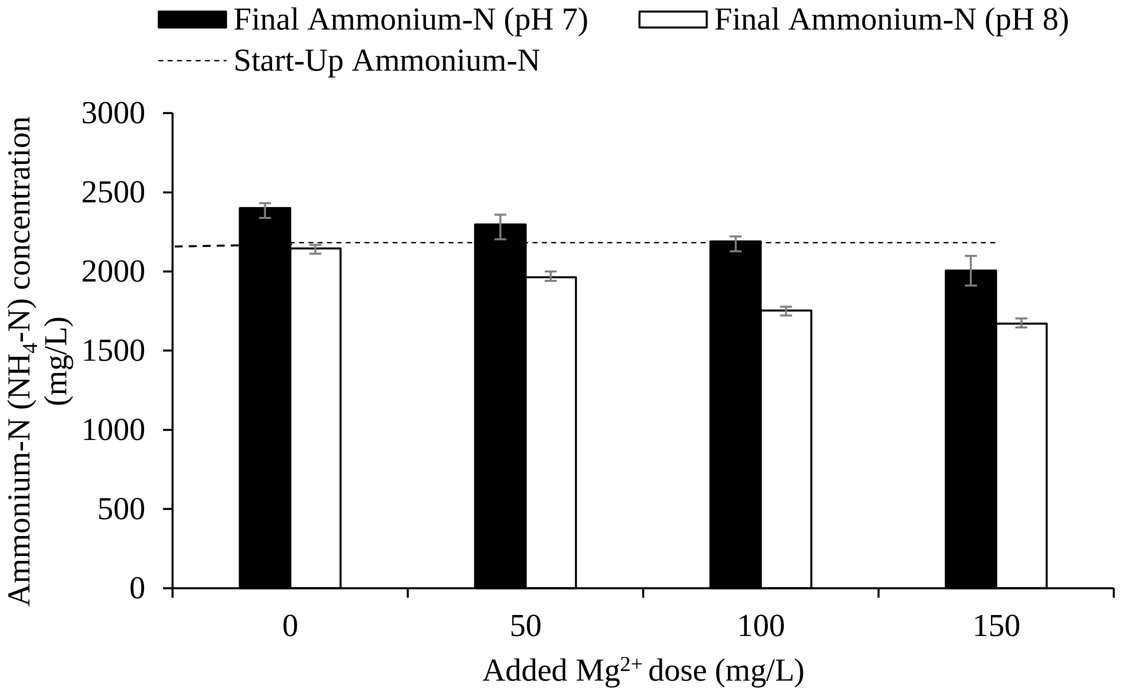

Figure 4.7: Effect of pH and Mg^{2+} addition to post-CAMBI sludge on NH_4-N concentration in effluent from bench-scale AD reactors. Mean start-up NH_4-N concentration 2182 ± 15.3 mg/L obtained from start-up NH_4-N concentrations measured daily after feeding the reactors and allowing 10mins of mixing. Error bars refer to standard errors.

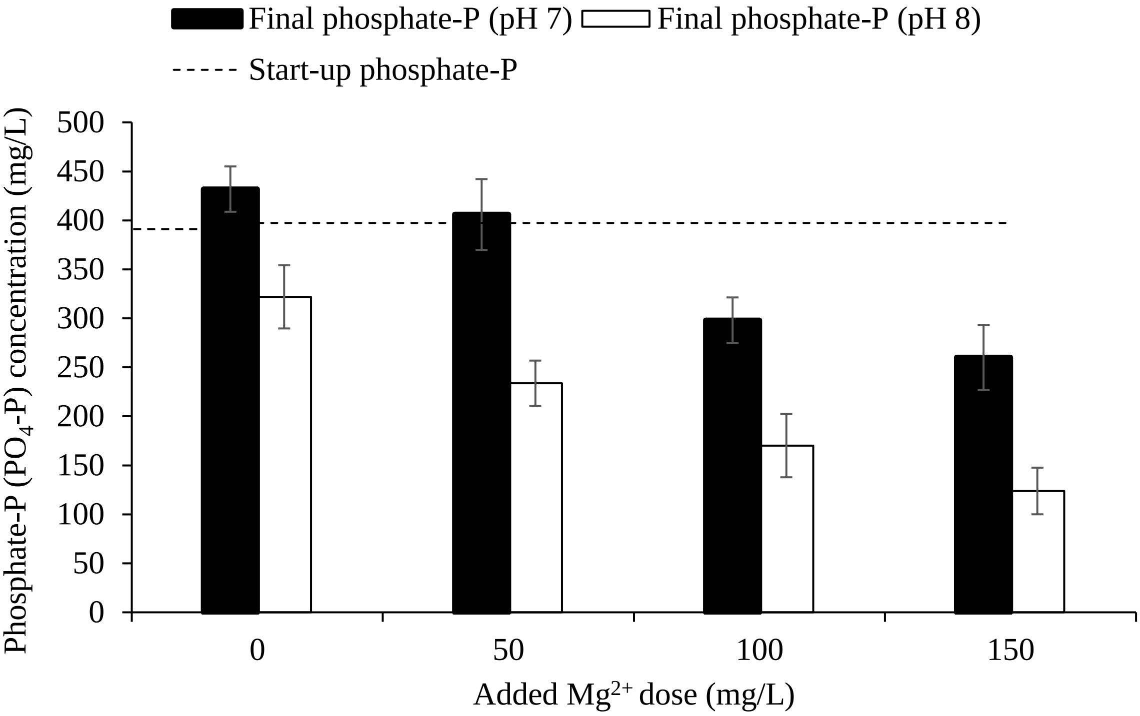

Figure 4.8: Effect of pH and Mg^{2+} addition to post-CAMBI sludge on PO_4-P concentration in effluent from bench-scale AD reactors. Mean start-up PO_4-P concentration 397 ± 11.7 mg/L obtained from start-up PO_4-P concentrations measured daily after feeding the reactors and allowing 10mins of mixing.Error bars refer to standard errors.

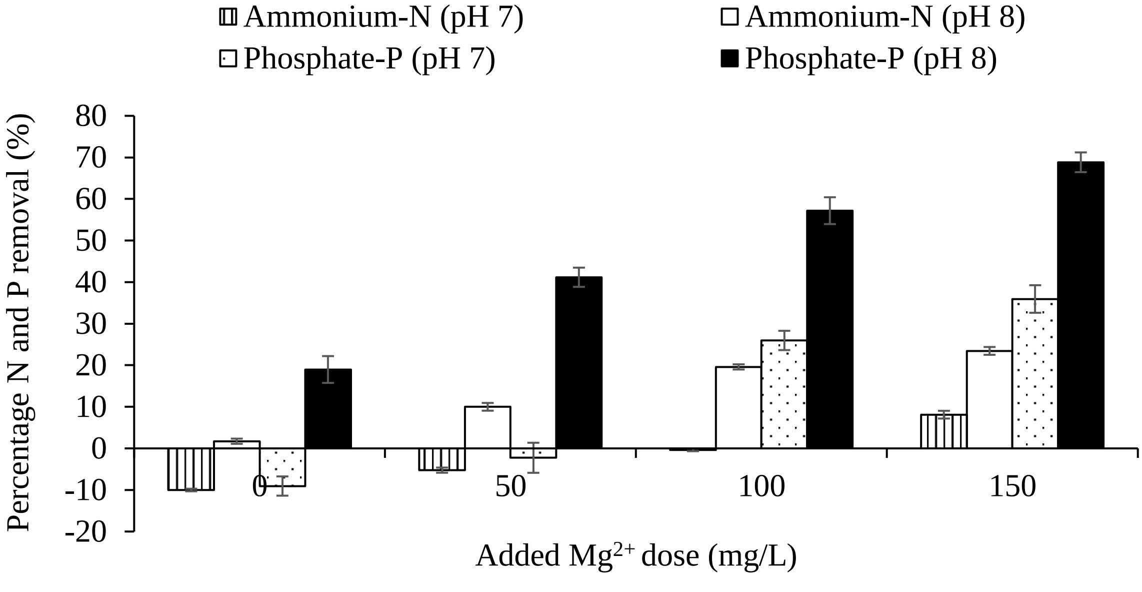

Figure 4.9: Effect of added Mg^{2+} dose to post-CAMBI sludge on Percentage NH_4-N and PO_4-P removal effluent from bench-scale AD reactors at pH 7 and 8. Error bars refer to standard errors.

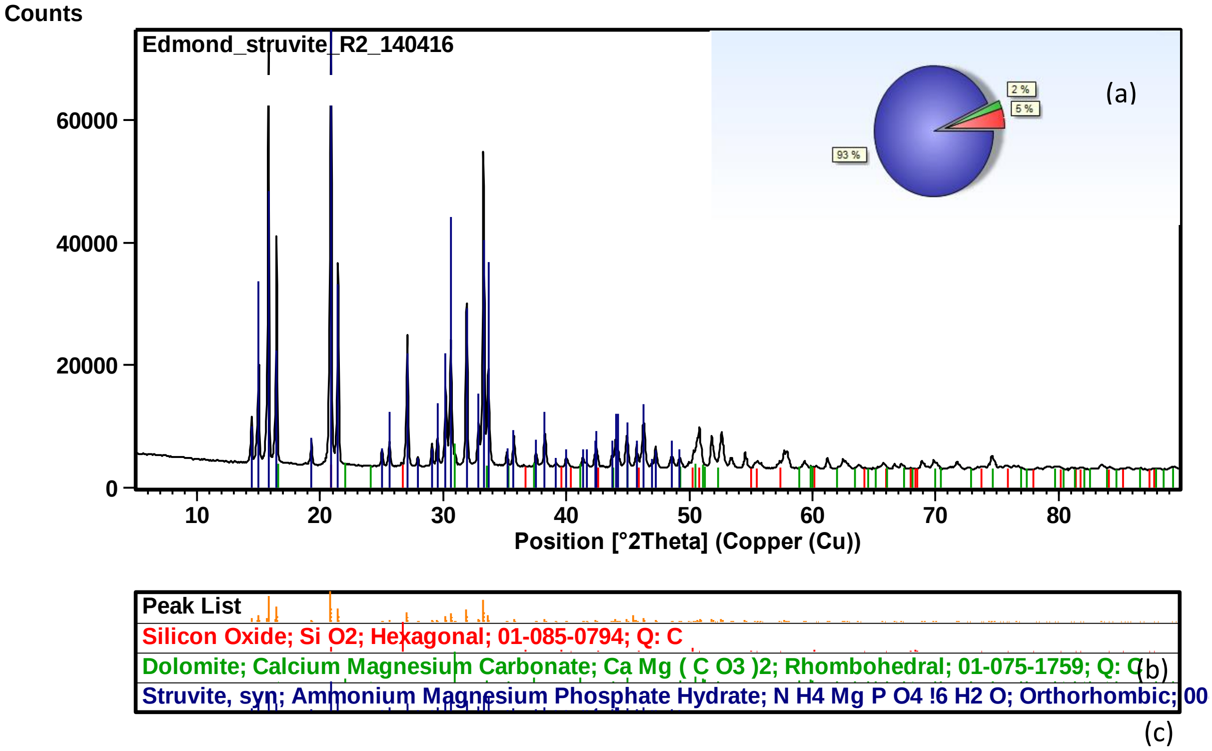

Figure 4.10: Diffractogram of XRD analysis showing how patterns in crystalline precipitate samples (following the dosing of 100mg/L Mg^{2+} in AD units) conform to standard struvite spectra. Where, (a): Sample spectrum; (b): Library spectrum; (c): Standard struvite spectrum

Another beneficial outcome of pH adjustment and dosing of Mg^{2+} into the AD units was increases in volatile solids removal and increased biogas/methane (CH_4) production. Both VS removal and methane (CH_4) production improved with increased pH and Mg^{2+} dose. Figures 4.11 and 4.12 shows there reductions in mean VS concentrations in the effluent from all AD reactors under the different experimental conditions. For instance, VS levels following AD dropped from 41500 ± 1880 (at start-up) to ~24500 ± 1630 mg/L (at pH 7 with no added Mg^{2+}), with further reductions to ~18900 ± 1259 mg/L (at pH 8 with 150mg/L of added Mg^{2+}). This translates to an increase in percentage VS removal in the AD units from 41.1 ± 1.1% (at pH 7 with no added Mg^{2+}) to 54.5 ± 1.2% (at pH 8 with 150mg/L of added Mg^{2+}; see Figure 4.12). These VS removal efficiencies are less than typical mean VS removal efficiencies (~60-65%) following the AD of sewage sludge at similar pH levels (Metcalf & Eddy, 2003). This may have been due to the fact that some of the readily degradable VS may have been broken down in CAMBI prior to the sludge being collected to feed the AD reactors. In addition, unlike the full-scale AD reactors, the bench-scale AD reactors had no recirculation system in place which could have enhanced VS destruction and digester performance as recommended by Torpey and Melbinger (1967).

A two-sample t-test analysis on the VS data shows that differences between VS removed at pH 7.0 versus 8.0 (with increasing Mg^{2+}) were significantly greater (P value = 0.014 at 95% confidence interval limit, DF = 32). However, a one-sample t-test analysis performed on the VS levels between the added Mg^{2+} doses at pH 8.0 revealed no statistical significance (P value = 0.821 at 95% confidence interval limit, DF = 32) between the mean VS levels between 100mg/L and 150mg/L of added Mg^{2+} doses. This implies increasing the added Mg^{2+} dose beyond 100mg/L does not yield any significant difference in VS removal.

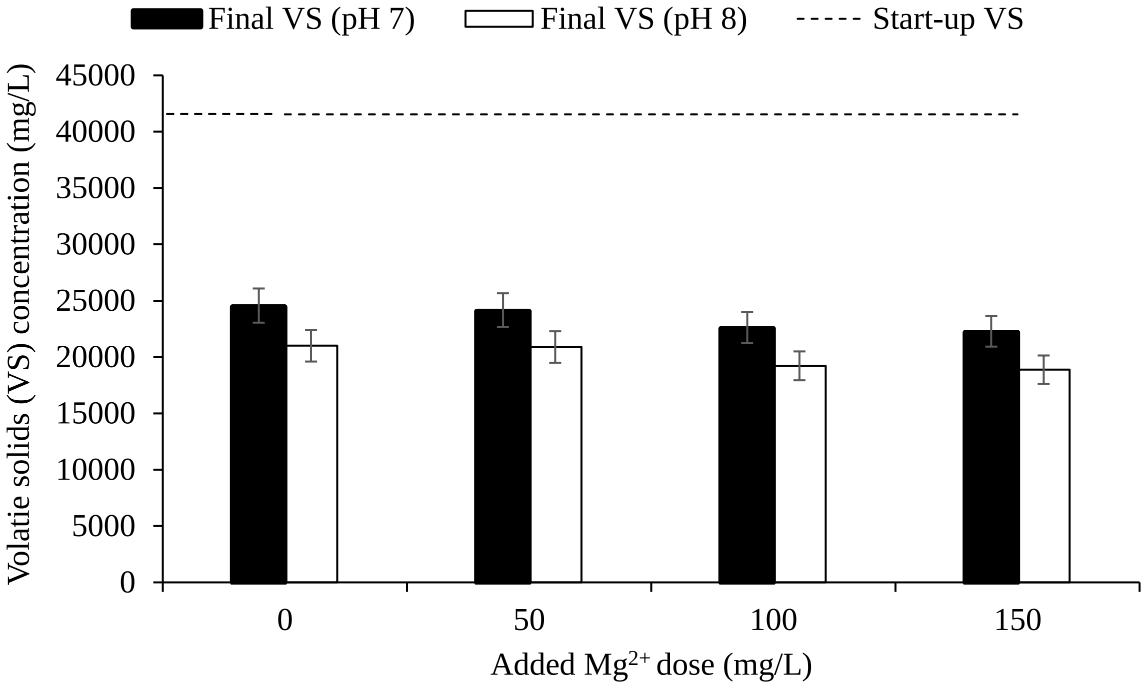

Figure 4.11: Effect of Mg^{2+} Dose on Volatile solids (VS) concentration in AD reactors at pH 7 and 8. Error bars refer to standard errors.

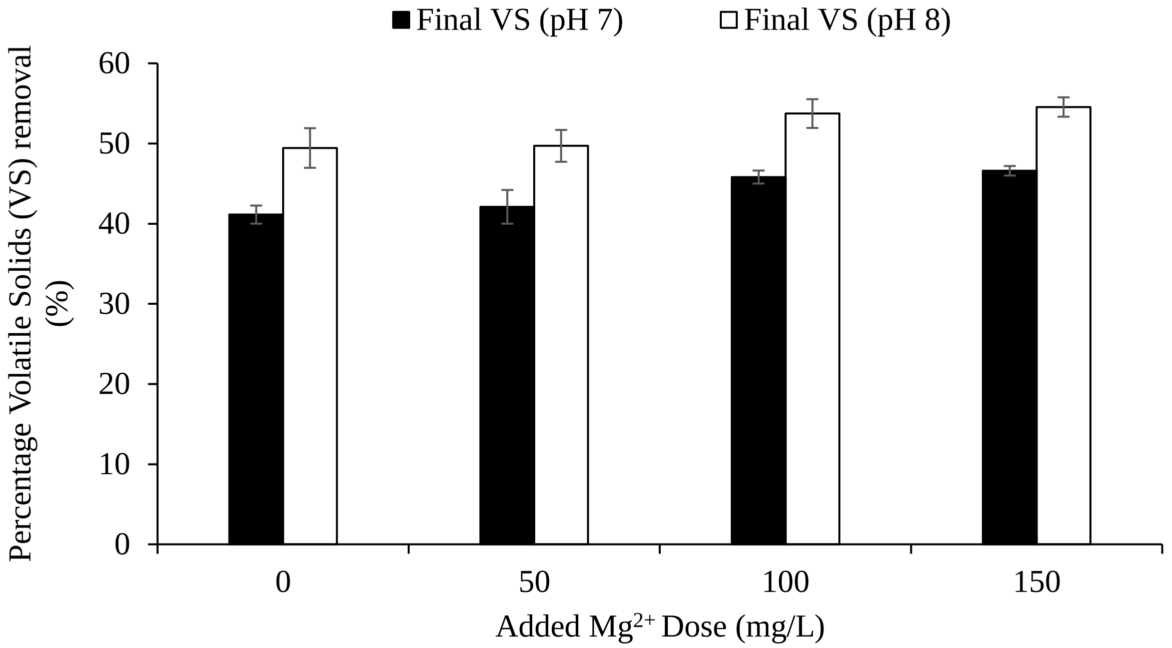

Figure 4.12: Effect of Mg^{2+} dose on Percentage VS removal in bench-scale AD reactors at pH 7 and 8. Error bars refer to standard errors.

Differences were also noted in biogas/CH_4 production rates among different pH and Mg^{2+} additions. Cumulative volume of CH_4 produced by the AD units is reported in Figure 4.13, and shows a range from 19500 ± 693 mL (for pH 7.0 with no added Mg^{2+}) to 23300 ± 151 mL (for pH 8.0 with 150 mg/L of added Mg^{2+}) after 60 days of operation. Similarly, Table 4.3 shows the mean level of CH_4 within the biogas also rose with higher Mg^{2+} addition and pH; i.e. percent CH_4 increased from ~70 % (pH 7.0, no Mg^{2+}) to ~78 % (pH 8.0, 150 mg/L Mg^{2+}).

Overall, ~19 % difference in CH_4 production were seen across AD units. Two-sample t-test comparisons of the cumulative CH_4 data shows significant differences between pH 7.0 and 8.0 (with increasing Mg^{2+}) (P value = 0.05 at 95% confidence interval limit, DF = 32), and between 0 and 50, and 50 and 100 mg/L Mg^{2+} additions, but differences between 100 mg/L and 150 mg/L were not statistically significant (P value = 0.0696 at 95% confidence interval limit, DF = 16), suggesting Mg^{2+} additions above 100 mg/L Mg^{2+} may not provide added benefit. Observed increases in CH_4 production roughly parallels increases in NH_4-N removal (i.e. with increasing Mg^{2+} dose), which implies increases in CH_4 might have been due to reduced ammonia toxicity on the methanogens. This is consistent with reports that the addition of Mg^{2+} into AD systems was antagonistic to ammonia inhibition in the systems, and could potentially increase biogas production by 10 % (Kugelman & McCarty, 1964; Chen *et al.,* 2008). Furthermore, it has also been reported that high concentrations of Mg^{2+} (upto 720 mg/L Mg^{2+}) resulted in *Methanosarcinae*-dominated AD reactors (Ahring *et al.*, 1991; Scmidt & Ahring, 1993).

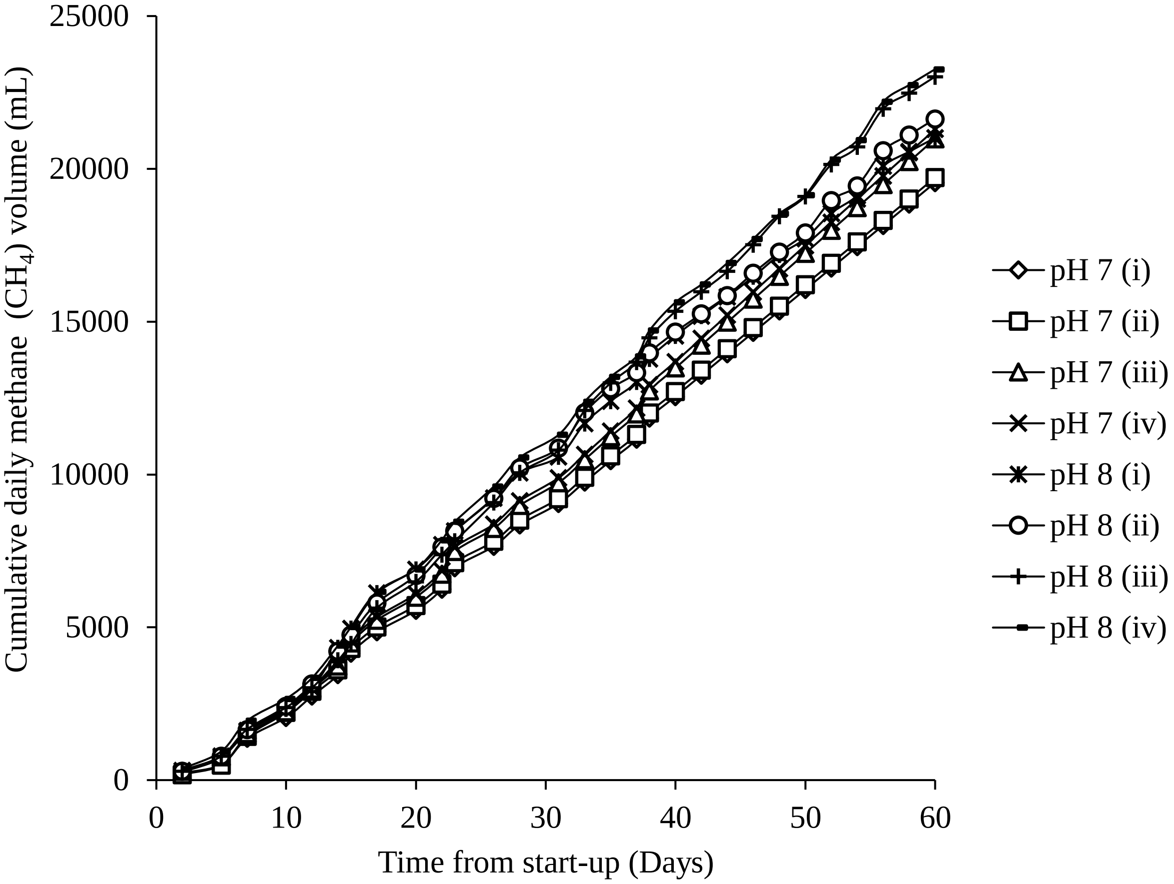

Figure 4.13: Effect of Mg^{2+} Dose on cumulative CH_4 production by bench-scale AD reactors at pH 7 and 8. Where (i), (ii), (iii), and (iv) refer to bench-scale AD reactor experimental conditions with 0, 50, 100 and 150 mg/L Mg^{2+} added dose, respectively.

The average VS loading rate into the AD units was 2.27 kg VS/m^3.day and the average solids (TS) content of the sludge fed into the ADs was ~6.01%. This VS loading rate and solids content are consistent with full-scale operations at Bran Sands WWTP and typical ranges (i.e. 1.6 – 4.8 kg VS/m^3.day and 4.8 – 8% TS content, respectively) for effective mesophilic anaerobic digestion of sewage sludge (USEPA, 1979; Metcalf & Eddy, 2003). Following the increase in VS removal with increasing pH and Mg^{2+} dose, data in Table 4.3 show specific gas production per kilogram VS removed (VS_r) rose from 0.643 m^3/Kg VS (at pH 7.0, no Mg^{2+}) to 0.81 m^3/Kg VS (pH 8.0, 150mg/L Mg^{2+}) which shows ~29 % rise in CH_4 produced per VS removed. This rise highlights the theoretical potential to increase specific gas production per kilogram VS removed at Bran Sands WWTP (0.65 m^3/Kg VS); i.e. ~25 % increase might be possible if the pH of the AD unit was adjusted to 8 and 150mg/L of Mg^{2+} dosed into the unit.

The rise in specific gas production per kilogram VS removed could even be higher if the solids content in the feed-sludge were reduced towards lower boundaries of the typical range (4.0 to 7.0 %) reported by Metcalf & Eddy (2003) for the effective operation of mesophilic AD reactors. It should be noted that potential gas production levels per kilogram VS removed using at pH 8.0 and 150mg/L Mg^{2+} addition falls within expected ranges (i.e. 0.75 – 1.1 m^3/Kg VS) reported by Metcalf & Eddy (2003) and Turovskiy and Mathai (2006) for AD reactors operating at full capacity.

Table 4.4: Effects of pH and Mg addition on % CH_4 composition and Specific gas production per kilogram VS

Added Mg^{2+} Dose (mg/L)	**% CH_4 composition in biogas produced**		**Specific gas production per kilogram VS_r (m^3/kg.VS_r)**	
	pH 7	**pH 8**	**pH 7**	**pH 8**
0	70 ± 0.6*	71 ± 1.1	0.63	0.65
50	72 ± 2.4	74 ± 0.4	0.65	0.70
100	74 ± 0.1	77 ± 0.6	0.66	0.80
150	74 ± 1.3	78 ± 1.2	0.66	0.81

*The data presented in the Table represent mean values of 60 data points collected during the 60 days operation of the bench-scale AD reactors. *Uncertainty ranges in this study were presented as standard error of sample means*

4.3.4 Comparison of N and P recovery between pre-AD and post-AD Mg^{2+} dosing systems

It has been demonstrated in both the pre-AD and post-AD Mg^{2+} dosing experiments that Mg^{2+} ions appear to be a limiting factor for potential N and P recovery via struvite precipitation. Table 4.5 shows despite the addition of Mg^{2+} (upto 150 mg/L) and adjusting the pH in both the bench-scale AD reactors and post-AD Jar test units to 7 and 8, the IAP in the effluent from the experiments was still greater than the K_{sp} value for struvite. This was probably due to the relative high NH_4-N levels in the treated effluent. However, it was observed that adjusting the pH in the bench-scale AD reactors to 8 and adding upto 150 mg/L of Mg^{2+} could potentially reduce the IAP in digested sludge by one order of magnitude; i.e. from 3.14 x 10^{-6} (see Figure

3.12) to 2.3 x 10^{-7} (see Table 4.5). In addition, adjusting the pH in the post-AD Jar test experiments to 8 and adding upto 150 mg/L of Mg^{2+} showed that IAP in digested sludge and RLs could potentially be reduced by approximately three orders of magnitude and one order of magnitude, respectively; i.e. from 3.14 x 10^{-6} and 6.86 x 10^{-8} (see Figure 3.12) 9.3 x 10^{-9} (see Table 4.5), respectively. This suggests that Mg dosing and pH adjustment in both scenarios could potentially reduce possible struvite-related scaling problems downstream of the AD units. It was also shown at pH 7 and 8 that the amount of N and P that can potentially be recovered is almost proportional to the addition of Mg^{2+}. In addition, a statistical significant difference was observed among the mean NH_4-N and PO_4-P levels, and added Mg^{2+} doses in both the pre-AD and post-AD Mg^{2+} dosing experiments. Further, extrapolation of linear regression predicts that excess Mg^{2+} may be required to remove all the available PO_4-P from solution, which is consistent with past reports that showed the presence of excess Mg^{2+} can increase P removal (Shin and Lee, 1997; and Matsumiya *et al.*, 2000). However, data also show that mean residual PO_4-P levels were not significantly different between 100 mg/L and 150 mg/L Mg^{2+} doses at pH 8 (for both AD and post-AD Mg^{2+} additions).

These suggest dosing needs, but the question is whether it is better to add Mg^{2+} to the AD units or post-AD units. NH_4-N and PO_4-P removal levels using post-AD Mg^{2+} dosing were higher than that of Mg^{2+} dosing to AD reactors at similar pH and added Mg^{2+} doses (see Figures 4.5 and 4.9). However, the difference in the mean NH_4-N and PO_4-P removal rates between the post-AD and AD reactor Mg^{2+} additions were not statistically significant (i.e. P value = 0.134 for NH_4-N removal and P value = 0.280 for PO_4-P removal at 95% confidence limit). This suggests post-AD may be better, but the differences are not significantly better, especially considering potentially improved CH_4 production per VS removed with added Mg^{2+} to the AD units. This will be examined in the next section.

One qualification to the above observations is needed. The relatively higher NH_4-N and PO_4-P removal efficiencies in post-AD Mg^{2+} dosing experiments compared to that of the AD Mg^{2+} dosing experiments may be associated with a greater chance for CO_2 stripping in the former units (which was done in open beakers) compared to the latter (being conducted in completely sealed units). This is because the higher potential for CO_2 stripping in the former resulted in a further increase in pH which further enhanced potential for N and P removal via struvite precipitation. Also, AD units were continuously stirred with very minimal allowance for settling (hence precipitated of NH_4-N and PO_4 remains in suspension) compared to the "Jar Test" scenario (i.e. the post-AD Mg^{2+} dosing experiments) where the 10-mins mixing period was followed by a 3-hours settling phase for effective struvite precipitation. This is consistent with the observations of Wang *et al.* (2006) and Ariyanto *et al.* (2014), which reported that mixing speed significantly influenced nucleation and crystal growth. Furthermore, the jar test experiments were conducted at room temperature which falls within the typical temperature range (15 – 35˚C) for effective struvite precipitation and the determination of struvite solubility constants (K_{sp}) (Hanhoun *et al.*, 2011), whereas the ADs were operated at 38 – 40 ˚C (which could potentially disintegrate the struvite crystals) (Marchi *et al.*, 2015).

Therefore, the post-AD and AD experiments were not performed identically, but are still suggestive of the pros and cons of different Mg^{2+} addition strategies. Overall, post-AD addition of Mg^{2+} appears to be superior to AD dosing of Mg^{2+} primarily due to greater levels of NH_4-N and PO_4-P removal, but comparative value also depends on economic considerations and ease of P recovery, and other benefits like more methane production which are reviewed below.

Table 4.5: Effects of pH and Mg addition on IAP in effluent from bench-scale AD and post-AD Jar test experiments

Added Mg^{2+} dose (mg/L)	**IAP in effluent from bench-scale AD experiments**		**IAP in effluent from post-AD Jar test experiments**	
	pH 7	**pH 8**	**pH 7**	**pH 8**
0	3.4×10^{-6}	1.7×10^{-6}	3.8×10^{-6}	1.5×10^{-6}
50	2.9×10^{-6}	8.9×10^{-7}	3.3×10^{-6}	6.6×10^{-7}
100	2.1×10^{-6}	4.1×10^{-7}	2.0×10^{-6}	5.0×10^{-8}
150	8.3×10^{-7}	2.3×10^{-7}	7.7×10^{-7}	9.3×10^{-9}

Results are mean IAP values obtained from AD and Jar test experiments over a 60-days period

4.4 Economic Analysis on struvite control in AD and post-AD systems

Munch and Barr (2001) and Roeleveld *et al.* (2004) report that ‘controlled’ struvite crystallisation is an economically feasible technology in the removal/recovery of N and P from RLs. With the aim of this study being to extend the application of this technology to the removal/recovery of N and P in sludge (i.e. in- and post-AD), a cost-benefit analysis (CBA) was performed in order to also ascertain the economic viability. A CBA generally stems from the notion that a technology (or process change) is only economically feasible if all benefits outweigh the all relevant costs; i.e. net profit (NP) > 0. NP refers to difference between benefits and costs (AMBIO, 2011). It is commonly referred to as the sum of the internal benefits, IBs and the external benefits, EBs of a project (see Equation 21);

NP = IBs + EBs **Equation 23**

Therefore, one needs to tally all the IBs and EBs to compare the cost-benefits of different possible operational solutions. However, in the context of this study, only the IBs for N and P recovery in AD reactors and post-AD systems were considered, due to the complexities involved involved in determining the EBs which are beyond the scope of this thesis.

4.4.1 Internal Benefits (IBs) of potential N and P recovery in AD and post-AD systems

IBs are factors that are directly linked to the nutrient recovery process and can be expressed in monetary units as they have attributable market value. IBs of potential N and P recovery is calculated as the difference between internal income (IN_c) and internal costs (ICs). Some typical IN_c associated with N and P recovery include the sale of recovered products as well as potential savings such as: (i) reduced sludge generation, and reduced cost of sludge management; (ii) reduced operation cost for carbon required for biological P-removal due to a reduced P-load via RLs recycled to the head of the WWTP); (iii) reduced aeration cost for N-removal (i.e. due to reduced N-load recycled via RLs to the head of the WWTP; and (iv) reduced maintenance of clogged pump/pipes due to reduced uncontrolled struvite crystallization. Geerts *et al.* (2015) also reported other potential savings of N and P recovery in sludge such as: reduced sludge disposal cost due to improved dewaterability, and also lower polymer use in sludge dewatering processes.

ICs refer to the total investment costs associated with inclusion of the nutrient recovery processes. Possible ICs associated with N and P recovery include: (i) Investment in the main installation (i.e. mixing and crystallization units); (ii) operation man-hours; (iii) investment in chemical storage and dosing (i.e. to facilitate bulk delivery and minimise cost of chemical-dosing); (iv) Cost of $MgCl_2$ (as Mg^{2+} source) and NaOH (for pH adjustment); (v) Investment in struvite purification (since precipitation takes place in sludge); and (vi) additional system maintenance costs.

IBs can be expressed as shown in Equations 22 and 23 below (AMBIO, 2011):

IBs = IN_c – ICs **Equation 24**

$$\text{IBs (£)} = \sum_{t=0}^{T}[(\boldsymbol{APR_t \; x \; SPP_t}) + (\boldsymbol{ASR_t x CSM_t}) + (\boldsymbol{ARR_t \; x \; CR_t}) + (\boldsymbol{ADR_t \; x \; CCD_t}) - (\boldsymbol{IC_t})]$$ **Equation 25**

Where: APR_t *= Annual volume of P recovered (kg);* SPP_t *= present selling price of recovered P (£/kg);* ASR_t *= Annual volume of reduction of sludge generation (kg);* CSM_t *= present cost of sludge management (£/kg);* ARR_t *= Annual volume reduction of reagents (kg);* CR_t *= present cost of reagents (£/kg);* ADR_t *= Annual volume reduction of uncontrolled P deposit (kg);* CCD_t *= present cost of cleaning P deposit (£/kg);* IC_t *= Investment cost (£)*

CBA for P-recovery systems is generally expressed as cost per kilogramme of P recovered as cost per kilogram of P recovered. Using mean start-up PO_4-P concentrations of 397 ± 11.7 mg/L and 439 ± 14.8 mg/L for AD and post-AD Mg^{2+} addition systems, respectively, about 69 ± 2.4% and 89 ± 1.1% removal of PO_4-P occurs (based on experimental data). This translates into an average of ~90.3 ± 2.1 tonne/yr and ~128.4 ± 2.6 tonnes/yr of P being that could potentially be recovered as struvite in AD and post-AD systems at Bran Sands WWTP, respectively (see Figure 4.14), extrapolated from the NH_4-N and PO_4-P daily loads presented on the 2013 N and P balances (see Figure Figures 3.5, 3.8 and 3.9). Like NH_4-N and PO_4-P concentrations, Figure 4.14 showed that potentially recoverable N and P loads in sludge increased with increase in Mg^{2+}doses. In addition, potentially recoverable N and P loads were higher in post-AD systems than in AD Mg^{2+}dosing experiments. This was expected as it was consistent with observed NH_4-N and PO_4-P removal efficiencies (see Figures 4.5 and 4.9). Further, Tables 4.6 and 4.7 show that post-AD recovery systems can be relatively more cost-effective than nutrient recovery in AD reactors.

The CBA of potentially extrapolating the dosing experiments in full-scale AD reactors and post-AD systems at Bran Sands WWTP is summarised in Tables 4.6 and 4.7. The factors and rates used in establishing the CBAs are presented on Tables B4 and B3, respectively. Assuming that, P loads in the feed into the recovery systems remained constant, Tables 4.6 and 4.7 show that potential revenue generated from the sales of the recovered P (as struvite) in both the AD and post-AD systems, increased to about four fold from systems with 150 mg/L of added Mg^{2+} compared to those with no added Mg^{2+} at pH 8; i.e. from £62,210 to £225,850 and £69, 370 to £320,990 in AD and post-AD systems, respectively. The revenue was calculated based on the recommendations of Dockhorn (2009) that recovered P from sludge can be sold at €3/kg P recovered (i.e. £2.5/kg P recovered (see Tables B4 and B3). The potential revenue from the sales of recovered P (with > 50 mg/L added Mg^{2+}) from Bran Sands WWTP is greater than the maximum potential revenue of £76000 reported by Jaffer *et al.* (2002) through P recovery from 400m^3 per day digester liquors at Slough WWTP. This could be as a result of the the relatively higher P load in digested sludge at Bran Sands

WWTP available for recovery. In addition, the potential revenue from the sales of recovered P (as struvite) from Bran Sands WWTP using post-AD recovery is 1.3 times the cost of chemicals for recovery, implying dosing Mg^{2+} in post-AD systems is a potentially profitable technology.

A basic economic comparison between the current situation at Bran Sands WWTP, the different tested Mg^{2+}dosing experiments and the theoretical Mg^{2+}dose (~255 mg/L; see Figure B.2) required to recover all the PO_4-P in digested sludge is reported in Table 4.7. Generally a statistically significant difference (P value < 0.05 at 95% confidence interval limit) was seen between the current situation at Bran Sands WWTP and the various Mg^{2+}dosing experiments. Overall, Table 4.7 suggests that it is more cost effective to leave things as they currently are at Bran Sands as opposed to adjusting the pH in digested sludge to 8.0 with no added Mg^{2+}. However, as expected the internal benefits of adjusting pH to 8.0 increased with added Mg^{2+} ≥50 mg/L; probably due to the increased potential for N and P recovery, and potential reduction in scaling and related maintenance costs. On the other hand, Table 4.7 also suggests that although adding the theoretical Mg^{2+}dose of ~255 mg/L could potentially recover all the available P in digested sludge and subsequently eradicate the potential for scaling, the CBA between this Mg^{2+}dose was not statistically significantly (P value < 0.05 at 95% confidence interval limit) different from that of 150 mg/L added Mg^{2+}dose. Overall a potential 31% increase internal benefits could be realised by increasing the added Mg^{2+}dose from 150 mg/L to 255 mg/L.

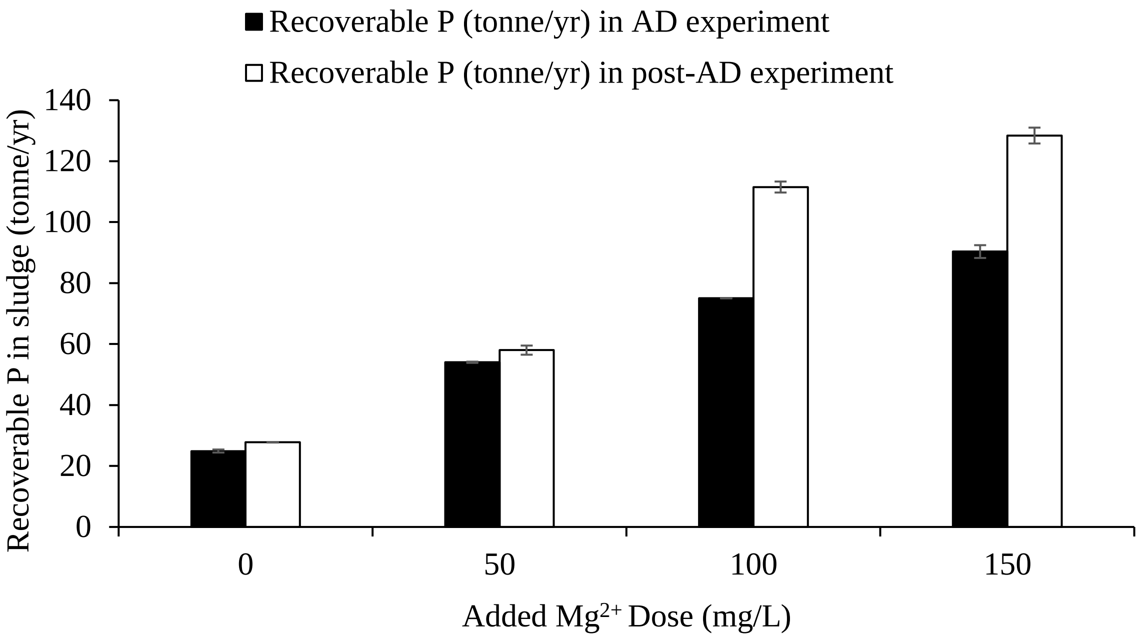

Figure 4.14: Comparison between recoverable P in sludge from AD reactors and post-AD systems at pH 8. Results show potential annual mean recoverable PO_4-P in sludge at Bran Sands WWTP extrapolated from data from the AD reactor and post-AD Mg-dosing experiments at pH 8. Error bars refer to standard errors.

Table 4.6: Cost benefit analysis for potential N and P recovery in AD reactors

Added Mg^{2+} Dose (mg/L)	**0.0**	**50**	**100**	**150**
Internal Costs (£/yr)				
Investment/Modification Costs	29,580	29,580	29,580	29,580
Operation and Maintenance costs	124070	90090	65570	47730
Financial Costs (£/yr)				
Cost of 5N NaOH	263,520	263,520	263,520	263,520
Cost of Mg_2Cl	0.0	12200	24410	36620
Internal Incomes (£/yr)				
Struvite Sale	62,270	135,080	187,630	225,850
Savings in maintenance/replacement Costs	20,930	54,910	79,440	97,270
Savings on sludge management	54,060	117,270	162,890	196,070
Savings on nitrification cost	1390	8070	15,810	18,920
Savings on acetic acid for denitrification	3540	20,540	40,260	48,170
Energy savings (from increased biogas)**	15,400	24,000	48,500	48,550
Internal Benefits (£/yr)				
Internal Income - Internal Costs	-259,580	-35,520	151,450	257,380
Internal Benefits (without income from the increased biogas; £/yr)	-337,250	-194,600	-84,680	-17,020

***This energy saving can be negatively affected by reductions in sludge production on site which could result in a decrease in biogas production. Factors and rates used for establishing this CBA are presented on Table B4 (See Appendix B). Financial costs are also included in Total Benefits calculations.*

Table 4.7: Cost benefit analysis for potential N and P recovery in post-AD systems

Added Mg^{2+} Dose (mg/L)	Present situation at Bran Sands	0.0	50	100	150	255
Internal Costs (£/yr)						
Investment Cost	0	118,300	118,300	118,300	118,300	118,300
Operation and Maintenance costs	145,000	136,810	101,510	39080	19,380	0
Financial Costs (£/yr)						
Cost of 5N NaOH	0	209,330	209,330	209,330	209,330	209,330
Cost of Mg_2Cl	0	0	12700	25300	38000	64500
Internal Incomes (£/yr)						
Struvite Sale	0	69,370	145,000	278,790	320,990	362,530
Savings in maintenance/replacement Costs	0	8190	43490	105,920	125,620	145,000
Savings on sludge management	0	60,220	125,890	242,030	278,670	314,730
Savings on nitrification cost (aeration cost savings)	0	3810	16,060	28,500	32,580	46,460
Savings on acetic acid for denitrification	0	9690	40,890	72,570	82,970	118,310
Internal Benefits (£/yr)						
Internal Income - Internal Costs	-145,000	-313,160	-70,510	335,800	455,820	594,900

Factors and rates used for establishing this CBA are presented on Table B3 (See Appendix B). Financial costs are also included in Total Benefits calculations.

The investment costs (i.e. cost of purchase and installation of the reactors) pertaining to post-AD systems are estimated from Montag *et al.* (2009), which reports €1,417,700 (i.e. £1,181,500) as the investment cost of P recovery from the effluent and sludge from a WWTP serving a population of 100,000 p.e. This involves civil works (i.e. equipment, auxiliary facilities, machinery, etc.). From Table 4.7, it can be seen that spreading this cost over a period of 10 years for the various Mg^{2+} doses, the ICs involved in P-recovery in post-AD systems could potentially decrease from ~£ 464,440 /yr. (with no added Mg) to £ 385,010 /yr. (pH 8, 150mg/L added Mg^{2+}). Table 4.6 also shows ICs for P-recovery in AD systems decrease with increasing Mg^{2+} dose. However, both the investment cost and ICs for the AD experiments were less than that of the post-AD systems. Reasons being that only ~25% of the investment cost for post-AD P-recovery systems was required to retrofit existing AD systems.

A two-sample t-test analysis shows that differences among mean ICs for AD and post-AD systems are statistically significant (P value = 0.028 at 95% confidence interval limit, DF = 32). Furthermore, a one-sample t-test analysis show statistically significant differences among mean ICs for the different Mg doses in AD systems (P value = 0.024 at 95% confidence interval limit, DF = 32), and among mean ICs for the different Mg doses in the post-AD systems (P value = 0.008 at 95% confidence interval limit, DF = 32).

The decrease in ICs with increasing Mg dose in both the AD and post-AD recovery systems can be linked to the decrease in operation and maintenance cost with increasing Mg dose. This is consistent with reports (Berg *et al*., 2006; Dockhorn, 2009; Molinos-Senante *et al.*, 2011; Sharp *et al.*, 2013; Mudragada *et al.*, 2014) that N and P recovery through ‘controlled’ struvite recovery could potentially reduce operation and maintenance costs at WWTPs. In addition, adjusting the pH to 8 without the addition of Mg into post-AD sludge in ‘designated’ reactor could potentially reduce the reported current cost (£145, 000 per year) dealing with struvite-related problems (i.e. replacement of clogged pipes and pumps) at Bran Sands WWTP by £8190 (i.e. ~6% reduction). Morever, Table 4.7 shows that savings in struvite-related operation and maintenance costs could possibly reach £125,620 in post-AD recovery systems with the addition of 150 mg/L Mg^{2+} at pH 8. The Operations and maintenance costs were estimated based reports by Berg *et al.* (2006), Dockhorn, (2009) and Molinos-Senante *et al.* (2011), that maintenance costs of systems used for P-recovery from digested sludge cost about €2.14/kg P in effluent. This includes; the cost of repairs, cleaning, washing and separation of struvite from sludge.

These savings would not have been realized without acknowledging the impact of chemical costs. As expected, chemicals costs increased with increasing Mg^{2+} dose probably due to the increasing Mg^{2+} load requirements and its corresponding costs. For instance, ~48% (i.e. £38,000) of the difference in ICs between the post-AD systems with no additional Mg^{2+} and those with 150 mg/L of added Mg^{2+}, is as result of potential expenses incurred in purchasing Mg_2Cl (see Table 4.7). However, the highest chemical costs in all experiments is that pertaining to the purchase of NaOH for pH adjustment. For instance, Table 4.7 shows that the annual cost of NaOH in post-AD recovery systems to adjust pH 8 could potentially account for ~85 - 95% of chemical costs. This is consistent with reports by Jaffer *et al.* (2002) that the cost of NaOH for pH adjustment in P-recovery from digester liquors at Slough WWTPs could account for upto 97% of chemical costs. However, Battistoni (2004) reported that this cost can be reduced by incorporating air stripping for pH adjustment to reduce NaOH cost.

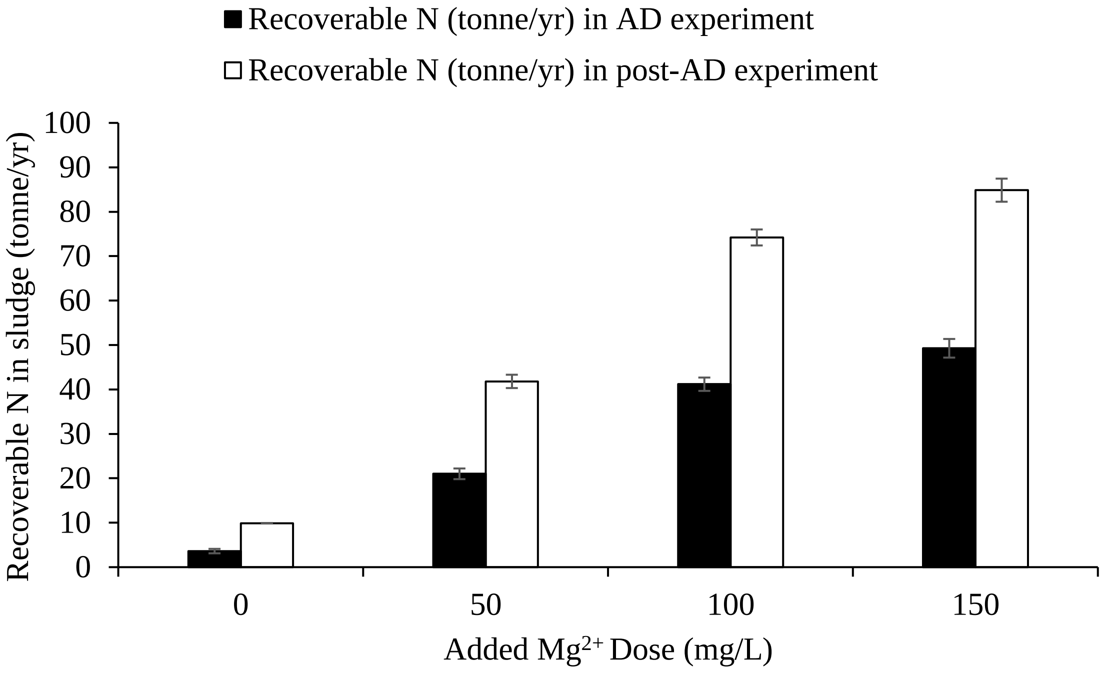

Figure 4.15: Comparison between recoverable NH_4-N in sludge from AD reactors and post-AD systems at pH 8. Results show potential annual mean recoverable NH_4-N in sludge at Bran Sands WWTP extrapolated from data from the AD reactor and post-AD Mg-dosing experiments at pH 8.Error bars refer to standard error of means.

Tables 4.6 and 4.7 further show that IBs of both the AD and post-AD recovery systems also increase with increasing Mg dose. This is partially associated with increases in the amount of P and N recovered with increasing Mg-dose as shown on Figures 4.14 and 4.15, respectively. It is also noted that as more P and N are recovered with increase in Mg dose, savings are made on the cost of: (i) maintenance/replacement of pipelines and equipment affected by ‘uncontrolled’ struvite crystallization; (ii) sludge management resulting from the reduction in N and P recycled via RLs to the activate sludge treatment units and also reduction in digested sludge volume to be dewatered; and (iii) energy needed for nitrification and the purchase of acetic acid for denitrification. For instance, at a rate of 2.0 g SS/g P released (reported by Jeanmaire & Evans, 2001) as the rate of reduction of sludge production following P recovery (as struvite), reductions in sludge productions of around ~181 tonnes SS/yr and 257 tonnes SS/yr of biomass can be achieved in AD and post-AD recovery systems. An added benefit of N and P recovery in AD units was the increase in biogas production which when converted to energy, can potentially offset some of the annual energy costs on site. Up to 8.3% (i.e. an additional annual energy equivalence of ~510 MWH) increase in biogas production was observed in ADs with 150 mg/L of added Mg compared to the current annual average biogas produced a Bran Sands WWTP. However, this can be offset by reductions in sludge production as indicated above. Morever, Table 4.6 shows that excluding potential energy savings resulting from increased biogas production at Bran Sands WWTP could result in negative IBs following N and P recovery in AD reactors, implying this could neither be a cost-effective solution for struvite control nor recovery at Bran Sands WWTP.

All potential cost savings and revenues from the sale of recovered N and P will contribute to the IBs of each of the systems, although cost savings were generally greater with increasing Mg dose. For instance, Table 4.6 shows that ~67% reduction in current annual costs (~£145,000/yr) required for addressing struvite-related problems at Bran Sands WWTP could be achieved by recovering N and P in AD reactors (assuming pH 8.0, 150 mg/L Mg^{2+}). Whereas in Table 4.7, it can be seen that maintenance cost savings could be up to ~87% in post-AD nutrient recovery systems operated at pH 8.0 with 50 mg/L of added Mg^{2+} dose. Interestingly, these savings dropped to 10% in post-AD recovery systems operated at pH 8.0 with 150 mg/L of added Mg^{2+} dose, probably due to comparatively higher operations costs needed to cope with excessive struvite formation in high Mg^{2+} post-AD recovery systems. This suggests that increasing the Mg dose too high in post-AD recovery systems might cease to be cost effective. This also could be true for N and P recovery in AD units as there was very small difference (15%) between the savings on maintenance costs in systems with 100 mg/L of added Mg and those with 150 mg/L of added Mg at pH 8. In addition, the difference (~26%) in IBs between post-AD systems with 100 mg/L Mg^{2+} versus 150 mg/L Mg^{2+} (at pH 8.0) further confirms that increasing the Mg^{2+} dose beyond 100mg/L is not likely cost effective.

Overall, the IBs of recovering N and P in AD units are less than post-AD systems except in systems with no added Mg^{2+} dose where the IBs in AD units are higher than in post-AD systems. For instance, the IBs involved in recovering N and P in post-AD systems with 150 mg/L of added Mg was about 1.5 times greater than that AD recovery systems with 150 mg/L of added Mg at pH 8 (see Tables 4.6 and 4.7). Given the current operational settings and results of the tests simulated at the same scale as operations at Bran Sands WWTP, a selling price of £500/tonne struvite recovered is enough to guarantee a discounted payback of 10 years, 9 years, 8 years or 7 years following the installation of N and P recovery in ADs operating at pH 8.0 with 0.0 mg/L, 50 mg/L, 100 mg/L or 150 mg/L of added Mg^{2+}, respectively. At the same struvite selling price, the possible discounted payback time will be for post-AD 10 years, 8 years, 6 years or 5 years following the installation of N and P recovery in post-AD systems operating at pH 8 with 0.0 mg/L, 50 mg/L, 100 mg/L or 150 mg/L of added Mg^{2+}, respectively.

Finally, Table 4.7 shows that if the CBA on N and P recovery in post-AD systems is based on IBs alone, not all the operating conditions are economically feasible. However, incorporating EBs, could make all assessed conditions appear economically feasible due to the overall positive values. But for the purpose of this study only potential IBs relevant to Bran Sands WWTP were considered due to complexities involved in estimating potential EBs.

4.5 Fate of struvite in sludge

Currently there exists two different techniques for separating struvite from sludge: i.e. separation with a hydrocyclone (as in the Ebara process), and gravity settling (as in the AirPrex system) (Shepherd *et al.*, 2009; Bergmans, 2011; Andrea *et al.*, 2015). With a hydrocyclone, heavier (larger) particles (e.g. struvite crystals) are directed to the wall, for them to flow towards the underflow exit; whereas the lighter (smaller) organic particles/biomass are simultaneously directed towards the overflown exit of the crystallization tank (see Figure 4.16). The main advantage of struvite/sludge separation in a hydrocyclone is the comparatively small footprint. However, it is more sensitive to scaling and clogging problems than a simple gravity settler.

With gravity settling, large struvite crystals are collected from the sedimentation zone and washed; while the smaller crystals are usually recycled in the reaction zone in order to stimulate crystal growth (see Figure 4.17). This is because, the larger the final crystal size, the easier the crystals can be separated and washed.

Both approaches above entail an extra installation/retrofitting and maintenance costs. However, these costs could potentially be offset by the sale of the recovered crystals. On the other hand, if both approaches are not cost effective, the sludge struvite can be left in the sludge and sold to fertiliser companies as raw materials or to farmers at a higher value. In addition some of the small crystals from the sludge can be sold to companies (like Ostara and Airprex) as 'seed' material to stimulate crystal growth.

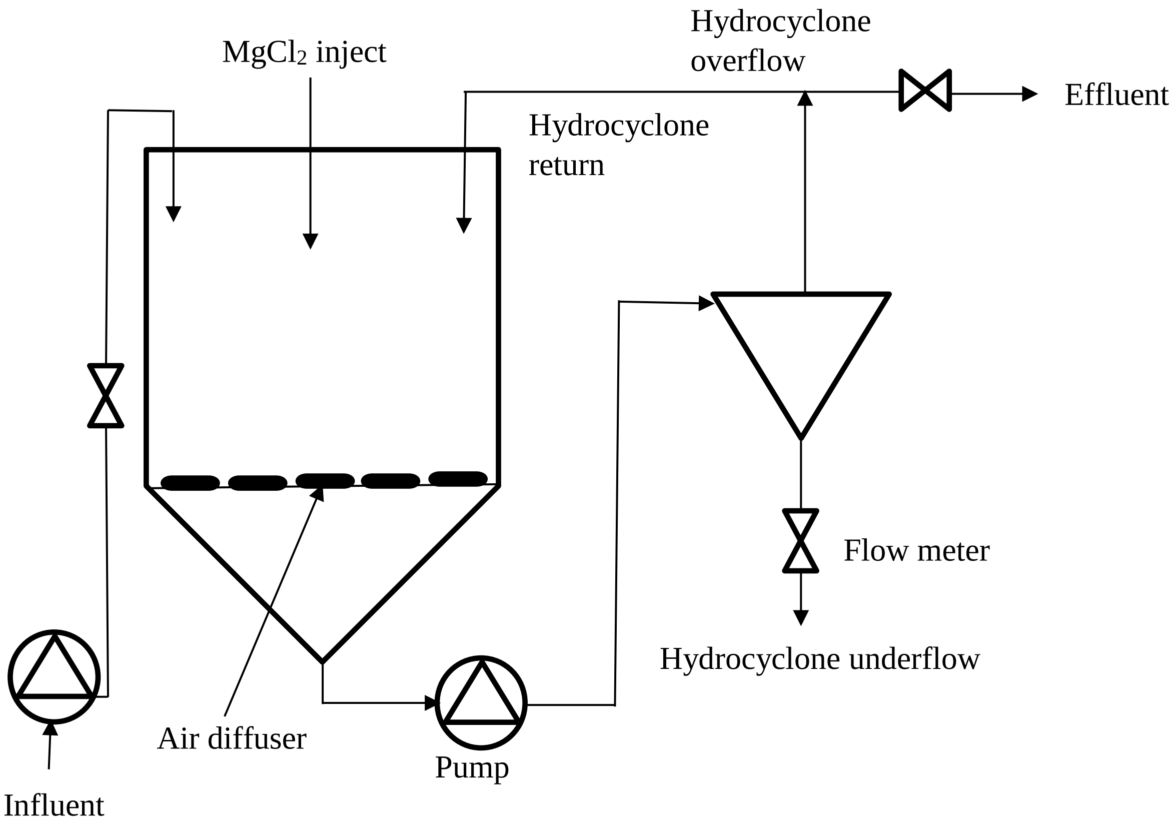

Figure 4.16: Struvite/Sludge separation in a hydrocyclone (Shepherd *et al.*, 2009)

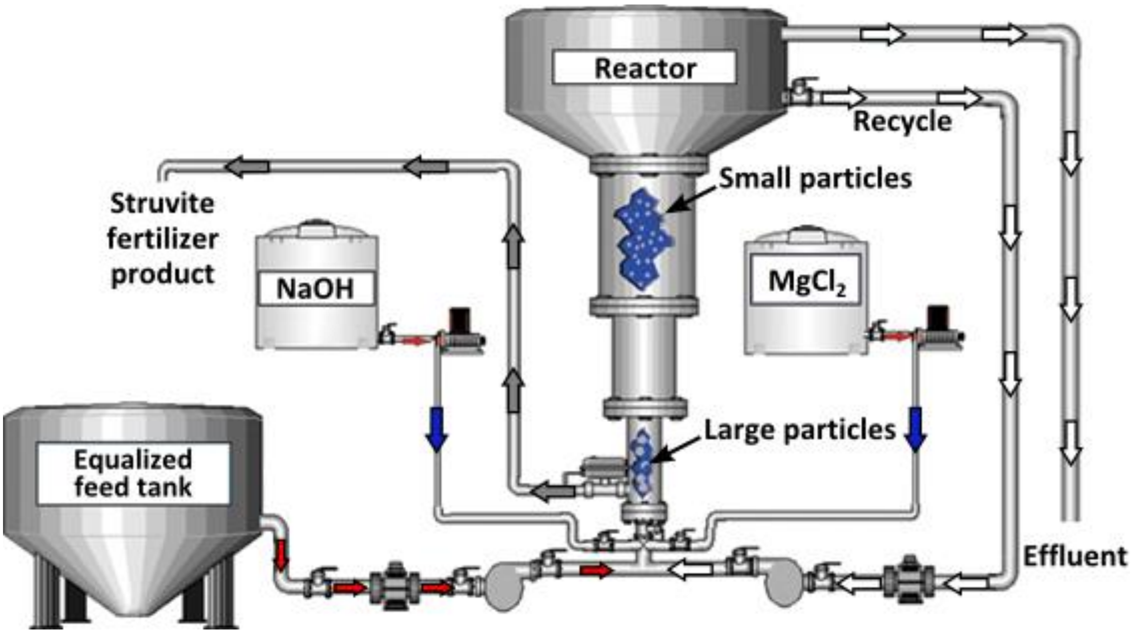

Figure 4.17: A pumped-flow dosing gravity settler for struvite/sludge separation (Capodaglio *et al.*, 2015)

4.6 Conclusion

There is growing interest in research towards the development of AD processes capable of producing effluents with reduced P and N levels due to the cost of treating nutrient-rich RLs and increasingly stringent regulations limiting the discharge of N and P into aquatic environments. This study assessed the feasibility of recovering N and P (as struvite) in AD units by the addition of Mg^{2+} (as $MgCl_2$) and pH adjustment. The study also provided analysis on the effect of Mg^{2+} addition and pH adjustment on the performance of the ADs (in terms of CH_4 production and VS removal). Experiments involving Mg^{2+} addition and pH adjustment in post-AD sludge (i.e. digested sludge) also were conducted to compare P- and N-removal rates with that of the P-recovery experiments in the AD units.

In both AD- and post-AD recovery systems, N and P recovery was found to increase with increasing Mg^{2+} doses and pH levels, although recovery rates were higher with post-AD addition compared with direct addition to AD units. However, both options significantly reduced soluble P and N levels in the RL systems, which would reduce the potential for scaling resulting from ‘uncontrolled’ struvite precipitation in processes downstream of the AD. Both approaches, also would potentially reduce energy and other associated costs involved in treating N and P recycled to the head of the works via RLs. Although not examined here from an operational perspective, it was shown that recovering N and P as struvite could generate income through their potential sale. Finally, Mg addition and recovering N and P in AD units increased biogas production.

IBs derived from N and P recovery in ADs and post-AD systems also were favourable towards Mg dosing in both AD and post-AD recovery systems. However, the IBs, EBs and NP for N and P recovery in ADs was less than that of post-AD systems, although there was no statistically significant difference in the financial benefits between N and P recovery in ADs and post-AD.

Operational and CBA data suggest the addition of 100 mg/L Mg^{2+} as the most the most likely dose for cost effective N and P recovery in both approaches. However, 100 mg/L Mg^{2+}addition in post-AD sludge was seen to be the most favourable N and P recovery option. Hence based on evidence from this study, the addition of up to 100 mg/L Mg^{2+} in digested sludge in a designated tank immediately after the AAD at Bran Sands, has been recommended for N and P recovery as struvite, reduction of scaling and maintenance costs, and reduction of N and P load in RLs on-site.

Despite the success in potentially reducing some of the NH_4-N load in downstream of the AD using Mg dosing and pH adjustment, the residual NH_4-N concentration in RLs is still not likely sustainable for existing systems at Bran Sands WWTP. Hence a cost effective polishing step might be necessary, especially for N, prior to the RLs being recycled to the ETW. Although other options were considered, aerobic granular reactors and treatment was assessed for this purpose, which are described in detail in Chapters 5 and 6.

Chapter 5: The application of Aerobic Granular Technology in N-removal from RLs

5.1 Introduction

Aerobic granulation technologies (AGT) are a relatively new wastewater treatment option that has been successfully applied in treating different industrial and domestic wastewaters (Liu & Tay, 2002; Yang *et. al.,* 2004a; Jing, 2008; Bindhu and Madhu, 2013). The technology has some advantages. For example, it provides the possibility of more compact WWTPs due to the fact that N, P and COD removal can be achieved simultaneously within a single SBR unit (de Kreuk *et al.,* 2005; Pronk *et al.,* 2015). The simultaneous removal of N, P and COD in AGT occurs via a carefully sequenced, batch-fed process that selectively promotes the growth of microorganisms in granules; a process called biogranulation, which is the self-immobilisation of microorganisms into spherical granules (through cell-to-cell interactions governed by biological, physical and chemical phenomena). Recent success of the technology has suggested it as a potential substitute for conventional wastewater treatment because of its smaller footprint, lower energy requirement, and their economics (De Kreuk and van Loosdrecht, 2006; Pronk *et. al.,* 2013). However, limited operational data on successful full-scale applications has been published in scientific literature (de Bruin *et al.*, 2004; de kreuk *et al.*, 2005; Coma *et al.*, 2012; Show *et al.*, 2012; Morales *et al.*, 2013; Pronk *et al.*, 2015). This is partly because the technology was rapidly commercialised and it is often wrapped in secrets relative to optimal conditions to promote granulation.

The development of aerobic granules (AGs) was first reported by Mishima and Nakamura (1991) in a continuous aerobic upflow sludge blanket reactor. AGs are commonly characterised by a spherical compact structure with average diameter of 1 – 8 mm, high settling velocities (about 12 – 157 m/h) and low sludge volume index (SVI) (Etter and Wilderer, 2001; De Bruin *et. al.,* 2004; Wang *et. al.,* 2007; Bindhu and Madhu, 2013). AGT has since been studied in sequencing batch reactors (SBRs) and has been utilised in treating both high and low strength wastewaters (with organics, P, N and toxic substances) (De Kreuk *et. al.*, 2005a; Buitron and Moreno-Andrade, 2011). De Kreuk *et al.* (2005) reported that AGT is most effective using a SBR-process with a constant working volume and promoting oxygen gradients within the structure of the AGs using alternating aeration periods. This could be a potential drawback in the technology as considerations for a ‘balance tank’ to deal with variable flow may be most likely in full-scale applications.

The technology has been tested at laboratory scale (Beun *et al.*, 1999; Tay *et al.*, 2002; Zeng *et al.*, 2003), pilot-scale (Liu *et al.*, 2010; Isanta *et al.*, 2012; Wei *et al.*, 2012; Morales *et al.*, 2013; Li *et al.*, 2014), and later scaled-up in to treat industrial and domestic sewage at demonstration plants in Gansbaai, South Africa and Frielas, Portugal (Pronk *et. al.*, 2015). The first AGT full-scale application was applied by Royal HaskoningDHV in 2010 using the Nereda technology at Epe WWTP, Netherlands (van der Roest *et al.*, 2011; Giesen *et al.*, 2013) and, soon thereafter in 2013 at Garmerwolde WWTP, Netherlands (Pronk *et al.*, 2015).

Beyond domestic wastewater treatment, AGT also has been successful in treating industrial wastewater with high N-loads (especially from abattoirs and animal farms) (Cassidy and Belia, 2005; Kishida, 2009; Giesen *et al.*, 2013). Therefore, interest has been stimulated in extending the technology to RLs with high N-concentrations and less biodegradable organic matter. In fact, Cydzik-Kwiatkowska *et al*. (2013) successfully applied AGT to remove NH_4-N (via nitrification) from both diluted and undiluted RLs with NH_4-N concentrations of 260 mg/L and 474 mg/L, respectively. The RLs had relatively low COD/N ratio of 1.4 and less biodegradable organics (BOD/COD = 0.4). In both scenarios, carbohydrates and carbonates were added to the RLs in theoretical concentrations required for nitrification. However, most of the NH_4-N removed from the RLs was simply oxidised to nitrite, with nitrification efficiency seen to have increased from 90% (in diluted RLs at cycle time of 6 h) to 93% (in undiluted RLs at cycle time of 8 h). Despite this, there is still very limited information on AGT on ‘real’ RLs. Furthermore, the RLs often have very high levels of NH_4-N (i.e. >1000mg-N/L), even after some N and P removal during and post-AD (see Chapter Four). Therefore, effective nitrification with AGT systems would be particularly attractive for RLs.

This study was undertaken to assess AGT with SBRs to treat elevated NH_4-N in RLs from Bran Sands WWTP. The goal, however, was ultimately to treat ‘real’ RLs and, if successful, develop nitrogen loading rates and define operating conditions that might be used in subsequent pilot-scale testing. Specifically, the study assessed NH_4-N removal rates in SBRs using: (i) three different feed types (i.e. synthetic wastewater, synthetic wastewater + RLs, and RLs only); (ii) three different COD/NH_4-N ratios (0.9, 2.5 and 4.1); and (iii) three different N-loading rates (0.2, 0.6 and 1.0 g NH_4-N/d). Using these conditions, the utility of AGT systems for Bran Sands and RLs were determined for future applications. One of the considerations made during the AGT experiments was to centrifuge RLs samples (where necessary) to reduce TSS levels in reactor feed below recommended maximum TSS levels (i.e. 8 kg/m^3) for full-scale applications as reported by Pronk *et al.* (2015). This is to minimise the inhibitory effects of excess TSS concentration (i.e. >8 kg/m^3) on AGT performance.

5.2 Methodology

5.2.1 Experimental Set-up and Operation

Three column glass reactors (each with a conical base) were used as SBRs. Each reactor had a working volume of 1.2 L (44.7cm tall with a 5.7 cm ID; see Figure 5.1). Influent to each reactor was introduced at the bottom and effluent was withdrawn from a port located ~ 3.7 cm above the middle of the reactor (providing a volumetric exchange ratio of 44%).

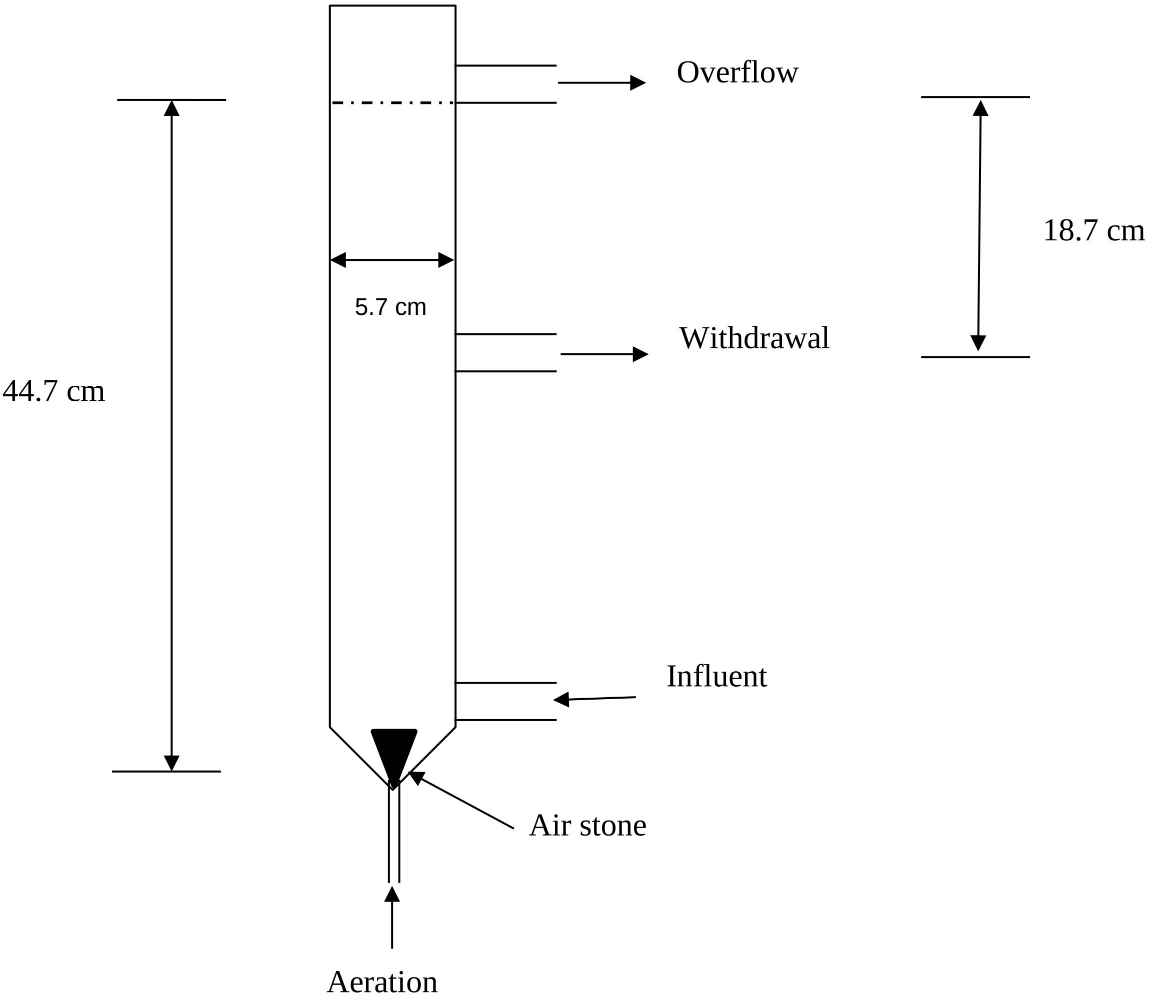

Figure 5.1: Schematic of the SBR

The reactor configuration was chosen following reports that almost all studies on AGT successfully produced aerobic granules only in column column SBRs (Tay *et al.*, 2003; de Kreuk *et al.*, 2005; Liu *et al.*, 2005; Cydzik-Kwiatkowska *et al*., 2013; Pronk *et al.*, 2013; Zhu *et al.*, 2013). The SBRs were provided air using porous air-stone diffusers, at a superficial air velocity of 2.4 cm/s; with mean DO levels maintained at 2.1 mg/L (which is within the 2-6 mg/L range for mature AGs; Yang *et al.*, 2003; Tsuneda *et al.*, 2004). Liu *et al.* (2005) reported that in a column SBR, air and/or liquid flow are subject to an upflow pattern that creates a relatively homogenous circular flow and a localized vortex along the height of the reactor, which in turn subjects microbial aggregates into circular hydraulic attrition. Circular flow was reported to be one of the almost 'regular circular' shape of the granules (Liu and Tay, 2002). Higher height to diameter (H/D) ratios in SBR, were also reported to enhance circular flow trajectory with more effective hydraulic attrition for microbial aggregates. Further, improvements in oxygen transfer in SBR were reported at high H/D ratios, which paved the way for reactors with relatively smaller footprints (Beun *et al.*, 2002). Hence, the chosen H/D ratio of ~8 for the reactors in the current study which is within the range (H/D >4; Zhu *et al.*, 2008; Li *et al.*, 2014; Awang and Shaabang, 2015) for the production of compact aerobic granules.

All reactors were operated in a sequence-batch mode with a six-hour cycle time comprised of 3 minutes of feeding, 349 minutes aeration, 5 minutes settling, and 3 minutes withdrawal (see Figure 5.2). The six-hour cycle time was chosen following reports that cycle times <6 hr saw the washing out of slow-growing nitrifying bacteria while in higher cycle times (>6 hr), less compact granules with poor settleability were formed (Tay *et al.*, 2002; Pronk *et al.*, 2013; Wei *et al.*, 2014). The cyclic operation was controlled by two 2-channel digital time switches (RS Electricals). Settling time of 5 minutes and volumetric exchange ratio of 44% were chosen following reports of improved nitrification and granular stability at settling times ≤5 minutes and volumetric exchange ratios ≥40%. The hydraulic retention time (HRT) in the SBRs was set at 14.4 hr to ensure better NH_4-N removal. Experiments were conducted at room temperature (20 ± 2°C), with no pH control to make the systems simpler to run and minimise the use of complex control systems.

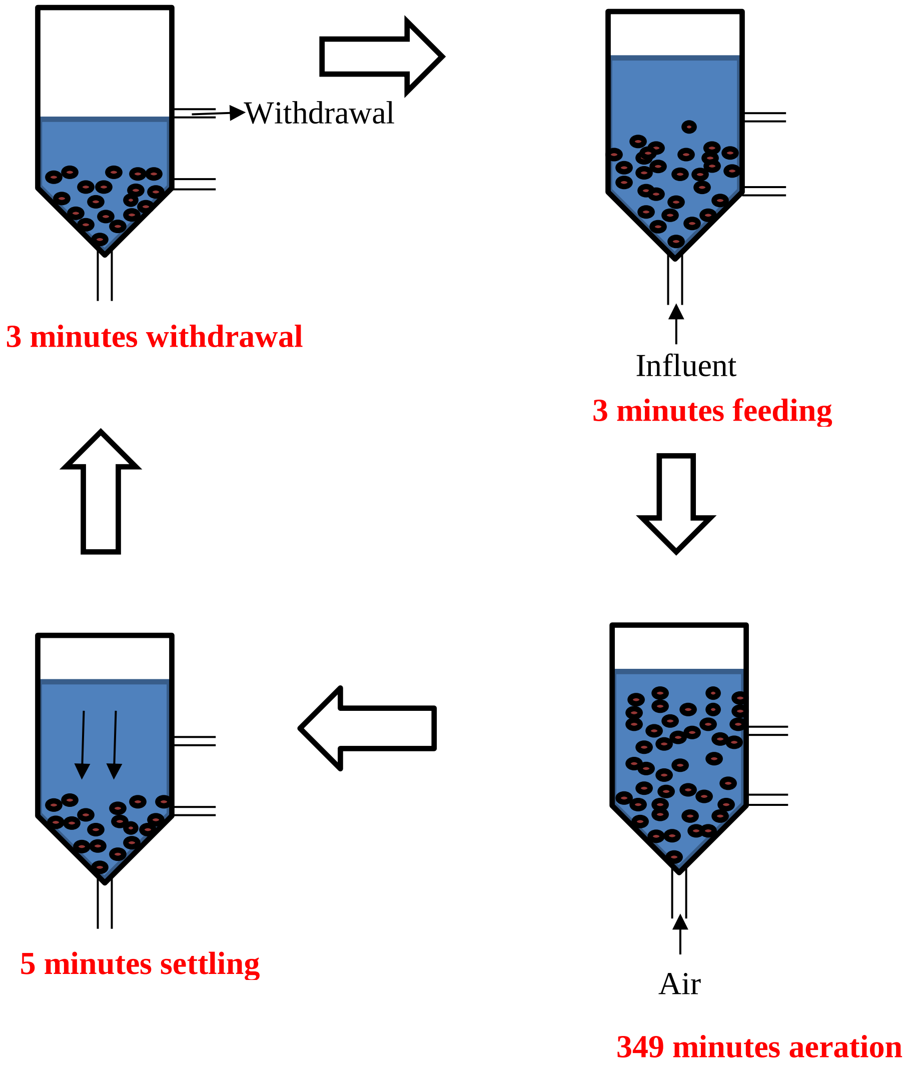

Figure 5.2: Schematic of the mode of operation of the SBRs

5.2.2 Media/Influent and seeding sludge

Each of the SBRs was inoculated by 250 mL of fresh activated sludge from the nitrification unit at Bran Sands WWTP. The inoculating sludge had an SVI of 142 mL/g and mixed suspended solids (MLSS) of 6980 mg/L.

Synthetic wastewater was prepared from a stock solution with the following composition: 64 mM sodium acetate as carbon source, 3.6 mM $MgSO_4.7H_2O$, 4.7 mM KCl, 35.4 mM NH_4Cl as N source, 4.2 mM K_2HPO_4 and 2.1 mM KH_2PO_4 as P source Pronk *et al.* (2013), and 10 mL/L trace elements solution (adopted from Vishniac and Santer, 1957; Tay *et al.*, 2001).

5.2.3 Developing Aerobic granules and operational strategy

The growth, development and maintenance of mature AGs (particularly those with nitrifying qualities) in the reactors was a rigorous process that was guided by observations and data from previous reports (Tay *et al.*, 2002; Cydzik-Kwiatkowska *et al*., 2013; Pronk *et al.*, 2013; Wei *et al.*, 2014). Each SBR was first seeded with 250 mL of nitrifying sludge from Bran Sands WWTP and the remaining operating volume was made up with synthetic wastewater (i.e. 1 in 7 dilution of the stock solution), and then allowed to adapt to ambient conditions in the lab. As noted earlier, the reactors were run at room temperature, with no pH control. Each reactor was then fed with synthetic wastewater in batch cycles. Influent COD:NH_4-N ratio was maintained at ~2.5 (with mean influent COD, NH_4-N and PO_4-P concentrations being 250 ± 5.6 mg/L 100 ± 4.1 mg NH_4-N/L and 25 ± 1.6 mg PO_4-P/L, respectively) during this start-up stage.

COD, NH_4-N and PO_4-P removal (see Figures C.1, C.2, C.3, C.4 and C.5 in APPENDIX C) were closely monitored alongside signs of granulation (i.e. biofilms with ~1 mm diameter and higher settling velocity; i.e. >9 m/h for activated sludge flocs). Nitrification activity was also monitored (through effluent NO_2-N and NO_3-N concentrations). During this initial granule formation stage (after ~19 days from start-up), granular sludge was collected once from the reactor showing the most effective nitrification activity and added to boost nitrification in the reactor where performance was comparatively poor.

Following the emergence of mature granules (around Day 55; see Figure 5.3), and stable COD and NH_4-N removal, alongside evidence of effective nitrification (i.e. NO_2-N oxidation to NO_3-N in the reactors), influent COD and NH_4-N concentrations were then increased to 760 ± 7.4 mg/L and 300 ± 2.2 mg-NH_4-N/L, respectively, while maintaining the COD:NH_4-N ratio at ~2.5. Following further improvements in COD and NH_4-N removal, nitrification activity and the granulation process, influent COD and NH_4-N concentrations were then increased to 1250 ± 5.9 mg/L and 500 ± 7.6 mg-NH_4-N/L, respectively, while maintaining the COD:NH_4-N ratio at ~2.4-2.5 (see Figure 5.6). However, following fluctuations in granule stability, COD and NH_4-N removal, and nitrification activity, influent COD and NH_4-N concentrations were lowered accordingly to enhance granular stability and performance.

With stability regained, the reactors were then gradually weaned over to RLs, by feeding them with a mixture of synthetic wastewater and RLs. Prior to their introduction into the reactors, the RLs were centrifuged in 1-L sterile plastic centrifuge bottles using a Heraeus Cryofuge 5500i centrifuge (supplied by Thermo Scientific) for 20 mins at 4400 rpm. This was to to reduce TSS levels in reactor feed below recommended maximum TSS levels (i.e. 8 kg/m^3) for

AGT reactors as reported by Pronk *et al.* (2015). The RLs were introduced in the following order; 5%, 10% and 20% of the influent volume (resulting in mean influent NH_4-N concentrations of ~100 mg/L, 300 mg/L and 500 mg/L, respectively). As the start-up phase, the COD/NH_4-N ratio was maintained at ~2.5, with occasional supplementation of granules from better performing to the poorer performing reactors (10% of seed v/v).

Following the formation of granules, three different feeding conditions (i.e. synthetic wastewater, synthetic wastewater + RLs, and RLs) were studied. Within each feeding condition, three N loading rates (NLRs); 0.2, 0.6, and 1.0 g NH_4-N/Lday corresponding to 100, 300, and 500 mg/L NH_4-N, respectively, were studied. Further, COD/NH_4-N ratios 1.0, 2.5, and 4.0 were assessed relative to NH_4-N removal. In summary, the study was split into three major stages referring to the feeding conditions outlined above. The first stage involved feeding the SBRs with synthetic wastewater, during which the different NLRs and COD/NH_4-N ratios were studied. The next stage was during the gradual weaning phase, with NLRs and COD ratios studied as in the first stage. And in the final stage, the SBRs were only fed RLs. However, in the final stage, only two NLRs and just one COD/ NH_4-N ratio were studied, since the only possible COD/ NH_4-N ratio for RLs was 1.2 from Bran Sands WWTP during the study.

5.2.4 Analytical Procedures

Chemical analyses of wastewater (influent and effluent) and biomass in the reactors were performed during the SBR cycle to observe for changes over time. Samples of the influent and effluent were collected, centrifuged, filtered through sterile syringe filters with 0.2 µm pore sizes and analysed for NH_4-N, NO_2-N, NO_3-N, COD and PO_4-P on a bi-weekly basis (Mondays and Fridays) as described in Chapter 3. Mixed liquor samples were collected from the reactors after the aerobic phase and analysed for SVI by standard methods (2710 D; APHA, 2005). SVI_{30}, SVI_{10} and SVI_5 referring to SVI measurements after 30, 10 and 5 minutes settling, respectively) were measured on Days 0 and after 200 (APHA, 2005). VSS and TSS were measured on mixed liquors and effluent samples from the reactors. The soluble COD and NH_4-N concentrations were measured using Merck Spectroquant test kits (www.analytical-test-kits.com) supplied by VWR; while the PO_4^{3-}, NO_2^- and NO_3^- concentrations were determined using Ion chromatography (IC) (APHA, 2005). TSS and VSS were analysed following Standard Methods for the Examination of Water and Wastewater (Methods 2540B and E, respectively) (APHA, 2005). The temperature, pH and dissolved oxygen (DO) levels were measured using HACH meters with temperature, pH and DO probes, respectively.

Granular sludge physical properties were assessed based on SVI (APHA, 2005), a simple free settling test and particle size distribution. Granular sludge samples (100 mL per reactor) were collected from the reactors fortnightly and placed in a 1L measuring cylinder filled with distilled water (a simplified version of the procedure used in Cydzik-Kwiatkowska *et al.*, 2009). The granules were monitored as they settled down the column and were partitioned according to their settling velocities. The measurements were based on reports (Liu *et al.,* 2003; De Bruin et. al., 2004; Qi *et al.*, 2004; Wang *et. al.,* 2007; Show *et al.,* 2012) that settling velocities of aerobic granules are typically between 12 and 157 m/h. After the settling test, some of the granules were recovered and returned to their respective reactors, while some were used for microscopic analysis.

The external structure of the granules was analysed using microscopy (Olympus BX41M, Tokyo, Japan). Granular sludge samples after collection, were first washed with phosphate buffer (pH 7.6), after which the granular sludge samples were separated into different diameters (using 0.45 mm, 0.9 mm, 1.25 mm, 2 mm, 2.5 mm, 3.2 mm, and 4 mm stainless steel standard mesh sieves) according to the wet-sieve procedure described in Zhu *et al.* (2013). An Image analysis system (Image-Pro Plus, V4.0, Media Cybernetics) with the Olympus BX41M microscope was used to analyse the granules following procedures in Liu *et al*. (2007) and Wagner *et al.* (2015).

5.2.5 Data Analysis

Data obtained from laboratory analyses for NH_4 removal were first tested for normality. After the data was confirmed as normal distribution, statistical analysis (i.e. parametric tests e.g. t-test, Pearson Correlation) using SPSS were performed to determine whether there were statistically significant differences between NH_4-N removal rates as a function of feed types, NLRs and operating COD/N ratios. Statistical tests (t-test, Pearson Correlation) were also performed on NO_3-N, NO_2-N data in order assess the impact of NLRs on effective autotrophic nitrification activity in the reactors. Statistical significance was based on 95% confidence limit (i.e., $p>0.05$ = Not statistically significant; $p<0.05$ = statistically significant). Uncertainty ranges in this study were presented as standard error of sample means.

5.3 Results and Discussion

5.3.1 Aerobic granules formation with RLs

The SBRs were operated for 250 days to assess NH_4-N removal during the formation and maturation of aerobic granules with RLs. To promote the formation of AGs capable of simultaneous NH_4-N and PO_4-P removal, the cycle time and volumetric exchange ratio for the SBRs were initially set at 6 hr and 44 %, respectively. This cycle time was suggested by work from Tay *et al.* (2002), Pronk *et al.* (2013), and Wei *et al.* (2014), which reported 6 hr as the best cycle time for the selective incorporation of nitrifying and denitrifying bacteria in AGs. Their reports also indicate cycle times below 6 h often resulted in wash out of nitrifying bacteria (which are usually slow-growing) in biomass with 'low settleability', while cycle times above 6 h often resulted in AGs with smaller sizes, less compact and poor settleability. Furthermore, settling times (5 min) and 44 % exchange volume were chosen to maximise the probability of retaining nitrifying bacteria in AGs. Previous reports (i.e. Liu *et al.,* 2004; Qin *et al.,* 2004 a, b; Wang *et al.,* 2004) indicated stable granules tended to be observed at settling times less than 5 min (with optimal nitrification at 5 min) and volumetric exchange ratios >40 %.

The formation of AGs was evident from the changes in particle size and shape (see Figure 5.3), with mean particle size increasing from ~ 300 ± 3.3 µm at start-up (i.e. day 0) to ~1500 µm (Figure 5.4) on day 55 (although initial granulation was seen after 19 days of operation). The morphology of the formed AGs was nearly spherical, with a clear outline (without filamentous growth) and a dense structure, as opposed to the fluffy, loose, irregular morphology of the seeding nitrifying activated sludge flocs.

Aerobic granulation is a slow process, and has been reported to take between 140 to 400 days to achieve 80 – 90 % granulation in treatment systems (Ni *et al.,* 2009; Liu *et al.,* 2010; Wagner and Costa, 2013; Wagner *et al.,* 2015). In this study, ~ 85 % of granulation was observed after 200 days of operation. Further, ~99 % of the AG mass formed had diameters between 500 µm and 4000 µm. In addition, mean settling velocity for the granules increased from ~2 m/h at start-up to ~44.9 m/h after 200 days of aerobic granulation.

Further evidence of aerobic granulation was the increase in TSS and VSS in the mixed liquor, and decrease in SVI over the experimental period (see Figure 5.5). Figure 5.5 shows that after start-up of the aerobic granulation process, TSS and VSS initially decreased from ~2300 mg/L and ~930 mg/L to ~2200 mg/L and ~890 mg/L, respectively, before increasing to ~13300 mg/L and ~10400 mg/L, respectively. The difference between the solids content in the SBRs at start-up and after 250 days of operation was statistically significant (p value = 0.001 at 95% confidence interval limit, DF = 26). On the other hand, SVI decreased from ~142 mL/g on day 0 to ~25 mL/g on day 250, which is a statistically significant reduction (p value = 0.024 at 95% confidence interval limit, DF = 26).

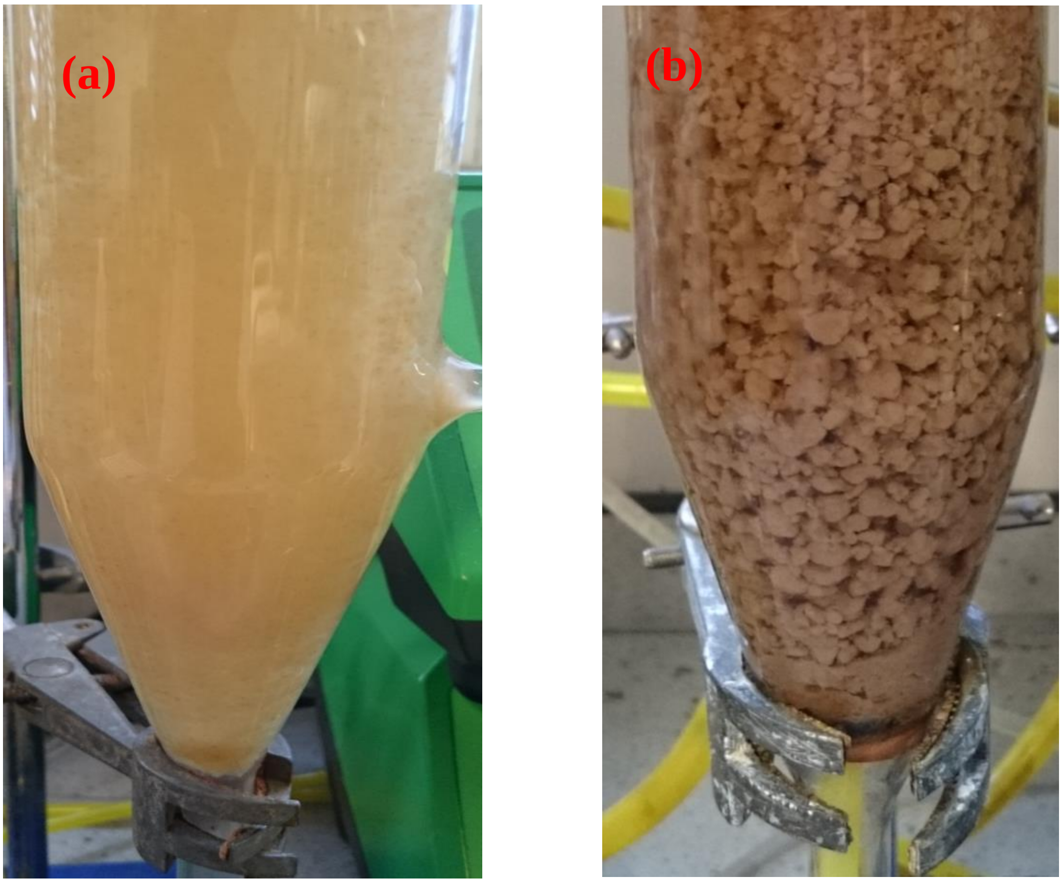

Figure 5.3: Aerobic granular sludge in SBR: (a) At start-up; (b) After 55 days operation

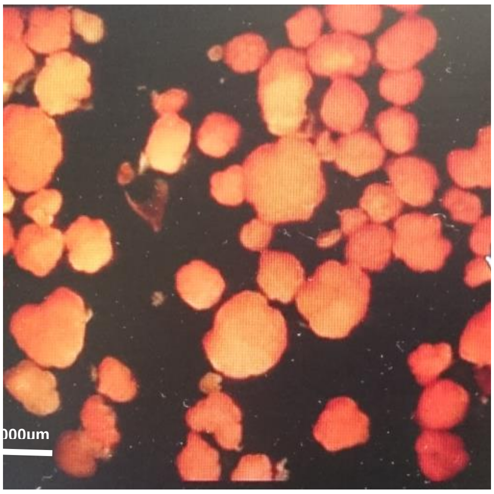

Figure 5.4: Image of Aerobic granules from SBR after 55 days operation: Microscopy

These observations are consistent with Wei *et al.* (2014) who found that TSS and VSS in treated effluent decreased after using aerobic granulation to treat high strength ammonia wastewater in SBRs, whereas SVI also was observed to decrease throughout the entire granulation process. The SVI_{30}/SVI_{10} ratio on Day 200 was ~95% (after rising from ~54% on Day 0), which indicated a completely granulated system according to recommendations in De Kreuk *et al.* (2007) and Wagner *et al.* (2015). The initial decrease in TSS and VSS at day 10 (see Figure 5.5) was linked to potential washout of poor settling sludge, while the decrease in the SVI resulted from improved settleability of sludge following agglomeration of 'flocs' to form larger dense compact AGs. The slight decrease (~2 %) in TSS after day 200, was probably linked to disintegration in AGs following the long operation of the SBRs and low COD/NH_4-N ratios (~1.2) as during that period the feeding condition was RLs only (with mean COD/NH_4-N ratios in RLs from Bran Sands WWTP being 1.2). This is consistent with Tay *et al.* (2002) and Show *et al.* 2012 who both reported that prolonged operation of aerobic granulation systems could result in the disintegration of AGs and subsequent deterioration in performance.

TSS in treated effluent was also observed to decrease (from ~285 mg/L at start-up to 35 mg/L on day 250) following aerobic granulation. This is similar to the observations of Wagner *et al.* (2015) where it was reported that TSS in treated effluent after granule formation in SBRs treating domestic wastewater also fell, from ~300 mg/L to 45 mg/L after 150 days of operation. Overall, these data indicate AGs truly were formed here, which has not always been successful in previous efforts at granule development with some wastes.

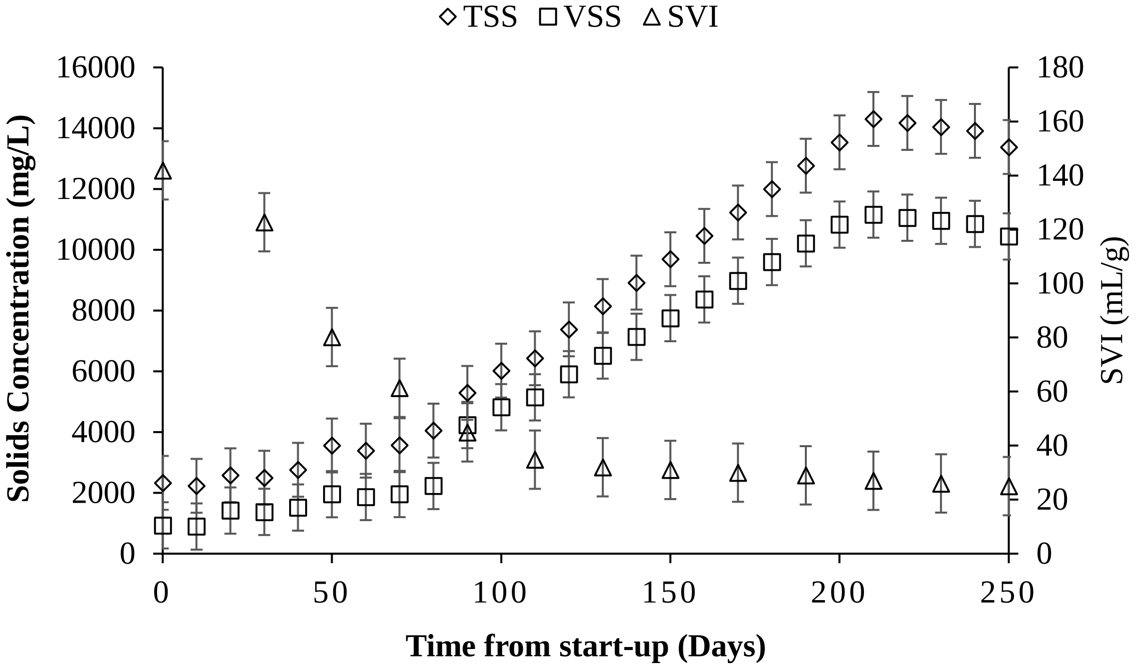

Figure 5.5: Variations in TSS, VSS and SVI during the aerobic granulation process in the SBRs. The data presented represent mean TSS, VSS and SVI values from the three identical SBRs operated (Error bars refer to standard errors). Feeding conditions in the SBRs were synthetic wastewater, synthetic wastewater + RLs, and RLs for the 150 days, Day 150 to 202, and day 202 to 250, respectively.

5.3.2 N Removal by aerobic granulation

Wagner *et al.* (2015) reported that during the aerobic granulation, N is transformed via three distinct mechanisms; i.e. assimilation, nitrification and denitrification. Table 5.1 shows variations in % NH_4-N removal as a direct consequence of changing NLRs, COD/NH_4-N ratios and feeding conditions in the three reactors here. Initial range-finding experiments were conducted using synthetic wastewater and synthetic wastewater mixed with RLs to verify granules could be formed and assess general trends between NLRs and treatment performance. One hundred percent RLs input also were assessed, but that was only at the previous optimal NLR and the COD/NH_4-N ratio (i.e. 1.2) typical of RLs from Bran Sands WWTP.

In general, NH_4-N removal rate increased with increasing COD/NH_4-N ratio in all NLRs and feeding conditions with mean % NH_4-N removal rising from 47 ± 0.5% (at NLR of 0.2 g NH_4-N/Ld with COD/NH_4-N ratio of 1.0) to 80 ± 3.0% (at NLR of 0.6 g NH_4-N/Ld with COD/N ratio of 4.0) in synthetic wastewater, and from 82 ± 3.0% (at NLR of 0.2 g NH_4-N/Ld with COD/NH_4-N ratio of 1.0) to 98 ± 0.4% (at NLR of 0.6 g NH_4-N/Ld with COD/NH_4-N ratio of 4.0) in Synthetic water + RLs. Overall, the difference in mean NH_4-N removal rate relative to COD/NH_4-N ratio alone was not statistically significant ($p = 0.471$). However, the combined effect of COD/NH_4-N ratio, NLR and feed conditions on NH_4-N removal rates in the reactors was statistically significant ($p = 0.008$).

This increase in % NH_4-N removal rate with increasing COD/NH_4-N ratio is probably due to increased stability of the AGs (i.e. the presence of mostly matured AGs typically >1 mm in diameter; Liu *et al.*, 2004) with increasing COD/NH_4-N ratio. This is consistent with the observations of Wan and Sperandio (2009) and Luo *et al.* (2014) who reported that reducing COD/NH_4-N ratio (to as low as 1.0) in treating industrial wastewaters with AG systems, resulted in the disintegration of granules and consequently reduced performance. However, with high COD loads (e.g. 6 kg COD/m^3.d), granules can become unstable and easily dominated by filamentous strains due to poor mass transfer as reported by Zheng *et al.* (2006). From Table 5.1, it can be seen across each of the NLRs, the highest N-removal was seen at COD/NH_4-N ratio 4.0. This implies that based on the observations made in this study, N-removal in AG systems requires COD/NH_4-N ratio in the reactor feed of between 2.5 and 4.0, but ideally closer to 4.0. Although t-test analysis using SPSS between data at COD/NH_4-N ratios of 2.5 and 4.0 did not show any statistically significant difference (i.e. P-value >0.05 at 95% confidence limit).

Table 5.1: Effect of Feed condition, nitrogen loading rate (NLR) and COD/NH_4-N ratio on NH_4-N removal from aerobic granulation reactors with synthetic wastewater and a mixture of synthetic wastewater and actual RLs from Bran Sands WWTP

NLR (g NH_4-N/Ld)	**COD/NH_4-N ratio**	**% NH_4-N removal**		
		Synthetic wastewater	**Synthetic wastewater + RLs**	**RLs**
0.2	1.0	47 ± 0.5*	82 ± 3.0	-
	1.2	-	-	47 ± 1.3
	2.5	48 ± 3.0	87 ± 2.3	-
	4.0	50 ± 1.5	93 ± 0.6	-
0.6	1.0	77 ± 0.7	90 ± 3.1	-
	1.2	-	-	64 ± 1.6
	2.5	79 ± 0.2	93 ± 1.7	-
	4.0	80 ± 3.0	98 ± 0.4	-
1.0	1.0	75 ± 2.2	73 ± 0.2	-
	1.2	-	-	-
	2.5	77 ± 0.9	77 ± 0.7	-
	4.0	79 ± 1.0	81 ± 2.1	-

*The data correspond to mean NH_4-N removal rate in the three identical SBRs. Only RLs data have been presented for COD/NH_4-N ratio of 1.2 since this is the mean COD/NH_4-N ratio for RLs samples from Bran Sands WWTP. Whereas COD/NH_4-N ratios 1.0, 2.5 and 4.0 were the test COD/NH_4-N ratios. NLRs 0.2, 0.6 and 1.0 g NH_4-N/Ld refer to 100, 300, and 500 mg/L NH_4-N concentrations in SBR feed. No data exist for RLs at NLR 1.0 g NH_4-N/Ld as pre-trial experiments at this NLR disrupted the stability and performance of the SBRs. *Uncertainty ranges in this study were presented as standard error of sample means*

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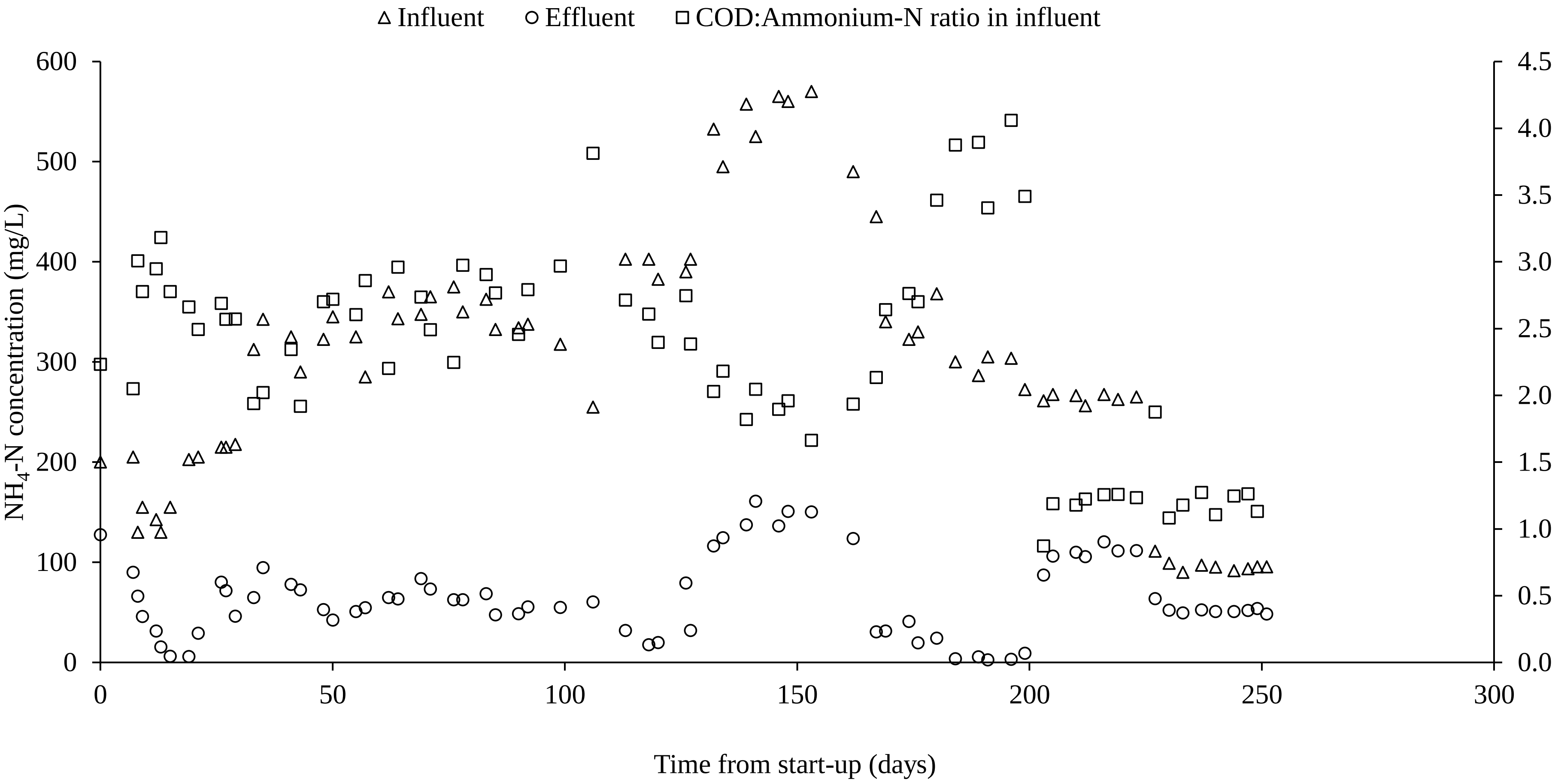

Figure 5.6: Effects of COD:NH_4-N ratio on mean NH_4-N levels in effluent from aerobic granulation SBRs over time. Data presented are mean NH_4-N concentrations in effluent from the three SBRs, for the various COD/NH_4-N ratios for the NLR and feed conditions presented. (Error bars refer to standard errors, n=30). NH_4-N concentrations in feed at NLRs 0.2, 0.6 and 1.0 g NH_4-N/Ld correspond to 100, 300, and 500 mg/L, respectively.

Actual effluent NH_4-N concentrations from the SBRs with aerobic granulation varied with feed conditions and NLR. Figure 5.7 summarises mean NH_4-N levels in effluent from the SBRs. It should be noted that a NLR of 1.0 g NH_4-N/Ld for 100% RLs was not tested because data with the synthetic and mixed feds implied the reactors would be susceptible to toxicity effects.

For all feeding conditions, mean effluent NH_4-N levels generally increased with increasing NLR, although removal rates were still comparatively high. This was expected as it is common that higher influent NH_4-N levels result in higher effluent NH_4-N levels. The lowest (i.e. ~12 ± 0.8 mg/L) and highest (~114 ± 7.8 mg/L) effluent NH_4-N levels were seen in synthetic wastewater plus RLs at NLRs 0.2 g NH_4-N/Ld and 1.0 g NH_4-N/Ld, respectively. However, these levels are still very low compared with influent NH_4-N levels.

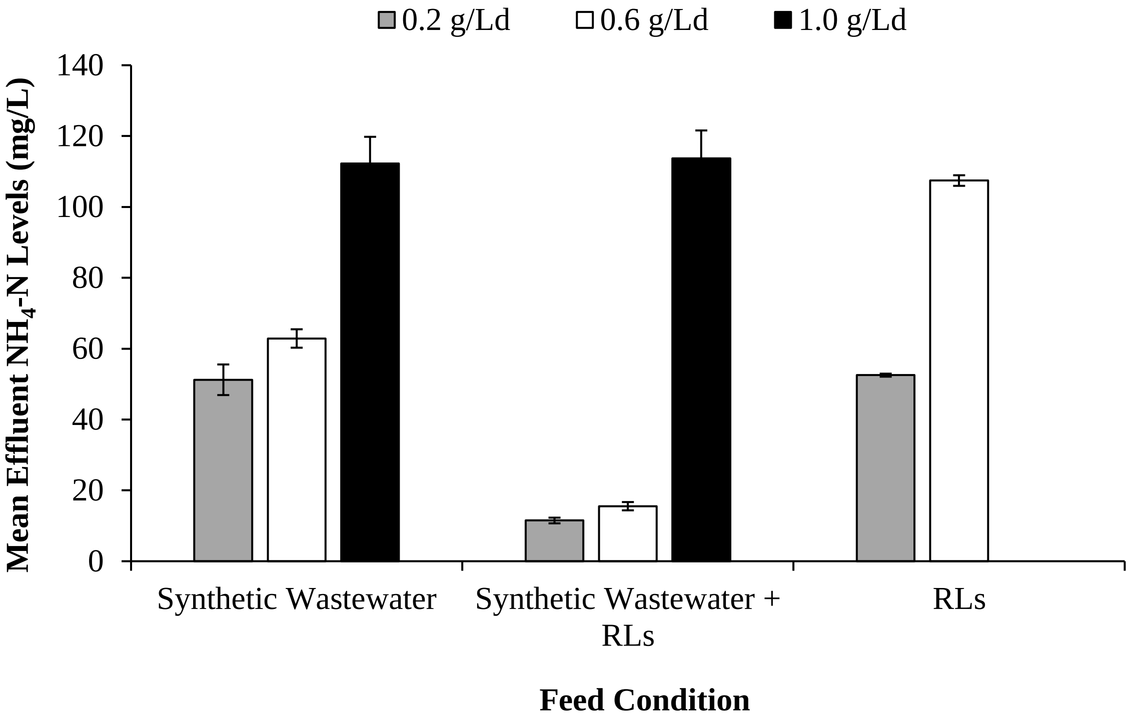

Figure 5.7: Effects of feed Condition on mean NH_4-N levels in effluent from aerobic granulation SBRs. Data presented are mean NH_4-N concentrations in effluent from the three SBRs, for the various COD/NH_4-N ratios for the NLR and feed conditions presented. (Error bars refer to standard errors, n=30). NH_4-N concentrations in feed at NLRs 0.2, 0.6 and 1.0 g NH_4-N/Ld correspond to 100, 300, and 500 mg/L, respectively.

Mean effluent NH_4-N levels roughly corresponded to the patterns of % NH_4-N removal shown on Figure 5.8. Overall, data show that NH_4-N removal rates by aerobic granulation were highest when the SBRs were fed with synthetic wastewater + RLs. This was probably due to the fact that experiments during this feeding regime were conducted between days 148 and 202 (i.e., the middle of the operating period), when most of the AGs formed were matured and stability in the SBRs was optimum. Table 5.2 shows that the difference in NH_4-N removal rates between 100% synthetic wastewater and synthetic wastewater + RLs feed conditions was statistically significant (i.e. $p<0.05$) for most of the NLRs. Figure 5.7 also shows that across each of the feeding conditions, % NH_4-N removal was highest at NLR of 0.6 g NH_4-N/Ld; with the highest % NH_4-N removal (~95 ± 1.1 %) seen at NLR of 0.6 g NH_4-N/Ld when the SBRs were fed with synthetic wastewater + RLs.

In contrast, NH_4-N removal decreased to ~77 ± 1.1 % when NLR was increased from 0.6 to 1.0 g NH_4-N/Ld during the synthetic + RLs feeding regime, probably due to toxicity, or high NH_4-N levels, for the SBRs. NH_4-N removal further decreased (to ~47 ± 0.4%) when the SBRs were first switched to a 100% RLs feed; i.e. at NLR 0.2 g NH_4-N/Ld. This could have resulted from disintegration of AGs when the COD/NH_4-N ratio was reduced to 1.2. However, when NLR increased to 0.6 g NH_4-N/Ld with 100% RLs, % NH_4-N removal rate improved to ~64 ± 0.6% (see Figure 5.8). This implies poorer performance at 0.2 g NH_4-N/Ld was not likely due to toxic effects of other components in the 100% RLs, but more likely under-feeding which potentially had a negative effect on effective granulation. This is a very promising result because it implies that AGT can work with 100% RLs. However, the key in practice will be identifying the “optimal” NLR for a particular application and allowing the reactors to slowly acclimate to real RL feed.

Overall, with % NH_4-N removal rate increasing when NLR increased from 0.2 and 0.6 g NH_4-N/Ld and decreasing when NLR was increased from 0.6 to 1.0 g NH_4-N/Ld, a suitable NLR for effective NH_4-N removal for Bran Sands RLs is between 0.2 and 1.0 g NH_4-N/Ld. Interestingly, past work (Cydzik-Kwiatkowska *et al.*, 2013) on other types of wastes indicated NLRs between 0.4 and 0.6 g NH_4-N/Ld are effective and work here confirms these rates also are suitable for RLs.

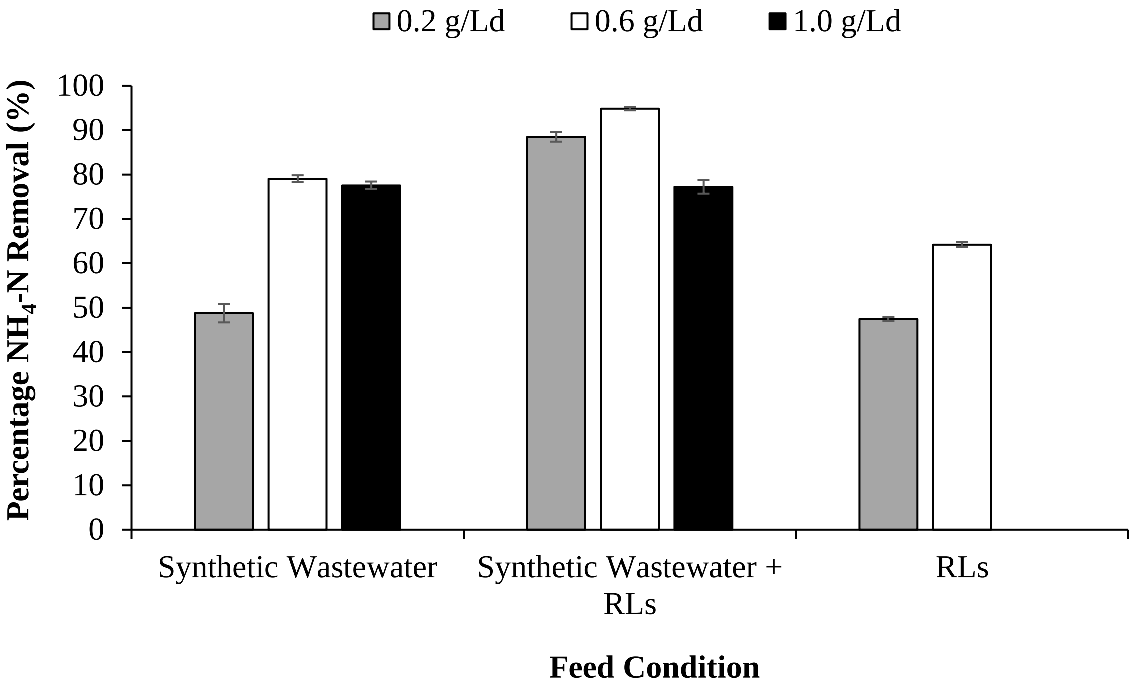

Figure 5.8: Effects of Feed condition on mean NH_4-N removal rate by aerobic granulation technology. NH_4-N removal data presented is a mean for the various COD/NH_4-N ratios for the different NLR and feed conditions presented. (Error bars refer to standard errors, n=30).

Table 5.2: Statistical Significance analysis on NH_4-N removal rates in the SBRs relative to NLR and feed condition

Feed conditions	**NLR (g NH_4-N/Ld)**	***P-values (at 95% confidence limit)***					
		Synthetic wastewater			**Synthetic wastewater + RLs**		
		0.2	**0.6**	**1.0**	**0.2**	**0.6**	**1.0**
Synthetic wastewater	**0.2**	1.00	0.001	0.001	0.007	0.003	0.007
	0.6	0.001	1.00	0.335	0.120	0.027	0.570
	1.0	0.001	0.335	1.00	0.093	0.024	1.00
Synthetic wastewater + RLs	**0.2**	0.007	0.003	0.007	1.00	0.207	0.078
	0.6	0.120	0.027	0.570	0.207	1.00	0.015
	1.0	0.093	0.024	1.00	0.078	0.015	1.00

$P>0.05$ = No statistical significance; $P<0.05$ = Statistical significance, n=30

The relative effect of COD/NH_4-N ratio, NLR and feed conditions also was assessed on N transformation in the AG SBRs (i.e., nitrification). Mean NO_3-N and NO_2-N levels under different operating conditions are summarised in Table 5.3 as a function of NLR and feed conditions. NO_x-N levels generally decreased as COD/NH_4-N ratio increased, probably due to improved competitive advantage of nitrifiers over heterotrophs within the granules (which breakdown COD). For instance, at a NLR of 0.6 g NH_4-N/Ld with the synthetic wastewater + RLs feed, NO_3-N levels in the effluent fell from 111 ± 2.5 mg/L (at COD/NH_4-N ratio of 1.0) to 87 ± 2.6 mg/L (at COD/N ratio of 4.0); i.e. a decrease of ~22 %. Overall, the difference in mean NO_3-N levels relative to COD/NH_4-N for the different feed was statistically significant ($p = 0.001$). This is consistent with other studies (Carrera *et al.*, 2004; Wu *et al.*, 2012), which observed nitrification levels improve in AG systems at lower COD/N ratios due to improved competition by autotrophic nitrifiers relative to resident heterotrophs. Table 5.3 also suggests complete oxidation of NO_2-N to NO_3-N at a NLR of 0.2 g NH_4-N/Ld with a synthetic wastewater + RL feed (for seemingly all COD/NH_4-N ratios). This probably results from the low NH_4-N loading rate, and the high stability and performance of the AGs at this point in the study.

Table 5.3: Effect of Feeding condition, NLR and COD/NH_4-N ratio on NO_3-N and NO_2-N levels in effluent from aerobic granulation SBRs

NLR (g NH_4-N/Ld)	COD/NH_4-N	NO_x-N levels (mg/L)					
		Synthetic wastewater		Synthetic wastewater + RLs		RLs	
		NO_3-N	NO_2-N	NO_3-N	NO_2-N	NO_3-N	NO_2-N
0.2	1.0	25 ± 0.9*	22 ± 0.7	63 ± 2.5	0.0	-	-
	1.2	-	-	-	-	43 ± 0.7	0.0
	2.5	19 ± 2.5	18 ± 0.5	27 ± 0.8	0.1	-	-
	4.0	11 ± 0.3	16 ± 1.2	25 ± 1.7	2.0	-	-
0.6	1.0	56 ± 1.8	13 ± 2.7	111 ± 2.5	7.06 ± 0.5	-	-
	1.2	-	-	-	-	95 ± 6.2	5.6 ± 3.1
	2.5	47 ± 3.0	11 ± 0.4	94 ± 5.1	5.35 ± 1.3	-	-
	4.0	45 ± 0.5	6 ± 0.2	87 ± 2.6	4.24 ± 0.2	-	-
1.0	1.0	44 ± 0.2	108 ± 4.5	35 ± 3.5	100 ± 3.3	-	-
	1.2	-	-	-	-	-	-
	2.5	40 ± 1.0	127 ± 2.1	27 ± 2.4	82 ± 1.9	-	-
	4.0	32 ± 0.9	139 ± 1.7	25 ± 0.2	64 ± 2.7	-	-

*These are mean NO_3-N and NO_2-N levels in effluent from the 3 SBRs. Only RLs data were presented for COD/NH_4-N ratio of 1.2 since this is the mean COD/NH_4-N ratio for RLs at Bran Sands WWTP. No data exist for RLs at NLR 1.0 g NH_4-N/Ld as pre-trial experiments at this NLR disrupted the stability and performance of the SBRs. *Uncertainty ranges in this study were presented as standard error of sample means*

Overall, nitrification was observed in all feeding conditions, probably due to good biomass retention in the systems once granules had been formed. Figures 5.9 and 5.10 show variations in NO_3-N and NO_2-N levels in effluent from the aerobic granulation SBRs, respectively, under different NLRs and feed conditions. The difference in mean NO_3-N levels relative to NLR for the different feed was statistically significant ($p = 0.004$). Like % NH_4-N removal data (in Table 5.1 and Figure 5.8), Figure 5.9 shows that nitrification was always greatest at 0.6 g NH_4-N/Ld as demonstrated by the high NO_3-N build-up, nitrification was “complete” only at the lowest NLR. In addition, Table 5.4 shows that differences in mean NO_3-N levels between NLRs 0.2 and 0.6 g NH_4-N/Ld were statistically significant ($p<0.05$). For instance, the difference in mean NO_3-N levels between NLRs 0.2 and 0.6 g NH_4-N/Ld with synthetic wastewater feed was statistically significant ($p = 0.010$). This further suggests the suitability of NLRs approaching 0.6 g NH_4-N/Ld as best for effective NH_4-N removal (via nitrification). Alternatively, low NO_3-N levels (see Figure 5.9) and comparatively higher NO_2-N levels were seen at NLR 1.0 g NH_4-N/Ld for the different feed conditions (excluding RLs where no testing was conducted at this NLR). This suggests potential inhibition of nitrite-oxidising bacteria (NOB) at the highest NH_4-N loading rate, which is confirmed by qPCR quantification of NOB and ammonia oxidising bacteria (AOB) in the reactors (see Chapter 6). This is consistent with Zanetti *et al.* (2012) who found elevated free NH_4-N loadings suppress NOB growth, while increasing the relative abundance of AOB.

Overall, Figures 5.9 and 5.10 show that relatively high NO_3-N levels and lower NO_2-N levels were seen when real RLs were in the feed relative to without RLs. For example, higher NO_3-N levels were seen at NLRs of 0.2 and 0.6 g NH_4-N/Ld when the reactors were fed with synthetic wastewater + RLs (i.e. 40 ± 1.5 mg/L and 100 ± 7.9 mg/L, respectively) and RLs (i.e. 43 ± 0.2 mg/L and 94 ± 6.2 mg/L, respectively) relative to levels observed for the same NLRs when synthetic wastewater alone was the feed (i.e. 17 ± 4.8 mg/L and 48 ± 3.1 mg/L, respectively). Table 5.4 shows that the differences in effluent NO_3-N levels between the feed conditions were generally statistically significant ($p<0.05$).

The highest NO_2-N levels (125 ± 1.6 mg/L) was seen at NLR 1.0 g NH_4-N/Ld with synthetic wastewater feed. The difference in mean NO_2-N levels between feed with real RLs and those without RLs also was statistically significant ($p = 0.001$). The relatively higher nitrification activity in feeding conditions with RLs component was linked to the effect of the low COD/NH_4-N ratio (i.e. 1.2) of RLs on the growth performance of autotrophic nitrifiers. It was also linked to the low biodegradability of the COD in RLs (compared to the easily biodegradable COD (i.e. acetate) in synthetic wastewater) which could potentially have inhibited the growth of heterotrophs; thereby promoting the competitive advantage of nitrifiers (see Chapter 6).

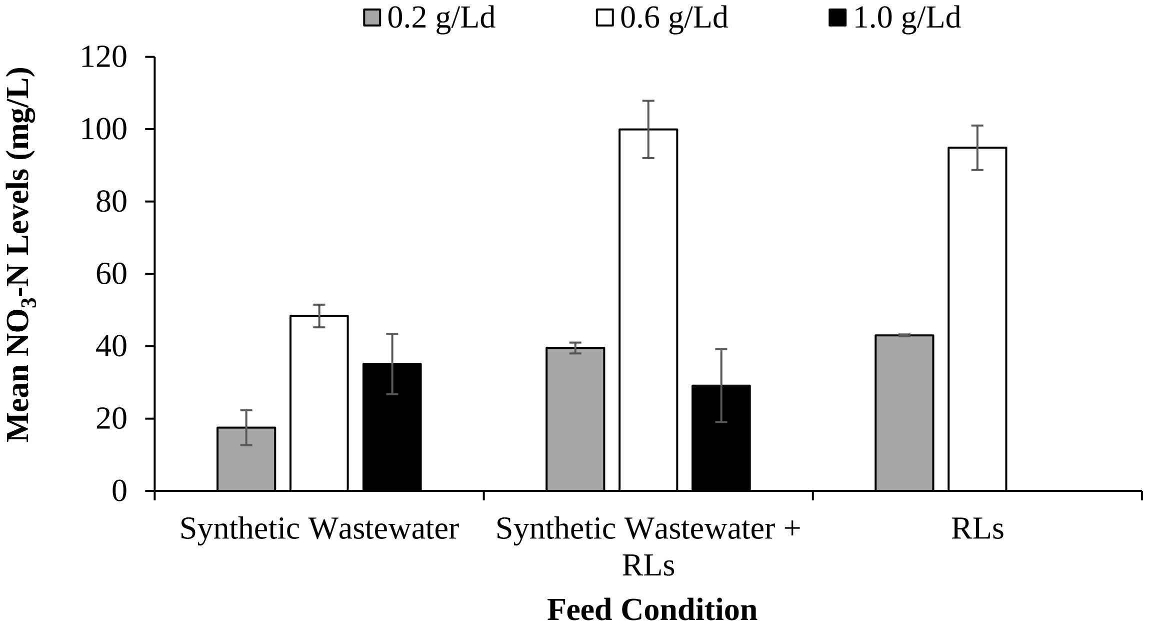

Figure 5.9: Effects of Feed condition on mean NO_3-N levels in effluent from aerobic granulation SBRs. NO_3-N data presented correspond to the mean NO_3-N concentration in the effluent from the three SBRs for the various COD/NH_4-N ratios for the different NLR and feed conditions presented. (Error bars refer to standard errors, n=30).

Table 5.4: Statistical Significance analysis on NO_3-N levels in effluent from the reactors relative to NLR and feed condition

Feed conditions	**NLR (g NH_4-N/Ld)**	***P-values (at 95% confidence limit)***					
		Synthetic wastewater			**Synthetic wastewater + RLs**		
		0.2	**0.6**	**1.0**	**0.2**	**0.6**	**1.0**
Synthetic wastewater	**0.2**	1.00	0.010	0.032	0.264	0.002	0.126
	0.6	0.010	1.00	0.117	0.481	0.026	0.021
	1.0	0.032	0.117	1.00	0.982	0.018	0.130
Synthetic wastewater + RLs	**0.2**	0.264	0.002	0.126	1.00	0.026	0.539
	0.6	0.481	0.026	0.021	0.026	1.00	0.013
	1.0	0.982	0.018	0.130	0.539	0.013	1.00

$P>0.05$ = No statistical significance; $P<0.05$ = Statistical significance, n=30

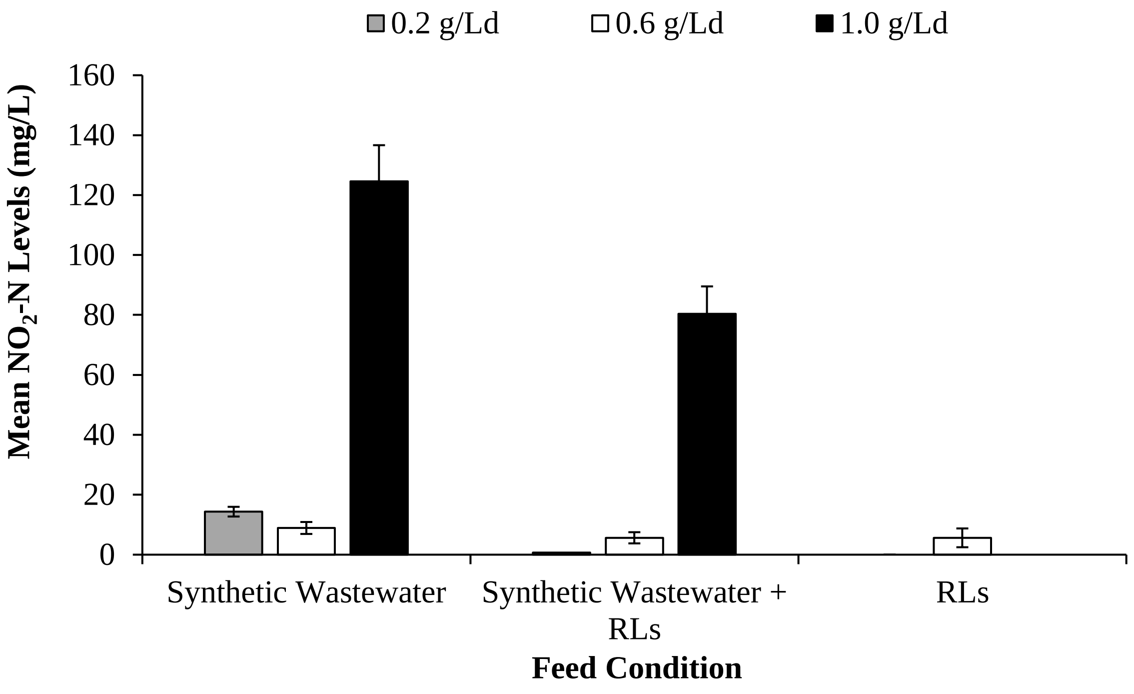

Figure 5.10: Effects of Feed condition on mean NO_2-N levels in effluent from aerobic granulation SBRs. NO_2-N data presented correspond to the mean NO_2-N concentration in the effluent from the three SBRs for the various COD/NH_4-N ratios for the different NLR and feed conditions presented. (Error bars refer to standard errors, n=30).

5.3.3 P Removal by aerobic granulation

Figure 5.11 summarises PO_4-P removal rates in AG systems relative to NLR and feed condition. Like NH_4-N, PO_4-P removal rates generally increased with synthetic wastewater feed between NLRs 0.2 and 0.6 g NH_4-N/Ld (except with 100% RLs feed), but decreased between NLRs 0.6 and 1.0 g NH_4-N/Ld. For example, PO_4-P removal efficiency increased from 2 ± 1.5% (at a NLR of 0.2 g NH_4-N/Ld) to 11 ± 0.5% (at a NLR of 0.6 g NH_4-N/Ld), and then decreased to -12 ± 2.8% (at a NLR of 1.0 g NH_4-N/Ld). The decrease in PO_4-P removal rates at NLRs >0.6 gNH_4-N/Ld was probably due to the inhibitory effects of growing nitrite levels according to Saito *et al.* (2004) and Peng *et al.* (2011) who both reported that excessive nitrite concentrations (i.e., >5.0 mg NO_2-N/L) and its derivatives in the mixed liquors inhibited the growth of phosphate accumulating organisms (PAOs).

PO_4-P removal rates was highest at each NLR for the reactors fed with synthetic wastewater, and lowest when fed with 100% RLs. The difference in PO_4-P removal rates relative to NLR and feed condition, also was statistically significant ($p = 0.001$). The relatively higher PO_4-P removal rates with synthetic wastewater feed was probably due to its comparatively higher easily biodegradable COD (i.e. acetate) content which could have potentially promoted the growth of PAOs, as opposed to the low biodegradability of the complex organic compound making up the COD in RLs. This is consistent with previous studies (Sathasivan *et al.,* 1999; Oehmen *et al.,* 2007) which reported that reactors fed with acetate showed comparatively higher PO_4 accumulating activities and improved PAO abundance than with feeds containing other substrates.

Figure 5.11 shows potential P-release (based on the negative in PO_4-P removal efficiencies) in the AG SBRs for all feeding conditions and NLRs except synthetic wastewater feeding condition at NLRs 0.2 and 0.6 g NH_4-N/Ld. Given that SBRs were first operated on synthetic wastewater (as follows: at NLR 0.2 g NH_4-N/Ld for the first 30 days, at NLR 0.6 g NH_4-N/Ld for the next 60 days and at NLR 1.0 g NH_4-N/Ld for the next 16 days), then synthetic wastewater + RLs; and finally 100% RLs, it is possible that the potential release of P observed in the feed conditions after synthetic wastewater feed condition at 0.6 g NH_4-N/Ld could have resulted potential local dissolved oxygen and pH gradients in the mature dense spherical aerobic granules. This is consistent with reports by Liu & Tay (2004) and Adav *et al.* (2008) that the dense-spherical structure of aerobic granules promote local pH and DO gradients due to transfer limitations and biological reactions (e.g. nitrification and denitrification). In addition, De Kreuk *et al.* (2005), Lemaire (2007) and Mañas, *et al.* (2011)

reported that biological induced precipitation of P as hydroxyl-apatite ($Ca_5(PO_4)_3(OH)$) may occur in aerobic granules, but as the granules grow denser with time DO and pH gradients may be created across the granular structure which may cause the anaerobic release of accumulated P and resolubilization of the constituent ions of the precipated compound. It is assumed that a similar biologically induced P precipitation may have occurred in the granules during the early granulation stages (i.e. when the SBRs when operated on synthetic wastewater at NLRs 0.2 and 0.6 g NH_4-N/Ld), however as the granules grew denser with time, local conditions became conducive for the resolubilization P from the precipitated compound. Hence the observed negative P-removal efficiency in the SBRsin feed conditions beyond synthetic wastewater feeding condition at NLRs 0.2 and 0.6 g NH_4-N/Ld.

Overall, this study shows that PO_4-P removal through the separate treatment of RLs using AGT, was not as good as NH_4-N removal. However, combining Mg addition post-AD and AGT can effectively remove NH_4-N and PO_4-P.

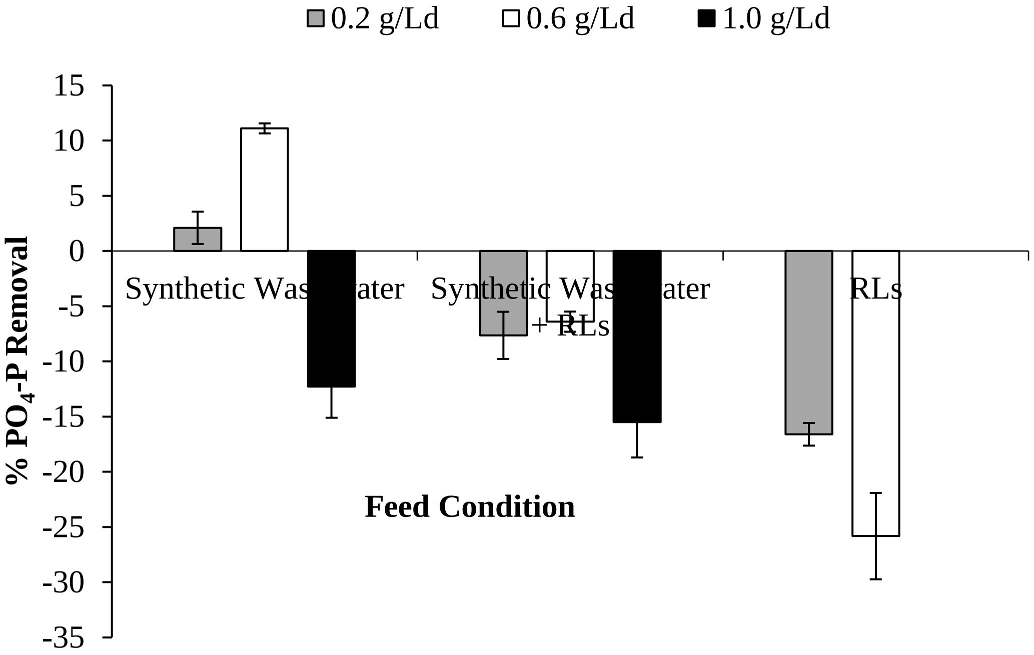

Figure 5.11: Effects of Feed condition on mean PO_4-P removal by aerobic granulation SBR. PO_4-P data presented correspond to the mean PO_4-P concentration in the effluent from the three SBRs for the various COD/NH_4-N ratios for the different NLR and feed conditions presented. (Error bars refer to standard errors, n=30).

5.3.4 Economic analysis of N-removal from RLs by aerobic granulation

A simple cost analysis was performed to assess the relative value of separate treatment (i.e. for NH_4-N removal) of RLs for Bran Sands WWTP. AGT was compared with nitrification in activated sludge systems (currently used). As background, Fux (2003) estimated the cost of conventional nitrification for a full-scale activated sludge (CAS) plant is 8.0 €/kg$N_{removed}$ (i.e. 6.67 £/kg$N_{removed}$). Gray (2004) reported that, theoretically *Nitronomas* require ~3.43g of molecular oxygen to oxidise 1g of ammonia to nitrite while *Nitrobacter* require a further 1.14g to oxidise 1g of nitrite to nitrate. Additionally, Montgomery and Bourne (1966) estimated that the oxygen equivalent of ammonia and nitrite assimilated as cell material during synthesis were at 0.2g and 0.02g, respectively. Therefore, to oxidise the 4.7 tonnes of NH_4-N load in wastewater (RLs inclusive; see Figure 3.9) entering the CAS units daily at Bran Sands WWTP to nitrate (with considerations of potential assimilation into cells) will theoretically require ~20.4 tonnes of molecular oxygen; ~46% of which can be attributed to nitrifying the 2.14 tonnes NH_4-N/day in RLs (i.e. RL1 and RL2 ; see Figure 3.9) if treated separately via CAS. Additionally, Shi (2011), reported 0.09 kWh/m^3 as the specific energy/electricity consumption for nitrification at a typical municipal sewage treatment plant. This implies that potential combined daily energy consumption of nitrifying wastewater entering the CAS units at Bran Sands WWTP is ~ 9205.11 kWh/day; of which ~4234.3 kWh/day can be attributed to NH_4-N removal from RLs via nitrification.. On the other hand, De Bruin *et al.* (2004), van Loosdrecht and De Kreuk, (2004), and de Kreuk (2006) indicate that AGT requires only 25% of the surface area and 70% of energy required for full-scale CAS systems.

Using these and other assumptions factors (presented on Table C.1), Table 5.5 summarises the cost of treating RLs at Bran Sands via CAS; estimated cost of separate treatment of RLs (without any addition of COD) prior to it being returned to CAS for polishing. Individual cost items were estimated based on the unit cost of NH_4-N removal from RLs (expressed in some cases as £/kg$N_{removed}$). From previous data (see Figure 3.9), the NH_4-N load in RLs to be treated is 2.14 tonne/d and the design NLR is assumed to be 0.6 g NH_4-N/Ld, according to laboratory work here. $N_{removed}$ for costs involved in CAS has been estimated using 95 % NH_4-N removal which is the current mean N-removal rates via CAS at Bran Sands WWTP. Also this removal rate refers to the minimum required to comply with limits (250 mg NH_4-N/L) in the discharge consent for the site. While 64 % and 95 % NH_4-N removal are used for cost estimates involved in treating RLs (without additional COD) and subsequent final treatment via CAS.

Investment cost for treating RLs via CAS is estimated using 1.35 €/kgN$_{removed}$ (i.e. 1.08 £/kgN$_{removed}$) as reported in Fux (2003). While ~35% discount iss applied to this investment to treating RLs via AGT, since De Bruin *et al.* (2004), van Loosdrecht and De Kreuk, (2004), and de Kreuk (2006) report that AGT requires a 25 – 35% discount applied to the surface area needed for CAS. However, there is an additional investment cost of a 'balancing tank' to help AGT SBRs deal with flow variations and relatively high suspended solids content in RLs. For instance, Table 5.5 shows that investment cost for treating RLs via CAS is £1,669,600/yr, while that for the separate treatment of RLs (without additional COD) via AGT is £1,124,800/yr with additional £300,400/yr for returning the RLs to CAS in order to achieve 95 % N-removal. This implies the investment cost involved in the separate treatment of RLs (without additional COD) via AGT prior to recycling to CAS at Bran Sands WWTP could potentially be reduced by ~14.6 % (i.e. £244,400/yr). This saving is slightly less than half of the 25 – 35% discount/savings reported by De Bruin *et al.* (2004), van Loosdrecht and De Kreuk, (2004), and de Kreuk (2006) for AGT compared to CAS. This could be due to the fact that retrofitting the current treatment system on site may need some time for the AG microbial community to acclimatise before the technology becomes substantially profitable/cost effective. In addition, this investment savings can be offset by the addition, of the cost of a balancing tank which as mentioned earlier will be necessary to deal with varying flows and solids in RLs. Another advantage of treating RLs separately prior to recycling them to CAS units compared to their direct recycling back to the CAS units is that they could potentially reduce the potential for uncontrolled struvite precipitation. For instance, separately treating RLs could potentially reduce the IAP for struvite component ions from 9.3 x 10^{-9} after post-AD Mg addition of Mg (i.e. 150mg/L Mg^{2+}) to 1.7 x 10^{-10} which is closer to the k_{sp} value for struvite.

Operation cost for treating RLs is estimated based on the following cost items; energy (required for nitrification), maintenance, and sludge disposal costs. Their costs are estimated based on assumptions presented on Table C.1. Overall, it can be seen that potential savings of ~18% (i.e. £327,800/yr) can be made from the separate treatment of RLs (without COD addition) via AGT prior to recycling to CAS compared to directly recycling RLs to CAS. This savings is similar to the 25% operational cost savings reported by De Bruin *et al.* (2004), van Loosdrecht and De Kreuk, (2004), and de Kreuk (2006) as one of the major benefits of AGT over CAS.

Table 5.5: Cost Estimate and comparison of NH_4-N treatment costs for RLs under three treatment scenarios

Cost Item	Current costs of treating RLs via CAS at Bran Sands WWTP (£/yr)	Estimated costs of treating RLs (without COD addition) via AGT (£/yr)	Estimated costs of treating RLs (with COD addition) via AGT (£/yr)
Investment Cost	1,669,600	1,124,800	300,400
Balancing tank	-	Unknown cost**	Unknown cost**
Operation cost			
Energy costs (for nitrification)	382,700	193,400	137,800
Maintenance (Analysis, Replacement/Repair)	618,400	416,600	222,500
Sludge Disposal	463,800	0	166,900
Total Costs	**3,134,500**	**1,734,800***	**827,500***

*******No reasonably practicable unit cost for balance tank was found in literature explored.*
**Amount could increase when the cost of the balancing tank is included*

Finally, the separate treatment of RLs (without COD addition) via AGT can be a potentially cost effective technology for the separate treatment of NH_4-N in RLs with a potential 18% savings in overall costs (excluding the cost of a balancing tank) compared to CAS. However, with only 64% NH_4-N removal, the effluent will need additional polishing. Hence recycling the effluent to CAS will incur an additional total cost of £827,500/yr, thereby bringing up the total cost to £2,562,300/yr.

5.4 Conclusion

AGT was seen to be an effective biological treatment option for NH_4-N removal from RLs. NH_4-N removal via nitrification was observed to increase with increasing COD/NH_4-N ratios in all tested feed conditions. Overall, a comparison between the different feeding conditions showed that NH_4-N removal by AGT was highest when the SBRs were fed with synthetic wastewater + RLs. NH_4-N removal rates for each of the feeding conditions was seen to rise between NLR of 0.2 and 0.6 g NH_4-N/Ld. Nitrification activity was also seen to be highest at NLR of 0.6 gNH_4-N/Ld, especially with RLs as one of the feed components.

The most positive result is that removing NH_4-N from RLs without synthetic feed may be feasible and employing a NLR of between 0.2 and 0.6 g NH_4-N/Ld might be a good starting point for pilot-scale testing. Finally, a simple cost analysis shows that separate treatment of RLs via AGT with the addition of COD could be a cost-effective technology for NH_4-N removal from RLs. However, the overall any potential financial savings realised compared to the current practice of directly recycling RLs back to CAS units at Bran Sands WWTP may be affected by the need for a balancing tank to deal with varying flows and a mechanism to separate some of suspended solids from RLs which could be a potential hindrance to the smooth performance of the technology. The AGT experiments also showed a potential to further reduce the possibility of ‘uncontrolled’ struvite precipitation downstream of the AD, particularly in RLs conveying lines following the dewatering of digested sludge. In addition, this also highlights reduced NH_4-N loads being recycled to the CAS units with other associated problems (e.g. failing compliance, scaling, high energy consumption). The one element not discussed in Chapter 5 is the associated microbial communities, which will be examined in greater detail in Chapter 6.

Chapter 6: Effects of feeding conditions and nitrogen loading rates on nititrifying communities in Aerobic Granular Systems

6.1 Introduction

Aerobic granule (AG) systems are increasingly being used to remove nutrients and organic compounds from industrial, toxic and municipal wastewaters, at both high and low loading rates (Khan *et al.,* 2013; Szabó *et al.,* 2016). AGs are self-mobilized microbial aggregates characterized by compact structures and good settleability, which helps increase biomass retention and reduce suspended solids in treated effluent. In terms of AGs capable of nitrification, they have been found to have layered structures, which facilitate the coexistence of nitrifying (in the outer aerobic layers), and denitrifying (in the inner anoxic/anaerobic layer) strains within the same aggregates (Gieseke *et al.,* 2001; Winkler *et al.,* 2013; Pronk *et al.,* 2015). This coexistence of different microbial populations in AGs has been shown to facilitate the simultaneous removal of varied N, P and COD from wastewater (de Kreuk *et al.,* 2005).

Biological N-removal via AGT is driven by the following groups of bacteria; denitrifying bacteria, ammonia oxidising bacteria (AOB), nitrite oxidising bacteria (NOB), and anaerobic AOB (anammox) (Caffaz *et al.,* 2008; Cervantes, 2009; Gustavsson, 2010). Previous work has shown that certain factors, including volumetric exchange ratio, settling time, and hydrodynamic shear force, primarily influence the performance of aerobic granulation systems (Tay *et al.,* 2004; Liu *et al.,* 2007; Adav *et al.,* 2009; Zhu *et al.*, 2013; Bindhu and Madhu, 2014). These factors have often laid emphasis on process performance, and the chemical and physical parameters of AGs, but with limited understanding on their effect on microbial community assembly in the AGs. Further, there is very limited information on how feed conditions affect bacterial community composition in aerobic granular sludge, which is actively performing nitrification.

Therefore, this study aimed at using AGT in SBRs to explore nitrification activity and bacterial community dynamics during; (i) three different feeding conditions (i.e. synthetic wastewater, synthetic water + RLs, and RLs only); and (ii) three different N-loading rates (0.2, 0.6 and 1.0 g NH_4-N/Ld). Within this chapter, selected microbial levels are quantified, especially related to the relative abundance of NOB and AOB in reactors with different feeds and NLRs, and with differing nitrification performance.

6.2 Methodology

6.2.1 Experimental Set-up and Operation

Three identical column glass reactors were used as SBRs and operated for 250 days under NLRs and different feed conditions as presented in Section 5.2.1 in Chapter 5. Tables 6.1 and 6.2 summarise the feed and operating conditions for the reactors, respectively throughout the study.

Table 6.1: Test feed conditions for the reactors

Test	Time (Days)	Feed Condition	Parameters			
			Influent NH_4-N (mg/L)		NLR (g NH_4-N/Ld)	Influent COD (mg/L)
I	100	Synthetic wastewater	a)	100	0.2	250
			b)	300	0.6	760
			c)	500	1.0	1250
II	100	Synthetic wastewater + RLs	a)	100	0.2	250
			b)	300	0.6	760
			c)	500	1.0	1250
III	50	RLs	a)	100	0.2	120
			b)	300	0.6	360

Table 6.2: Operating conditions for the reactors

Operating Conditions	Unit	Value
H/D ratio	-	8
Cycle Time	hr	6
Feed time	min	3
Aeration time	min	349
Settling time	min	5
Withdrawal time	min	3
Volumetric exchange ratio	%	44
Mean DO level	mg/L	2.1
HRT	hr	14.4

6.2.2 Media/Influent and seed sludge

Table 6.3 shows the components of the stock solution (recommended by Vishniac and Santer, 1957; Tay *et al.*, 2001; Pronk *et al.*, 2013) used for preparing the synthetic wastewater used in the study. The reactors were seeded with nitrifying sludge from Bran Sands WWTP as shown in Section 5.2.2 in Chapter 5. The influent parameters were as presented in Table 6.1.

Table 6.3: Media/stock solution Composition

Chemical	Unit	Value
sodium acetate	mM	64
$MgSO_4.7H_2O$	mM	3.6
KCl	mM	4.7
NH_4Cl	mM	35.4
K_2HPO_4	mM	4.2
KH_2PO_4	mM	2.1
Trace elements solution (Vishniac and Santer, 1957; Tay *et al.*, 2001)	mL/L	10

6.2.3 DNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

50 mL mixed liquor samples were collected (approximately from the centre of each reactor during aeration/mixing, by manually drawing it up through a 9-mm-internal-diameter-sampling-tube connected to a 100ml syringe) two times a week (on Mondays and Fridays) and stored at -20˚C. The samples were then sent to Strathclyde University, Glasgow, for DNA extraction and qPCR analysis. DNA from the samples were extracted and purified by MoBio PowerClean Soil DNA kit (Cambio, Cambridge, UK). Before extraction, 5 – 10 mL samples were centrifuged (10X g; for 10 min) to remove excess liquid. DNA was extracted directly from pelleted suspended solids according to manufacturer's instructions. A FastPrep24 (supplied by MP Biomedical) was used for cell disruption.

Organisms assayed include total eubacteria, AOB, ammonia oxidising archaea (AOA), and two major species groupings of NOB. Quantification was performed using qPCR (BioRad iQ; BioRad, Hercules, CA) on purified community DNA.

Consumables used for qPCR analysis include: Bio-Rad 96-well qPCR green plates (PN:HSP-9645), Bio-Rad SSoFast EvaGreen mix Supermix (PN: 172-5202 for 1000 x 20 µL reactions), Bio-Rad 96-well plate microseal 'B' film (PN: MSB1001) and 500 nMprimer concentrations. Prior to carrying out qPCR, all equipment were cleaned with 70% ethanol and transferred into a microbial flow cabinet (which had also been cleaned with 70% ethanol) to prevent contamination. All samples (including standards) were diluted 1:100 with molecular grade water, as reactions were predetermined to be most efficient at those samples concentrations. All standards, samples and No Template Controls (NTCs) were done in triplicate. Instead of the 3 µL DNA used in the sample and standards, it was replaced with 3 µL of filtered sterile water in the NTCs so as to ensure no contaminants were present that could have caused amplification. The qPCR mix were prepared as below. The master mix for all the qPCR reactions was first prepared in a single, before the qPCR mix. The volume of the master mix prepared was slightly more than required in order to cater for pipetting errors.

<u>qPCR Mix (10 µL reaction):</u>

$$\left.\begin{array}{l}\left.\begin{array}{l}\text{5 }\mu\text{L SSoFast EvaGreen Supermix,}\\ \text{0.5 }\mu\text{L Forward Primer (dilution 1:10),}\\ \text{0.5 }\mu\text{L Reverse Primer (dilution 1:10),}\\ \text{1 }\mu\text{L Water (filtered sterilised)}\end{array}\right\}\text{ master mix}\\ \text{3 }\mu\text{L DNA (standards or samples) or 3 }\mu\text{L filtered sterilised water (NTC).}\end{array}\right\}\text{ qPCR mix}$$

7 µL of the master mix was manually dispensed into each of the wells (needed) in the qPCR plates. This was followed by the addition of 3 µL of standard, sample or filtered sterilised water (for NTC) in the appropriate wells and manually mixed using the pipette. Once the plate was ready, the “microseal” film was placed on top of it by using the perforated handles in order to avoid touching the film, thereby preventing the transfer of dirt on to the seal that could have caused fluorescence and thus amplification during the run. Lens cleansing tissue was then used to rub the seal so it sticks to the plate well with no gaps to avoid evaporation during the qPCR cycle. The qPCR plate was then loaded onto the thermal cycler of the Bio-Rad instrument and run as follows: 50˚C for 2 mins, polymerase activation at 95˚C for 10 mins, 40 cycles with melting at 94˚C for 20 secs annealing at specific temperatures (see Table 6.4) for 1 min, and elongation at 72˚C for 40 secs. Standards and post-analytical melting curves (Smith *et al.*, 2004) were generated to verify PCR reactions quality and quantify results.

Gene assays and primer sequences were based on methods previously developed as presented in Table 6.4:

Table 6.4: Oligonucleotide primers and probe sequences used in the study

Target Organism	Primer/Probe	Sequence (5’-3’)	Annealing temp (˚C)	Reference
‘Total’ bacteria	1055f	ATGGCTGTCGTCAGCT	50	Harms *et al.* (2003)
	1392r	ACGGGCGGTGTGTAC		
	16STaq1115	HEX-CASCGAGCGCAACCC-TAMRA		
AOA	Arch-amoAF	STAATGGTCTGGCTTAGACG	60	Chen *et al.* (2008)
	Arch-amoAR	GCGGCCATCCATCTGTATGT		
AOB	AmoA-1F	GGAGRAAAGCAGGGGATCG	60	Chen *et al.* (2008)
	AmoA-2R	CCCCTCKGSAAAGCCTTCTTC		
Nitrobacter spp.	Nitro-1198f	ACCCCTAGCAAATCTCAAAAAACCG	58	Graham *et al.* (2007); Knapp and Graham (2007)
	Nitro-1423r	CTTCACCCCAGTCGCTGACC		
	Nitro-1374Taq	FAM-AACCCGCAAGGAGGCAGCCGACC-TAMRA		
Nitrospira spp.	Nspra-675f	GCGGTGAAATGCGTAGAKATCG	58	Graham *et al.*, 2007; Knapp and Graham, 2007
	Nspra-746r	TCAGCGTCAGRWAYGTTCCAGAG		
	Nspra-723Taq	FAM-CGCCGCCTTCGCCACCG-TAMRA		

No signals were seen for AOA. Single primer/probe sets were used to quantify ‘total’ eubacteria and AOB, while NOB abundance was the combined signal of *Nitrospira spp.* and *Nitrobacter spp.* primer/probe sets. Standards included purified plasmids containing cloned 16S rRNA genes including *Nitrospira defluvii* (#DQ059545; Spieck *et al.*, 2006), *Nitrospira moscoviensis* (#X82558; Maixner *et al.*, 2006), *Nitrobacter spp.* (GenBank #DQ388518;

Hawkins *et al.,* 2006) and unspecified AOB (Graham *et al.,* 2007; Knapp & Graham, 2007). Probes and primers were used instead of hydrolysis dyes (e.g. SYBR green) due to reports (Bustin *et al.,* 2005) that the former is more reliable than the latter. QPCR results were reported as 16S rRNA gene abundances based on the assumption that one rRNA operon exists per NOB and AOB cell (Klappenbach *et al.,* 2001; Knapp & Graham, 2007).

qPCR is a technique that enables fast, quantitative and reliable microbial analysis. The technique was chosen due to its ability to identify amplified fragments during the PCR process (i.e. it measures the PCR products during the exponential phase) as opposed to the traditional PCR which measures the product at the end (i.e. at the plateau phase). Another advantage of qPCR is that the entire PCR process (from amplification to analysis) takes place in the same tube; whereas with traditional PCR, the product needs to be moved, manipulated into other forms and analysed possibly via agarose gel electrophoresis, which is not only time consuming but could also increase chances of contamination. In addition, gel electrophoresis has been reported to be unsuitable for laboratory purposes (Bustin *et al.,* 2005; Bustin, 2010; Taylor *et al.,* 2010).

6.2.4 Data Analysis

All gene-abundance data were log-transformed and tested for normality prior to statistical analyses using SPSS. Statistical tests used include Mann-Whitney t-test and Spearman-Rho correlation analyses (for data which was not normal), and t-test and Pearson bivariate correlation analyses (for normal data). Statistical significance (at 95% confidence limit) between the abundances of NOB and AOB relative to feed conditions and NLRs was tested. Uncertainty ranges in this study were presented as standard error of sample means.

6.3 Results and Discussions

The abundance of total bacteria, AOB and NOB were measured by quantifying the 16S-rRNA gene copies per volume of the reactors under different NLR and feed compositions in the AG SBRs. The mean “total bacteria” abundance in the reactors under different feed conditions and NLRs are summarised in Figure 6.1. Regardless of the feed conditions, the abundance of total eubacteria was always highest at a NLR of 0.6 gNH_4-N/Ld (except during the RLs feeding condition), with the highest abundances (1.20×10^{10} bacteria gene copies/mL) being observed with synthetic wastewater feed alone. This basically means that bacterial abundance was highest when the reactors were being fed the most biodegradable feed at an optimal loading rate, which is consistent with expectations. High eubacterial gene abundances are consistent with the high biomass levels, which are summarised in Chapter 5. A positive correlation (R = 0.68) was seen between increased biomass retention and total eubacterial abundance in the reactors. No statistical significance was seen between mean total eubacteria abundance in the different feed conditions (Mann Whitney t-test and t-test; P>0.05; see Table 6.5).

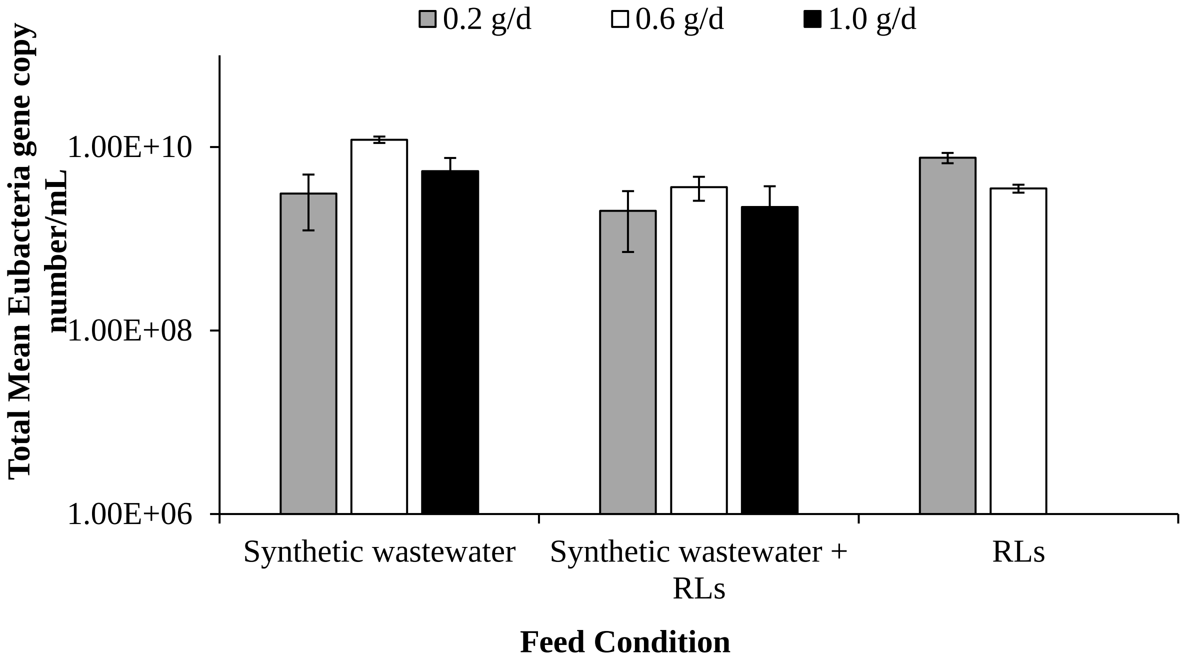

Figure 6.1: Variations in Total mean eubacteria abundance in aerobic granulation SBRs due to varying feed conditions. The y-axis is shown in a Logarithmic scale. Data summarises mean eubacteria data from the mean of different COD/NH_4-N ratios in Chapter 5. Error bars refer to standard errors.

Table 6.5: Statistical Significance analysis for total eubacteria abundance in the reactors

Feeding conditions	***P-values (at 95% confidence limit)***		
	Synthetic wastewater	Synthetic wastewater + RLs	RLs
Synthetic wastewater	1.00	0.26	0.74
Synthetic wastewater + RLs	0.26	1.00	0.40
RLs	0.74	0.40	1.00

P>0.05 = No statistical significance; P<0.05 = Statistical significance

Figure 6.2 shows the time profile of total mean eubacteria abundance in the SBRs. The population was seen to fluctuate with time and in some instances around Day 100 and between Days 150 and 200 it was seen to increase with increase in COD/NH_4-N, i.e. the eubacteria abundance incraesed with COD:NH_4-N = 4.0. In addition, during the period of Day 100 and Day 150-200, the SBRs were being fed at NLR of 0.6 gNH_4-N/Ld. This coincides with observations in Figures 5.7, 5.9, 5.10 and 6.2 which showed better performance in the SBRs for the various feed types in terms of NH4-N removal, nitrification activity and bacterial growth, respectively, at NLR 0.6.

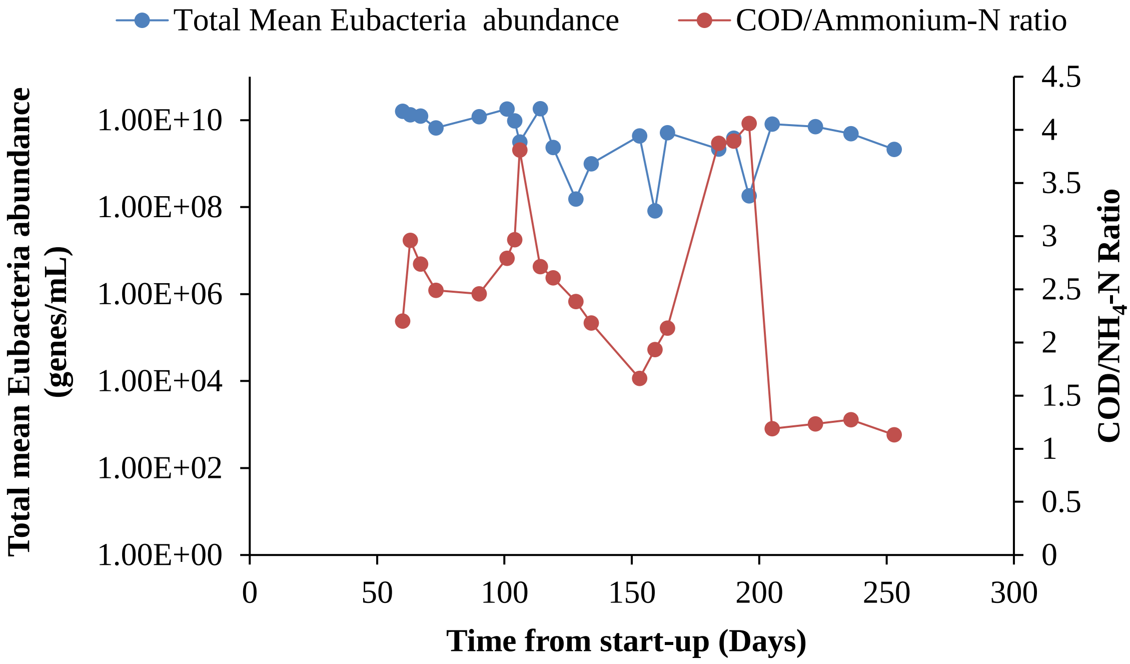

Figure 6.2: Variations in Total mean eubacteria abundance in aerobic granulation SBRs with time and varying COD/NH_4-N ratios. The y-axis is shown in a Logarithmic scale. Error bars refer to standard errors of means

The appearance of both NO_3-N and NO_2-N following NH_4-N removal (see Figures 5.9, 5.10 and 5.7, respectively) in the reactors suggested that autotrophic nitrification occurred, although it is likely that heterotrophic assimilation may have also contributed to NH_4-N removal. A further understanding of the autotrophic nitrifying bacteria community (e.g. relative abundances of AOB and NOB) is presented in the following sections.

6.3.1 AOB relative abundance

Figure 6.3 shows the variations in the abundance of AOB in the aerobic granular reactors during the different feed conditions and NLRs. Like the abundance of total bacteria, the mean abundance of AOB was seen to be relatively higher at NLR 0.6 gNH_4-N/Ld for the different feeding conditions. However, overall, the highest abundance of AOB (2.87 x 10^8 AOB gene copies/mL) was seen during synthetic wastewater feeding condition at NLR 1.0 gNH_4-N/Ld. This feeding phase was within the first 140 days as and the peak AOB abundance is clearly shown in Figure 6.4. This was probably due to the high NH_4-N load which could have favoured the proliferation of AOB in the reactor. The difference between mean abundances of AOB at NLR 0.2 and 0.6 gNH_4-N/Ld was statistically significant (t-test; P = 0.02; 95% confidence limit) during the different feeding conditions. However, Table 6.6 shows no statistical significance (t-test; P>0.05) between the mean AOB abundances for the feeding conditions. A positive correlation (R = 0.51) was also observed between suspended solids, total bacteria abundance and the abundance of AOB in the reactors.

Mean abundance of AOB was expected to be highest in feeding conditions with RLs components, with the hope that the relatively low COD:NH_4-N ratio of the RLs component will contribute to the competitive advantage of AOB over heterotrophic bacteria. However, relatively higher mean abundances were seen with synthetic wastewater feed alone (Figure 6.3), probably due to lower potential toxicity effects of the feed on the microbial community compared to RLs (which could potentially contain toxic chemicals of industrial origin). This could also be linked to the unpredictability of AOB assembly in bioreactors as reported in Graham *et al.,* 2007.

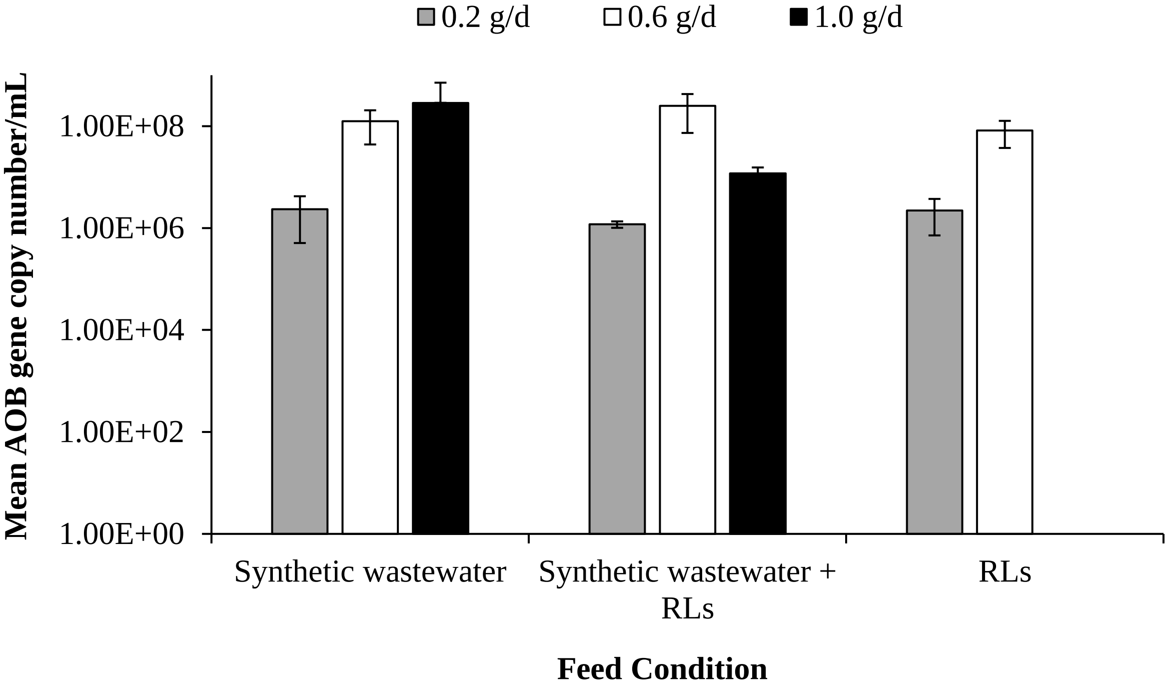

Figure 6.3: Variations in mean abundance of AOB in aerobic granulation SBRs due to varying feed conditions. The y-axis is shown in a Logarithmic scale. Data presented are means from various COD:NH_4-N ratios. Error bars refer to standard errors

Table 6.6: Statistical Significance analysis for AOB abundance in the reactors

Feeding conditions	***P-values (at 95% confidence limit)***		
	Synthetic wastewater	Synthetic wastewater + RLs	RLs
Synthetic wastewater	1.00	0. 69	0.41
Synthetic wastewater + RLs	0.69	1.00	0.67
RLs	0.41	0.67	1.00

P>0.05 = No statistical significance; P<0.05 = Statistical significance

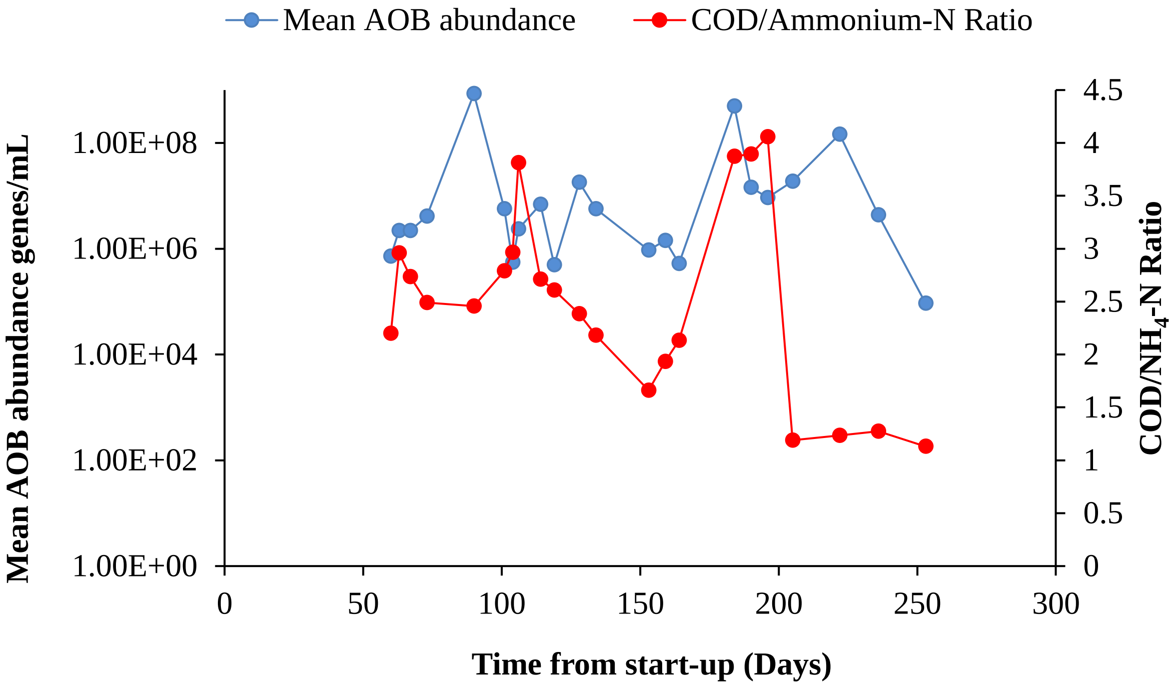

Figure 6.4: Variations in Total mean AOB abundance in aerobic granulation SBRs with time and varying COD/NH4-N ratios. The y-axis is shown in a Logarithmic scale.

Variations in the relative abundance of AOB to Eubacteria in the reactors (presented in Figure 6.5), was also consistent with the AOB abundance data presented in Figure 6.3. The relative abundance of AOB was measured as a percentage of the ratio between the mean abundance of AOB and total bacterial abundance. Like the AOB abundance, the relative abundance of AOB for each of the feeding conditions was lowest at NLR 0.2 gNH_4-N/Ld, with the overall lowest mean relative abundance of AOB (0.08%) seen during the RLs feeding phase. This was linked to the comparatively low NH_4-N load which could have reduced the competitive growth advantage of AOB abundance over other bacterial populations in the community. While the relative abundance of AOB was relatively higher at NLR 1.0 gNH_4-N/Ld for the various feeding conditions, although the highest relative abundance (11.5 ± 0.6%) was at NLR 0.6 gNH_4-N/Ld during the synthetic wastewater + RLs feeding condition. The high AOB relative abundance at NLR 1.0 gNH_4-N/Ld is consistent with NO_2-N accumulation in the aerobic granular reactors as shown Figure 5.10 in Chapter 5. This is also consistent with reports (Zanetti *et al.,* 2012) that high free NH_4-N load suppresses NOB growth, while the relative abundance of AOB is enhanced. Figures 6.3 and 6.5 show that both the mean AOB gene counts/mL and mean relative abundances of AOB for the different feeding conditions increased between NLR 0.2 to 0.6 gNH_4-N/Ld, with an increase of up to two orders of magnitude seen during the synthetic wastewater + RLs feeding condition.

Unlike, the abundance of AOB shown in Figure 6.3, relative abundances of AOB were seen be relatively higher in feeding conditions with RLs component, probably due the relatively low COD:N ratio of the RLs component suppressing the growth of heterotrophs over autotrophic bacteria (i.e. AOB).

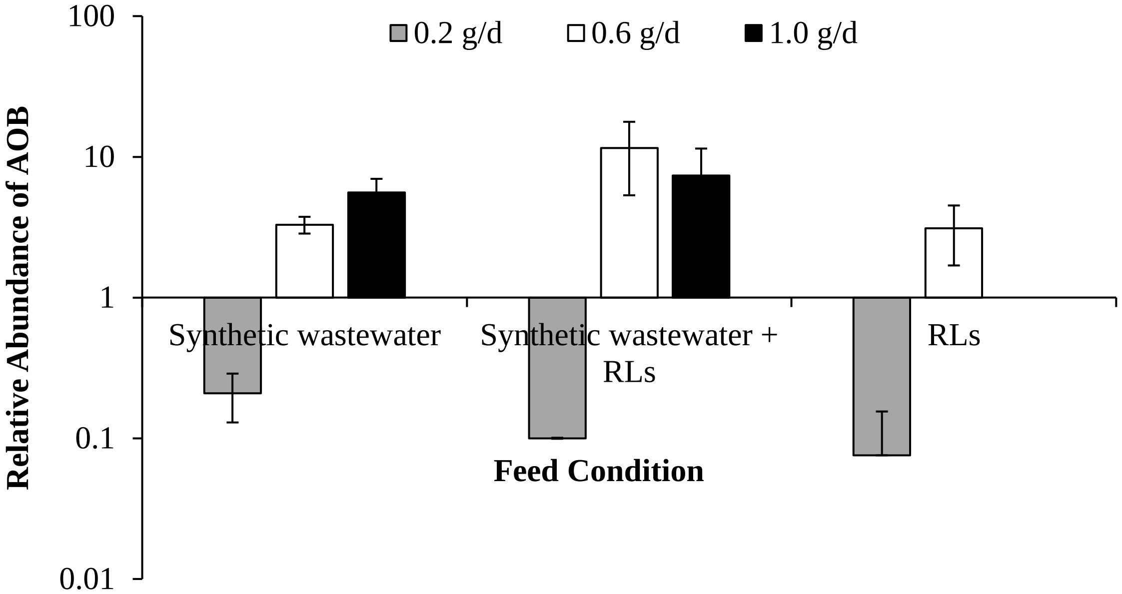

Figure 6.5: Variations in relative abundance of AOB to eubacteria in aerobic granulation SBRs due to varying feed conditions. The y-axis is shown in a Logarithmic scale. Error bars refer to standard errors

6.3.2 NOB relative abundance

Figure 6.6 shows variations in the abundance of NOB with varying feeding conditions and NLRs. As with mean AOB abundance, mean NOB abundance was also seen to increase from 0.2 to 0.6 gNH_4-N/Ld for each of the feeding conditions, with the highest value (6.98×10^6 NOB gene copies/mL) seen during the synthetic wastewater + RLs feeding condition. However, mean NOB abundance was seen to decrease from NLR 0.6 to 1.0 gNH_4-N/Ld, with the least mean value (8.60×10^5 NOB gene copies/mL, i.e. a decrease of approximately one order of magnitude) seen during the synthetic wastewater feeding condition. This is consistent with the increase in nitrification activity (as demonstrated by NO_3-N accumulation) between NLRs 0.2 and 0.6 gNH_4-N/Ld, and a decrease observed between NLR 0.6 and 1.0 gNH_4-N/Ld (see Figure 5.9 in Chapter 5).

This consistency with NOB and AOB abundances (see Figure 6.3) shows that NH_4-N removal via nitrification in the reactors were primarily by autotrophic nitrifying bacteria. This is also consistent with reports (Graham *et al.,* 2007; Szabó *et al.,* 2016) that N-removal via nitrification is a direct consequence of the mutualistic guild of AOB and NOB. Further, suspended solids concentration, abundances of total bacteria, AOB and NOB, and nitrification performance showed a weak positive correlation ($R = 0.48$). This further confirmed the effect of the mutualistic relationship between AOB and NOB in the aerobic granules on the performance of the reactors, due to reports (Rittman and McCarty, 2003; Graham *et al.,* 2007) that NOB primarily depend on AOB for their preferred electron donor, while AOB rely on NOB for the removal of toxic NO_2-N. Like the mean abundances of "total bacteria" and AOB, no statistical significance (t-test; $P>0.05$) was seen between the mean abundances of NOB (see Table 6.7).

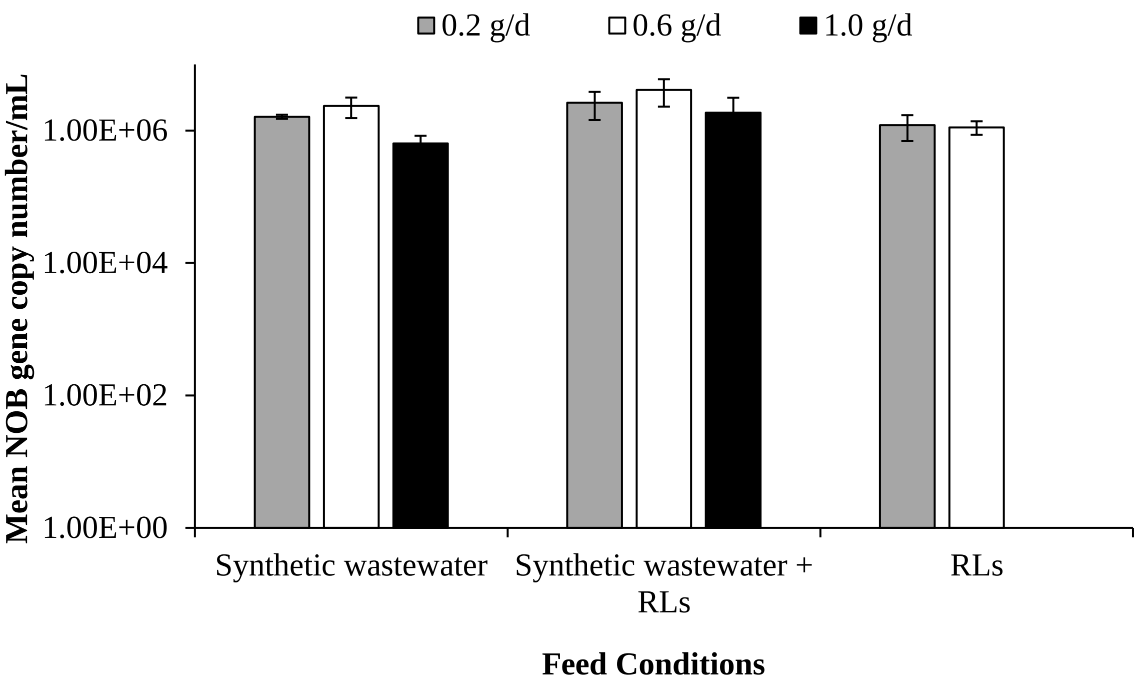

Figure 6.6: Variations mean abundance of NOB in aerobic granulation SBRs due to varying feed conditions. The y-axis is shown in a Logarithmic scale. Error bars refer to standard errors of means

Table 6.7: Statistical Significance analysis for NOB abundance in the reactors

Feeding conditions	***P-values (at 95% confidence limit)***		
	Synthetic wastewater	Synthetic wastewater + RLs	RLs
Synthetic wastewater	1.00	0. 43	0.55
Synthetic wastewater + RLs	0.43	1.00	0.22
RLs	0.55	0.22	1.00

P>0.05 = No statistical significance; P<0.05 = Statistical significance

Two major species (*Nitrobacter spp.* and *Nitrospira spp.*) were observed following qPCR analysis of AG samples from the reactors. Table 6.8 shows variations in mean abundances of *Nitrobacter* and *Nitrospira* following variations in feeding conditions and NLRs; while Figures 6.7 and 6.8 show time profiles of *Nitrobacter* and *Nitrospira* abundances with varying COD/NH_4-N ratios. Overall, *Nitrobacter spp.* dominated *Nitrospira spp.* during all feeding conditions and NLRs, except at NLR 0.2 gNH_4-N/Ld during the synthetic wastewater feeding phase where *Nitrospira spp*. constituted on average ~87.6% (i.e. 6.24 x10^6 gene copy number/mL) of the total NOB population. The lowest mean *Nitrospira spp*. abundance (5.67 x 10^2 gene copy number/mL, i.e. ~0.01% of the total NOB population) was seen at NLR 0.2 gNH_4-N/Ld during the synthetic wastewater + RLs feeding condition. *Nitrobacter spp.* were most common during feeding conditions incorporating RLs component, with this genus making up >99% of the NOB gene copy number/mL at most of the NLRs. The highest mean *Nitrobacter spp.* abundance (6.24 x 10^6 gene copy number/mL, i.e. ~99.99% of the total NOB population) was seen at NLR 0.2 gNH_4-N/Ld during the synthetic wastewater + RLs feeding condition; while the least (8.86 x 10^5 gene copy number/mL, i.e. ~12.4% of the total NOB population) was seen at NLR 0.2 gNH_4-N/Ld during the synthetic wastewater feeding condition.

The differences in *Nitrobacter* and *Nitrospira* abundances observed can be linked to variations in environmental conditions and operational controls. *Nitrospira* was particularly less dominant at high NLRs (i.e. 1.0 gNH_4-N/Ld; see Table 6.8) whereby NO_2-N levels were high (see Figure 5.10 in Chapter 5). However, the lowest *Nitrospira* abundance was seen at NLR 0.2 gNH_4-N/Ld during the synthetic wastewater feeding condition, probably due to errors in the quantitative methodologies. The inverse relationship seen between the abundance of *Nitrospira* and NO_2-N levels is consistent with observations in Huang *et al.* (2010) and ter Haseborg *et al.* (2010). On the other hand, the overall dominance of *Nitrobacter* during the different feeding conditions can be linked to the high NH_4-N concentrations (100 mg/L, 300 mg/L and 500 mg/L represented by NLRs of 0.2, 0.6, and 1.0 gNH_4-N/Ld, respectively) in the influent into the reactors which became oxidised to NO_2-N and eventually to NO_3-N. This is supported by the K/r hypothesis in previous studies (Schramm *et al.,* 1999; Kim and Kim, 2006) which reported that *Nitrospira* is often a K-strategist adapted to low N (i.e. NH_4-N and NO_2-N) levels, while *Nitrobacter* are frequently r-strategists primarily dependent on abundance of substrate (i.e. NO_2-N) concentration.

Furthermore, temperature (i.e. room temperature; 24°C on average in the pilot Lab) could also have favoured the dominance of *Nitrobacter* over *Nitrospira*. This too is supported by observations in Huang *et al.* (2010) that *Nitrobacter* was dominant at relatively lower temperatures (24 - 25°C), while *Nitrospira* were the dominant NOB at relatively higher temperatures (29 - 35°C). In addition, the relatively long aeration period (i.e. 349 min; with mean DO levels of 2.1 mg/L and max DO levels of ~9.0 mg/L) in the reactors also favoured the dominance of *Nitrobacter* over *Nitrospira*. This is supported by reports that *Nitrobacter* are significantly positively correlated to DO levels, whereas the abundance of *Nitrospira* showed significant negative correlation to DO (Schramm *et al.*, 2000; Blackburne *et al.*, 2007; Huang *et al.*, 2010). The relatively high HRT (i.e. 14.4h) could also have been the reason behind the predominance of *Nitrobacter* as reported in Huang *et al.* (2010) where a significant positive correlation was seen between HRT and *Nitrobacter* abundance. Another potential reason behind the predominance of *Nitrobacter* could be linked to acetate (i.e. the primary COD source) in the synthetic wastewater. This notion is supported by reports (van Loosdrecht *et al.*, 2012) that *Nitrobacter* was the dominant NOB in acetate-fed aerobic granular systems.

Table 6.8: Variations in mean *Nitrobacter* and *Nitrospira* abundances in aerobic granulation SBRs due to varying feeding conditions and NLRs.

NLRs (g.NH_4-N/Ld)	**No. of *Nitrobacter* genes copies/mL**			**No. of *Nitrospira* genes copies/mL**		
	Synthetic wastewater	**Synthetic wastewater + RLs**	**RLs**	**Synthetic wastewater**	**Synthetic wastewater + RLs**	**RLs**
0.2	8.86 x 10^5	6.20 x 10^6	1.32 x 10^6	6.24 x 10^6	5.67 x 10^2	2.99 x 10^3
0.6	4.34 x 10^6	5.25 x 10^6	1.31 x 10^6	2.29 x 10^5	1.74 x 10^6	6.61 x 10^3
1.0	7.79 x 10^5	1.85 x 10^6	-	8.19 x 10^4	1.65 x 10^4	-

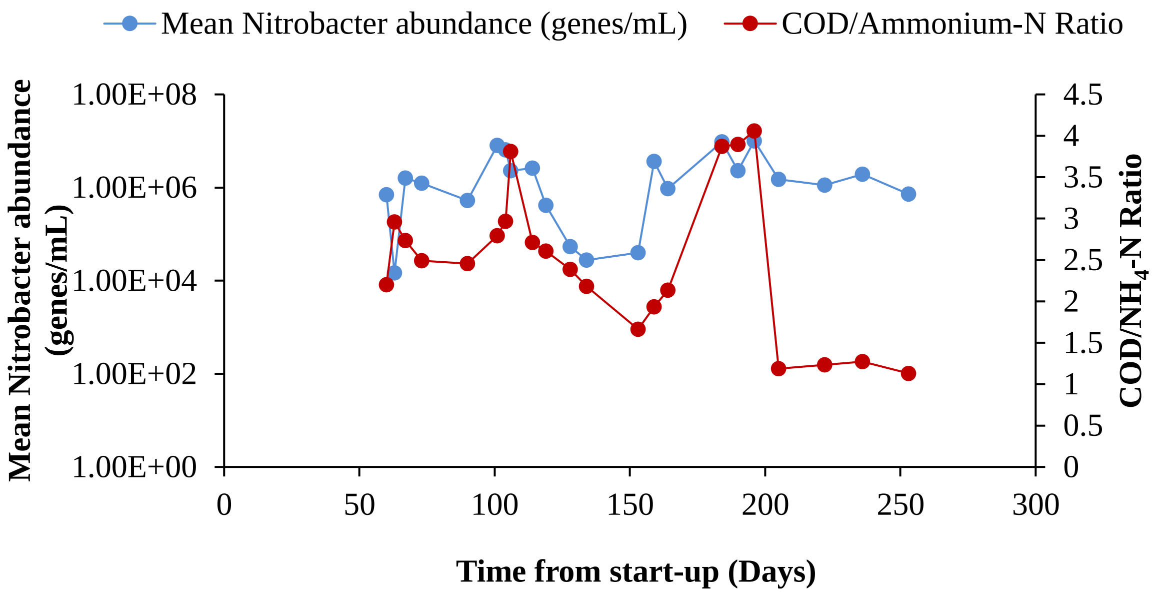

Figure 6.7: Variations in Total mean Nitrobacter abundance in aerobic granulation SBRs with time and varying COD/NH4-N ratios. The y-axis is shown in a Logarithmic scale.

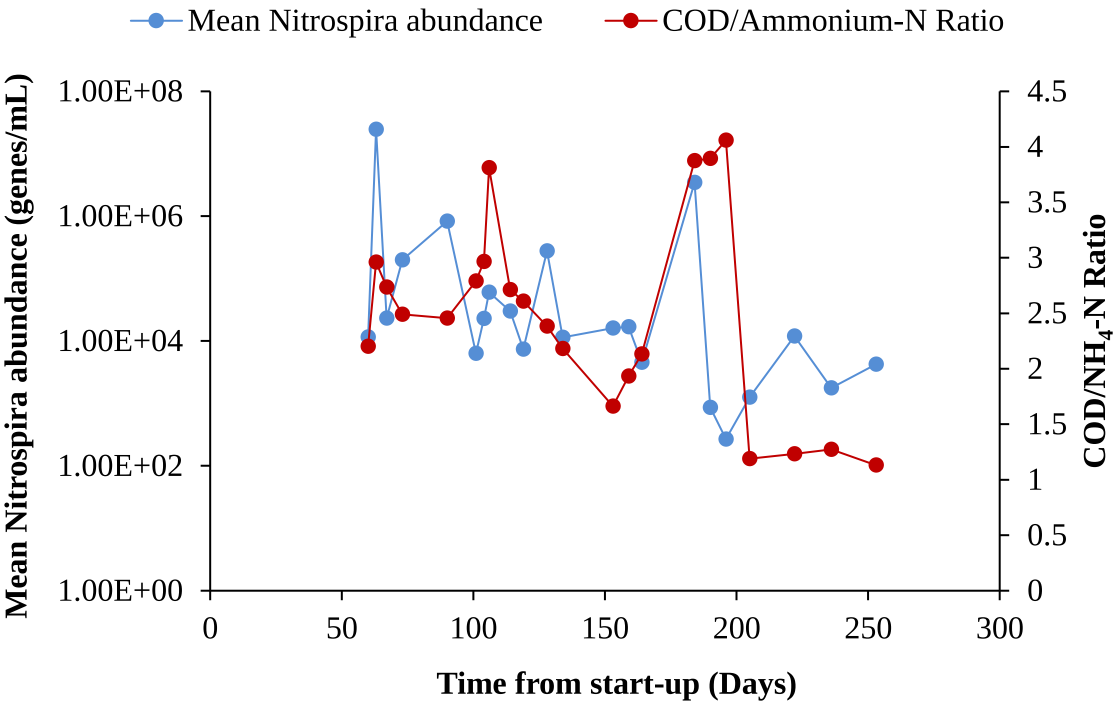

Figure 6.8: Variations in Total mean Nitrospira abundance in aerobic granulation SBRs with time and varying COD/NH4-N ratios. The y-axis is shown in a Logarithmic scale.

Variations in mean relative abundances of NOB in the SBRs are presented on Figure 6.9. As seen with NOB abundances, relative abundances were highest at 0.6 gNH4-N/Ld for all feeding conditions. This was also consistent with NO3-N levels in the effluent from the reactors. NOB relative abundances were also higher in feeding conditions with RLs component, with the highest (10.9 ± 0.02%) and least (0.04 ± 0.01%) relative abundance seen at NLR 0.6 gNH4-N/Ld during the synthetic wastewater + RLs feeding phase and NLR 0.2 gNH4-N/Ld during the synthetic wastewater feeding phase, respectively. This was probably due to the relatively higher competitive advantage of autotrophic nitrifiers as a result of the low COD:NH4-N ratio in RLs compared to relatively higher COD:NH4-N ratio and more readily biodegradable COD in synthetic wastewater which potentially favour the proliferation of heterotrophs.

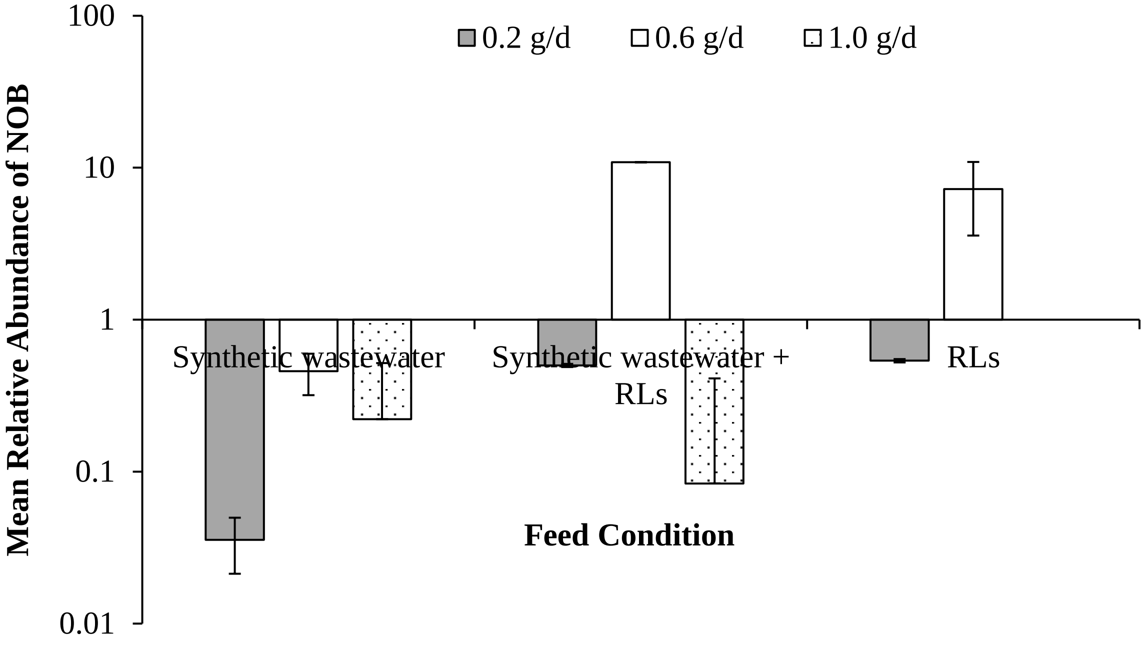

Figure 6.9: Variations in mean relative abundance of NOB to Eubacteria in aerobic granulation reactors with varying feed conditions. The y-axis is shown in a Logarithmic scale. Error bars refer to standard errors

The relatively low abundances of AOB and NOB could (see Figures 6.3 and 6.5) be as a result of the fact that autotrophic nitrifiers are generally slow-growers compared to heterotrophic bacteria. This is consistent with the reports (Rittman and McCarty, 2003; Szabó *et al.*, 2016) that COD processing microorganisms have comparatively higher specific growth rates in complex organic media. This is also consistent with observations in Graham *et al.* (2007) that NOB and AOB were relatively less abundant than other bacteria (e.g. heterotrophs or denitrifiers) in nitrifying systems.

Figure 6.6 shows variations in mean NOB/AOB ratio in the reactors for the various feeding conditions and NLRs. Elevated NOB/AOB ratio (higher than the typical ratio for autotrophic nitrification systems of 0.5 (Daims *et al.*, 2001; Gieseke *et al.*, 2003)) were seesin feeding conditions with RLs component. The observed mean NOB/AOB ratio is similar to the ratio for lab-scale aerobic granular sludge systems (in SBR) reported in van Loosdrecht *et al.* (2012). As expected, the least NOB/AOB ratio was seen at NLR 1.0 gNH$_4$-N/Ld due to the excessive NH$_4$-N load that could have potentially favoured the growth of AOB, while suppressing the growth of NOB. However, NLRs unexpectedly showed the highest NOB/AOB ratios for the various feeding conditions.

Based on the similarity in the unexpected NOB/AOB ratios with that reported in van Loosdrecht *et al.* (2012), two mechanisms (i.e. the “ping-pong” effect and “nitrite loop” theory) might explain the unexpected observations. The ping-pong effect describes a mechanism whereby mixotrophic *Nitrobacter* use acetate (as C source and electron donor) to reduce excess NO_3-N, and subsequently uncoupling *Nitrobacter* growth from the direct supply of nitrite by AOB. Whereas the “nitrite loop” theory describes a scenario whereby during simultaneous nitrite oxidation-nitrate reduction, if partial denitrification (i.e. nitrate to nitrite) occurs, it will result in accumulated nitrite, which could then be used by NOB, and consequently, uncoupling their growth from AOB. In the context of this thesis, given that more microbial analysis will be required to demonstrate the presence of mixotrophic NOBs, it is quite difficult to ascertain if the ping-pong effect is the mechanism behind unexpected NOB/AOB ratios. However, it’s possible both mechanisms occur in alternate circumstances ; for example the ping-pong effect may have been dominate during feeding regimes with synthetic wastewater input (which contains acetate) that could have favoured the proliferation of mixoptrphic NOBs. While during the feeding regime with 100% RLs, the nitrite loop effect could have set in.

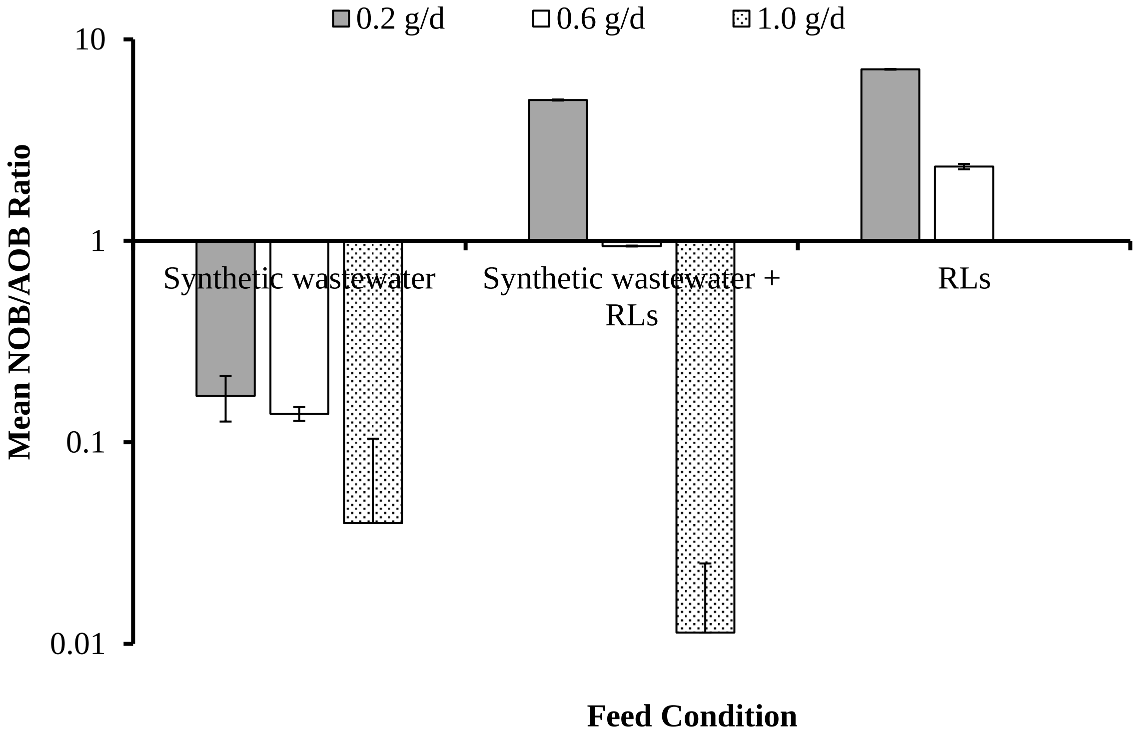

Figure 6.10: Variations in mean NOB/AOB ratio in aerobic granulation reactors with varying feed conditions. The y-axis is shown in a Logarithmic scale. Error bars refer to standard errors of means.

6.4 Conclusion

During the study, AOB and NOB were successfully grown and sustained in aerobic granular sludge reactors during three different feeding conditions and NLRs. Positive correlations were seen between AOB and NO_2-N levels, and between NOB and NO_3-N levels in the reactors. AOB abundances and relative abundances were seen to increase with increase in NLRs. While NOB abundances and relative abundances was seen to peak at NLR 0.6 gNH_4-N/Ld for the different feed conditions.

These observations support recommendations in Chapter 5 in that a NLR 0.6 gNH_4-N/Ld can be used as the ‘guide’ design NLR for effective NH_4-N removal via autotrophic nitrification in aerobic granulation systems. However, there was not net removal of N, as N just changes form from NH_4-N to NO_3-N and NO_2-N. The study also showed that *Nitrobacter sp.* was the dominant NOB (not *Nitrospira sp.*) during the different feed conditions and NLRs. This selective microbial assembly in the NOB community was linked to the potential effect of environmental factors (e.g. acetate as C-source, temperature DO, NH_4-N and NO_2-N levels,) and operational controls (i.e. HRT). Unexpectedly high NOB/AOB ratios were seen during feeding conditions with RLs component (excluding at NLR 1.0 gNH_4-N/Ld where AOB was expected to be significantly higher than NOB).

Chapter 7: Conclusion and Recommendations

7.1 Overall Conclusion

The main drivers of NH_4-N and PO_4-P removal/recovery are legal compliance; operational and financial challenges; environmental concerns (e.g. eutrophication and offsetting phosphate rock mining); and potential economic gains (through the sales of recovered N and P, and reduced maintenance cost). However, the overall aim of this thesis was to assess and determine the effect of 'controlled' precipitation of struvite in sludge (through Mg addition and pH adjustment) and the application of aerobic granular sludge technology on 'free' NH_4-N and PO_4-P levels in RLs to mitigate scaling problems at Bran Sands WWTP.

NWL's Bran Sands WWTP has been experiencing a plethora of N and P problems (e.g. scaling in pipes and pumps, and occasional performance deterioration in some treatment processes) associated with nutrient-rich RLs. This is very common on WWTPs treating nutrient-rich (N and P) wastewaters, especially those treating sludge (often rich in N and P) via AD (Caffaz *et al.*, 2008; Malamis *et al.*, 2014). With the relatively relaxed consent for P and N at Bran Sands WWTP compared to sites with similar characterics, the situation is worsened by inadequate monitoring, however, the company is keen on resolving the in the most appropriate manner. Existing treatment technologies (biological and physicochemical) for NH_4-N and PO_4-P removal/recovery from wastewater which could be potentially applied to the treatment of RLs, were reviewed. However, research gaps highlighted the necessity for a comprehensive mass balance of NH_4-N and PO_4-P as the first step towards quantifying the problem, identifying potential points for intervention and also as a practical guide in choosing the most reasonable treatment option.

The N and P mass balances showed that there was an increase in NH_4-N and PO_4-P in CAMBI following thermal hydrolysis and in the AD units associated to the anaerobic biodegradation of organic matter with the increased potential for resolubilisation of struvite component ions that promote struvite formation and re-entry into the ETW. Specifically, NH_4-N and PO_4-P loads of 2.23 tonne/d and 0.36 tonne/d respectively, are returned to the ETW via post-AD RLs. Based on 2013 data, about 13% of NH_4-N, 40% of PO_4-P and 88% of Mg^{2+} in digested sludge precipitated out of solution (in post-AD processes) into the solid phase, which caused scaling in equipment and precipitates in digested sludge cake (due to the operating conditions that favour struvite formation). The recycled NH_4-N to the ETW accounts for ~ £224,260/yr in additional energy costs to reduce NH_4-N to consent levels (250 mg/L) at Bran Sands WWTP. In addition, scaling problems linked to the 'uncontrolled' struvite crystallization in equipment reported on site occurred between the AD and the digested sludge centrifuge. Furthermore, using $K_{sp} = 10^{-13}$, Figure 3.12 shows that IAP for struvite component ions was greater than K_{sp} in all RSTC units sampled during the mass balance exercise by a factor of $>10^4$. In addition, t-test analyses showed that mean IAP for each of the sampled treatment units was statistically significantly different from K_{sp} (p-value < 0.05 at 95% confidence interval limit). The high IAP values in the RSTC units could have been as a result of the significantly high concentrations of the struvite component ions resulting from a potential build-up within the systems and potentially high organic loading rates. IAP values also went up even higher after CAMBI. There were even further increases in the AD. IAP data presented alongside the Mg^{2+}, NH_4-N and PO_4-P loads and the pH in the various RSTC treatment units, led to recommendations for P-recovery trials (in AD and post-AD) through Mg dosing presented in Chapter 4. The high IAP, suitable pH and stoichiometrically favourable Mg^{2+}:PO_4-P molar ratio in AD units and in digested sludge, were good indicators for cost-effective N and P recovery through 'controlled' struvite precipitation.

Although struvite formation was problematic, it also highlighted this location as a suitable place for N and P recovery as struvite. However, data also show these areas are relatively low in Mg^{2+} (i.e. in digested sludge), which suggests that Mg^{2+} levels are rate-limiting relative to struvite formation reactions. Hence, it was decided to perform complementary experiments to determine the feasibility of using pH adjustment and Mg addition (in AD and post-AD processes) to promote N and P recovery/removal, both to reduce scaling problems, but also to reduce N and P loadings in RLs recycled to the ETW. Experiments assessing the effect of different Mg addition, to AD units and post-AD RLs, on N and P levels are presented in Chapter 4

In both AD- and post-AD recovery systems, NH_4-N and PO_4-P recovery was found to increase with increasing Mg^{2+} doses and pH levels, although recovery rates were higher with post-AD addition (i.e. ~89%; see Figure 7.2) compared with direct addition to AD units (i.e. 69%; see Figure 7.1). Both recovery options significantly reduced NH_4-N and PO_4-P levels in the RLs, which would reduce the potential for scaling resulting from 'uncontrolled' struvite precipitation in processes downstream of the AD. For instance, adjusting the pH in the post-AD Jar test experiments to 8 and adding upto 150 mg/L of Mg^{2+} showed that IAP in digested sludge and RLs could potentially be reduced by approximately three orders of magnitude and one order of magnitude, respectively; i.e. from 3.14×10^{-6} and 6.86×10^{-8} (see Figure 3.12)

9.3×10^{-9}. Both approaches, also would potentially reduce energy and other associated costs involved in treating NH_4-N and PO_4-P recycled to the head of the works via RLs. Although not examined here from an operational perspective, it was shown that recovering NH_4-N and PO_4-P as struvite could generate income through their potential sale. It was also observed that Mg addition and recovering NH_4-N and PO_4-P in AD units increased biogas production, although this could be offset by the reduction in the sludge production from the ETW due to reduced N and P loads recycled to the CAS units at Bran Sands WWTP.

Despite the success in potentially reducing some of the NH_4-N load in downstream of the AD using Mg dosing and pH adjustment, the residual NH_4-N concentration in RLs was still not likely sustainable for existing systems at Bran Sands WWTP. Hence a cost effective polishing step might be necessary, especially for NH_4-N, prior to the RLs being recycled to the ETW. Although other options were considered, aerobic granular reactors and treatment was assessed for this purpose.

Aerobic granular technology was seen to be an effective treatment option for NH_4-N removal from RLs. NH_4-N removal via nitrification was observed to increase with increasing COD/N ratios in the different feeding conditions. Overall, a comparison between different feeding conditions showed that NH_4-N removal by aerobic granular technology was highest when the reactors were fed with synthetic wastewater + RLs. % NH_4-N removal for each of the feeding conditions was seen to rise between NLRs of 0.2 and 0.6 gNH_4-N/Ld. Nitrification activity was also seen to be highest at N loading rate of 0.6 gNH_4-N/Ld, especially in feed conditions with ‘real’ RLs as one of the components. The AGT experiments also showed a potential to further reduce the possibility of ‘uncontrolled’ struvite precipitation downstream of the AD, particularly in RLs conveying lines following the dewatering of digested sludge. For instance, separately treating RLs could potentially reduce the IAP for struvite component ions from 9.3×10^{-9} after post-AD Mg addition of Mg (i.e. 150mg/L Mg^{2+}) to 1.7×10^{-10} which is closer to the k_{sp} value for struvite. In addition, Figure 7.3 also show a further 64% reduction in NH_4-N during the separate treatment of RLs using AGT at NLR 0.6 g NH_4-N, prior to being recycled to CAS units in the ETW at Bran Sands WWTP.

AOB and NOB were successfully grown and sustained in aerobic granular sludge reactors during three different feeding conditions and NLRs. Positive correlations were seen between AOB and NO_2-N levels, and between NOB and NO_3-N levels in the reactors. AOB abundances and relative abundances were seen to increase with increase in NLRs. While NOB abundances and relative abundances was seen to peak at NLR 0.6 gNH_4-N/Ld for the different feeding conditions. This was also seen to be consistent with the nitrification performance during the feeding conditions. This further supports recommendations in Chapter 5 that based on observations from the studies, NLR 0.6 gNH_4-N/Ld can be used as the ‘guide’ design NLR for effective N removal via autotrophic nitrification in aerobic granulation systems. The study also showed that *Nitrobacter sp.* was the dominant NOB (not *Nitrospira sp.*) during the different feeding conditions and NLRs. This selective microbial assembly in the NOB community was linked to the potential effect of environmental factors (e.g. acetate as C-source, temperature DO, NH_4-N and NO_2-N levels,) and operational controls (i.e. HRT). Unexpectedly high NOB/AOB ratios were seen during feeding conditions with RLs component (excluding at NLR 1.0 gNH_4-N/Ld where AOB was expected to be significantly higher than NOB).

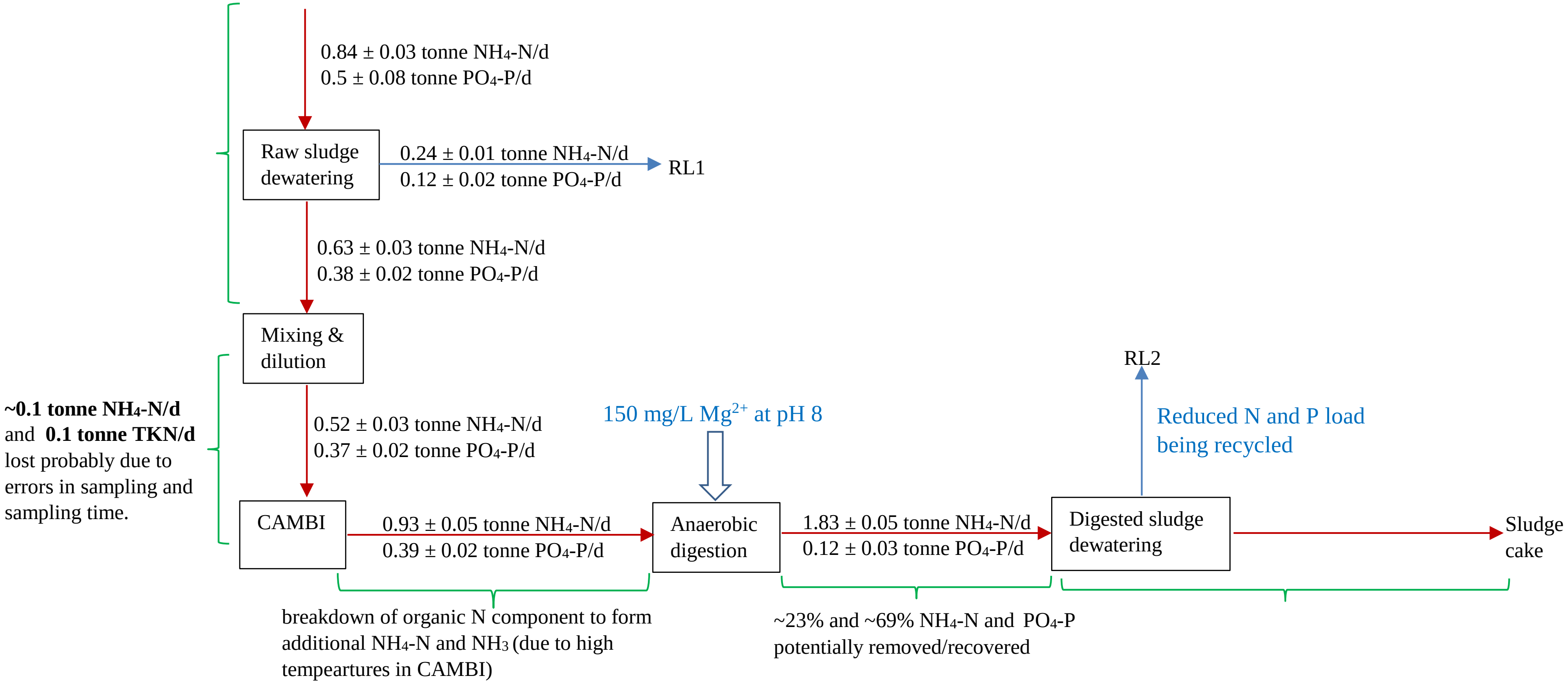

Figure 7.1: Potential Mass flow of NH_4-N and PO_4-P in sludge treatment processes at Bran Sands following 150 mg/L Mg^{2+} dosing in AD at pH 8

(Uncertainty ranges in this study were presented as standard error of sample means)

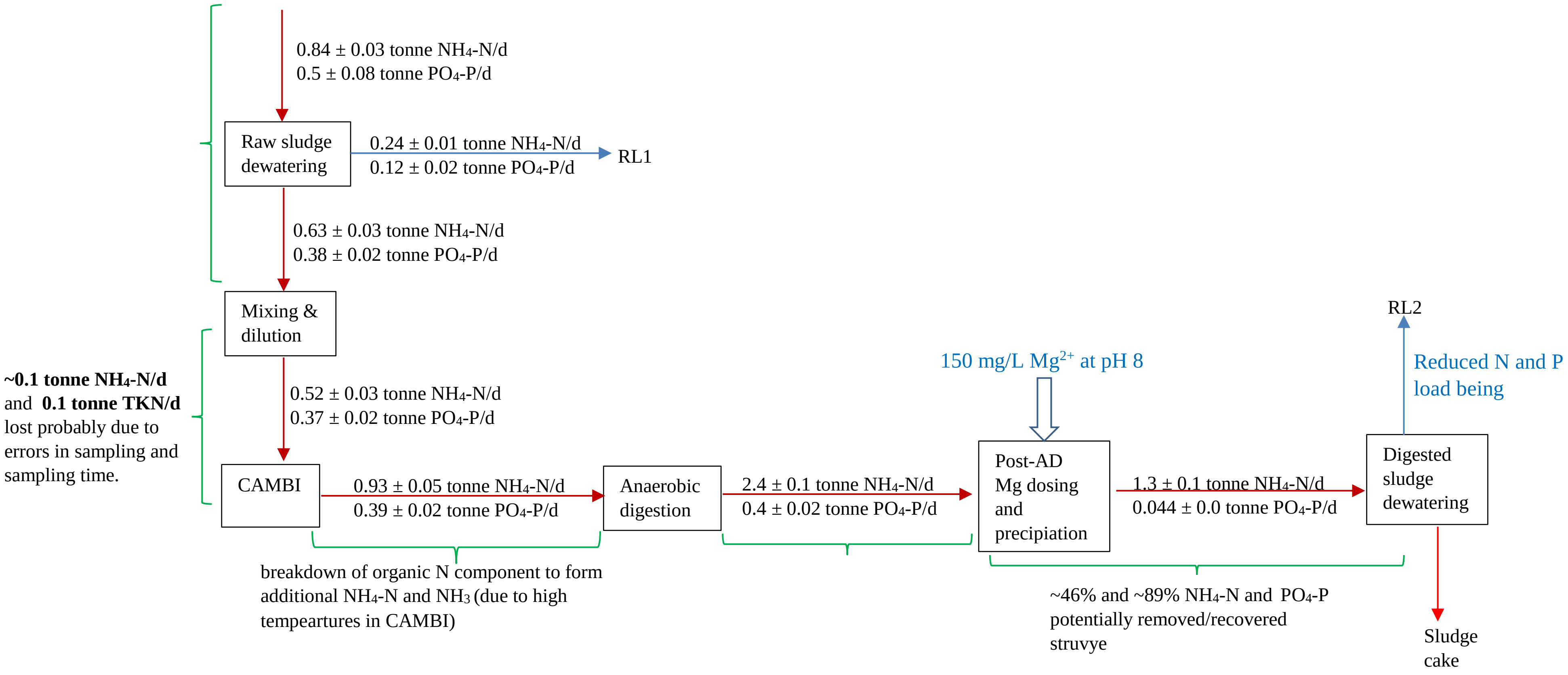

Figure 7.2: Potential Mass flow of NH_4-N and PO_4-P in sludge treatment processes at Bran Sands WWTP after post-AD Mg^{2+} dosing at pH 8

(Uncertainty ranges in this study were presented as standard error of sample means)

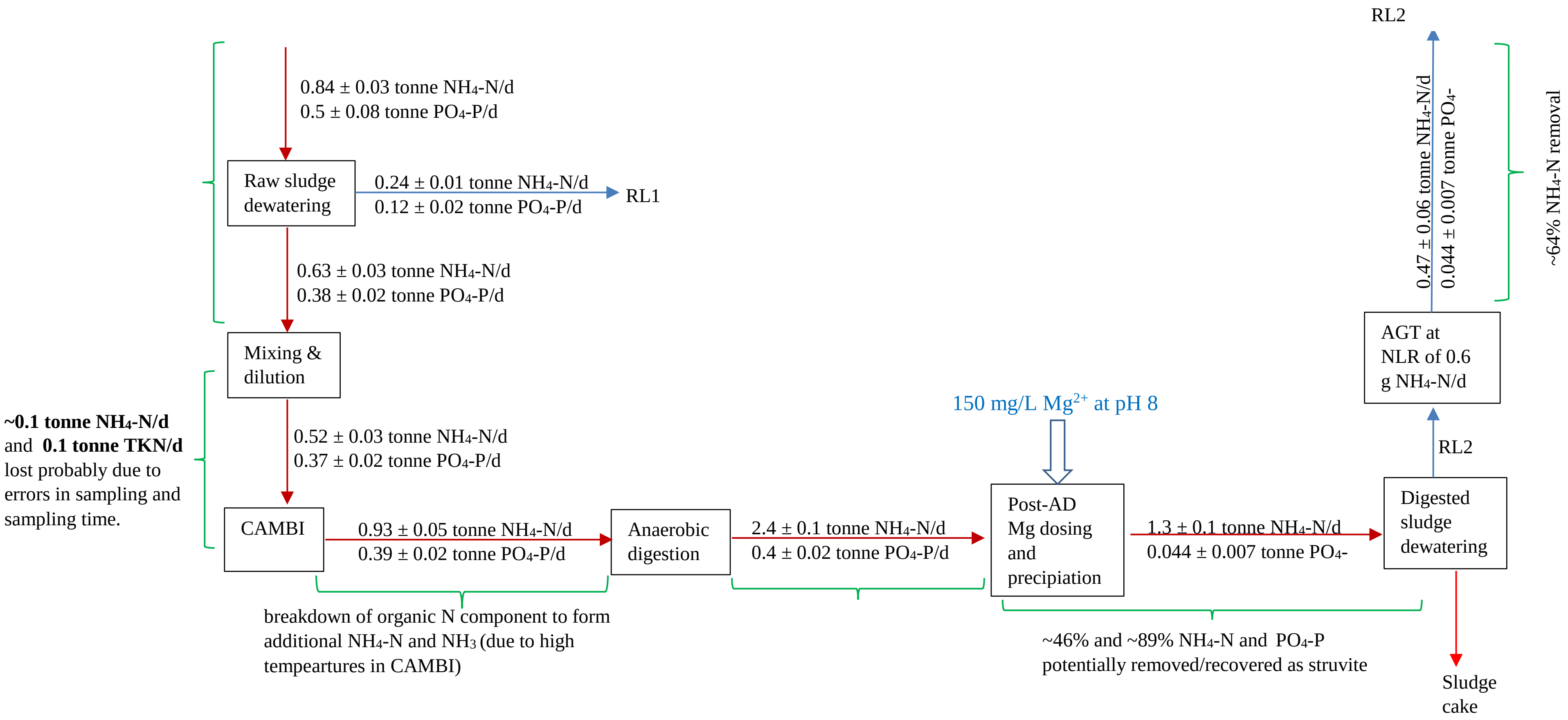

Figure 7.3: Mass flow of NH_4-N and TKN in sludge treatment processes at Bran Sands WWTP after post-AD Mg^{2+} dosing at pH 8 and separate treatment of NH_4-N in RLs using AGT at NLR 0.6 gNH_4-N/d.

(Uncertainty ranges in this study were presented as standard error of sample means)

7.2 Future Work

Some of the major difficulties faced during the studies especially with respect to establishing the mass balance, was the absence of historical data (flow, PO_4-P, volume of foreign sludge, methane production, etc.) and even when available there were so many irregularities. Also differences in measuring methods for similar parameters (e.g. NH_4-N) measured at Bran Sands and at Newcastle University meant there was slight differences in some of the data, although the differences were not significant. For the purpose of analysis, the mean was used to minimise these differences. Recommendations have also been made to NWL on parameters to be measured/monitored at least on a daily basis to facilitate future mass balance exercises. A more comprehensive mass balance template for all treatment processes at Bran Sands WWTP and other sites should also be developed for a better understanding of the transfer of material through the individual units and their interdependence.

Following the successful lab-scale pH adjustment and Mg dosing experiments for NH_4-N and PO_4-P recovery in AD units and designated post-AD units, field trials are recommended. Also, due to the unique circumstances at Bran Sands WWTP (i.e. its ETW treats both industrial and municipal wastewaters, its AAD treats both indigenous and foreign sludge, and RLs from sludge treatment is recycled to the ETW without pre-treatment), pilot trials are recommended to see if lab-scale outcomes can be replicated. Pilot trials for P recovery AD units and post-AD systems can be done using Mg doses between 50 and 100 mg/L at pH 8.0. Considerations will also be made on potentially separating the recovered P in sludge into a product of better quality. This considerations will be facilitate the drafting of a much more comprehensive cost benefit analysis.

RLs from the two recovery options mentioned above can then be treated via aerobic granulation at NLRs between 0.2 gNH_4-N/Ld (i.e. 100 mg/L NH_4-N) and 0.6 gNH_4-N/Ld (i.e. 300 mg/L NH_4-N). Aerobic granules from the lab experiments have been stored and can be used for seeding the pilot reactors. Further molecular analysis needs to be carried out in order to identify and quantify the species of AOB and other microoganisms present in the aerobic granular sludge, and how their abundances vary during the different feeding conditions. It is hoped that this future study will provide a better understanding of the operating conditions for aerobic granulation systems and how they affect microbial community assembly and process performance. Further, it is hoped that the work will not only provide a site-specific solution but may provide practical bases for extensive application throughout the wastewater treatment industry.

APPENDICES

APPENDIX A

This section presents raw data from NWL (i.e. Table A.1) and from analysis carried out on in the Environmental Engineering lab at Newcastle (i.e. Tables A.2 to A.7). Flow data on Tables A.6, A.7 and A.8 were collected from the SCADA system during sampling. However, some of the flow meters were occassionally flooded which may have resulted in faulty readings, hence the disparity in some of the flows. Data on Tables A.2 to A.8 was to complete the 2013 N, P and Mg mass balance while data on Table A.1 was used for comparison purposes only. Data on Table A.1 had already been transformed to 2 significant figures prior to being collected, while data on Tables A.2 to A.8 were written to the nearest integer.

Table A.1: NH_4-N, TSS and TKN data on RL2 obtained from Bran Sands WWTP for 2013.

Date	NH_4-N (mg/L)	TSS (mg/L)	TKN (mg/L)
06-Feb-13	2000	1600	1900
08-Feb-13	2000	2400	2300
11-Feb-13	1600	7100	2700
12-Feb-13	1400	7600	2100
13-Feb-13	1400	2700	2000
14-Feb-13	1400	19000	2400
16-Feb-13	1400	1900	1700
17-Feb-13	2100	4200	2200
18-Feb-13	1200	15000	1900
19-Feb-13	1300	7600	1900
20-Feb-13	1600	3200	2300
21-Feb-13	1500	5900	2000
22-Feb-13	1200	1000	1500
25-Feb-13	2100	8900	2700
26-Feb-13	1900	9300	2400
27-Feb-13	1400	14000	2100
28-Feb-13	1600	8500	2100
01-Mar-13	1700	1900	2100
03-Mar-13	1800	1900	2200
04-Mar-13	1800	2100	2300
05-Mar-13	1800	2000	2200
07-Mar-13	1700	4200	2000
08-Mar-13	1500	2300	2000
09-Mar-13	2100	6900	2100
10-Mar-13	1600	7600	2100
11-Mar-13	1600	2400	2000
12-Mar-13	630	1600	800
13-Mar-13	220	550	290
14-Mar-13	1400	1300	1900
15-Mar-13	1700	6900	2200
16-Mar-13	1700	8200	2000

17-Mar-13	1800	6600	2100
18-Mar-13	1500	7600	1900
19-Mar-13	970	4500	1200
20-Mar-13	1600	8500	2000
21-Mar-13	1600	7900	2200
22-Mar-13	1500	6000	1900
23-Mar-13	1300	3900	1700
26-Mar-13	990	6500	1800
27-Mar-13	1600	5300	1800
28-Mar-13	1700	4200	1900
29-Mar-13	1700	6500	1900
30-Mar-13	2200	5700	5600
31-Mar-13	1400	4900	1600
01-Apr-13	2100	3400	2100
02-Apr-13	1700	3300	2000
03-Apr-13	1700	6400	2000
04-Apr-13	1000	2800	1300
05-Apr-13	1400	3800	1700
06-Apr-13	1900	7300	2100
07-Apr-13	1500	4300	1800
08-Apr-13	780	1800	910
09-Apr-13	680	5500	1900
10-Apr-13	2300	6900	2100
11-Apr-13	1100	6100	2100
12-Apr-13	2300	7400	2100
13-Apr-13	2300	4700	2000
14-Apr-13	1700	6200	2000
15-Apr-13	2400	2800	2100
16-Apr-13	1800	5900	1800
17-Apr-13	640	2500	940
18-Apr-13	1000	1600	1300
19-Apr-13	1800	5700	2000
20-Apr-13	1900	7500	2000
21-Apr-13	2300	4200	2100
22-Apr-13	1200	4000	1400
23-Apr-13	1900	4200	2100
24-Apr-13	1100	3500	1400
25-Apr-13	1500	4000	2100
26-Apr-13	990	2800	1300
27-Apr-13	690	2700	870
28-Apr-13	2600	7000	2200
29-Apr-13	1400	6200	1800
30-Apr-13	1300	4000	1700
01-May-13	1300	5600	1600
02-May-13	1600	7400	2000
03-May-13	1600	12000	2200
05-May-13	2200	9600	2200
06-May-13	2400	8100	2400
07-May-13	1500	5500	2000
08-May-13	1200	5200	1400
09-May-13	1700	7100	2200
10-May-13	1600	4200	2100

11-May-13	1600	1800	2100
12-May-13	1500	1700	1700
13-May-13	1700	3800	2100
14-May-13	1600	2000	2000
15-May-13	1200	1900	1000
16-May-13	1400	2100	1900
17-May-13	1500	4700	1900
18-May-13	1600	2700	1700
19-May-13	2000	2900	1900
20-May-13	1200	4100	1800
21-May-13	1400	2500	1700
22-May-13	1600	1300	1800
23-May-13	1500	1400	1800
24-May-13	1400	1000	1700
25-May-13	1500	680	1700
26-May-13	1300	970	1600
27-May-13	1400	2000	1600
28-May-13	1400	2400	1800
31-May-13	1400	3700	1800
05-Jun-13	1700	2100	2000
06-Jun-13	1700	5000	2100
07-Jun-13	1400	560	1700
08-Jun-13	2000	680	1800
09-Jun-13	2200	700	1700
10-Jun-13	1400	1400	1600
11-Jun-13	1400	1500	1800
12-Jun-13	1700	3200	2000
13-Jun-13	1600	7100	1900
14-Jun-13	1600	6200	1900
15-Jun-13	1800	5200	2000
16-Jun-13	1600	3000	1900
17-Jun-13	610	2800	1900
18-Jun-13	1800	5900	1800
19-Jun-13	1500	19000	2300
20-Jun-13	1500	5800	2100
22-Jun-13	2500	4000	2500
23-Jun-13	2400	5300	2400
24-Jun-13	1700	4700	2300
25-Jun-13	1700	1800	2200
26-Jun-13	1700	2900	2300
27-Jun-13	1600	2500	2200
28-Jun-13	1700	3300	2100
29-Jun-13	1800	5300	2300
30-Jun-13	1700	4200	2100
01-Jul-13	1600	3500	1900
02-Jul-13	1300	14000	1600
03-Jul-13	1500	7000	2100
04-Jul-13	1400	4800	1800
05-Jul-13	1500	7500	2000
06-Jul-13	2200	7200	2100
07-Jul-13	1500	2200	2000
08-Jul-13	1100	950	1500

10-Jul-13	1500	800	2000
12-Jul-13	1500	700	1800
13-Jul-13	1500	1500	2000
14-Jul-13	1600	2600	2100
15-Jul-13	1800	2200	1900
16-Jul-13	1300	1500	1600
18-Jul-13	1800	5200	2100
19-Jul-13	1700	5400	2200
20-Jul-13	1900	6200	2200
22-Jul-13	1900	1500	2300
23-Jul-13	1600	1200	200
24-Jul-13	1200	2500	1500
25-Jul-13	1700	9000	2100
26-Jul-13	1800	8600	2300
27-Jul-13	1400	5300	1800
28-Jul-13	1500	3100	1900
29-Jul-13	1600	4800	2300
30-Jul-13	1400	2200	2100
31-Jul-13	1000	1100	1400
01-Aug-13	1600	2900	2100
02-Aug-13	1800	2500	2600
03-Aug-13	2000	3600	2500
04-Aug-13	1800	1900	2300
05-Aug-13	1400	1900	1800
06-Aug-13	1200	3500	1700
07-Aug-13	1500	2200	2200
08-Aug-13	1500	1200	2000
09-Aug-13	1200	1500	1700
10-Aug-13	1800	2000	2500
11-Aug-13	1700	1600	2500
12-Aug-13	1600	3000	2400
13-Aug-13	1800	2800	2600
14-Aug-13	1800	2000	2500
15-Aug-13	1700	8800	2500
16-Aug-13	1400	7500	2100
17-Aug-13	1900	4600	2400
18-Aug-13	1800	6500	2400
19-Aug-13	1800	3400	2400
20-Aug-13	1700	1900	2300
21-Aug-13	1700	1700	2200
28-Aug-13	1900	2400	2600
29-Aug-13	1700	1600	2200
30-Aug-13	1800	3800	2500
31-Aug-13	2300	3100	2700
02-Sep-13	1600	1800	2200
04-Sep-13	1500	1200	2000
05-Sep-13	1600	1700	2200
06-Sep-13	1200	2000	2100
07-Sep-13	1000	2200	2600
08-Sep-13	2000	1800	2700
09-Sep-13	2000	1300	2700
10-Sep-13	1700	1400	2200

11-Sep-13	1800	1500	2400
12-Sep-13	1900	1200	2500
13-Sep-13	1700	2300	2500
15-Sep-13	1900	4700	2600
16-Sep-13	1700	1100	1900
17-Sep-13	1900	1300	2500
18-Sep-13	1800	1300	2200
19-Sep-13	1900	1000	2300
20-Sep-13	1900	1600	2500
21-Sep-13	1900	1200	2300
22-Sep-13	1700	1000	2200
23-Sep-13	1400	1400	1800
24-Sep-13	2000	2600	2600
25-Sep-13	2000	3500	2600
26-Sep-13	2000	2300	2500
27-Sep-13	1700	1900	2500
28-Sep-13	2400	1900	2600
29-Sep-13	2200	2200	2600

Abbreviations in Tables A.2, A.3, A.4, A.5 and A.8 are as follows: RS=Raw sludge; RL1=Raw sludge Liquors; DRS=Dewatered Raw sludge; PMD=Post-mixing & dilution; PC=Post-Cambi; DS=Digested sludge; RL2=Digested sludge liquors; DS Cake=Digested sludge cake.

Table A.2: NH_4-N data (in mg/L) used to complete the 2013 N mass balance around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	241	110	502	436	862	3500	1950	2422
21/02	184	103	385	306	742	2470	1376	1709
07/03	205	99	428	349	765	2980	1660	2062
21/03	193	77	403	183	476	2788	1553	1929
04/04	229	91	477	373	724	3087	1720	2136
18/04	315	108	656	359	702	4044	2253	2798
02/05	204	115	425	311	762	2418	1347	1673
16/05	195	86	406	336	586	2226	1240	1540
06/06	217	112	452	383	751	2761	1538	1910
20/06	230	73	480	400	683	3105	1730	2149
04/07	177	100	369	350	778	2190	1220	1515
18/07	170	72	355	347	734	1858	1035	1285
01/08	228	93	476	389	716	2712	1511	1877
15/08	241	99	502	491	702	3279	1827	2269
05/09	212	112	443	425	683	2773	1545	1919
19/09	236	90	493	437	762	3262	1817	2257
03/10	221	105	460	406	742	2908	1620	2012
17/10	214	142	450	347	704	2601	1449	1800
07/11	189	100	390	424	765	2675	1490	1851
21/11	188	97	390	254	517	2405	1340	1664
06/12	216	70	451	333	651	2242	1249	1551

Table A.3: TKN data (in mg/L) used to complete the 2013 N mass balance around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	2048	140	5393	4918	5308	7721	1962	26046
21/02	1569	107	4131	4022	4669	7861	1605	25399
07/03	1745	120	4596	4475	5094	7296	2786	24231
21/03	1697	116	4331	4217	4894	8048	2082	27756
04/04	1947	133	5127	4992	5794	8092	1992	26182
18/04	2615	179	7052	6866	6969	7591	2740	25629
02/05	1733	119	4563	4443	4756	8265	1773	27172
16/05	1405	96	4364	4249	4531	8079	1695	24815
06/06	1909	131	4862	4433	4746	7956	1769	22155
20/06	2085	143	5160	4953	5050	6955	1977	26112
04/07	1506	103	3965	3860	4481	7706	2041	20102
18/07	1512	104	3816	3716	4313	7567	1483	22835
01/08	1877	129	5111	4660	5409	7474	1860	26573
15/08	2010	138	5393	5050	6094	8140	2095	26658
05/09	1808	124	4762	4636	5081	6451	1850	25528
19/09	1947	133	5293	5053	5981	6947	2056	23479
03/10	1877	129	4945	4814	5588	6621	1921	21588
17/10	1758	120	4795	4669	5019	7482	1863	20578
07/11	1859	127	4231	4120	4781	6955	2644	28578
21/11	1537	105	4215	4103	4763	6939	2237	21548
06/12	1840	126	4845	4717	5075	7528	1882	22677

Table A.4: PO_4-P (in mg/L) data used to complete the 2013 P mass balance around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	160	58	338	322	373	552	245	996
21/02	118	43	250	238	275	407	180	735
07/03	130	47	275	262	304	450	199	809
21/03	125	45	264	252	292	431	192	778
04/04	171	62	361	344	399	591	262	1065
18/04	148	54	313	298	346	511	226	922
02/05	98	35	208	197	229	338	150	610
16/05	90	33	191	181	210	311	138	560
06/06	112	41	236	226	262	387	171	698
20/06	127	48	272	257	297	439	194	792
04/07	130	47	275	262	310	450	199	809
18/07	120	43	254	241	281	415	183	747
01/08	145	53	307	292	338	500	222	903
15/08	151	55	319	304	353	522	230	940
05/09	115	42	244	232	268	398	176	716
19/09	129	47	272	259	301	445	197	803
03/10	119	43	252	240	278	412	181	741
17/10	118	43	250	238	275	407	180	735
07/11	107	39	227	215	249	370	164	667
21/11	121	44	256	244	282	418	186	753
06/12	135	49	286	271	315	467	206	840

Table A.5: Mg^{2+} data (in mg/L) used to complete the 2013 Mg mass balance around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	65	26	159	144	161	133	12	111
21/02	52	21	127	117	129	106	9	89
07/03	60	24	146	135	149	122	11	103
21/03	45	18	110	99	112	92	8	77
04/04	49	20	120	108	122	100	9	84
18/04	41	16	100	93	103	84	7	70
02/05	30	12	73	66	74	61	5	51
16/05	27	11	66	60	67	55	5	46
06/06	52	21	127	117	129	106	9	89
20/06	57	23	139	126	142	117	10	97
04/07	52	21	127	117	129	106	9	89
18/07	44	18	107	99	110	91	8	75
01/08	53	21	129	117	133	108	10	91
15/08	59	24	144	132	147	120	11	101
05/09	51	20	122	111	124	102	9	86
19/09	55	22	134	123	136	113	10	94
03/10	43	17	105	96	108	88	8	74
17/10	37	15	90	84	92	75	7	63
07/11	60	24	146	135	149	122	11	103
21/11	51	20	124	114	127	105	9	87
06/12	65	26	159	144	161	133	12	111

Table A.6: Combined Industrial effluent data used to complete the N and P mass balance around the ETW at Bran Sands WWTP.

Date	NH_4-N (mg/L)	PO_4-P (mg/L)	Flow (m^3/d)	Total Influent Flow (m^3/d)
07/02	613	25	8228	152328
21/02	483	21	8460	122355
07/03	1395	53	4202	104802
21/03	1770	71	3815	92274
04/04	1583	66	2466	99272
18/04	1746	74	7377	91369
02/05	408	19	9060	102279
16/05	338	14	9455	103981
06/06	373	15	6695	113329
20/06	629	25	12463	100202
04/07	463	19	12503	111531
18/07	464	19	14862	101221
01/08	358	15	4945	96295
15/08	369	14	6329	122626
05/09	303	20	7295	85612
19/09	499	13	10098	85834
03/10	254	15	11477	87735
17/10	321	10	9393	167526
07/11	378	13	9217	174474
21/11	573	18	10739	121685
06/12	439	23	8208	97949

Table A.7: Treated effluent data used to complete the 2013 N and P mass balance around the ETW at Bran Sands WWTP.

Date	NH_4-N (mg/L)	PO_4-P (mg/L)	Flow (m^3/d)
07/02	0.07	22	166320
21/02	0.029	19	132906
07/03	0.35	46	120639
21/03	42.7	63	107028
04/04	51.1	58	116218
18/04	62.2	65	106189
02/05	0.18	17	116652
16/05	0.014	12	109446
06/06	0.63	13	125604
20/06	1.1	23	99271
04/07	1.04	17	107318
18/07	0.43	19	104373
01/08	18	10	121956
15/08	61.6	14	139309
05/09	12.4	15	102491
19/09	1.01	10	98459
03/10	0.75	14	102274
17/10	0.56	9	185311
07/11	0.35	9	9227
21/11	0.275	16	6165
06/12	6.33	19	5033

Table A.8: Flow data (in m^3/d) used to complete the 2013 mass balances around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	4171	2662	1509	1557	1401	971	1286	121
21/02	3514	2277	1237	1276	1248	773	1983	189
07/03	4471	3065	1406	1451	1206	894	727	133
21/03	3875	2770	1105	1138	1024	863	1248	177
04/04	4315	3085	1230	1266	1239	969	1267	154
18/04	3375	2112	1263	1303	1073	840	1093	167
02/05	4053	2853	1200	1239	1115	924	1099	198
16/05	4288	2616	1672	1740	1666	1024	1223	227
06/06	2712	1634	1078	1124	1012	1016	810	214
20/06	3820	2430	1390	1431	1188	827	1312	220
04/07	4347	2884	1463	1516	1364	1013	1429	210
18/07	3993	2446	1547	1561	1305	884	1267	169
01/08	4547	2603	1944	1968	1871	813	1441	196
15/08	3664	2736	928	946	851	895	1303	188
05/09	2380	1190	1190	1229	1006	796	1056	164
19/09	3996	2611	1385	1434	1391	881	870	170
03/10	4285	2854	1431	1479	1231	715	1349	182
17/10	3107	1725	1382	1413	1272	869	1347	189
07/11	4284	2577	1707	1740	1666	949	1294	206
21/11	4753	2775	1978	2006	1805	967	709	218
06/12	4726	2937	1789	1839	1755	1053	1426	227

Table A.9: NH_4-N load data (in tonne NH_4-N/d) used to complete the 2013 mass balances around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	1.0031	0.2928	0.7576	0.6781	1.2075	3.3985	2.5077	0.2931
21/02	0.6475	0.2345	0.4757	0.3898	0.9259	1.9093	2.7286	0.3230
07/03	0.9165	0.3034	0.6015	0.5069	0.9224	2.6641	1.2068	0.2742
21/03	0.7484	0.2133	0.4453	0.2086	0.4877	2.4060	1.9381	0.3414
04/04	0.9867	0.2807	0.5869	0.4719	0.8970	2.9913	2.1792	0.3289
18/04	1.0614	0.2281	0.8291	0.4675	0.7536	3.3970	2.4625	0.4673
02/05	0.8248	0.3281	0.5098	0.3850	0.8500	2.2342	1.4804	0.3313
16/05	0.8345	0.2250	0.6788	0.5852	0.9764	2.2794	1.5165	0.3496
06/06	0.5880	0.1830	0.4876	0.4306	0.7598	2.8052	1.2458	0.4087
20/06	0.8791	0.1774	0.6675	0.5730	0.8116	2.5678	2.2698	0.4728
04/07	0.7688	0.2884	0.5400	0.5301	1.0607	2.2185	1.7434	0.3182
18/07	0.6796	0.1761	0.5494	0.5418	0.9582	1.6425	1.3113	0.2172
01/08	1.0364	0.2421	0.9247	0.7650	1.3403	2.2049	2.1774	0.3679
15/08	0.8812	0.2709	0.4659	0.4649	0.5977	2.9347	2.3806	0.4266
05/09	0.5055	0.1333	0.5275	0.5224	0.6872	2.2073	1.6315	0.3147
19/09	0.9433	0.2350	0.6823	0.6264	1.0604	2.8738	1.5808	0.3837
03/10	0.9449	0.2997	0.6586	0.5999	0.9133	2.0792	2.1854	0.3662
17/10	0.6645	0.2450	0.6168	0.4905	0.8949	2.2603	1.9518	0.3402
07/11	0.8084	0.2577	0.6725	0.7374	1.2743	2.5386	1.9281	0.3813
21/11	0.8934	0.2692	0.7755	0.5085	0.9334	2.3256	0.9501	0.3628
06/12	1.0212	0.2056	0.8065	0.6120	1.1429	2.3608	1.7811	0.3521

Table A.10: TKN load data (in tonne TKN/d) used to complete the 2013 mass balances around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	8.5422	0.3727	8.1377	7.6569	6.9969	7.5841	2.5231	3.2226
21/02	5.5135	0.2436	5.1105	5.1319	5.8269	6.9846	3.1827	4.9128
07/03	7.8019	0.3678	6.4618	6.4932	7.2640	6.8406	1.2984	4.0947
21/03	6.5759	0.3213	4.7856	4.7986	7.0115	6.4934	2.0991	4.3728
04/04	8.4013	0.4103	6.3064	6.3195	5.1788	5.6434	2.5239	5.4140
18/04	8.8256	0.3780	8.9067	8.9459	7.5507	7.5364	2.9948	5.1090
02/05	7.0238	0.3395	5.4754	5.5045	5.7489	7.9409	1.9485	4.6181
16/05	6.0246	0.2511	7.2965	7.3932	7.2150	6.1769	2.0730	5.7740
06/06	5.1772	0.2141	5.2407	4.9831	4.2078	8.3241	1.4329	4.7452
20/06	7.9647	0.3475	7.1727	7.0879	5.8310	8.9324	2.5938	3.0046
04/07	6.5466	0.2971	5.8015	5.8524	6.1121	5.7542	2.2021	5.4914
18/07	6.0374	0.2544	5.9036	5.8001	5.6285	7.1532	1.8790	5.1551
01/08	8.5347	0.3358	9.9350	9.1717	10.1202	6.6374	2.6803	4.6803
15/08	7.3646	0.3776	5.0045	4.9665	5.1860	6.5108	2.7298	5.8157
05/09	4.3030	0.1476	5.6672	5.6982	5.4133	7.5430	1.9536	4.3986
19/09	7.7802	0.3473	7.3308	7.3896	7.3196	5.3583	1.7887	3.6114
03/10	8.0429	0.3682	7.0759	7.1192	6.8788	6.3040	2.5914	3.5630
17/10	5.4621	0.2070	6.6273	6.5969	6.8930	4.8949	2.5095	3.4322
07/11	7.9640	0.3273	7.2225	7.1684	7.9651	6.7533	2.1273	5.5611
21/11	7.3054	0.2914	8.3364	8.2309	8.5972	6.8090	1.1606	4.6455
06/12	8.6958	0.3701	8.6677	8.6740	9.6086	7.7680	2.6837	5.9697

Table A.11: PO_4-P load data (in tonne PO_4-P/d) used to complete the 2013 mass balances around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	0.6674	0.1544	0.5106	0.5007	0.5225	0.5363	0.3148	0.1206
21/02	0.4147	0.0979	0.3088	0.3032	0.3432	0.3146	0.3569	0.1389
07/03	0.5812	0.1441	0.3864	0.3796	0.3664	0.4021	0.1445	0.0876
21/03	0.4844	0.1247	0.2917	0.2868	0.2993	0.3723	0.2390	0.1377
04/04	0.7379	0.1913	0.4443	0.4360	0.4942	0.5723	0.3321	0.1240
18/04	0.4995	0.1140	0.3956	0.3878	0.3708	0.4293	0.2471	0.1140
02/05	0.3972	0.0999	0.2491	0.2438	0.2553	0.3124	0.1646	0.0608
16/05	0.3859	0.0863	0.3190	0.3153	0.3503	0.3180	0.1691	0.1270
06/06	0.3037	0.0670	0.2548	0.2536	0.2652	0.3933	0.1388	0.1494
20/06	0.4851	0.1166	0.3786	0.3675	0.3524	0.3631	0.2551	0.1742
04/07	0.5651	0.1355	0.4020	0.3966	0.4144	0.4557	0.2840	0.1699
18/07	0.4792	0.1052	0.3936	0.3765	0.3664	0.3665	0.2317	0.1262
01/08	0.6593	0.1380	0.5972	0.5739	0.6331	0.4068	0.3196	0.1770
15/08	0.5533	0.1505	0.2962	0.2872	0.3002	0.4669	0.3002	0.1467
05/09	0.2737	0.0500	0.2899	0.2846	0.2694	0.3166	0.1855	0.1174
19/09	0.5155	0.1227	0.3773	0.3717	0.4186	0.3922	0.1716	0.1366
03/10	0.5099	0.1227	0.3606	0.3550	0.3421	0.2943	0.2448	0.1349
17/10	0.3666	0.0742	0.3449	0.3357	0.3499	0.3537	0.2425	0.1389
07/11	0.4584	0.1005	0.3871	0.3738	0.4150	0.3514	0.2124	0.1374
21/11	0.5751	0.1221	0.5056	0.4887	0.5094	0.4039	0.1317	0.1641
06/12	0.6380	0.1439	0.5109	0.4987	0.5535	0.4914	0.2936	0.1208

Table A.12: Mg^{2+} load data (in tonne Mg^{2+}/d) used to complete the 2013 mass balances around the sludge treatment line at Bran Sands WWTP.

Date	RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
07/02	0.2711	0.0692	0.2399	0.2242	0.2257	0.1290	0.0154	0.0134
21/02	0.1827	0.0478	0.1571	0.1493	0.1613	0.0821	0.0178	0.0168
07/03	0.2683	0.0736	0.2053	0.1959	0.1793	0.1090	0.0080	0.0137
21/03	0.1744	0.0499	0.1216	0.1127	0.1142	0.0796	0.0100	0.0136
04/04	0.2114	0.0617	0.1476	0.1367	0.1513	0.0969	0.0114	0.0129
18/04	0.1384	0.0338	0.1263	0.1212	0.1102	0.0709	0.0077	0.0117
02/05	0.1216	0.0342	0.0876	0.0818	0.0829	0.0563	0.0055	0.0101
16/05	0.1158	0.0288	0.1104	0.1044	0.1121	0.0560	0.0061	0.0104
06/06	0.1410	0.0343	0.1369	0.1315	0.1308	0.1080	0.0073	0.0190
20/06	0.2177	0.0559	0.1932	0.1803	0.1682	0.0969	0.0131	0.0213
04/07	0.2260	0.0606	0.1858	0.1774	0.1762	0.1076	0.0129	0.0187
18/07	0.1757	0.0440	0.1655	0.1545	0.1432	0.0801	0.0101	0.0127
01/08	0.2410	0.0547	0.2508	0.2303	0.2484	0.0877	0.0144	0.0178
15/08	0.2162	0.0657	0.1336	0.1249	0.1250	0.1077	0.0143	0.0190
05/09	0.1214	0.0238	0.1452	0.1364	0.1246	0.0808	0.0095	0.0141
19/09	0.2198	0.0574	0.1856	0.1764	0.1896	0.0991	0.0087	0.0160
03/10	0.1843	0.0485	0.1503	0.1420	0.1329	0.0626	0.0108	0.0135
17/10	0.1150	0.0259	0.1244	0.1187	0.1171	0.0652	0.0094	0.0119
07/11	0.2570	0.0618	0.2492	0.2349	0.2477	0.1157	0.0142	0.0212
21/11	0.2424	0.0555	0.2453	0.2287	0.2300	0.1012	0.0064	0.0190
06/12	0.3072	0.0764	0.2845	0.2648	0.2827	0.1399	0.0171	0.0252

Table A.13: Ionic activity product (IAP) data used to complete the 2013 IAP Vs K_{sp} compaisons around the sludge treatment line at Bran Sands WWTP.

RS	RL1	DRS	PMD	PC	DS	RL2	DS Cake
6.09454E-08	4.04191E-09	6.58186E-07	4.91427E-07	1.26162E-06	6.25602E-06	1.39579E-07	6.52748E-06
2.75492E-08	2.2663E-09	2.97006E-07	2.06936E-07	6.42418E-07	2.60251E-06	5.43158E-08	2.72459E-06
3.89582E-08	2.72105E-09	4.18253E-07	3.00611E-07	8.41926E-07	3.98073E-06	8.84168E-08	4.18795E-06
2.6472E-08	1.51974E-09	2.85149E-07	1.11427E-07	3.78295E-07	2.70208E-06	5.79787E-08	2.81594E-06
4.66848E-08	2.74951E-09	5.0396E-07	3.37814E-07	8.59404E-07	4.4423E-06	9.88547E-08	4.65479E-06
4.65006E-08	2.27368E-09	5.00968E-07	2.4197E-07	6.07138E-07	4.24869E-06	8.68788E-08	4.40184E-06
1.45782E-08	1.1769E-09	1.56874E-07	9.83338E-08	3.16159E-07	1.214E-06	2.45768E-08	1.26893E-06
1.15236E-08	7.60673E-10	1.24575E-07	8.90988E-08	2.01946E-07	9.21284E-07	2.08842E-08	9.66024E-07
3.0769E-08	2.34971E-09	3.30891E-07	2.46415E-07	6.19522E-07	2.76694E-06	5.77964E-08	2.89157E-06
4.05941E-08	1.96374E-09	4.43019E-07	3.15684E-07	6.99192E-07	3.89322E-06	8.19474E-08	4.02125E-06
2.91319E-08	2.40497E-09	3.13861E-07	2.60803E-07	7.43889E-07	2.55038E-06	5.31663E-08	2.65875E-06
2.18971E-08	1.35789E-09	2.35561E-07	2.01957E-07	5.51289E-07	1.70117E-06	3.68968E-08	1.75384E-06
4.26795E-08	2.52215E-09	4.59309E-07	3.23132E-07	7.84111E-07	3.56443E-06	8.1647E-08	3.75763E-06
5.2208E-08	3.18421E-09	5.62272E-07	4.79848E-07	8.86936E-07	5.01523E-06	1.12825E-07	5.24844E-06
3.03511E-08	2.2924E-09	3.20999E-07	2.66284E-07	5.52395E-07	2.72986E-06	5.95232E-08	2.87784E-06
4.08101E-08	2.26754E-09	4.38137E-07	3.39325E-07	7.61898E-07	3.9812E-06	8.73435E-08	4.15321E-06
2.74951E-08	1.87025E-09	2.96723E-07	2.27705E-07	5.42436E-07	2.55175E-06	5.72968E-08	2.68826E-06
2.27513E-08	2.23173E-09	2.44285E-07	1.688E-07	4.33984E-07	1.93449E-06	4.44868E-08	2.03134E-06
2.95189E-08	2.2807E-09	3.17847E-07	2.99448E-07	6.90299E-07	2.94129E-06	6.556E-08	3.09811E-06
2.82627E-08	2.07992E-09	3.02778E-07	1.71535E-07	4.53241E-07	2.56246E-06	5.45874E-08	2.65514E-06
4.62013E-08	2.173E-09	4.98816E-07	3.16685E-07	8.06011E-07	3.38578E-06	7.52029E-08	3.52565E-06

Calculating energy cost of treating NH_3 in RL2 separately (using first principles)

Estimating the energy cost of treating NH_3 **(**in RL2 prior to recycling them to the ETW) to consent levels – 250 mg/L for Bran Sands WWTP

NH_4-N Concentration in RL2 at Bran Sands = 1546 mg/L

Daily Flow = 1216 m^3/d

Daily load = $\frac{1216\ x\ 1920}{1000000}$ = 1.9 tonne NH_4-N/d

Amount to reduce to attain consent level = 1920 – 250 = 1296 mg/L

Daily load to be removed = $\frac{1216\ x\ 1296}{1000000}$ = 1.6 tonne NH_4-N/d

Using Energy cost for treating NH_3 (3.2 kWh/kg NH_3 removed (EPA, 2007) at £0.12/kWh:

Energy cost of removing 1.6 tonne NH_4-N/d = 3.2 x 1.6 x 1000 x 0.12 = £614.4/d or £224,260/yr

APPENDIX B

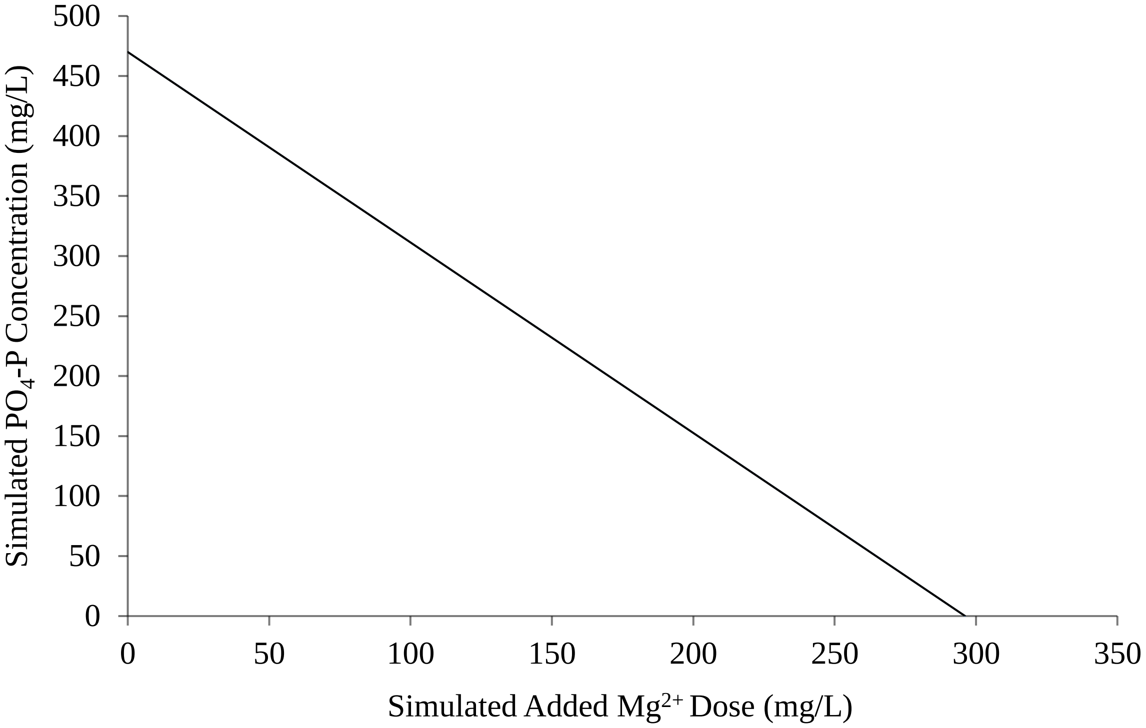

Figure B.1: Simple Linear Regression of the effect of added Mg^{2+} dose on on theoretical PO$_4$-P concentration in digested sludge at pH 7

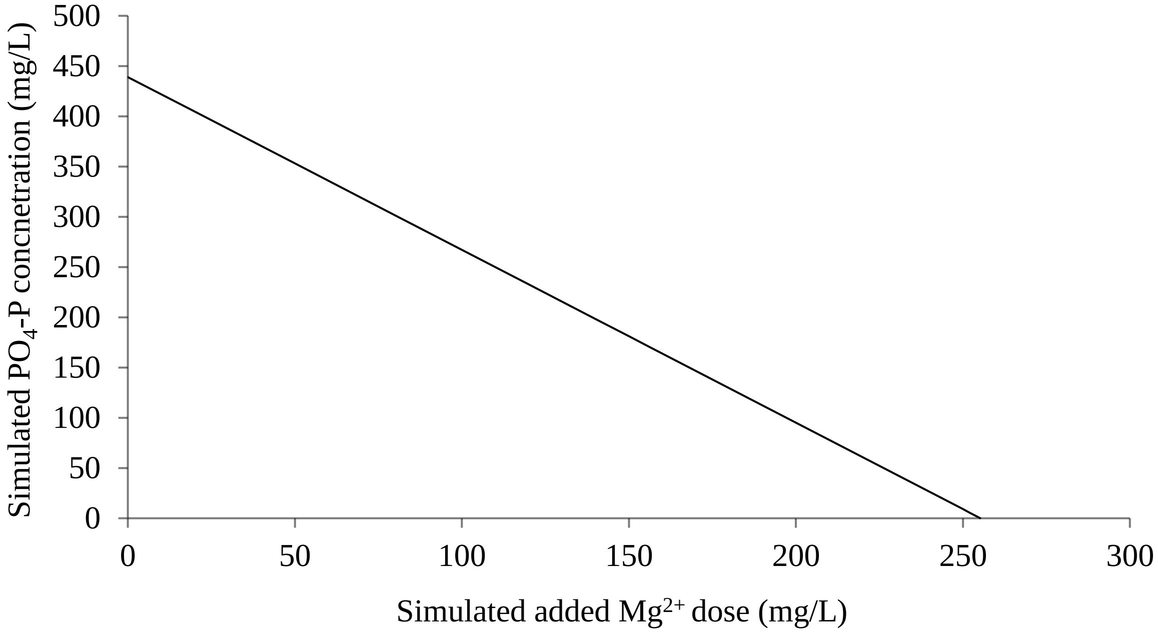

Figure B.2: Simple Linear Regression of the effect of added Mg^{2+} dose on theoretical PO$_4$-P concentration in digested sludge at pH 8

(*Results linear regression model for mean 'soluble' PO$_4$-P concentrations in effluent from Jar test experiments carried out on digested sludge samples*)

Table B.1: Effect of pH and Mg^{2+} dosage on daily NH_4-N and PO_4-P concentration in AD units

Days	pH 7 + 0mg/L Mg^{2+}	pH 7 + 50 mg/L Mg^{2+}	pH 7 + 100 mg/L Mg^{2+}	pH 7 + 150 mg/L Mg^{2+}	pH 8 + 0mg/L Mg^{2+}	pH 8 + 50 mg/L Mg^{2+}	pH 8 + 100 mg/L Mg^{2+}	pH 8 + 150 mg/L Mg^{2+}
2	2250	2150	2030	2005	1800	1700	1650	1600
5	2100	2000	2000	1995	1790	1710	1660	1610
7	2200	2090	2070	2000	1810	1720	1655	1605
10	2050	2070	2000	1990	1780	1760	1650	1600
12	2150	2100	2020	2010	1790	1724	1655	1610
14	2250	2150	2030	2005	1794	1730	1654	1607
15	2100	2000	2000	1995	1800	1700	1660	1620
17	2200	2090	2070	2000	1790	1710	1650	1610
20	2050	2070	2000	1990	1810	1720	1660	1600
22	2150	2100	2020	2010	1780	1760	1655	1610
23	2050	2070	2000	2000	1790	1724	1650	1605
26	2150	2100	2020	1990	1794	1730	1655	1600
28	2250	2150	2030	2010	1780	1700	1654	1610
31	2100	2000	2000	2005	1790	1710	1660	1607
33	2250	2150	2000	1995	1794	1720	1655	1620

35	2100	2000	2070	2010	1800	1760	1654	1610
37	2200	2090	2000	2005	1790	1724	1660	1600
38	2050	2070	2020	1995	1810	1730	1650	1610
40	2150	2100	2030	2000	1790	1700	1660	1607
42	2250	2070	2000	1990	1794	1710	1655	1620
44	2100	2100	2070	2010	1800	1720	1650	1610
46	2200	2090	2000	2000	1790	1760	1655	1600
48	2050	2070	2020	1990	1810	1724	1660	1610
50	2150	2100	2020	2010	1780	1730	1655	1605
52	2050	2070	2030	2005	1790	1760	1650	1600
54	2150	2100	2000	1995	1794	1724	1655	1610
56	2250	2150	2000	2000	1780	1730	1654	1607
58	2100	2000	2070	1990	1790	1700	1660	1620
60	2250	2150	2020	2010	1794	1710	1655	1610

Table B.2: Effect of Mg^{2+} Dose on daily cumulative CH_4 production by AD units at pH 7 and 8

Days	pH 7 + 0 mg/L Mg^{2+}	pH 7 +50 mg/L Mg^{2+}	pH 7 + 100 mg/L Mg^{2+}	pH 7 + 150 mg/L Mg^{2+}	pH 8 + 0 mg/L Mg^{2+}	pH 8 + 50 mg/L Mg^{2+}	pH 8 + 100 mg/L Mg^{2+}	pH 8 + 150 mg/L Mg^{2+}
2	226.0341	181.321	290.5295	258.3562	315.6543	289.0805	293.6375	370.1476
5	508.5075	495.7509	768.7815	729.116	781.4635	784.2488	779.6093	939.1451
7	1368.161	1437.418	1530.98	1592.381	1626.682	1651.423	1663.37	1944.186
10	2052.53	2213.673	2236.396	2293.756	2336.62	2414.72	2378.755	2660.077
12	2752.53	2913.673	2986.396	3053.756	3000.89	3146.262	3021.606	3347.379
14	3452.53	3613.673	3736.396	3813.756	4335.436	4220.17	3922.56	4404.612
15	4152.53	4313.673	4486.396	4573.756	4957.19	4754.885	4454.996	5012.165
17	4852.53	5013.673	5236.396	5333.756	6123.121	5794.974	5632.666	6167.724
20	5552.53	5713.673	5986.396	6093.756	6897.7	6695.431	6491.495	6897.648
22	6252.53	6413.673	6736.396	6853.756	7703.585	7626.833	7376.989	7853.507
23	6952.53	7113.673	7486.396	7613.756	8155.324	8154.41	7812.701	8454.271
26	7652.53	7813.673	8236.396	8373.756	9234.991	9221.392	9079.225	9609.041
28	8352.53	8513.673	8986.396	9133.756	10061.68	10211.37	10053.4	10555.08
31	9052.53	9213.673	9736.396	9893.756	10581.14	10865.32	10797.62	11301.77
33	9752.53	9913.673	10486.4	10653.76	11667.32	12023.99	12098.81	12383.91
35	10452.53	10613.67	11236.4	11413.76	12400.33	12805.16	12993.8	13195.54

37	11152.53	11313.67	11986.4	12173.76	13025.47	13333.03	13674.98	13867.17
38	11852.53	12013.67	12736.4	12933.76	13788.25	13981.82	14470.11	14713.43
40	12552.53	12713.67	13486.4	13693.76	14538.55	14665.77	15339.62	15633.48
42	13252.53	13413.67	14236.4	14453.76	15190.64	15257.81	15981.58	16217.72
44	13952.53	14113.67	14986.4	15213.76	15816.4	15856.38	16647.4	16922.56
46	14652.53	14813.67	15736.4	15973.76	16475.27	16592.54	17522.47	17713.55
48	15352.53	15513.67	16486.4	16733.76	17183.7	17275.49	18454.9	18530.38
50	16052.53	16213.67	17236.4	17493.76	17722.96	17912.44	19095.82	19138.92
52	16752.53	16913.67	17986.4	18253.76	18548.47	18967.81	20147.24	20301.28
54	17452.53	17613.67	18736.4	19013.76	19124.59	19449.05	20724.35	20937.74
56	18152.53	18313.67	19486.4	19773.76	20090.18	20594.52	21967.14	22200.35
58	18852.53	19013.67	20236.4	20533.76	20561.85	21111.8	22478.38	22744.96
60	19552.53	19713.67	20986.4	21293.76	21014.72	21638.1	23017.61	23260.81

Working out volume of 5N NaOH needed for the cost benefit analysis of Struvite dosing experiments

In AD reactors

Original pH = 7.7
Target pH = 8
pH = -Log [H^+]
[H^+] = $10^{-7.7}$ ions
NaOH conc = 5 N
Vol of sludge (V_1) = 905000 L/d
concentration (C_1) = 9.98×10^{-8} N
pOH = 6
[H^+] = $10^{-6.0}$ ions
concentration (C_2) = 0.000005
Vol of NaOH (V_2) = ??
C_1V_1 = C_2V_2
Vol of NaOH (V_2) = 18057.12395 L/d per year = 6590850 L/yr
Mass of NaOH needed = 722.284958 kg/d per year = 263634 Kg/yr

In post-AD recovery systems

Original pH = 7.8
Target pH = 8
pH = -Log [H^+]
[H^+] = $10^{-7.8}$ ions
NaOH conc = 5 N
Vol of sludge (V_1) = 905000 L/d
concentration (C_1) = 7.92×10^{-8} N
pOH = 6
[H^+] = $10^{-6.0}$ ions
concentration (C_2) = 0.000005
Vol of NaOH (V_2) = ??
C_1V_1 = C_2V_2
Vol of NaOH (V_2) = 14343.28339 L/d per year = 5235298 L/yr
Mass of NaOH needed = 573.7313357 kg/d per year = 209411.9 kg/yr

Calculation of P removal and recovery

P removal = $\frac{\boldsymbol{(Start\!-\!up\ P\ concentration\ -Final\ P\ concentration\ )\ x\ 100}}{\boldsymbol{Start\!-\!up\ P\ concentration}}$

Recoverable P (tonne/yr)= $\frac{\boldsymbol{(Start\!-\!up\ P\ concentration\ -Final\ P\ concentration)x\ Volumetric\ Flow\ x\ 365}}{\boldsymbol{1000000}}$

For Example; Potential Recoverable P from digested sludge at pH 8 with 100 mg/L added Mg^{2+};

Recoverable P (tonne/yr) = $\frac{\boldsymbol{\left(439\frac{mg}{L} - 101.41\frac{mg}{L}\right)x\frac{905m^3}{d}\ x\ 365}}{\boldsymbol{1000000}}$ = **111.5 tonne P/yr**

P removal = $\frac{\boldsymbol{(439\frac{mg}{L} - 101.41\frac{mg}{L})\ x\ 100}}{\boldsymbol{439\frac{mg}{L}}}$ = **~77**

Table B.3: Factors and rates used for establishing the cost benefit analysis for post-AD Mg^{2+} dosing at Bran Sands WWTP

Unit cost	Items	Added Mg^{2+} Dose (mg/L)
	Internal Costs (£/yr)	
a) €1419773 as investment cost for P-recovery systems from WWTPs serving ≥100,000p.e. (Montag et al, 2009) spread over 10 years	Investment Cost	(Total Investment cost) ÷ 10 ÷ 1.2. The amount is same for all Mg^{2+} doses
b) Unit Operation and Maintenance costs of €1400/ton P in treated effluent (Berg *et al*, 2006; Dockhorn, 2009)	Operation and Maintenance costs	(Unit cost x Annual residual P in treated effluent for the various added Mg^{2+} dose experiments) ÷ 1.2
	Financial Costs (£/yr)	
c) 209410 kg needed at a cost of £24.99/25kg NaOH commercial grade (from Chemiphase (serial No: 5240258/16/48/11; www.chemiphase.co.uk)	Cost of 5N NaOH	(Mass of required NaOH) x 24.99 ÷ 25kg. The amount is same for all Mg doses
d) \$300/ton of Mg_2Cl (EC No.: 232-094-6; www.americanelements.com/magnesium-chloride)	Cost of Mg_2Cl	(Unit cost) x (mass of $MgCl_2$ required for the various Mg^{2+} doses) ÷ 1.35
	Internal Incomes (£/yr)	
e) €3/kg of P recovered (Dockhorn, 2009)	Struvite Sale	(Unit cost x Annual Recoverable P for the various Mg^{2+} doses) ÷ 1.2
f) £145000/yr spent on dealing with struvite problems at Bran Sands (Bran Sands Annual Site report, 2015)	Savings in maintenance/replacement Costs	(Current Annual cost of dealing with struvite at Bran Sands) – (Operation and Maintenance costs for the various Mg^{2+} doses)
g) \$6.5/lb (or \$2.93/kg) P removed (Doyle and Parsons, 2002)	Savings on sludge management	(Unit cost x Annual Recoverable P for the various Mg^{2+} doses) ÷ 1.35
h) Energy cost for treating NH_3 (3.2 kWh/kg NH_3 removed (EPA, 2007) at £0.12/kWh (http://www.businessenergy.com/electricity); Hurkett, 2016)	Savings on nitrification cost	(Unit cost x Annual Recoverable N for the various Mg^{2+} doses) x 0.12
i) \$2.93/lb (or \$1.32/kg) NO_x-N removed (Katehis and Metcalf & Eddy, 2006)	Savings on acetic acid for denitrification	(Unit cost x Annual Recoverable N for the various Mg^{2+} doses) ÷ 1.35
	Internal Benefits (£/yr)	
	Internal Income – (Internal Costs + Financial costs)	(Sum of Internal Income for the various Mg^{2+} doses) – (Sum of Costs for the various Mg^{2+} doses)

Exchange Rate: £1 = \$1.35; £1 = €1.2 (www.oanda.com)

Table B.4: Factors and rates used for establishing the cost benefit analysis for Mg^{2+} dosing in AD reactors at Bran Sands WWTP

Unit cost	Items	Added Mg^{2+} Dose (mg/L)
	Internal Costs (£/yr)	
a) 25% of €1419773 as investment cost for P-recovery systems from WWTPs serving ≥100,000p.e. (Montag et al, 2009) spread over 10years	Investment Cost	(Total Investment cost) ÷ 10 ÷ 1.2. The amount is same for all Mg^{2+} doses
b) Unit Operation and Maintenance costs of €1400/ton P in treated effluent (Berg *et al*, 2006; Dockhorn, 2009)	Operation and Maintenance costs	(Unit cost x Annual residual P in treated effluent for the various added Mg^{2+} dose experiments) ÷ 1.2
	Financial Costs (£/yr)	
c) 263630kg needed at a cost of £24.99/25kg NaOH commercial grade (from Chemiphase (serial No: 5240258/16/48/11; www.chemiphase.co.uk)	Cost of 5N NaOH	(Mass of required NaOH) x 24.99 ÷ 25kg. The amount is same for all Mg doses
d) \$300/ton of Mg_2Cl (EC No.: 232-094-6; www.americanelements.com/magnesium-chloride)	Cost of Mg_2Cl	(Unit cost) x (mass of $MgCl_2$ required for the various Mg^{2+} doses) ÷ 1.35
	Internal Incomes (£/yr)	
e) €3/kg of P recovered (Dockhorn, 2009)	Struvite Sale	(Unit cost x Annual Recoverable P for the various Mg^{2+} doses) ÷ 1.2
f) £145000/yr spent on dealing with struvite problems at Bran Sands (Bran Sands Annual Site report, 2015)	Savings in maintenance/replacement Costs	(Current Annual cost of dealing with struvite at Bran Sands) – (Operation and Maintenance costs for the various Mg^{2+} doses)
g) \$6.5/lb (or \$2.93/kg) P removed (Doyle and Parsons, 2002)	Savings on sludge management	(Unit cost x Annual Recoverable P for the various Mg^{2+} doses) ÷ 1.35
h) Energy cost for treating NH_3 (3.2 kWh/kg NH_3 removed (EPA, 2007) at £0.12/kWh (http://www.businessenergy.com/electricity))	Savings on nitrification cost	(Unit cost x Annual Recoverable N for the various Mg^{2+} doses) x 0.12
i) \$2.93/lb (or \$1.32/kg) NO_x-N removed (Katehis and Metcalf & Eddy, 2006)	Savings on acetic acid for denitrification	(Unit cost x Annual Recoverable N for the various Mg^{2+} doses) ÷ 1.35
j) Energy savings from increased biogas production	Energy savings from increased biogas production	
	Internal Benefits (£/yr)	
	Internal Income – (Internal Costs + Financial costs)	(Sum of Internal Income for the various Mg^{2+} doses) – (Sum of Costs for the various Mg^{2+} doses)

Exchange Rate: £1 = \$1.35; £1 = €1.2 (www.oanda.com)

APPENDIX C

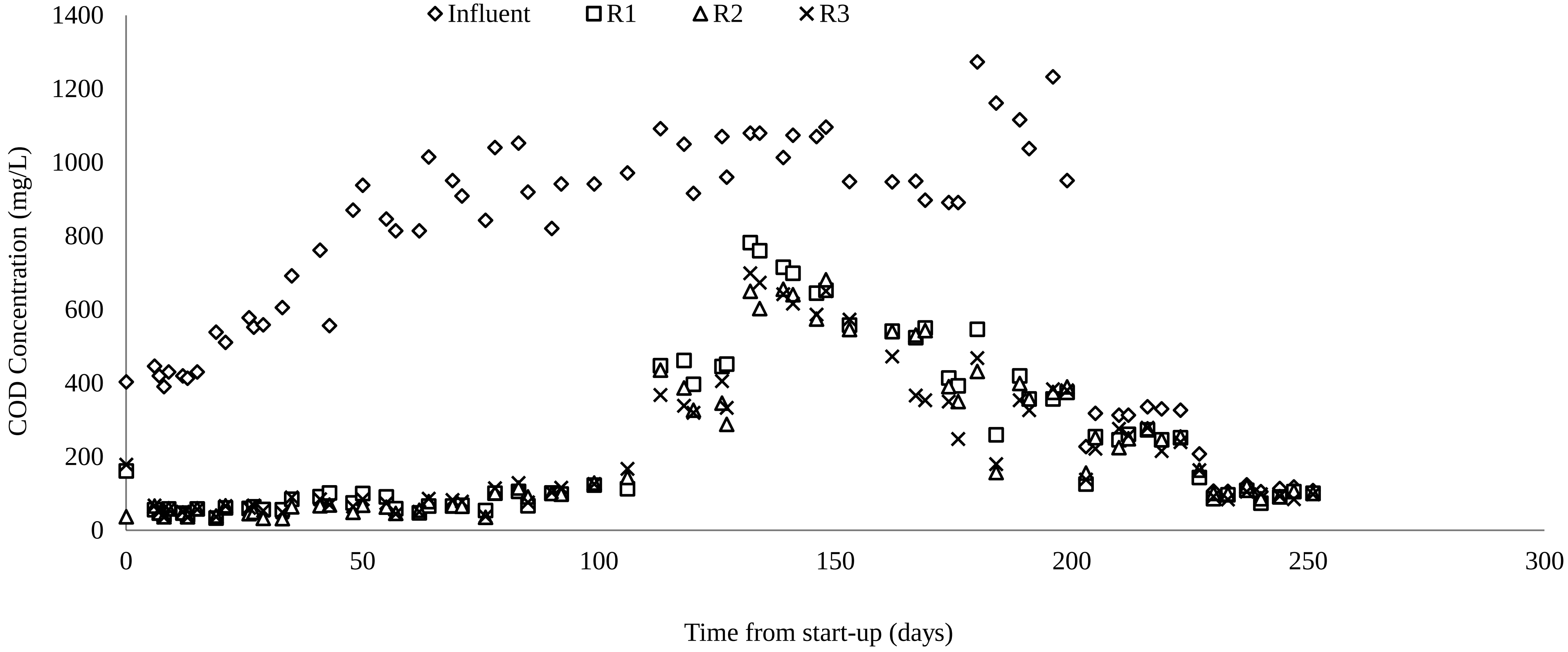

Figure C.1: Influent and effluent COD concentrations during the operation of three identical aerobic granular sludge reactors (R1, R2, and R3) over a period of 250 days.

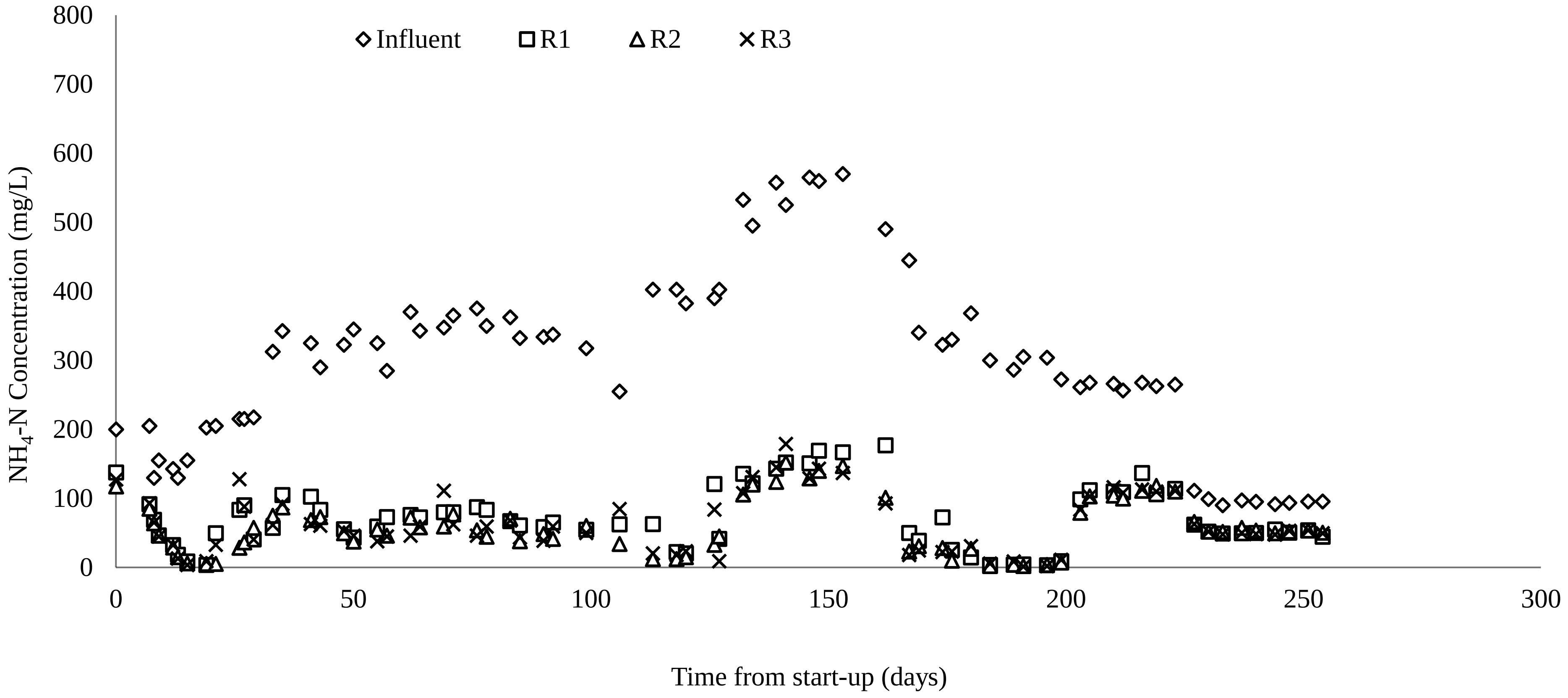

Figure C.2: Influent and effluent NH4-N concentrations during the operation of three identical aerobic granular sludge reactors (R1, R2, and R3) over a period of 250 days.

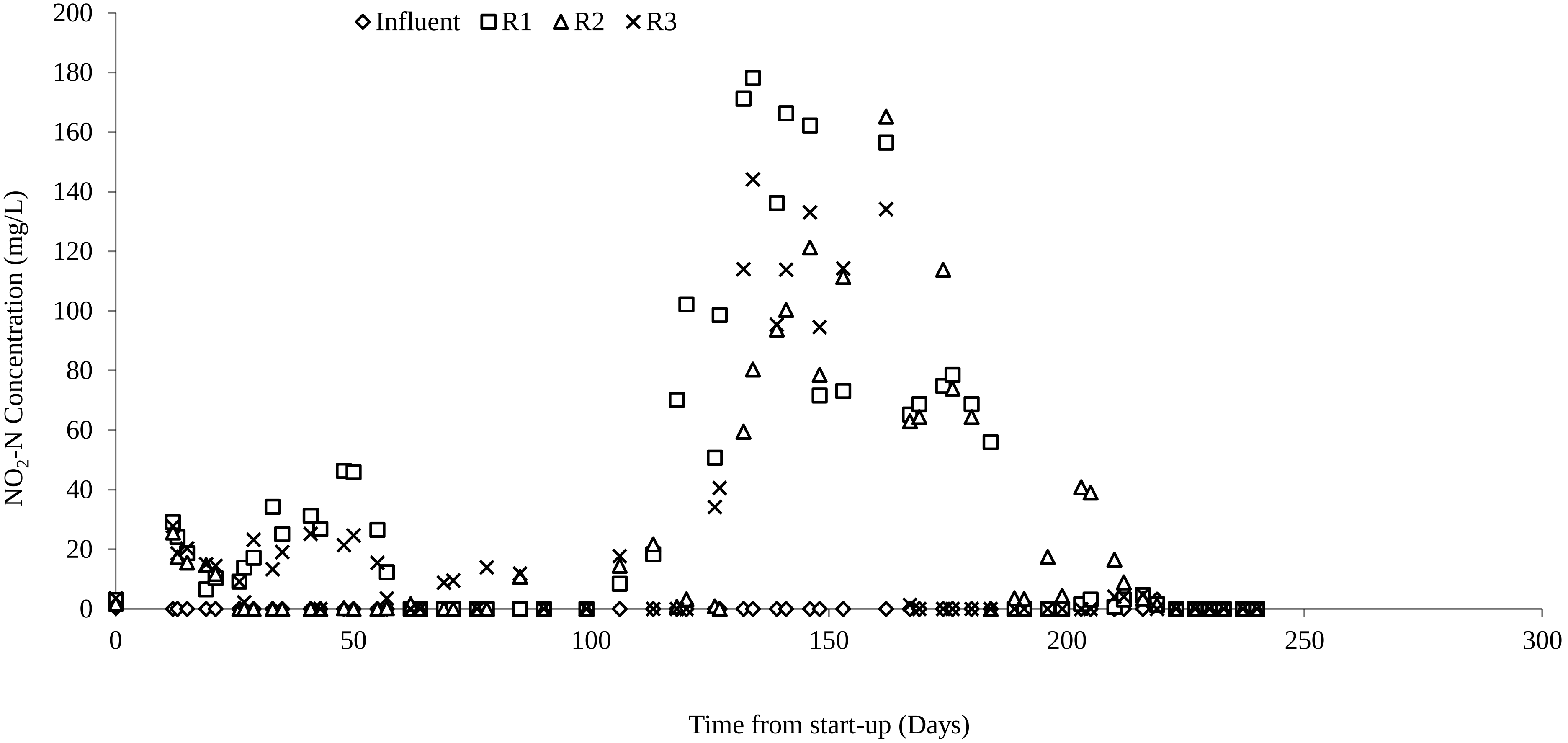

Figure C.3: Influent and effluent NO₂-N concentrations during the operation of three identical aerobic granular sludge reactors (R1, R2, and R3) over a period of 250 days.

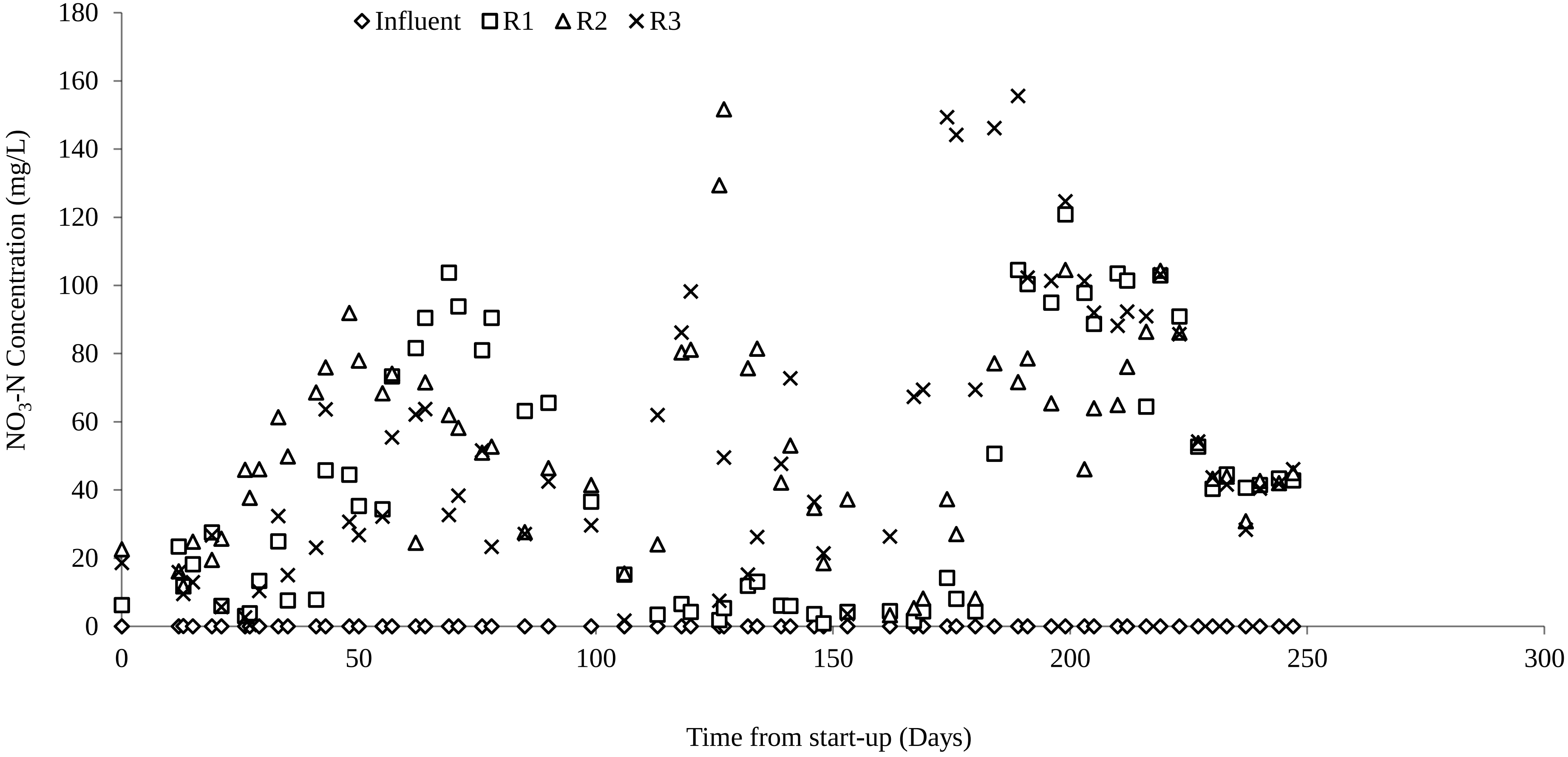

Figure C.4: Influent and effluent NO₃-N concentrations during the operation of three identical aerobic granular sludge reactors (R1, R2, and R3) over a period of 250 days.

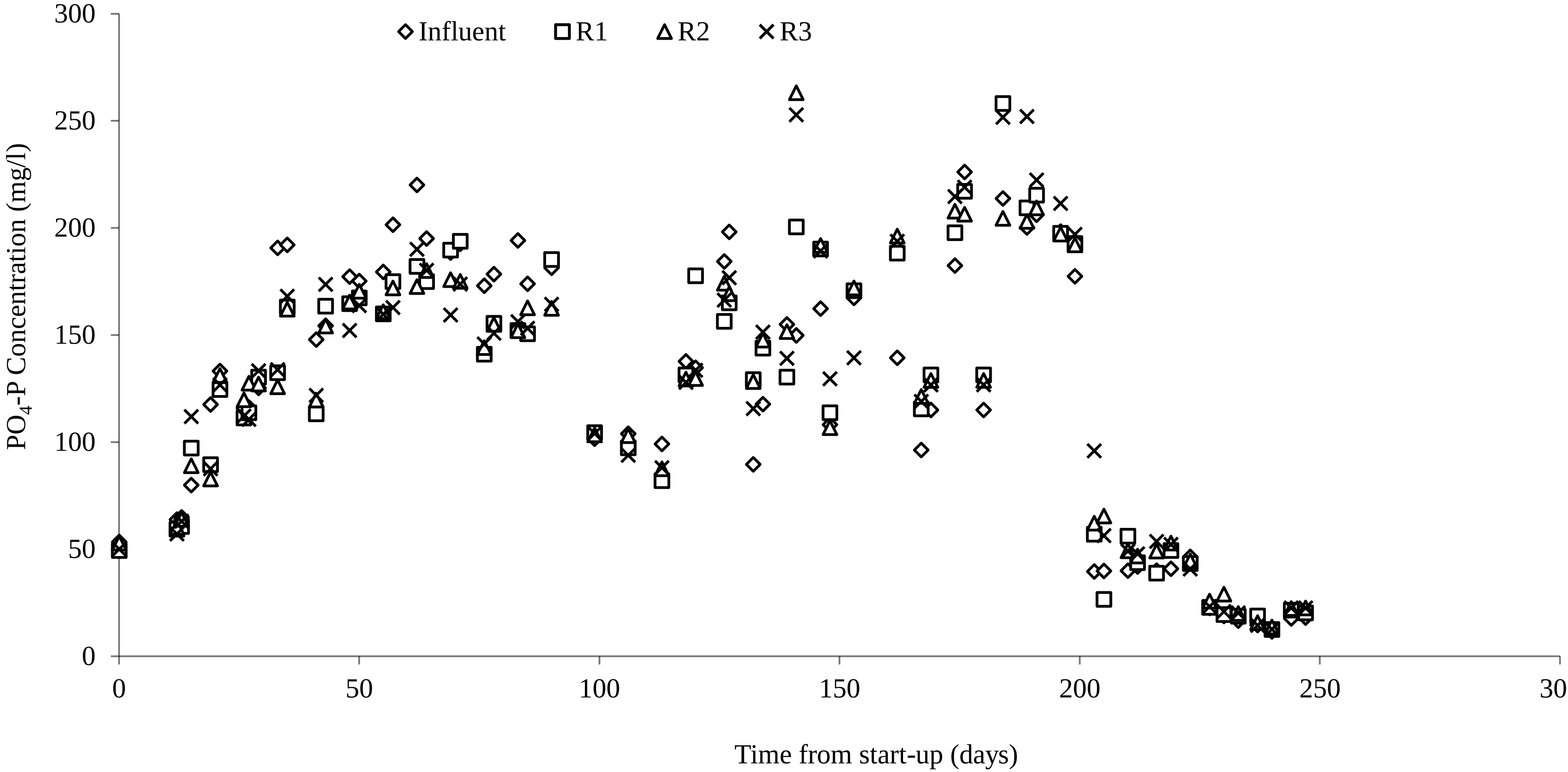

Figure C.5: Influent and effluent PO4-P concentrations during the operation of three identical aerobic granular sludge reactors (R1, R2, and R3) over a period of 250 days.

Table C.1: Factors/assumptions used in Cost Estimate and comparison of NH_4-N treatment costs for RLs under three treatment scenarios

Cost Item	Current costs of treating RLs via CAS at Bran Sands WWTP (£/yr)	Estimated costs of treating RLs (without COD addition) via AGT (£/yr)	Estimated costs of treating of RLs via CAS post-AGT (£/yr)
Investment Cost (at a unit cost of 1.35 €/kgN$_{removed}$; Fux, 2004)	Unit cost x Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency x 2 units ÷ 1.2	Unit cost x Annual NH_4-N load in RLs only x 64% NH_4-N removal efficiency x 2 units ÷ 1.2	Unit cost x post-AGT Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency x 2 units ÷ 1.2
Balancing tank	-	Unknown cost	-
Operation cost			
Energy costs (at a unit nitrification cost of 0.09 kWh/m^3; 12p/kWh electricity; Shi, 2011; Hurkett, 2016)	Unit cost x Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency x unit cost of kWh electricity in UK	Unit cost x Annual NH_4-N load in RLs only x 64% NH_4-N removal efficiency x unit cost of kWh electricity in UK	Unit cost x post-AGT Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency x unit cost of kWh electricity in UK
`Maintenance (Analysis, Replacement/Repair; at a unit cost of 1.0 €/kgN$_{removed}$; Fux, 2004; De Kreuk, 2006)	Unit cost x Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency ÷ 1.2	Unit cost x Annual NH_4-N load in RLs only x 64% NH_4-N removal efficiency ÷ 1.2	Unit cost x post-AGT Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency ÷ 1.2
Sludge Disposal (at a unit cost of 0.75 €/kgN$_{removed}$; Fux, 2004; De Kreuk, 2006)	Unit cost x Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency ÷ 1.2	***	Unit cost x Annual NH_4-N load in RLs only x 95% NH_4-N removal efficiency ÷ 1.2
Total Costs	**Sum of all costs**	**Sum of all costs**	**Sum of all costs**

Exchange Rate: £1 = €1.2 (www.oanda.com)

****Sludge disposal not as frequent as in CAS; no reasonably applicable cost of Balancing tank was not found literature searched.*

REFERENCES

Aage, H.K., Andersen, B.L., Blom, A., Jensen, I. (1997). The solubility of struvite. Journal of Radioanalytical and Nuclear Chemistry. 223(1): 2213-215.

Abma, W., Schultz, C.E., Mulder, J.W., van Loosdrecht, M.C.M., van der Star, W.R.L., Tokutomi, T. (2007). The Advance of Anammox. Water. 21: 36-37.

Abbona, F., Boistelle, R. (1979). Growth morphology and crystal habit of struvite crystals (MgNH4PO4.6H2O). Journal of Crystal Growth. 46: 339-354.

Adav, S.S., Lee. D.J. (2008). Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. Journal of Hazardous Materials. 154: 1120-1126.

Adav, S.S., Lee. D.J., Lai, J.Y. (2009). Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. Bioresource Technology. 100: 5359-5361.

Ahring, B.K., Alatriste-Mondragon, F., Westermann, P., Mah, R.A. (1991). Effects of cations on *Methanosarcina thermophila* TM-1 growing on moderate concentrations of acetate: production of single cells. Applied Microbiology & Biotechnology. 35: 686–689.

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. (2004). Essential Cell Biology, 4th Ed. Garland Science. pp 10-15.

Ali, M.I., Schneider, P.A., Hudson, N. (2005). Thermodynamics and solution chemistry of struvite. Journal of the Indian Institute of Science. 85: 141-149.

Ali, M.I. (2007). Struvite crystallization in fed-batch pilot scale and description of solution chemistry of struvite. Trans IChemE, Part A. Chemical Engineering Research and Design. 85 (A3): 344-356.

AMBIO. (2011). Economic feasibility study for phosphorus recovery processes. Royal Swedish Academy of Sciences. 40: 408-416.

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). (2005). Standard methods for the examination of water and wastewater. (21st Ed.) Washington DC.

Andreottola, G., Ragazzi, M., Foladori, P., Villa, R., Langone, M., Rada, E.C. (2012). The unit integrated approach for OFMSW treatment. U.P.B. Scientific Bulletin, Series C – Electrical Engineering and Computer Science. 74(1): 19-26.

Anthoisen, A.C., Loehr, R.C., Prakasam, T.B., Srinath, E.G. (1976). Inhibition of nitrification by ammonia and nitrous acid. Journal of the Water Pollution Control Federation. 48: 835-852.

Arnold, E., Böhm, B., Wilderer, P.A. (2000). “Application of activated sludge and biofilm sequencing batch technology to treat reject water from sludge and dewatering systems: A comparison.” Water Science & Technology. 41 (1): 115-122.

Ariyanto, E. S., Sen, T.K., Ang, H.M. (2014). The influence of various physico-chemical process parameters on kinetics and growth mechanism of struvite crystallization. Advanced Powder Technology. 25 (2): 682-694.

Auckley, J. (2000). The Rainbows of Crane Creek. Available online at http://mdc.mo.gov/conmag/2000/01/rainbows-crane-creek. (Accessed: 30th January 2016).

Awang, N.A., Shaaban, G.M. (2015). The impact of reactor height/diameter (H/D) ratio on aerobic granular sludge (AGS) formation in sewage. Jurnal Teknologi (Sciences & Engineering). 77 (32): 95-103

Babic-Ivancic, V., Kontrec, J., Kralj, D., Brecevic, L. (2002). Precipitation diagram of struvite and dissolution kinetics of different struvite morphologies. Croatica chemical acta. 75: 89-106.

Barak, P., Stafford, A. (2006). Struvite: A recovered and recycled phosphorus fertilizer. Proceedings of the 2006 Wisconsin Fertilizer, Aglime and Pest Management Conference, Vol. 45.

Barat, R., Montoya, T., Seco, T., Ferrer, J. (2005). The role of potassium, magnesium and calcium in enhanced biological phosphorus removal treatment plants. Environmental Technology. 26: 983-992.

Barker, P.S., Dold, P.L. (1996). Denitrification behaviour in biological excess phosphorus removal activated sludge systems. Water Resources. 30: 769-780.

Barnard, J.L. (2006). Requirements for achieving effluent phosphorus of less than 0.1 mg/L. Session P1 in WERF, 2006.

Barnard, J.L. (2009). Elimination of eutrophication through resource recovery, International Conference on nutrient recovery from wastewater streams, IWA Publishing, London.

Bassett, H., Bedwell, W.L. (1933). Studies of phosphates. Part I. Ammonium magnesium phosphate and related compounds. Journal of Chemical Society. 854-871.

Battistoni, P. Boccadoro, R., Fatone, F., Pavan, P. (2004). Auto-nucleation and crystal growth of struvite in a demonstrative fluidized bed reactor. In: Proceedings of the

International Conference on struvite: ite role in phosphorus recovery and reuse, Cranfield (UK).

Benisch, M., Clark, C., Sprick, R.G., Baur, R. (2000). Struvite deposits: a common and costly nuisance, WEF Operations Forum.

Berg, U., Knoll, G., Kaschka, E., Weidler, P.G., Nüesch, R. (2006). Is phosphorus recovery from wastewater feasible? Environmental Technology. 28: 165-172.

Bergmans, B.J.C., Veltman, A.M., van Loosdrecht, M.C.M., van Lier, J.B., Rietveld, L.C. (2011). Struvite recovery from digested sludge. Dissertation: Delft University. Delft. The Netherlands

Bergmans, B.J.C., Veltman, A.M., van Loosdrecht, M.C.M., van Lier, J.B., Rietveld, L.C. (2014). Struvite formation for enhanced dewaterability of digested waster sludge. Environmental Technology. 35 (5): 549-555.

Beun, J.J., Hendriks, A., van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J. (1999). Aerobic granulation in a sequence batch reactor. Water Resources. 33 (10): 2283-2290.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J. (2000a). Aerobic granulation. Water Science and Technology. 41: 41-48.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J. (2002). Aerobic granulation in a sequencing batch airlift reactor. Water Research. 36: 702-712.

Bhuiyan, M.I.H., Mavinic, D.S., Beckie, R.D. (2007). A solubility and Thermodynamic study of struvite. Environmental Technology. 28 (9): 1015-1026.

Bindhu, B.K., Madhu, G. (2013): Influence of Organic Loading Rates on Aerobic Granulation Process for the Treatment of Wastewater. Journal of Clean Energy Technologies. 1 (2).

Björlenius, B. (1994). Nitrous oxide emissions from municipal wastewater treatment plants (In Swedish). Naturvårdsver-ket Report. No. 4309.

Blackburne, R., Vadivelu, V.M., Yuan, Z.G., Keller, J. (2007). Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. Water Research. 41 (14): 3033-3042.

Bloech, H. (2005). European Union Legislation on wastewater treatment and nutrient removal. In Proceedings of the IWA Special Conference: “Nutrient Management in wastewater treatment and recycle streams.” Lemtech Konsult, Krakow, Poland.

Blöch, H. (2005). European Union Legislation on wastewater treatment and nutrient removal. Foundation for water research, Allen House, The Listons Road, Marlow, Bucks. United Kingdom.

Bonmati, A., X. Flotats (2003). Air stripping of ammonia from pig slurry: characterisation and feasibility as a pre- or post-treatment to mesophilic anaerobic digestion. *Waste Management.* 23(3): 261-272.

Borgerding, J. (1972). Phosphate deposits in digestion systems. Journal of Water Pollution Control Federation. 44: 813-819.

Bortone, G., Saltarelli, R., Alons, V., Sorm, R., Wanner, J., Tilche, A. (1996). Biological anoxic phosphate removal – the DEPHANOX process. Water Science & Technology. 34:119-128.

Bouropoulos, C.C., Koutsoukos, P.G. (2000). Spontaneous precipitation of struvite from aqueous solutions. Journal of Crystal Growth. 213: 381-388.

Bridger, G.L., Salutsky, M.L., Starosika, R. (1961). Metal ammonium phosphates as fertilizer. 140th Meeting of the American Chemical Society, 1-19, Chicago, (USA).

Britton, A., Prasad, R., Balzer, B., Cubbage, L. (2009). Pilot testing and economicevaluation of struvite recovery from dewatering centrate at HRSD's NansemondWWTP. In: International conference on nutrient recovery from wastewaterstreams. Vancouver, Canada, 193–202.

Buchanan, J.R., Mote, C.R., Robinson, R.B. (1994). Struvite control by chemical treatment. ASAE. 37 (4): 1301-1308.

Buitrón, G., Moreno-Andrade, I. (2011). Biodegradation kinetics of a mixture of phenols in a sequencing batch moving bed biofilm reactor under starvation and shock loads. Journal of Chemical Techniques in Biotechnology. 86 (5): 669-674.

Burns, J.R., Finlayson, B. (1982). Solubility Product of Magnesium Ammonium Phosphate Hexahydrate at various temperatures. Journal of Urology. 128 (2): 426-428.

Bustin, S.A., Benes, V., Nolan, T., Pfaff, M.W. (2005). Quantitative real-time RT-PCR – a perspective: Review. Journal of Molecular Endocrinology. 34:597-601.

Bustin, S.A. (2010). Developments in real-time PCR research and molecular diagnostics. Expert Review of Molecular Diagnostics. 10 (6): 713-715.

Cacho, R.J.A. (2005). Anaerobic digestion of excess municipal sludge. Optimization for increased solid destruction. Ph.D Thesis in Environmental Engineering. University of Cincinnati, Cincinnati, OH.

Caffaz, S. Bettazzi, E., Scaglione, D., Lubello, C. (2008). An integrated approach in a municipal WWTP: anaerobic codigestion of sludge with organic waste and nutrient removal from supernatant. Water Science & Technology. 58: 669-676.

Capdevielle, A., Sýkorová, E., Biscans, B., Béline, F., Daumer, M. (2013). Optimization of struvite precipitation in synthetic biologically treated swine wastewater.

Determination of the optimal process parameters. *Journal of Hazardous Materials*. 244-245: 357-369.

Capodaglio, A.G., Hlavínek, P., Rabioni, M. (2015). Physico-chemical technologies for nitrogen removal from wastewaters: A Review. Ambiente & Agua - An interdisciplinary Journal of Applied Science. 1980-1993.

Carrera, J., Vicent, T., Lafuente, J. (2004). Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater. Process Biochemistry. 39: 2035-2041.

Cassidy, D.B., Belia E. (2005). Nitrogen and phosphorus removal from an abattoir wastewater in a SBR with aerobic granular sludge. Water Research. 39: 4817-4823.

Cecchi, F., Battistoni, P., Boccadoro, R. (2003). Phosphate crystallization process for P-recovery applied at Teviso municipal wastewater treatment plant (Italy). Strada le Gazie. University of Verona.

Celen, I., Türker, M. (2001). Recovery of ammonia as struvite from anaerobic digester effluents. Environmental Technology. 22: 1263-1272.

Cervantes, F.J. (2009). Environmental technologies to treat nitrogen pollution. London, UK: IWA Publishing.

Chambers, R.G. (1998). Input and output indicators. In index numbers: essays in honour of Sten Malmquist, ed. R. Färe, S. Grosskopf, and R. Russell. Boston: Kluwer Academic Publishers.

Chen, Y., Jiang, W., Liang, D.T., Tay, J.H. (2007). Aerobic granulation under the combined hydraulic and loading selection pressures. Bioresource Technology. 99: 7444-7449.

Chen, X.P., Zhu, Y.G., Xia, Y., Shen, J.P., He, J.Z. (2008). Ammonia-oxidizing archaea: important players in paddy soil? Environmental Microbiology. 10: 1978-1987.

Coma, M., Verawaty, M., Pijuan, M., Yuan, Z., Bond, P.L. (2012). Enhancing aerobic granulation for biological nutrient removal from domestic wastewater. Bioresource Technology. 103 (1): 101-108.

Cordell, D., Drangert, J.O., White, S. (2009). The story of phosphorus: global food security and food for thought. Global Environmental Change. 19: 292-305.

CRU (2003). The competitiveness of phosphate fertilizer producers to 2020: Coping with changing production costs, resource availability and market structures. CRU Group international of British sulphur consultants. Confidential.

Cydzik-Kwiatkowska, A., Bialowiec, A., Wojnowska-Baryla, I., Smoczyński, L. (2009). Characteristics of granulated activated sludge fed with glycerine fraction from biodiesel production. Archives of Environmental Protection. 35: 41-52.

Cydzik-Kwiatkowska, A., Wojnowska-Baryla, I. (2011). Nitrifying granules cultivation in a sequencing batch reactor at a low organics-to-total nitrogen ratio in wastewater. Folia Microbiology. 56: 201-208.

Cydzik-Kwiatkowska, A., Zielinska, M., Bernat, K., Wojnowska-Baryla, I., Truchan, T. (2013). Treatment of high-ammonium anaerobic digester supernatant by aerobic granular sludge and ultrafiltration processes. Chemosphere. 90: 2208-2215.

Czerwionka, K., Makinia, J., Pagilla, K. (2008). Effluent dissolved organic nitrogen (DON) studies in Poland. WEFTEC pre-conference workshop "WERF Nutrient Removal Challenge", 18 – 22nd October 2008, Chicago (USA).

Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H., Wagner, M. (2001). In-situ characterization of Nirospira-like nitrite oxidizing bacteria active in wastewater treatment plants. Applied and Environmental Microbiology. 67 (11): 5273-5284.

Daphne, L.H.X., Utomo, H.D., Kenneth, L.Z.H. (2011). Correlation between Turbidity and Total Suspended Solids in Singapore Rivers. *Journal of Water Sustainability.* 1 (3): 313–322.

De Araújo, M.J., Ducom, G., Achour, F., Rouez, M., Bayard, R. (2008). Mass Balance to assess the efficiency of a mechanical-biological treatment. Waste Management. 28 (10): 1791-1800.

de Bruin L.L.M., de Kreuk, M.K., van der Roest, H.F.R., Uijterlinde, C., van Loosdrecht, M.C.M. (2004). Aerobic granular sludge technology: an alternative to activated sludge? Water Science & Technology. 49: 1-7.

de Bruin L.L.M., Kraan, M.W., de Kreuk, M.K. (2007). Process and apparatus for the purification of wastewater. International patent – WO2007089141, DHV, The Netherlands.

de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M. (2005). Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. Biotechnology and Bioengineering. 90 (6): 761-769.

de Kreuk, M.K. (2006). Aerobic Granular sludge – Scaling-up a new technology. PhD Thesis, Department of Biotechnology, Technical University Delft – The Netherlands. p 199.

de Kreuk, M.K., van Loosdrecht, M.C.M. (2006). Formation of aerobic granules with domestic sewage. Journal of Environmental Engineering. 132 (6): 694-697.

de Kreuk, M.K., de Bruin L.L.M. (2007). Aerobic Granule Reactor Technology. London, IWA Publishing.

De-Bashan, L.E., Bashan, Y. (2004). Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003). Water Research. 38 (19): 4222- 46.

De la Rubia, M.A., Walker, M., Heaven, S., Banks, C.J., Borja, R. (2010). Preliminary trials of in-situ ammonia stripping from source segregated domestic food waste digestate using biogas: effect of temperature and flow rate. Bioresource Technology. 101:9486-9492.

DEFRA. (2014). Water Framework Directive Implementation in England and Wales: new and updated standards to protect the water environment. May 2014.

Demirer, G.N., Uysal, A., Yilmazel, Y.D. (2010). The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. Journal of Hazardous Materials. 181: 248-254.

Di Iaconi, C., Pagano, M., Ramadori, R., Lopez, A. (2010). Nitrogen recovery from a stabilized municipal landfill leachate. Bioresource Technology. Elsevier Ltd. 101: 1732-1736.

Dockhorn, T. (2009). About the economy of phosphorus recovery. In International Conference on nutrient recovery from wastewater streams, ed. K. Ashley, D. Mavinic, and Koch. London, UK: IWA Publishing.

Doyle, J.D., Philip, R., Churchley, J. and Parsons, S.A. (2000). Analysis of struvite precipitation in real and synthetic liquors. Process Safety and Environmental Protection. 78: 480-488.

Doyle, J.D., Parsons, S.A. (2002). Struvite formation, control and recovery. Water Research. 36: 3925-3940.

EA, Environment Agency. (2014). Water company fined for sewage treatment works pollution. Press Release. First published: 11 June 2014.

Egle, L. (2014). (Comparative) Technical, Environmental and Economic Assessment of phosphorus recycling technologies from wastewater. Institute for water quality, Resource and Waste Management. Vienna University of Technology, Karlzplatz 13/226-1, 1040 Vienna, Austria.

Ekama, G.A., Wentzel, M.C., Sötemann, S.W. (2006). Mass balance-based plant-wide wastewater treatment plant models – Part 1: Biodegradability of wastewater organics under anaerobic conditions. Water SA. 32 (3):269-275.

Etterer, T., Wilderer, P.A. (2001). Generation and properties of aerobic granular sludge. Water Science & Technology. 43:19-26.

EU Regulation No. 259/2012 of the European Parliament and of the Council of 14 March 2012. Official Journal of the European Union.

Evans, T.D. (2007). Recovering ammonium and struvite fertilisers from digested sludge dewatering liquors. Proc. IWA Specialist Conference: Moving Forward – Wastewater biosolids sustainability. June 2007. Moncton, NB, Canada.

Färe, R., Grosskopf, S., Webber, W. (2006). Shadow prices and pollution costs in US agriculture. Ecological Economics. 43: 19-26.

Fatone, F., Dante, M., Nota, E. Di Fabio, S., Frison, N., Pavan, P. (2011). Biological short-cut nitrogen removal from anaerobic digestate in a demonstration sequencing batch reactor. Chemical Engineering Transactions. 24: 1135-40.

Forbes, C.M., O'Leary, N.D., Dobson, A.D., Marchesi, J.R. (2009). The contribution of 'omic' – based approaches to the study of enhanced biological phosphorus removal microbiology. FEMS Microbiology Ecology. 69: 1-15.

Fortsner, G. (2015). AirprexTM: Biosolids Treatment Optimization Process with the option of phosphate Recovery MWEA – Annual Biosolids Conference, March 2nd 2015.

Frison, N., Katsou, E., Malamis, S., Renzi, D., Fatone, F., Lijo, L. (2012a). Nitritation-denitritation and enhanced nitrite biological phosphorus removal from the liquid resulting from the codigestion of WAS and OFMSW. International Conference on Sustainable Solid Waste Management. Athens, Greece.

Frison, N., Lampis, S., Bolzonella, D., Pavan, P., Fatone, F. (2012b). Two-Stage start-up to achieve the stable via-nitrite pathway in a demonstration SBR for anaerobic codigestate treatment. Industrial &Engineering Chemistry Research. 51: 15423-15430.

Frison, N., Di Fabio, S., Cavinato, C., Pavan, P., Fatone, F. (2013). Best available carbon sources to enhance the via-nitrite biological nutrients removal from supernatants of anaerobic co-digestion. Chemical Engineering Journal. 215: 15-22.

Fuhs, G.W., Chen, M. (1975). Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. Microbiology Ecology. 2(2): 119-138.

Furukawa, K., Inatomi, Y., Qiao, S., Quan, L., Yamamoto, T., Isaka, K., Sumino, T. (2009). Innovative treatment system for digester liquor using Anammox process. Bioresource Technology. 100: 5437-43.

Fux, C. (2003). Biological nitrogen elimination of ammonium-rich sludge digester liquids. PhD Thesis, ETH Zurich Switzerland.

Fux, C., Lange, K., Faessler, A., Huber, P., Grueniger, B., Siegrist, H. (2003). Nitrogen removal from digester supernatant via nitrite – SBR or SHARON? Water Science and Technology. 48 (8): 9-18.

Fux, C., Siegrist, H. (2004). Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economic considerations. Water Science & Technology. 50 (10): 19-26.

Fux, C., Velten, S., Carozzi, V., Solley,D., Keller, J. (2006). Efficient and stable nitritation and denitritation of ammonium-rich sludge dewatering liquor using an SBR with continuous loading. Water Research. 40 (14): 2765-2775.

Gao, M., Yang, M., Li, H., Yang, Q., Zhang, Y. (2003). Comparison between a submerged membrane bioreactor and a conventional activated sludge system on treating ammonia bearing inorganic wastewater. Journal of Biotechnology. 108: 265-269.

Gao, H., Scherson, Y.D., Wells, G.F. (2014). Towards energy neutral wastewater treatment: methodology and state of the art. *Environmental Science: Processes & Impact.* 16 (6): 1223-1246. CODEN: ESPICZ; ISSN: 2050-7895.

Gaval, G., Pernelle, J.J. (2003). Impact of the repetition of oxygen deficiencies on the filamentous bacteria prolifereation in activated sludge. Water Resources. 37: 1991-2000.

Geerts, S., Marchi, A., Weemaes, M. (2015). Full-scale Phosphorus Recovery form digested wastewater sludge in Belgium – Part II: Economic Opportunities and Risks. Water Science & Technology. 71 (4): 495.

Gieseke, A., Purkhold, U., Wagner, M., Amann, R., Schramm, A. (2001). Community structure and activity dynamics of nitrifying bacteria in a phosphate removing biofilm. Applied Environmental Microbiology. 67 (3): 1351-1362.

Gieseke, A., Bjerrum, L., Wagner, M., Amann, R. (2003). Structure and activity of multiple nitrifying bacterial populations coexisting in a biofilm. Environmental Microbiology. 5 (5): 355-369.

Giesen, A. (2009). P-recovery with the Crystalactor – efficient water treatment without waste. DHV. Netherlands.

Giesen, A., de Bruin, L.M.M., Niermans, R.P., Van der Roest, H.F. (2013). Advancements in the application of aerobic granular biomass technology for sustainable treatment of wastewater. Water Practice and Technology. 8 (1): 47-54.

Graham, D.W., Knapp, C.W., Van Vleck, E.S., Bloor, K., Lane, T.B., Graham, C.E. (2007). Experimental demonstration of chaotic instability in biological nitrification. ISME J. 1: 385-393.

Gray, N.F. (2004). Biology of Wastewater Treatment. World Scientific: *Technology & Engineering*. P 117.

Guskin S., Marinsek-Logar, R. (2011). Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. *Process Safety and Environmental Protection*. 8(9): 61–66

Gustavsson, D.J.I. (2010). Biological sludge liquor treatment at municipal wastewater treatment plants – a review. VATTEN. 66: 179-192.

Gustavsson, D.J.I., la Cour Jansen, J. (2010). Dynamics of nitrogen oxides emissions from a full-scale sludge liquor treatment plant with nitritation. Water Science and Technology. 63 (12): 2838-2845.

Gustavsson, D.J.I., Nyberg, U., la Cour Jansen, J. (2010). Dynamics of nitrogen oxides emission form a full-scale sludge liquor treatment plant with nitritation. Water Science & Technology. 63 (12): 2838-2845.

Güven, D., Dapena, A., Kartal, B., Schmidt, M.C., Mass, B., van de Pas Schoonen, K, Sozen Sozen, S., Mendez, R., Op den Camp, H.J.M., Jetten, M.S.M., Strous, M. Schmidt, I. (2005). Propionate oxidation by and methanol inhibition of anaerobic ammonium oxidising bacteria. Applied and Environmental Micobiology. 71 (2): 1066-1971.

Hanhoun, M., Montastruc, L., Azzaro-Pantel, C., Biscans, B., Freche, M., Pibouleau, L. (2011). Temperature impact assessment on struvite solubility product: a thermodynamic modelling approach. Biochemical Engineering Journal. 167 (1): 50-58.

Harms, G., Layton, A.C., Dionisi, H.M., Gregory, I.R., Garrett, V.M., Hawkins, S.A., Robinson, K.G., Sayler, G.S. (2003). Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. Environmental Science and Technology. 37 (2): 343-351.

Hawkins, S.A, Robinson, K.G., Layton, A.C., Sayler, G.S. (2006). A comparison of ribosomalgene and transcript abundance during high and low nitrite oxidizing activity using a newly designed real-time PCR detection system targeting the Nitrobacter spp. 16S-23S intergenic spacer region. Environmental Engineering and Science. 23: 521-532.

Hawkins, S., Robinson, K., Layton, A., Sayler, G. (2010). Limited Impact of free ammonia on Nitrobacter spp. Inhibition assessed by chemical and molecular techniques. Bioresource Technology. 101 (12): 4513-4519.

Hedstrom, A. (2001). Ion exchange of ammonium in zeolites: a literature review. Environmental Engineering. 673-681.

Heffer, P., Prud'homme, M. (2014). Fertilizer outlook 2014-2018. In: 82nd IFA Annual Conference, Sydney.

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M., Heijnen, J.J. (1998). The SHARON process: an innovative method for nitrogen removal from ammonium-rich wastewater. Water Science & Technology. 37 (9): 135-142.

Henze, M., Harremoës, P., la Cour Jansen, J., Arvin, E. (2002). Wastewater Treatment: Biological and Chemical Processes, 3rd ed, Springer-Verlag, Berlin.

Henze, M. (2008). Biological Wastewater Treatment: Principles Modelling and Design. Edited by M. Henze, M.C.M. van Loosdrecht, G.A. Ekama and D. Brdjanovic. ISBN: 9781843391883. Published by IWA Publishing, London, UK.

Hernández, F., Urkiaga, A., De las Fuentes, L., Bis, B., Chiru, E., Balazs, B., Wintgens, T. (2006). Feasibility studies for water reuse projects: An economical approach. Desalination. 187: 253-261.

Hesselmann, R., Werlen, C., Hahn, D., van der Meer, J., Zehnder, A., (1999). Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. Systematic and Applied Microbiology. 22: 454-465.

Hobson, P.N., Wheatley, A.D. (1993). Anaerobic Digestion: A modern Theory and Practice. Elsevier: Applied Science. London, UK

Huang, J., Shang, C. (2006). Air Stripping. From: Handbook of Environmental Engineering, Vol. 4: Advanced Physicochemical Treatment Processes. Edited by: L.K. Wang, Y.T. Hung, and N.K. Shammas © The Humana Press Inc., Totowa, NJ.

Huang, Z., Gedalanga, P.B., Asvapathanagul, P., Olson, B. H. (2010). Influence of physico-chemical and operational parameters on Nitrobacter and Nitrospira communities in an aerobic activated sludge bioreactor. Water Research. 44: 4351-4358.

Hurkett, C. (2016). Watts the amount Eddie Hall could save on his electricity bill? *Journal of Interdisciplinary Science Topics.* 5:57-59

Isanta, E., Suarez-Ojeda, M.E., del Rio, A.V., Morales, N., Perez, J., Carrera, J. (2012). Long term operation of a granular sequencing batch reactor at pilot scale treating a low-strength wastewater. Chemical Engineering Journal. 198: 163-170.

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002). Potential Phosphorus recovery by struvite formation. Water Research. 36: 1834-1842.

Janus, H.M., van der Roest, H.F. (1997). Don't reject the idea of treating reject water. Water Science & Technology. 35 (10): 27-34.

Jardin, N., Thöle, D., Wett, B. (2006). Treatment of sludge return liquors: experiences from the operation of full-scale plants. In: Proceedings of the Water Environment Federation. WEFTEC. 5237-5255.

Jasinski, D., Tandoi, V. (1991). The applied microbiology of biological phosphate removal – Accomplishment and needs. Water Research. 25: 1471-1478.

Jenkins, D., Tandoi, V. (1991). The applied microbiology of biological phosphate removal – Accomplishment and needs. Water Research. 25: 1471-1478.

Jenicek, P., Svehla, P., Zabranska, J., Dohanyos, M. (2004). Factors affecting nitrogen removal by nitritation/denitritation. Water Science & Technology. 49 (5-6): 73-79.

Jiang, A., Zhang, T., Zhao, Q., Frear, C., Chen, S. (2010). Integrated Ammonia Recovery Technology in Conjunction with Dairy Anaerobic Digestion. CSANR Research Report 001.

Jing, H.O. (2008). Fundamental and application of aerobic granulation technology for wastewater treatment.

Jones, A.G. (2002). Crsytallization process system. Butterworth/ Heinemann, London, UK.

Jordão, E.P., Pessôa, C.A. (2011). Tratamento de Esgotos Domésticos (Domestic wastewater treatment), 6th ed. ABES, Rio de Janeiro.

Jorgensen, K.S., Pauli, A.S.L. (1995). Polyphosphate accumulation among denitrifying bacteria in activated sludge. Anaerobe. 1:161-168.

Joss, A., Salzgeber, D., Eugster, J. König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J., Siegrist, H. (2009). Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. Environmental Science & Technology. 43: 5301-5306.

Kampschreur, M.J., Poldermans, R., Kleerebezem, R., van der Star, W.R.L., Haarhuis, R., Abma, W.R., Jetten, M.S., van Loosdrecht, M.C.M. (2009a). Emission of nitrous oxide and nitric oxide from full-scale single stage nitritation-anammox reactor. Water Science & Technology. 60 (12): 3211-3217.

Kämpfer, P., Erhart, R., Beimfohr, C., Böhringer, J., Wagner, M., Amann, R. (1996). Characterization of bacterial communities from activated sludge: culture-dependent numerical identification versus in situ identification using group- and genus-specific rRNA-targeted oligonucleotide probes. Microobial Ecology. 32:101-121.

Kerrn-Jespersen, J.P., Henze, M. (1993). Biological phosphorus uptake under anoxic and aerobic conditions. Water Research. 27: 617-624.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dwason, L. (2011). The anaerobic digestion of solid organic waste. Waste Management. 31:1737-1744.

Khan, M.Z., Mondal, P.K., Sabir, S. (2013). Aerobic granulation for wastewater bioremediation. A review. Canadian Journal of Chemical Engineering. 91 (6): 1045-1058.

Kim, D.J., Seo, D. (2006). Selective enrichment and granulation of ammonia oxidizers in a sequencing batch airlift reactor. Processes in Biochemistry. 41: 1055-1062.

Kishida, N., Tsuneda, S., Kim, J.H., Sudo, R. (2009). Simultaneous nitrogen and phosphorus removal from high-strength industrial wastewater using aerobic granular sludge. Journal pf Environmental Engineering. 135: 153-158.

Klappenbach, J.A., Saxman, P.R., Cole, J.R., Schmidt, T.M. (2001). rrndb: the ribosomal RRNA operon copy number database. Nucleic Acids Research. 29: 181-184.

Kleeman, R. (2015). Sustainable Phosphorus Recovery from Waste. Engineering Doctorate Thesis. University of Surrey, UK.

Knapp, C.W., Graham, D.W. (2007). Nitrite-oxidizing bacteria guild ecology associated with nitrification failure in a continuous-flow reactor. FEMS Microbiology Ecology. 62 (2): 195-201.

Kobelev, A.P., Stefanovsky, S.V., Knyazev, O.A., Lashchenova, T.N., Ptashkin, A.G., Polkanov, M.A. (2006). Results of a 50% Waste Loading Vitrification Test Using the Cold Crucible Melter for Savannah River Site Waste. WM’06 Conference, February 26–March 2, 2006, Tucson, AZ. USA.

Korchef, A., Saidou, H., Ben, A.M. (2011). Phosphate recovery through struvite precipitation by CO2 removal: effect of magnesium, phosphate and ammonium concentrations. Journal of Hazardous Materials. 186: 602-613.

Kruk, D.J., Elektorowicz, M., Oleszkiewicz, J.A. (2014). Struvite precipitation and phosphorus removal using magnesium sacrificial anode. Chemosphere. 101: 28-33.

Kuba, T., Smolders, G., van Loosdrecht, M.C.M., Heijnen, J.J. (1993). Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. Water Science & Technology. 27: 241-252.

Kuba, T., van Loosdrecht, M.C.M., Heijnen, J.J. (1996). Phosphorus and nitrogen removal with minimum COD requirement by integration of denitrification in a two-sludge system. Water Research. 30: 1702-1710.

Kugelman, I.J., McCarty, P.L. (1964). Cation toxicity and stimulation in anaerobic waste treatment. *J. Water Pollut. Control Fed.* 37: 97–116.

Lahav, O., Telzhensky, M., Zewuhn, A., Gendel, Y., Gerth, J., Calmano, W., Birnhack, L. (2013). Struvite recovery from municipal-wastewater sludge centrifuge supernatant using seawater NF concentrate as a cheap Mg (II) source. Separation & Purification Technology. 108: 103-110.

Lamarre, B., Sherouse, D. (1994). Air stripping industrial wastewater. Pollution Engineering. 26 (9): 46.

Langereis, R., Geraats, B. (2013). Optimisation of anaerobic sludge treatment from enhanced biological phosphate removal by airprex struvite precipitation system. The 18th European Biosolids & Organic Resources Conference. 18th – 20th November, 2013. Manchester, UK.

Laridi, R., Auclair, J.C., Benmoussa, H. (2005). Laboratory and pilot-scale phosphate and ammonium removal by controlled struvite precipitation following coagulation and flocculation of swine wastewater. Environmental Technology. 26 (5): 525-536.

Laurich, F. (2003). Combnined quantity management and biological treatment of sludge liquor at Hamburg’s WWTP. Presentation at the 9th IWA Specialised Conference on Large WWTPs, Prague.

Le Corre, K.S., Valsami-Jones, E., Hobbs, P., Parsons, S.A. (2005). Impact of calcium on struvite crystal size, shape and purity. Journal of Crystal Growth. 283: 514-522.

Le Corre, K.S., Valsami-Jones, E., Hobbs, P., Jefferson, B., Parsons, S.A. (2006). Agglomeration of struvite crystals. Water Research. 41 (2): 419-425.

Le Corre, K.S., Valsami-Jones, E., Hobbs, P., Parsons, S.A. (2009). Phosphorus recovery from wastewater by struvite crystallization: A Review. Critical Reviews in Environmental Science and Technology. 39 (6): 433-477.

Ledezma, P., Kuntke, P., Buisman, C.J.N., Keller, J., Freguia, S. (2015). Source-separated urine opens golden opportunities for microbial electrochemical technologies. Trends in Biotechnology. 33: 214-220.

Lee, S.I., Weon, S.Y., Lee, C.W., Koopman, B. (2003). Removal of nitrogen and phosphate from wastewater by addition of bittern. Chemosphere. 51 (4): 265-271.

Lei, X., Sugiura, N., Feng, C., Maekawa, T. (2007). Pretreatment of anaerobic digestion effluent with ammonia stripping and biogas purification. Journal of Hazardous Materials. 145 (3): 391-397.

Li, J., Zhang, Z.J., Li, Z.R., Huang, G.Y., Abe, N. (2006). Removal of organic matter and nitrogen from distillery wastewater by a combination of methane fermentation and denitrification/nitrification processes. Journal of Environmental Science. 18: 654-659.

Li, Y., Zou, J., Zhang, L., Sun, J. (2014). Aerobic granular sludge for simultaneous accumulation of mineral phosphorus and removal of nitrogen via nitrite in wastewater.

Bioresource Technology. 154: 178-184.

Li, Z., Ren, X., Zuo, J., Liu, Y., Duan, E., Yang, J., Chen, P., Wang, Y. (2012). Struvite Precipitation for Ammonia Nitrogen Removal in 7-Aminocephalosporanic Acid Wastewater. Molecules. 17: 2126-2139.

Liao, P., Chen, A.; Lo, K. (1995). Removal of nitrogen from swine manure wastewaters by ammonia stripping, *Bioresour.Technol.* 54 (1): 17-20.

Liberti, L., Boari, G., Passino, R. (1979). Phosphate and ammonium recovery from secondary effluents by selective ion exchange with production of a slow release fertiliser, Water Research. 13 (6): 5-73.

Lind, B.B., Ban, Z., Byden, S. (2001). Volume reduction and concentration of nutrients in human urine. Ecological Engineering. 16: 561-566.

Ling, D. (2009). Experience from commissioning of full-scale DeAmmon plant at Himmerfjärden (Sweden). In: Proceedings of 2nd IWA specialised Conference on nutrient Management in wastewater treatment Processes. Lentech Konsulting ed., Lemtech Konsulting, Krakow, Poland, pp. 403-410.

Little, J.C., Marinas, B.J. (1997). Cross-flow versus counterflow air-stripping towers. Journal Environmental Engineering – ASCE. 123 (7): 668.

Liu, Y., Tay, J.H., (2002). The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. Water Research. 36: 1653.

Liu, Q.S., Tay, J.H., Liu, Y. (2003). Substrate concentration-independent aerobic granulation in sequential aerobic sludge blanket reactor. Environmental Technology. 24:1235-1243.

Liu, Y., Tay, J.H., (2004). State of the art of biogranulation technology for wastewater treatment. Biotechnology Advancements. 22 (7): 533-563.

Liu, Y., Wang, Z.W., Quin, L., Liu, Y.Q. (2005). Selection pressure-driven aerobic granulation in a sequencing batch reactor. Applied Microbiology and Biotechnology. 67:26-32.

Liu, Y., Tay, J.H., (2007). Cultivation of aerobic granules in a bubble column and an airlift reactor with divided darft tubes at low aeration rate. Biochemical Engineering Journal. 34: 1-7.

Liu, Y., Moy, B.Y.P., Tay, J.H., (2007). COD removal and nitrification of low strength domestic wastewater in aerobic granular sludge sequencing batch reactors. Enzyme and Microbial Technology. 42 (1): 23-28.

Liu, Y.Q., Moy, B., Kong, Y., Tay, J.H. (2010). Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment. Enzyme and Microbial Technology. 46 (6): 520-525.

Liu, Y., Kumar, S., Kwag, J.H., Ra, C. (2013). Magnesium ammonium phosphate formation, recovery and its application as valuable resources: A Review. Journal of Chemical Technology and Biotechnology. 88:181-189.

Liu, B., Giannis, A., Zhang, J., Victor, W.C., Chang, V.W.C., Wang, J.Y. (2013a). Characterization of induced struvite formation from source separated urine using seawater and brine as magnesium sources. Chemosphere. 93:2738-2747.

Maixner, F., Noguera, D.R., Anneser, B., Stoeker, K., Wegl, G., Wagner, M., Daims, H. (2006). Nitrite concentration influences the population structure of Nitrospira-like bacteria. Environmental Microbiology. 8: 1487-1495.

Malamis, S.,Katsou, E., Di Fabio, S., Bolzonella, D., Fatone, F. (2014). Biological nutrients removal from supernatant originating from the anaerobic digestion of the organic fraction of municipal solid waste. Critical Reviews in Biotechnology.34 (3): 244-257.

Malpei, F., Ficara, E., Canziani, R. (2008). Nitrogen removal from digestate: Conventional and advanced technologies. In: Adani, F., Schievano, A., Boccaile, G. eds. Anaerobic Digestion: Opportunities for Agriculture and Environment. Milan: University of Milan. 113-27.

Mamais, D., Pitt, P.A., Cheng, Y.W., Loiacono, J., Jenkins, D. (1994). Determination of ferric-chloride dose to control struvite precipitation in anaerobic sludge digesters. Water Environment Research. 66: 912 - 918.

Mañas, A., Biscans, B., Spérandio, M. (2011). Biologically induced phosphorus precipitation in aerobic granular sludge process. *Water Research*. 45: 3776 - 3786

Mao, Y., Graham, D.W., Tamaki, H., Zhang, T. (2015). Dominant and novel clades of Candidatus Accumulibacter phosphatis in 18 globally distributed full-scale wastewater treatment plants. Scientific Reports. 5: 11857.

Marchi, A., Geerts, S., Weemacs, M., Wim, S., Vanhoof, C. (2015). Full-scale phosphorus recovery from digested wastewater sludge in Belgium – part I: technical achievements and challenges. Water Science & Technology. – Special Issue on Full-scale Investigations. 71 (4): 487-494.

Marias, G.V.R., Loewenthal, R.E., Siebritz, I.P. (1983). Reviews: Observations supporting phosphate removal by biological excess uptake. Water Science &Technology. 15 (3-4): 15-41.

Marti, N., Bouzas, A., Seco, A., Ferrer, J. (2008). Struvite precipitation assessment in anaerobic digestion processes. Valencia, Spain. Chemical Engineering Journal. 141: 67-74.

Martins, A.M.P., Heijnen, J.J., van Loosdrecht, M.C.M. (2003). Effect of dissolved oxygen concentration on sludge settleability. Applied Microbiology and Biotechnology. 62: 586-593.

Matsumiya, Y., Yamasita, T., Nawamura, Y. (2000). Phosphorus removal from sidestreams by crystallization of magnesium-ammonium-phosphate using seawater. Journal of the Chartered Institution of Water and Environmental Management. 14: 291-296.

Matynia, A., Koralewska, J., Wierzbowska, B. (2006). The influence of process parameters on struvite continuous crystallization kinetics. Chemical Engineering Communications. 193: 160-176.

Mayer, M., Smeets, W., Braun, R., Fuchs, W. (2009). Enhanced ammonium removal from liquid anaerobic digestion residuals in an advanced sequencing batch reactor system. Water Science & Technology. 60: 1649-1660.

McSwain, B.S., Irvine, R.L., Wilderer, P.A. (2004a). The influence of settling time on the formation of aerobic granules. Water Science & Technology. 50:195-202.

McSwain, B.S., Irvine, R.L., Wilderer, P.A. (2004b). Effect of intermittent feeding on aerobic granule structure. Water Science & Technology. 49: 19-25.

Mehta, C.M., Khunjar, W.O., Nguyen, V., Tait, S., Batstone, D.J. (2015). Technologies to recover nutrients from waste streams. A Critical Review. Critical Reviews in Environmental Science and Technology. 45 (4): 385-427.

Meinhold, J., Arnold, E., Isaac, S. (1999). Effect of nitrogen on anoxic phosphate uptake in biological phosphorus removal activated sludge. Water Research. 33: 1871-1883.

Melkinia, J., Stensel, H.D., Czerwionka, K., Drewnowski, J., Zapero, D. (2009). Nitrogen transformations and mass balances in anaerobic/anoxic/aerobic batch experiments with full-scale biomasses from BNR activated sludge systems. Water Science & Technology. 60 (9): 2463-2470.

Mertooetomo, E., Valsaraj, K.T., Wetzel, D.M., Harrison, D.P. (1993). Cascade crossflow air stripping of moderately volatile compound using high air-to-water ratios. Water Research. 27 (7): 1139.

Metcalf and Eddy. (2004). Wastewater engineering, treatment and reuse (4th ed., international ed.), McGraw-Hill, New York.

Mino, T., Liu, W.T., Kurisu, F., Matsuo, T. (1995). Modelling glycogen storage and denitrification capability of microorganisms in enhanced biological phosphate removal processes. Water Science & Technology. 31: 25-34.

Mino, T., van Loosdrecht, M.C.M., Heijnen, J.J. (1998). Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Research. 32: 3193-3207.

Mishima, K., Nakamura, M. (1991). Self-immobilization of aerobic activated sludge – A pilot-study of the aerobic upflow sludge blanket process in munipicipal sewage-treatment. Water Science & Technology. 23 (4-6): 981-990.

Mishina, F., Sakai, Y., Ishiduka, S. (1999). MAP recovery in area-wide sewage sludge treatment plant. 36th Proceeding of Gesidokenkyuhappyokai. 923-925.

Moerman, W.H.M., de Danschutter, J., de Ru, B., Veltman, A., Verwoert, G., Reitsma, B.A.H., van den Bulk, J. (2012). Phosphate recovery nuisance control for struvite clogging sensitive wastewater or sludge. In: IWA Nutrient Removal and Recovery 2012, September 23-25, Harbin, China.

Molinos-Senante, M., Hernandez-Sancho, F., Sala-Garrido, R., Garrido-Baserba, M. (2011). Economic feasibility study for phosphorus recovery processes. Royal Swedish Academy of Sciences. AMBIO. 40: 408-416.

Montag, D., Gethke, K., Pinnekamp, J. (2009). Different strategies for recovering phosphorus: Technologies and costs. In International conference on nutrient recovery from wastewater streams, ed. K. Ashley, D. Mavinic, and F. Koch. London, UK: IWA Publishing.

Morales, N., Figueroa, M., Fra-Vazquez, A., Val Del Rio, A., Campos, J.L., Mosquera-Corral, A., Mendez, R. (2013). Operation of an aerobic granular pilot scale SBR plant to treat swine slurry. Process Biochemistry. 48 (8): 1216-1221.

Morgenroth, E., Sherden, T., van Loosdrecht, M.C.M., Heijnen, J.J., Wilderer, P.A. (1997). Aerobic granular sludge in a sequencing batch reactor. Water Research. 31 (12): 3191-3194.

Mosquera-Corral, A., de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M. (2005). Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor. Water Research. 39: 407-412.

Moy, B.Y.E., Tay, J.H., Toh, S.K., Liu, Y., Tay, S.T.L. (2002). High organic loading influences the physical characteristics of aerobic sludge granules. Letters in Applied Microbiology. 34: 407-412.

Mudragada, R., Kundral, S., Coro, E., Moncholi, M.E., Laha, S., Tansel, B. (2014). Phosphorus removal during sludge dewatering to prevent struvite formation in sludge digesters by full-scale evaluation. Journal of Water Process Engineering. 2: 37-42.

Mulder, J.W., Duin, J.O.J., Goverde, J., Poiesz, W.G., van Veldhuizen, H.M., vam Kempen, R., Roeleveld, P. (2006). Full-scale experience with SHARON process through the eyes pf the operators. In: Proceedings of the Water Environment Federation, WEFTEC 2006. 5256-5270.

Mullin, J.W. (1993). Crystallization. Butterworth-Heinemann, Oxford, UK.

Münch, E.V., Barr, K. (2001). Controlled struvite crystallization for removing phosphorus from anaerobic digester sidestreams. Water Research. 35: 151-159.

Muster, T.H., Douglas, G.B., Sherman, N., Seeber, A., Wright, N., Guezuekara, Y. (2013). Towards effective phosphorus recycling from wastewater: quantity and quality. Chemosphere. 91: 676-684.

Nancharaiah, Y.V., Mohan, S.V., Lens, P.N.L. (2016). Recent advances in nutrient removal and recovery in biological and bioelectrochemical systems. Bioresource Technology. 215:173-185.

Neethling, J.B., Benisch, M. (2004). Struvite control through process and facility design as well as operation strategy. Water Science & Technology. 49 (2): 191-199.

Neethling, J.B., Bakke, B., Benisch, M., Gu, A.Z., Stephens, H., Stensel, H.D., Moore, R. (2005). Factors influencing the reliability of enhanced biological phosphorus removal. Report by Water Research Foundation, 01-CTS-3.

Nelson, N.O., Mikkelsen, R.L., Hesterberg, D.L. (2003). Struvite precipitation in anaerobic swine lagoon liquid: effect of pH and Mg:P ratio and determination of rate constant. Bioresource Technology. 89: 229-236.

Ni, B.J., Xie, W.M., Liu, S.G., Yu, H.Q., Wang, G., Dai, X.L. (2009). Granulation of activated sludge in a pilot-scale sequencing batch reactor for the treatment of low strength municipal wastewater. Water Research. 43: 751-761.

Nirmalakhandan, N., Brennan, R.A., Speece, R.E. (1997). Predicting Henry's Law constant and the effect of temperature on Henry's law constant. Water Research. 31 (6): 1471.

Obaja, D., Macé, S., Costa, J., Sans, C., Mata-Alvarez, J. (2003). Nitrification, denitrification and biological phosphorus removal in piggery wastewater using a sequencing batch reactor. Bioresource Technology. 87: 103-111.

Oehmen, A., Lemos, P.C. Carvalho, G., Yuan, Z., Keller, J., Blackall, L.L., Reis, M.A.M. (2007). Advances in enhanced biological phosphorus removal: from micro to macro scale. Water Research. 41: 2271-2300.

OFWAT (2006). What is the cost of reducing ammonia, nitrates and BOD in sewage treatment works effluent? Oxera.

Ohlinger, K.N., Young, T.M., Schroeder, E.D. (1998). Predicting struvite formation in digestion. Water Research. 32: 3607-3614.

Ohlinger, K.N., Young, T.M., Schroeder, E.D. (1999). Kinetic effects on preferential struvite accumulation in wastewater. Journal of Environmental Engineering. 125: 730-737.

Omar, B.N., Entrena, M., Gonzáles-Muńoz, M.T. (1994). Effects of pH and phosphate on the production of struvite by Myxococcus xanthus. Geomicrobiology Journal. 12:81-90.

Pan, S., Tay, J.H., He, Y.X., Tay, S.T.L. (2004). The effect of hydraulic retention time on the stability of aerobically grown microbial granules. Letters of Applied Microbiology. 38: 158-163.

Parsons, S.A., Thornton, P., Pearce, A. (2007). Ammonium removal from digested sludge liquors using ion exchange. Water Research. 41: 433-443.

Parsons, S.A., Smith, J.A. (2008). Phosphorus removal and recovery from municipal wastewaters. Mineralogical Society of America. 4 (2): 109-112.

Peng, D., Bernet, N., Delgenes, J.P., Moletta, R. (1999). Aerobic granular sludge – A case report. Water Research. 33: 890-893.

Peng, Y.Z., Wu, C.Y., Wang, R.D., Li, X.L. (2011). Denitrifying phosphorus removal with nitrite by real-time step feed sequencing batch reactor. Journal of Chemical Technology and Biotechnology. 86: 541-546.

Petruzzlli, D., Dell'Erba, A., Liberti, L., Notarnicola, M., Sengupta, A.K. (2004). A phosphate-selective sorbent for the REM NUT process: field experiences at Massafra Wastewater. Reactive & Functional Polymers. 60: 195-202.

Pitman, A.R. (1999). Management of biological nutrient removal plant sludges – Change the paradigms? Water Research. 33 (5): 1141-1146.

Pognani, M., Barrena, R., Font, X., Sánchez, A. (2011). A complete mass balance of a complex xombined anaerobic/aerobic municipal source-separated waste treatment plant. Waste Management. 32: 799-805.

Pronk, M., Bassin, J.P., de Kreuk, M.K., Kleerebezem, R., van Loosdrecht, M.C.M. (2013). Evaluating the main and side effects of high salinity on aerobic granular sludge. Applied Microbiology and Biotechnology.

Pronk, M., Abbas, B., Al-zuhairy, S.H.K., Kraan, R., Kleerebezem, R., van Loosdrecht, M.C.M. (2015). Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. Applied Microbiology and Biotechnology. 1-12.

Pronk, M., de Kreuk, M.K., de Bruin, B., Kamminga, P., Kleerebezem, R., van Loosdrecht, M.C.M. (2015). Full scale performance of the aerobic granular sludge process for sewage treatment. Water Research. 84: 207-217.

Qi, L., Li, Y., Tay, J.H. (2004). Effect of settling time on aerobic granulation in sequencing batch reactor. Biochemical Engineering Journal. 21 (1): 47-52.

Rahman, M.M., Liu, Y., Kwag, J.H., Ra, C. (2011). Recovery of struvite from struvite from wastewater and its nutrient leaching loss in soil. Journal of Hazardous Materials. 186 (2-3): 2026-2030.

Rahaman, S., Mavinic, D.S., Meikleham, A., Naoko, E. (2014). Modelling phosphorus removal and recovery from anaerobic digester supernatant through struvite crystallization in a fluidized bed reactor. Water Research. 51: 1-10.

Rao, K. J., Vaidhyanathan, B., Ganguli M., Ramakrishnan, P.A. (1999). Synthesis of Inorganic Solids Using Microwaves. *Chemistry of Materials.* 11 (4): 882–895

Rawn, A.M., Perry, B, A., Pomeroy, R. (1937). Multiple stage sewage sludge digestion. American Society of Civil Engineers. 2116: 93-132.

Rittman, B.E. McCarty, P.L. (2003). Environmental Biotechnology: Principles and applications. McGraw-Hill: New York, USA.

RiverWatch (2015). How Is Phosphorus cycled through ecosystems? The RiverWatch Institute of Alberta. Canada.

Roberts, M. (2015). Fresh thinking on sludge. SludgeTech 2015 Conference Review. *Water & Sewerage Journal*. 55

Roeleveld, P., Loeffen, P., Temmink, H., Klapwijk, B. (2004). Dutch analysis for P-recovery from wastewater. Water Science & Technology. 49 (10): 191-199.

Rotthauwe, J.H., Witzel. K.P., Liesack, W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environmental Microbiology. 75: 4704-4712.

Rosenwinkel, K.H., Cornelius, A., Thöle, A. (2005). Full-scale application of the deammonification process for the treatment of sludge water. In: Proceedings of IWA specialised conference on nutrient management in wastewater treatment processes and recycle streams. Krakow, Poland. Pp. 483-491.

Ruiz, G., Jeison, D., Charmy, R. (2003). Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Res.* 37, 1371–1377.

Saito, T., Brdjanovic, D., van Loosdrecht, M.C.M. (2004). Effect of nitrite on phosphate uptake by phosphate accumulating organisms. *Water Research.* 38: 3760-3768.

Sanin, S.L., Sanin, F.D., Bryers, J.D. (2003). Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. *Process Biochemistry.* 38: 909-914.

Sarkar, A.K. (1990). Phosphate cement-based fast setting binders. *Journal of American Ceramic Society Bulletin.* 69: 234–238.

Sathasivan, A., Mino, T., Matsuo, T. (1993). A deeper look on acetic acid metabolism in biological phosphorus removal processes. *Proceedings of the Annual Conference of Japanese Society of Water and Environment.* 27.

Sathasivan, A. (2008). Biological Phosphorus Removal Processes for Wastewater Treatment, Water and Wastewater Treatment Technologies – Encyclopedia of Life Support Systems (EOLSS). Online at: www.desware.net/sample-chapters/d13/e6-144-10.pdf (Accessed on 8th April, 2016).

Satoh, H., Mino, T., Matsuo, T. (1992). Uptake of organic substrates and accumulation of polyhydroxalkanoates linked with glycolysis of intracellular carbohydrates under anaerobic conditions in the biological excess phosphate removal processes. *Water Science & Technology.* 26 (5-6): 933-942.

Sawayama, S., Tada, C., Tsukahara, K., Yagishita, T. (2004). Effect of ammonium addition on methanogenic community in a fluidized bed anaerobic digestion. *Journal of Bioscience and Bioengineering.* 97: 65 – 70.

Schmidt, J.E., Ahring, B.K. (1993). Effects of magnesium on thermophilic acetate-degrading granules in upflow anaerobic sludge blanket (UASB) reactors. *Enzyme Microbiol. Technol.* 15: 304–310.

Schmidt, J.E., Ahring, B.K. (1996). Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactor. *Biotechnology and Bioengineering*. 49 (3): 229–246

Schramm, A., de Beer, D., van den Heuvel, J.C., Ottengraf, S., Amann, R. (1999). Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. *Applied and Environmental Microbiology*. 65 (8): 3690-3696.

Schramm, A., De Beer, D., Gieseke, A., Amann, R. (2000). Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm. *Environmental Microbiology.* 2 (6): 680-686.

Schuiling, R. D., Andrade, A. (1999). Recovery of struvite from calf manure. *Environmental Technology*. 20: 765–768.

Schwarzenbeck, N., Erley, R., Wilderer, P.A. (2004). Aerobic granular sludge in a SBR system treating wastewater rich in particulate matter. *Water Science & Technology.* 49 (11 - 12): 41 - 48.

Seviour, R.J., Mino, T., Onuki, M. (2003). The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiology Reviews*. 27: 99–127.

Sharp, R., Vadiveloo, E., Fergen, R., Moncholi, M., Pitt, P., Wankmuller, D., Latimer R. (2013). A Theoretical and Practical Evaluation of Struvite Control and Recovery. *Water Environment and Research.* 85 (8).

Sharpley, A.N., Smith, S.J., Naney, J.W. (1987). The environmental impact of agricultural nitrogen and phosphorus use. *Journal of Agriculture and Food Chemistry.* 35:812-817.

Shepherd, T.A., Burns, R.T., Raman, D.R., Moody, L.B., Stalder, K.J. (2009). Performance of a pilot ‐ scale air sparged continuous Flow reactor and hydrocyclone for struvite Precipitation and removal from liquid swine manure. *Applied Engineering in Agriculture*. 25 (2): 257 ‐ 267

Shi, C.Y. (2011). Mass Flow and Energy Efficiency of Municipal Wastewater Treatment Plants. IWA Publishing. p. 58

Shin, H.S., Lee, S.M. (1997). Removal of nutrients in wastewater by using magnesium salts. *Environmental Technology*. 19: 283-290

Show, K.Y., Lee, D.J., Tay, J.H. (2012). Aerobic granulation: advances and challenges. *Applied Biochemistry and Biotechnology.* 167 (6): 1622 - 1640.

Shu, L., Schneider, P., Jegatheesan, V., Johnson, J. (2006). An economic evaluation of phosphorus recovery as struvite from digester supernatant. *Bioresource Technology.* 97 (17): 2211–2216.

Siegrist, H. (1996). Nitrogen removal from digestate supernatant – comparison of chemical and biological methods. *Water Science & Technology.* 34: 399–406.

Smith, M.S., Yang, Y.K., Knapp, C.W., Niu, Y., Peak, N., Hanfelt, M.M., Galland, J.C., Graham, D.W. (2004). Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. *Applied and Environmental Microbiology.* 70 (12): 7372-77.

Solley, D. (2006). Achieving low effluent nutrients with minimum energy and Residuals – Brisbane Water Alliance Case Study. *Enviro06 Conference,* Australia.

Spieck, E., Hartwig, C., McCormack, I., Maixner, F., Wagner, M., Lipski, A., Daims, H. (2006). Selective enrichment and molecular characterization of a previously uncultured Nitrospira-like bacterium from activated sludge. *Environmental Microbiologyy.* 8: 405–415

Starman, D. (2009). Opportunities for nutrient recovery in post digestion sludge handling: Analysis and feasibility study using municipal scale aerobic and anaerobic digesters. Scholar Commons, University of South Florida.

Stratful, I., Scrimshaw, M.D., Lester, J.N. (2001). Conditions influencing the precipitation of magnesium ammonium phosphate. *Water Research.* 35: 4191–4199.

Strous, M., Heijnen, J.J., Kuenen, J.G., Jetten, M.S.M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidising microorganisms. *Applied and Environmental Microbiology.* 50 (5): 589-596

Strous, M., Kuenen, J.G., Jetten, M.S.M. (1999). Key physiology of anaerobic ammonium oxidation. *Applied and Environmental Microbiology.* 65: 3248-3250.

Strous, M., Fuerst, J.A., Kramer, E.H.M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K.T., Webb R, Kuenen J.G., Jetten, M.S. (1999a). Missing lithotroph identified as new planctomycete. *Nature.* 400: 446–9.

Suzuki, K., Tanaka, Y., Kuroda, K., Hanajima, D., Fukumoto, Y. (2005). Recovery of phosphorous from swine wastewater through crystallization. *Bioresource Technology.* 96: 1544-1550.

Suzuki, K., Tanaka, Y., Kuroda, K, Hanajima, D, Fukumoto, Y. (2007). Removal and recovery of phosphorus from swine wastewater by demonstration crystallization reactor and struvite accumulation device. *Bioresource Technology.* 98: 1573-1578.

Szabó, E., Hermansson, M., Modin, O., Persson, F., Wilén, B. (2016). Effects of Wash-Out Dynamics on Nitrifying Bacteria in Aerobic Granular Sludge during Start-Up at Gradually Decreased Settling Time. *Water*. 8 (172): 1-11

Tay, J.H., Liu, Q.S., and Liu, Y. (2001). The effects of shear force on the formation, structure, and metabolism of aerobic granules. *Applied Microbiology and Biotechnology*. 57(1–2): 227–233.

Tay, J.H., Yang, S.F., Liu, Y. (2002). Hydraulic selection pressure-induced nitrifying granulation in sequencing batch reactors. *Applied Microbiology and Biotechnology*. 59: 332–337.

Tay, J.H., Liu, Q.S., Liu, Y. (2003). Shear force influences the structure of aerobic granules cultivated in sequencing batch reactor. 5th International conference on biofilm systems, 14–19 September, Cape Town, South Africa

Tay, J.H., Liu, Q.S., Liu, Y. (2004). The effect of upflow air velocity on the structure of aerobic granules cultivated in a sequencing batch reactor. Water Sci. Technol. 49: 35–40.

Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M., Nguyen, M. (2010). A practical approach to RT-qPCR—Publishing data that conform to the MIQE guidelines. *Mini-review.* Methods 50: S1–S5.

Teichgräber, B., Stein, A. (1994). Nitrogen elimination from sludge treatment reject water-comparison of the steam-stripping and denitrification processes. *Water Science & Technology.* 30 (6): 41-51

ter Haseborg, E., Zamora, T.M., Frohlich, J., Frimmel, F.H. (2010). Nitrifying microorganisms in fixed-bed biofilm reactors fed with different nitrite and ammonia concentrations. *Bioresource Technology.* 101 (6):1701-1706.

Thaler, J.O. (2011). Struvite Control without Chemicals - A Case Study. Michigan Water Environment Association Biosolids/IPP Joint Conference September 28, 2011

Thorndahl, U. (1993). “Nitrogen removal from returned liquors.” *Journal of the Institute of Water and Environmental Management.* 7: 492-496.

Torpey, W.N., Melbinger, R.N. (1967). Reduction of digested sludge volume by controlled recirculation. *Journal Water Pollution Control Federation.* 39 (1464).

Trigo, C., Campos, J.L., Garrido, J.M. & Méndez R. (2006). Start-up of Anammox process in a membrane bioreactor. *Journal of Biotechnology.* 126 (4): 475-487.

Tsuneda, S., Nagano, T., Hoshino, T., Ejiri, Y., Noda, N., Hirata, A. (2003). Characterization of nitrifying granules produced in an aerobic upflow fluidized bed reactor. *Water Research.* 37: 4965-4973.

Tsuneda, S., Ogiwara, M., Ejiri, Y., Hirata, A. (2006). High-rate nitrification using aerobic granular sludge. *Water Science & Technol*. 53: 147–154.

Turovskiy, I.S., Mathai, P.K. (2006). Wastewater Sludge Processing. John Wiley & Sons Inc. Hoboken, New Jersey.

Ueno, Y., and Fujii, M. (2001). Three years' experience of operating and selling recovered struvite from full-scale plant. *Environmental Technology.* 22: 1373–1381.

Uludag-Demirer, S., Demirer, G.N., Chen, S. (2005). Ammonia removal from anaerobically digested dairy manure by struvite precipitation. *Process Biochemistry.* 40 (12): 3667-3674.

UNFCCC. (1997). Kyoto Protocol to the United Nations Framework Convention on Climate Change. Kyoto, Japan

United States Environmental Protection Agency (USEPA). (1979). Process Design Manual for sludge Treatment and Disposal, EPA 625/1-79/011.

United States Environmental Protection Agency (USEPA). (2000). Ammonia Stripping. Wastewater Technology Fact Sheet. http://1.usa.gov/1m5xuGO.

USEPA (1979). Process design Manual sludge treatment and disposal, U.S. Environmental Protection Agency.

USEPA (2007). Advanced Wastewater Treatment to achieve low concentration of phosphorus. EPA 910-R-07-002. April, 2007.

Uysal, A., Demir, S., Sayilgan, E., Eraslan, F., Kucukyumuk, Z. (2014). Optimization of struvite fertilizer formation from baker's yeast wastewater: growth and nutrition of maize and tomato plants. *Environmental Science and Pollution Research.* 21: 3264–3274.

Vadivelu, V.M., Keller, J., Yuan, Z. (2007). Free ammonia and free nitrous acid inhibition on the anabolic and catabolic processes of nitrosomonas and nitrobacter. *Water Science and Technology.* 56 (7): 89-97.

van de Graaf, A.A., Mulder, A., Slijkhuis, H., Robertson, L.A., Kuenen, J.G. (1990). Anoxic ammonium oxidation. In: Christiansen C, Munck L, Villadsen J, eds. Proceedings of 5th European Congress on Biotechnology. Copenhagen, Denmark: Munkgaard International Publishers, 388–91.

van der Roest, H.F., de Bruin, L.M.M., Gademan, G., Coelho, F. (2011). Towards sustainable waste water treatment with Dutch Nereda® technology. *Water Practice and Technology.* 6 (3).

Van Dijk, J.C., Braakensiek, H. (1984). Phosphate removal by crystallization in a fluidized bed. *Water Science & Technology.* 17: 133–142.

van Dongen, U., Jetten, M.S.M., van Loosdrecht M.C.M. (2001). The SHARON-Anammox process for treatment of ammonium-rich wastewater. *Water Science and Technology.* 44 (1): 153-160.

Van Hulle, S.W.H., Vandeweyer, H.J.P., Meesschaert, B.D., Vanrolleghem, P.A., Dejans, P., Dumoulin, A. (2010). Review engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. *Chemical Engineering Journal.* 162: 1–20.

Van Loosdrecht, M.C.M., Heijnen, J.J. (1997). Importance of bacterial storage polymers in bioprocesses. *Water Science & Technology.* 35 (1): 41 - 47

Van Loosdrecht, M.C.M., De Kreuk, M.K. (2004). Method for the Treatment of Wastewater with Sludge Granules. Dutch and International patent - NL1021466C; O2004024638, Technische Universiteit Delft, The Netherlands.

Van Loosdrecht, M.C.M. (2008). Innovative nitrogen removal. In: Biological wastewater treatment: Principles, modelling and Design. IWA Publishing Eds. Henze M., van Loosdrecht M.C.M., Ekama, G. & Brdjanovic D. pp. 139-153

van Loosdrecht, M.C.M., Winkler, M.K.H., Bassin, J.P., Kleerebezem, R., Sorokin, D.Y. (2012). Unravelling the reasons for disproportion in the ratio of AOB and NOB in aerobic granular sludge. *Applied Microbiology and Biotechnology.* 94: 1657–1666

Vishniac, W., Santer, M. (1975). The Thiobacilli. *Bacteriological Reviews.* 21: 195–213.

Von Horn, J., Sartorius, C. F. (2009). Impact of Supply and Demand on the price development of phosphate (fertilizer). International Conference on Nutrient Recovery from Wastewater Streams, 10 – 13 May, 2009 in Vancouver (Canada) and published in: Ashley, K.; Mavinic, D. und Koch, F. (Hg.): International Conference on Nutrient Recovery from Wastewater Streams, London: IWA, pp. 45–54

Wäger-Bauman, F. (2011). Physical and biological methods for the treatment of the liquid fraction of anaerobic digester effluent [*PhD thesis*]. Vienna, Austria: University of Natural Resources and Applied Life Sciences.

Wagner, J., Costa, R.H.R. (2013). Aerobic granulation in a sequencing batch reactor using real domestic wastewater. *Journal of Environmental Engineering.* 139: 1391–1396.

Wagner, J., Guimarães, L.B., Akaboci, T.R.V., Costa R.H.R. (2015). Aerobic granular sludge technology and nitrogen removal for domestic wastewater treatment. *Water Science & Technology*. 71 (7): 1040-1046.

Walker, M., Iyer, K., Heaven, S., Banks, C.J. (2011). Ammonia removal in anaerobic digestion by biogas stripping: an evaluation of process alternatives using a first order rate model based on experimental findings. *Chemical Engineering Journal.* 178: 138–45.

Wan, J., Bessière, Y., Spérandio, M. (2009). Alternating anoxic feast/aerobic famine condition for improving granular sludge formation in sequencing batch airlift reactor at reduced aeration rate. *Water Research.* 43 (20): 5097–5108.

Wang, Q., Du, G., Chen, J. (2004). Aerobic granular sludge cultivated under the selective pressure as a driving force. *Processes in Biochemistry.* 39: 557–563.

Wang, J., Burken, J., Zhang, X. (2006). Effect of seeding materials and mixing strength on struvite precipitation. *Water Environment & Research.* 78 (2): 125–32.

Wang, S.G., Liu, X.W., Gong, W.X., Gao, B.Y., Zhang, D.H., Yu, H.Q. (2007). Aerobic granulation with brewery wastewater in a sequencing batch reactor. *Bioresources Technology*. 98(11): 2142–2147.

Wang, J.L., Peng, Y.Z., Wang, S.Y., Gao, Y.Q. (2008) Nitrogen removal by simultaneous nitrification and denitrification via nitrite in a sequence hybrid biological reactor. *Chinese Journal of Chemical Engineering.* 16: 778–784

Wastewater treatment in the United Kingdom (WWT-UK). (2012). Implementation of the European Union Urban Waste Water Treatment Directive – 91/271/EEC.

Wei, D., Si, W., Zhang, Y.F., Qiao, Z.M., Yao, Z.X., Zhao, W., Zhao, J., Chen, G.D., Wei, Q., Du, B. (2012). Aerobic granulation and nitrogen removal with the effluent of internal circulation reactor in start-up of a pilot-scale sequencing batch reactor. *Bioprocess and Biosystems Engineering.* 35: 1489–1496.

Wei, D., Shi, L., Yan, T., Zhang, G., Wang, Y., Du, B. (2014). Aerobic granules formation and simultaneous nitrogen and phosphorus removal treating high strength ammonia wastewater in sequencing batch reactor. *Bioresource Technology*. 171: 211–216

Weissenbacher, N., Takacs, I., Murthy, S., Fuerhacker, M., Wett, B. (2010). Gaseous nitrogen and carbon flux from a full-scale deammonification plant. *Water Environmental Research.* 82 (2): 169-175

Westerman, P.W., Bowers, K.E., Zering, K.D. (2010). Phosphorus recovery from covered digester effluent with a continuous-flow struvite crystallizer. *Transactions of the American Society of Agricultural and Biological Engineers.* 26 (1): 153-161.

Wett, B., Rostek, R., Rauch, W., Ingerle, K. (1998). pH-controlled reject-water-treatment. *Water Science & Technology.* 37 (12): 165-172.

Wett, B., Alex, J. (2003). Impacts of separate of rejection water treatment on the overall plant performance. *Water Science & Technology.* 48 (4): 139-146

Wett, B. (2006). Solved up-scaling problems for implementing deammonification of rejection water. *Water Science & Technology.* 53 (12): 121-128

Wett, B., Hell, M., Nyhuis, G., Puempel, T., Takacs, I., Murthy, S. (2010). Syntrophy of aerobic and anaerobic ammonia oxidisers. *Water Science and Technology.* 61 (8): 1915-1922

Wild, D., Siegrist, H. (1999). The simulation of nutrient fluxes in wastewater treatment plants with EBPR. *Water Research.* 33 (7): 1652-1662.

Williams, S. (1999). Struvite precipitation in the sludge treatment stream at Slough wastewater treatment plant and opportunities for phosphorus recovery. *Environmental Technology.* 20: 743-747

Williams, S. (1998). Adapting Strategies for Avoiding Struvite Build-Up in Plants and Transforming Them into Strategies for Recovery. 1st Phosphorus Recovery Conference. Warwick University, UK. 6th & 7th May 1998

Williams, S. (1999). Struvite precipitation in the sludge treatment stream at Slough wastewater treatment plant and opportunities for phosphorus recovery. *Environmental Technology.* 20: 743-747.

Winkler, M.K.H., Kleerebezem, R., de Bruin, L.M.M., Verheijen, P.J.T., Abbas, B., Habermacher, J., van Loosdrecht, M.C.M. (2013). Microbial diversity differences within aerobic granular sludge and activated sludge flocs. *Applied Microbiology and Biotechnology.* 97: 7447–7458.

Withers, P.J.A., Elser, J.J., Hilton, J., Ohtake, H., Schipper, W.J., van Dijk, K.C. (2015). Greening the global phosphorus cycle: how green chemistry can help achieve planetary P sustainability. *Green Chem.* 17: 2087-2099

Worrell, E., Phylipsen, D., Einstein, D., Martin, N. (2000). Energy Use and Energy Intensity of the U.S. Chemical Industry. U.D.O. Energy, ed., Environmental Energy

Technologies Division, Ernest Orlando Lawrence Berkeley National Laboratory, University of California.

Wu, Q., Bishop, P.L., Keener, T.C., Stallard, J., Stile, L. (2001). Sludge digestion enhancement and nutrient removal from anaerobic supernatant by $Mg(OH)_2$ application. *Water Science & Technology*. 44 (1): 161–166. IWA

Wu, Q., and Bishop, P.L. (2004). Enhancing struvite crystallization from anaerobic supernatant. *Journal of Environmental Engineering and Science.* 3: 21-29.

Wu, Q., Bishop, P.L., Keener, T.C. (2005). A strategy for controlling deposition of struvite in municipal wastewater treatment plants. *Water Environment Research.* 77 (2): 199–207

Wu, L., Peng, C., Peng, Y., Li, L., Wang, S., Ma, Y. (2012). Effect of wastewater COD/N ratio on aerobic nitrifying sludge granulation and microbial population shift. *Journal of Environmental Sciences.* 24 (2): 234–241

Xavier, L. D., Cammarota, M. C., Yokoyama, L., Junior I. V. (2014). Study of the recovery of phosphorus from struvite precipitation in supernatant line from anaerobic digesters of sludge. *Water Science & Technology.* 69 (7): 1546 – 1551

Yang, L., Alleman, J.E. (1992). Investigation of batchwise nitrite build-up by an enriched nitrification culture. *Water Science and Technology.* 26 (5-6): 997-1005.

Yang, S.F., Tay, J.H., Liu, Y. (2003). A novel granular sludge sequencing batch reactor for removal of organic and nitrogen from wastewater. *Journal of Biotechnology*. 106: 77–86.

Yang, S.F., Liu, Q.S. Tay, J.H., Liu, Y. (2004a). Growth kinetics of aerobic granules developed in sequencing batch reactors. *Letters of Applied Microbiology*. 38:106

Yang, S.F., Tay, J.H., Liu, Y. (2005). Effect of substrate nitrogen/chemical oxygen demand ratio on the formation of aerobic granules. *Journal of Environmental Engineering – ASCE.* 131: 86–92

Yang, Z., Peng, X.F., Chen, M.Y., Lee, D.J. (2007). Intra-layer flow in fouling layer on membranes. *Journal of Membrane Science.* 287: 280–286

Yu, Y., Lee, C., Kim, J., Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering.* 89 (6): 670-9.

Yuan, X., Gao, D. (2010). Effect of dissolved oxygen on nitrogen removal and process control in aerobic granular sludge reactor. *Journal of Hazardous Materials.* 178: 1041–1045

Zanetti, L., Frison, N., Nota, E., Tomizioli, M., Bolzonella, D., Fatone, F. (2012). Progress in real-time control applied to biological nitrogen removal from wastewater. A short review. *Desalination.* 286: 1–7.

Zeng, R.J., Lemaire, R., Yuan, Z., Keller, J. (2003). Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. *Biotechnology and Bioengineering.* 84: 170–178.

Zhang, Q., De Clippeleir, H., Su, C., Al-Omari, A., Wett, B., Vlaeminck, S. E., Murthy, S. (2016). Deammonification for digester supernatant pretreated with thermal hydrolysis: overcoming inhibition through process optimization. *Applied Microbiology and Biotechnology.* 100 (12): 5595–5606

Zheng, Y. M., Yu, H. Q., Liu, S. J., Liu, X. Z. (2006) “Formation and instability of aerobic granules under high organic loading conditions.” *Chemosphere*. 63: 1791–1800.

Zhu, L., Xu, X.Y., Luo, W.G., Tian, Z.J., Lin, H.Z., Zhang, N.N. (2008). A comparative study on the formation and characterization of aerobic 4-chloroaniline-degrading granules in SBR and SABR. *Applied Microbiology and Biotechnology*. 79 (5): 867–874

Zhu, L., Dai, X., Lv, M., Xu, X. (2013). Correlation analysis of major control factors for the formation and stabilization of aerobic granule. *Environmental Science and Pollution Research.* 20(5): 3165–3175.