CRANFIELD UNIVERSITY

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ROTATING BIOLOGICAL CONTACTORS OF THE FUTURE FOR ENHANCED AMMONIUM REMOVAL

School of Energy, Environmental Technology and Agrifood

PhD Thesis
Academic Year: 2013 – 2016

Supervisors: Professor Frédéric Coulon and Professor Bruce Jefferson

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This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

Rotating biological contactors (RBCs) are competitive wastewater treatment technologies for small wastewater treatment works (WWTs) due to their low energy, low maintenance and capacity for ammonium removal. Due to stricter environmental quality standards (EQS) as well as increased pressure on companies to cut cost and decrease energy consumption, RBC performance must be optimised to maintain competitiveness in the future. Optimisation can be achieved by altering operational parameters to yield a more conducive environment for ammonia oxidising microorganisms (AOM) to proliferate and increase their metabolic activity. The effects of altering these operational parameters was assessed using data from a range of sources, including fully operational WWTs. For instance, 2 fully operational WWTs were employed to test the effect of rotational speed, results indicated that increasing the speed from 1 rotation per minute (rpm) to 1.3 rpm enhanced the capacity for ammonium load removal. The effect of artificial aeration in another fully operational overloaded RBC led to significant improvement in ammonium load removal (46 %). Both routes to optimisation resulted in a thinner biofilm with less *Beggiatoa* (filamentous bacteria) present. It was concluded that retrofitting RBCs to implement these optimisation methods can be achieved with little expenditure, of approximately £10 000 life cycle cost. A novel performance robustness tool was used to identify any additional factors that significantly contributed to performance enhancement across a very large number (121) of fully operational WWTs, however no correlation could be found. High throughput DNA sequencing and quantitative PCR of RBC biofilm from 7 fully operational WWTs enabled an in depth investigation into AOM responsible for ammonium removal. Results indicated that ammonia oxidising archaea (AOA) generally dominated the biofilm, however ammonia oxidising bacteria (AOB) were more

competitive when exposed to lower loading. *Candidatus nitrososphaera* and *Nitrosomonas ureae* were the dominant AOA and AOB, respectively.

ACKNOWLEDGEMENTS

My greatest thanks must go towards my academic supervisor Frédéric Coulon and my industrial supervisor Andrea Wilson. Both supervisors went the extra mile to support me and provide indispensable feedback when I needed it. Without you the process would have been far more difficult and I will always be grateful for the investment you both made in me.

In memory of Andrea, I dedicate this thesis to you.

Bruce Jefferson, you played a huge role in helping shape my thesis and always gave excellent guidance in progress meetings.

Thank you to NERC and Severn Trent Water for funding my research and Cranfield University for good support over the years. There are too many people at Severn Trent to mention, but I would like to show extra gratitude towards Mark Jones, Pete Vale, Martin Horsley, Fiona Sutton, Russ Townsend, Nicole Jenkins and my student colleagues.

I must also acknowledge my family for essential support and Vanessa for being a continual reminder that there is more to life than finishing your PhD.

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LIST OF ABBREVIATIONS

5PL = 5 parameter logistics

ANOVA = analysis of variance

AOA = ammonia oxidising archaea

AOB = ammonia oxidising bacteria

AOM = ammonia oxidising microbes

AMO = ammonia mono-oxygenase

AMP = asset management plan

APHA = American Public Health Organisation

AS = Activated sludge

ATU = allylthiourea

BOD = biological oxygen demand

BZ = biozone

$CBOD_5$ = 5-day carbonaceous biochemical oxygen demand

COD = chemical oxygen demand

DEFRA = Department for environment, food and agriculture

DO = dissolved oxygen

E = effluent

EA = Environment Agency

EPS = extracellular polymeric substance

EQS = Environmental Quality Standard

FISH = fluorescence in situ hybridisation

FST = final settlement tank

GHG = greenhouse gas

HDPE = high density polyethylene

HRT = hydraulic retention time

IFAS = integrated fixed-film activated sludge

kWh = kilowatt hour

MAB = membrane aerated biofilm

N = nitrogen

NERC = Natural Environment Research Council

NH_4^+ = ammonium

NH_4OH = ammonium hydroxide

NO_2^- = nitrite

NO_3^- = nitrate

NOB = nitrite oxidising bacteria

OUR = oxygen uptake rate

OTR = oxygen transfer rate

OTU = operational taxonomic unit

PCR = polymerase chain reaction

PE = population equivalent

PST = primary settlement tank

qPCR = quantitative polymerase chain reaction

RBC = rotating biological contactor

RPI = robustness performance index

rpm = rotations per minute

STW = Severn Trent Water

TN = total nitrogen

TSS = total suspended solids

VSD = variable speed drive

VSS = volatile suspended solids

WFD = Water Framework Directive

WWT = Wastewater Treatment Works/Site

All abbreviations are re-introduced in the text on a chapter-by-chapter basis.

CHAPTER 1

INTRODUCTION

1 INTRODUCTION

1.1 Background

The water industry is encouraged to continuously maintain water quality and look for opportunities to enhance water quality in order to improve society's standard of living and protect the wider environment. Water pollution is a great problem, 40 % of rivers in China do not meet quality standards (Feng *et al.*, 2012) and this figure is around 10 % in the UK (UKTAG, 2013). Nitrogen pollution from human activities in aquatic ecosystems can cause eutrophication and severe water quality deterioration and has had a generally detrimental effect to ecosystems globally (Rockstrom *et al.*, 2009). Due to human activities the amount of reactive nitrogen (such as ammonia) released into the environment has more than doubled in half a decade (Galloway *et al.*, 2008). Large demand for nitrogen from industry, agriculture and domestic sources means that this is a serious global problem, for instance ammonia emissions in 2013 totalled 17.2, 3.1 and 3.2 Tg $N \cdot yr^{-1}$ for China, US and the EU respectively (Liu *et al.*, 2013). Nitrogen generally appears as organic nitrogen or more often as ammoniacal-nitrogen (66 – 90 % of total nitrogen) in wastewater (Sattayatewa *et al*, 2010; Gajewsko, 2011). In light of vast research on this topic which is briefly explained above, Governments recognise this serious problem and have introduced legislation to mitigate it. For example, the European Union Urban Waste Water Treatment Directive 91/271/EEC dictates that European Union member states must enforce their own environmental quality standards (EQS), otherwise known as effluent consents for ammoniacal-nitrogen. While this directive currently applies only to wastewater treatment works (WWTs) with a capacity > 10,000 population equivalent (PE), EQS are however often enforced at small treatment works (< 2,000 PE) and are becoming tighter. Meeting these tightening consents can incur significant expenditure, in terms of energy and both capital and operating cost, this is a growing concern (Ainger *et al.*, 2009). Incurring higher energy/financial costs is not a sustainable solution, particularly in small WWTs, which make up the largest share (79% of all WWTs) in the UK (DEFRA, 2013), where a low energy and low maintenance strategy through the use of passive technologies is encouraged (Brookes, 2013). Therefore, there is a need to optimise the performance of wastewater treatment technologies that are typically employed at small WWTs through upgrading/retrofitting

or by improving future designs. Increasing wastewater treatment efficacy at small WWTs in terms of ammonium removal would make a significant impact in the water industry, due to the reasons stated above. Rotating biological contactors (RBCs) represent a treatment technology that could achieve this whilst also minimising carbon footprint, energy requirement and financial cost. For instance, RBCs can be 35 % cheaper annually than trickling filters (a similar treatment technology) due to lower energy consumption, land use and operating costs (Upton *et al.*, 1995). Due to these attributes RBCs are widely employed, particularly in the UK by Severn Trent Water where they are utilised in around one third of all their WWTs.

RBCs were first implemented in the 1960s are commonly employed at small WWTs (Tchobanoglous *et al.*, 2013). They consist of several discs which present a large surface area for a natural biological community housed within a polymeric matrix to develop, which is capable of converting undesirable compounds in the wastewater into safer, and often redundant compounds (Husham *et al.*, 2012). The discs are partially submerged in the wastewater and are rotated at a constant speed using a common horizontal shaft. Unique to an RBC, rotation of the disks both enables effective mixing of bulk liquid (Rittman & McCarthy, 2001) and continuous supply of oxygen to the biofilm (Hassard *et al.*, 2014), which the community can utilise to metabolise ammonium in the wastewater. The rotation of the discs not only allows for oxygen delivery to the biofilm organisms within the RBC, but also the suspended biomass and related organisms in the bulk liquid. Around 55 % of the energy budget for wastewater treatment is used for artificial aeration (Ainger *et al.*, 2009) and so providing oxygen through another mechanism is a significant advantage for RBCs. Other advantages include: low land requirements, low capital requirements, low operating costs and suitability for decentralised, rural networks (Dutta *et al.*, 2007). In fact, it is estimated that RBCs require about 20-30 % less energy compared to a trickling filter system (Rodgers & Zhan, 2003), which require approximately 70 % of the electricity associated with the conventional activated sludge (AS) process (EPRI, 2002). Compared to AS, RBCs require less than half the operating expenditure (OPEX) and similar capital expenditure (CAPEX) (Labella *et al.*, 1972). Furthermore, RBCs are associated with 29% lower CAPEX than packed bed filters and are suited for decentralised water treatment systems which generally have lower OPEX

costs compared to the traditional centralised approach which may require specialist labour and process control (Fountoulakis *et al.*, 2009).

RBCs are very effective in small WWTs for the reasons outlined previously and optimising the treatment process in terms of ammonium removal is critical. Recent studies have investigated how optimising nutrient removal in fixed film systems can be achieved by altering operational parameters, such as media (Stephenson *et al.*, 2013) and rotational speed (Di Palma & Verdone, 2009; Downing & Nerenberg, 2008). These studies show that altering parameters can significantly impact treatment performance at lab-scale. The thesis aims to build upon these studies to answer the research question – Can the biological treatment process of an RBC be optimised to be more robust when complying with future ammonium effluent EQS regulations? Furthermore, numerous gaps in the current literature will be filled, such as, the lack of real industry-applicable data on RBCs, how fast an RBC biofilm can recover nitrifying activity after stress events and what microbial species constitute an RBC biofilm?

1.2 Aims & Objectives

The aim of this project is to investigate how various parameters within a rotating biological contactor system influences the RBC treatment process in terms of ammonium removal, with a specific focus on understanding how the biofilm is affected. It will then be possible to apply this understanding to enhance the treatment process.

To meet this aim, six objectives have been identified, each designed to address existing knowledge gaps.

1. To critically review parameters that influence ammonium removal in RBC fixed biofilms.
2. To quantify the robustness of RBC-containing WWTs with respect to ammonium removal and assess how robustness is influenced by consent shifts and operational attributes.
3. To evaluate the effect of rotational speed on treatment performance.
4. To assess the impact of artificial aeration on treatment performance.
5. Evaluate the diversity and abundance of nitrifying microorganisms in RBC biofilms using molecular techniques.

6. To recommend strategies that will positively impact on the RBC biofilm to improve treatment performance and yield an 'RBC of the Future.'

According to industry experts, effluent quality, costs and energy neutrality are the three factors which are most influential for wastewater treatment of the future (STOWA, 2010). Thus these objectives have been set to provide a better understanding of the underlying biofilm treatment process that will assist the industry for enhancing the performance of the RBC of the future.

1.3 Thesis Structure

The PhD thesis is written as a collection of chapters, with each chapter comprising of a paper written for journal publication that addresses the key objectives outlined previously. Each chapter contribute to the overall aim of the thesis, with the last chapter containing a business case for Severn Trent Water as well as summarising the industrial implications of the work. The PhD candidate was responsible for all experimental work undertaken and was the lead author of all papers, which were edited by Prof. Frédéric Coulon. It should be noted that Dr. Boyd McKew at the University of Essex contributed to chapter 6 with QIME analysis of DNA sequencing data.

A brief description for each chapter is provided hereinafter.

Chapter 2 – Review on the parameters influencing ammonium removal in rotating biological contactors: A literature review was carried out to understand how various RBC operational parameters influence ammonia removal, with particular focus on how this influences biofilm characteristics.

Chapter 3 – Assessing the robustness of small wastewater treatment systems performance: An evaluation of treatment robustness in terms of ammonium removal for over 100 fully operational RBC WWTs using 10 years of effluent quality data and which particular operational parameters contribute to robustness.

Chapter 4 – Effect of rotational speed on nitrification and energy consumption of rotating biological contactors: An assessment on the effect of rotational speed on ammonium removal and energy consumption on two full-scale parallel RBCs.

Chapter 5 – The impact of artificial aeration on ammonium removal in rotating biological contactors: An assessment on the effect of artificially aerating the second biozone on ammonium removal in an overloaded full-scale RBC.

Chapter 6 – Diversity and abundance of ammonia oxidising microbes in rotating biological contactors: Molecular techniques reveal the abundance and diversity of ammonia oxidising microbes in a range full-scale rotating biological contactors with different operating conditions.

Chapter 7 – A recommendation for an updated rotating biological contactor design: This chapter brings the other chapters together with a discussion and conclusion of the findings in the previous chapters (Figure 1), followed by a recommendation for updating and optimising RBC design and operation.

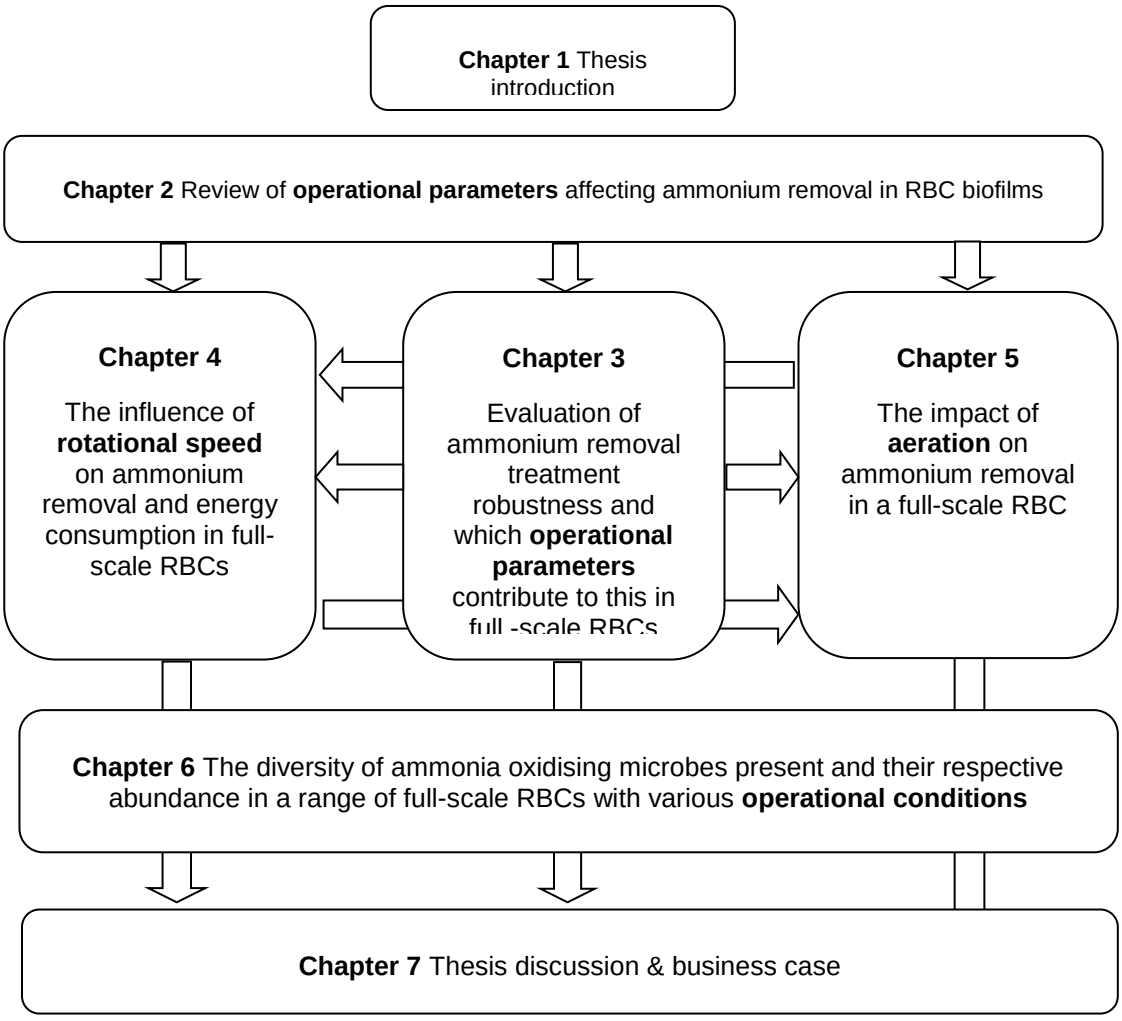

Figure 1. A flow chart to show how the thesis chapters' link with one another.

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CHAPTER 2

LITERATURE REVIEW

2 REVIEW ON THE PARAMETERS INFLUENCING AMMONIUM REMOVAL IN ROTATING BIOLOGICAL CONTACTORS

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Abstract

Rotating biological contactors (RBCs) are an efficient fixed film wastewater treatment technology commonly employed for ammonium removal in small wastewater treatment works. A great deal of studies have been carried out on the effect of operational parameters on ammonium removal in fixed biofilms. Recent advancements, such as in the field of molecular techniques has enabled closer investigation into how parameters affect species abundance, diversity and interactions. This review provides a critical insight into the key parameters influencing the biofilm in terms of ammonia removal. Recommendations and suggestions are further made on how parameters can be altered to optimise RBC performance and biofilm activity, via two strategies – biostimulation and bioaugmentation.

Keywords: Rotating Biological Contactor, Wastewater, Ammonia Removal, Biofilm

2.1 Introduction

Wastewater often contains nitrogen compounds of anthropogenic (e.g fertiliser) and natural origin (e.g urine) that may pose environmental and health risks. For instance, nitrogen pollution in aquatic ecosystems can cause eutrophication and critical water quality deterioration; large demand for nitrogen from industry, agriculture and domestic sources means that this is a serious global problem (Smith *et al.,* 1999). Pollution,

primarily with organic matter and ammonium contributes to around 40 % of rivers in China not meeting quality standards (Feng *et al.,* 2012) and this figure is around 10 % in the UK (UKTAG, 2013). Therefore, wastewater must be treated before being discharged into the environment. Typical wastewater treatment comprises a 4 step process including preliminary and primary treatment where the solids of the wastewater are removed, followed by a secondary treatment where further solids are removed, organic molecules are degraded, and ammonia is nitrified, and then a tertiary treatment where nitrogen, phosphorus and other chemicals are removed and/or disinfection using chlorine or UV exposure is carried out. The secondary treatment is almost exclusively based on a biological treatment system that utilises the metabolic capacity of microbes, this system is low cost allows for easy operation (Hibiya *et al.,* 2000). Two types of biological system can be used – fixed film (biofilms) or suspended (Tchobanoglous *et al.,* 2013). Popular fixed film technologies are trickling filters and biological aerated filters, whereas the most popular suspended film system is activated sludge. In a suspended system the organisms are free swimming and planktonic, they are suspended in solution with no definite structure. Fixed film systems on the other hand have a surface in which biofilms can attach and grow. Both systems rely on rich ecosystems of microbes and higher life forms to degrade and mineralise the organic and inorganic compounds contained in the wastewater. Fixed biofilm systems offer several advantages compared to the suspended system mainly because the surface of the fixed media encourages long-term residence of specialised microbes, rather than being carried away as would happened if the microbes were in a free-swimming planktonic state (Tawfik *et al.,* 2006). At these longer mean cell residence times the bacterial species diversity is reduced in biofilms due to the effect on competition between microbes, but nitrogen removal efficacy is maximised (Saikaly & Oerther, 2004; Tan *et al.,* 2008). Effective design of fixed-film systems relies upon promoting the selection of the correct microbes on the media (typically plastic). It is the authors view that promoting, whilst also maintaining the proliferation and activity of preferential microbes remains one of the greatest challenges in wastewater treatment optimisation.

This review investigates secondary treatment in fixed film systems, whilst focusing on rotating biological contactor (RBC) treatment technologies. RBCs consist of closely spaced, slowly rotating plastic disks on a shaft which is turned by a motor.

Microorganisms grow and colonise on the disks forming a biofilm that oxidize and mineralise organics and ammonium. During the rotation, about 40% of the surface area of the disk is usually submerged. The aeration required for the biofilm is provided by the rotation of the disks and the thickness of the biofilm is controlled by the shear forces of the rotation. These characteristics are advantageous as it reduces operational costs and prevents overgrowth of the biofilm which could limit the availability of dissolved oxygen and substrates to the microbes (Hassard *et al.,* 2015).

RBCs are simpler to operate than activated sludge systems and provide longer contact times between the bacteria and the wastewater components compared to the trickling filters, consequently they are capable of achieving low effluent sanitary consents for biological oxygen demand, total suspended solids and ammonia. While the start-up cost of RBCs can be high, the maintenance and energy costs are low and therefore RBCs are well suited secondary treatment systems for small WWTs (capacity $\leq$ 2000 population equivalent [PE]). Other advantages include low land requirements, low capital requirements and suitability for decentralised, rural networks (Dutta *et al.,* 2007). RBCs have great potential for wastewater treatment in the UK as 79% of WWTs are categorised as small, which favour employing low-energy, low-maintenance technologies like RBCs (DEFRA, 2012).

A great deal of studies has been carried out on the wastewater treatment process in fixed film systems. These studies have mainly focused on the aspect of process design and in particularly providing adequate oxygen in the system and ensuring the wastewater can pass through the system without limitation or any detrimental effects to the media. In this review, a critical insight into biofilm development as well as the key parameters influencing biofilm and RBC performance for ammonia removal is provided. Then recommendations and strategies for optimising biofilm treatment activity and RBC performance are proposed. Other reviews on RBCs (Hassard *et al.,* 2015; Cortez *et al.,* 2008; Patwardhan, 2003) have been published, however in the authors opinion these reviews have focused on evaluating the overall performance and potential of RBCs, without discussing the interaction between operational parameters (macro) and biofilm characterisation (micro), particularly changes in the dynamic microbial consortia.

2.2 Biofilm Development in Wastewater Treatment Systems

In the early stage of biofilm research biofilms were described as 'the adhesion and growth of a complex bacterial community enclosed within a polymer matrix, with the individual cells within the biofilm behaving very differently to their floating, planktonic counterparts (Costerton *et al.,* 1978). More recently they have been described as 'cells immobilized on a substratum and frequently embedded in an organic polymer matrix of microbial origin' (Characklis & Marshall 1990). A more comprehensive and up-to-date definition of a biofilm is 'a microbial derived sessile community characterized by cells that are irreversibly attached to either a substratum or to each other and are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription' (Donlan & Costerton, 2002).

2.2.1 Biofilm Development

Biofilms can form in a range of environments including of course, wastewater treatment media. Backed by studies in the literature, we have described biofilm development as a five-stage process including the conditioning layer, the bacterial cell attachment, the formation of a biofilm & production of EPS, biofilm maturation and detachment (Figure 1).

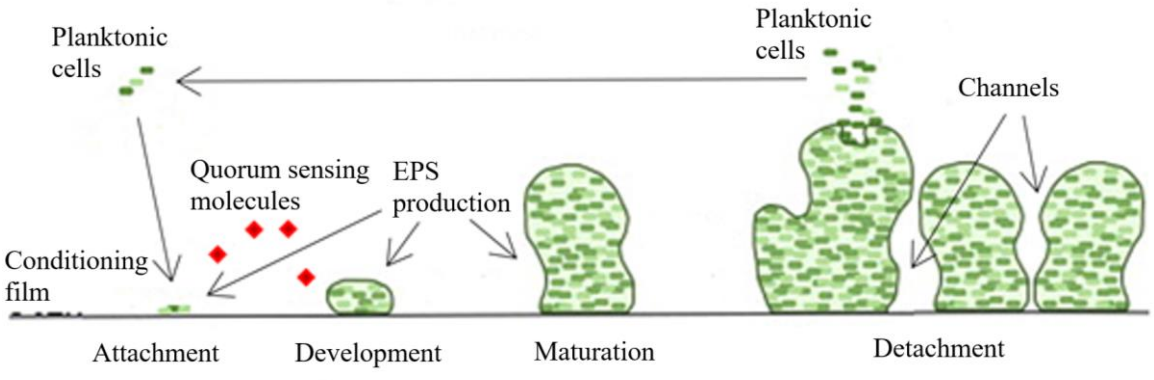

Figure 1. Overview of the biofilm lifecycle, from attachment to detachment.

2.2.1.1 Conditioning film

Before microbial colonisation of a surface occurs a conditioning film is often established, which quickly forms from the surrounding wastewater, within seconds to minutes (Yang *et al.*, 2016). It was theorised that this film can 'catalyse' bacterial attachment (Zobell & Allen, 1935), however evidence suggests this is not the case (Dunne, 2002; Percival *et al.*, 2011). It is known that the composition of the conditioning film can influence the efficacy of adhesion (Dunne, 2002), as it can compete with bacteria for adhesion sites on the surface leading to reduced adhesion (Foppen *et al.*, 2008); furthermore, it can inhibit the adhesion of specific bacterial species (Percival *et al.*, 2011). The film does however provide a favourable concentrated nutrient source and vital trace elements for biofilm proliferation, growth and reproduction (Bakker *et al.*, 2003). Membrane biofilms exhibit twice the concentration of bacteria, with six times the concentration of polysaccharides when formed upon a pre-conditioned film, rather than a non-conditioned membrane. It has also been reported that biofilms on non-conditioned membrane are patchier and scattered (Baek *et al.*, 2011). These studies provide evidence that a conditioning film may promote biofilm formation and growth. It is also known that the conditioning film can influence hydrophobicity (Nghiem *et al.*, 2008), surface roughness (Xu *et al.*, 2006) and surface charge (Hong & Elimelech, 1997) of the substratum. Our understanding on biofilm conditioning and development is progressing, however the presence and significance of this layer is often overlooked (Flemming & Ridgway, 2008) and further investigation is still needed on bacterial attachment and biofilm formation. Questions still remain as to whether specific components of the conditioning film confer advantages or disadvantages to biofilms and whether biofilm formation is influenced by the thickness of the conditioning film.

2.2.1.2 Attachment

Free-floating microbes can be transported to a surface by Brownian motion, sedimentation, convective mass transport or by actively moving towards the surface with cellular apparatus such as flagella (Habimana *et al.*, 2014). Cells must overcome an energy barrier surrounding the substrata before attachment, typically, the more electronegative a cell wall is, the harder it is for the cell to overcome this barrier (Subramani & Hoek, 2008).

Once near the substrata surface, it has been suggested that deviations in the shape of cell membrane/wall can trigger stress pathways which notify microbes when there is contact with solid surfaces (Otto & Silhavy, 2002; Morgenstein & Rather 2012). Upon sensing a surface, interaction with the surface is mediated by the type IV pilli (TFP) in a range of bacteria (O'Toole & Wong, 2016). At first the TFP are used to 'crawl' over the surface and retains the ability to retract. This stage is termed primary adhesion and is carried out via reversible and non-specific, often hydrophobic interactions (Conrad *et al.,* 2010; Habimana *et al.,* 2014; O'Toole & Wong, 2016).

In *Pseudomonas,* TFP interact with adhesion-type PilY1 proteins and upon contact with a surface PilY1 induces upregulation of c-di-GMP production which represses flagellum activity and therefore promotes transition into a biofilm (Heiniger *et al.,* 2010; Luo *et al.,* 2015). Eventually flagellum and TFP activity is permanently terminated and irreversible attachment occurs, meaning that the bacterial cells can no longer move away from the surface of the biofilm (Dunne, 2002). This sparks the upregulation of EPS production allowing a colony to grow exponentially (O'Toole & Wong, 2016).

Evidence suggests that in *Pseudomonas,* production of EPS polysaccharides may act as a web of 'guidance ropes' for the TFP during the initial stages of attachment which influences the location and extent of microcolony formation. Further to this, EPS polysaccharides may be crucial in irreversible attachment by acting as an adhesin and these polysaccharides may also upregulate c-di-GMP like the PilY1 protein and therefore promote transition into a biofilm (Jackson *et al.,* 2004; Irie *et al.,* 2012; Zhao *et al.,* 2013). The description of cell attachment so far has been from a Gammaproteobacteria perspective. Many species of Alphaproteobacteria on the other hand, have been shown to attach to surfaces with a stalk in conjunction with polysaccharide 'glue.' This glue can be dispensed very quickly upon sensing a surface (Tsang *et al.,* 20006; Wang *et al.,* 2014; Hoffman *et al.,* 2015).

The consensus is that the rate of bacterial adhesion in the initial stages of biofilm formation is not predictive of the amount of biofilm formed (Cerca *et al.,* 2005; Miller *et al.,* 2012). However, it may influence the amount of time needed for the biofilm to reach maturity (van Loosdrecht *et al.,* 2012) and has been shown to greatly influence the type of community that develops (Zhang *et al.,* 2009). Investigating the mechanisms behind

biofilm attachment in a wastewater environment is very difficult as this depends on, among other parameters, cell surface charge, cell surface hydrophobicity and type of EPS present (Habimana *et al.,* 2014). Therefore, cell adhesion efficacy changes depending on the cell species and strains that are present. It would be of interest to explore how wastewater biofilms change depending on the initial colonising species and how treatment performance is affected. For instance, would nitrifiers be more abundant and active if they formed a biofilm where other bacteria of a symbiotic nature had first colonised a surface?

2.2.1.3 Development

As previously described, the production of EPS is very important during the initial attachment stage. Following this stage the EPS matrix confers many advantages and plays a crucial role. In terms of volume, approximately 90 % of a wastewater biofilm volume is made up of EPS, whereas microbial cells and inorganic compounds only represent 8 % and 2 % respectively (Derlon *et al.,* 2016). EPS is variable and consists of long thin strands of biopolymers including polysaccharides, proteins, lipids, uronic acid, humic substances, extracellular DNA, and many other components (Arundhati & Paul, 2008). Polysaccharides are generally thought to be the dominant component in biofilms, however the proportion of EPS components is highly dependent on the microbial species present (Cegelski, 2016).The type of EPS in a biofilm and amount synthesised is dependent on wastewater composition and reactor conditions such pH and temperature (Zehra & Belma, 2008). EPS influences the structure of the biofilm and acts as a skeleton which supports and binds the bacteria cells. It allows other bacterial cells to adhere, contributes to their aggregation and provides many other functions (Schlafer & Meyer, 2016). The addition of DNase and the subsequent disappearance of *Pseudomonas* biofilms highlights the importance of eDNA in the EPS matrix. This component plays a key role in initial attachment and biofilm formation by possibly cross-linking with EPS polysaccharides which stabilises the biofilm (Whitchurch *et al.*, 2002; Jennings *et al.*, 2015).

EPS polysaccharides can confer specific functions such as cohesion, enhanced water retention, the capacity to adsorb and concentrate organic and inorganic compounds as well as trace elements, biocide protection and protection from predatory protozoa

(Flemming *et al.*, 2000; Flemming & Wingender, 2010; Arciola *et al.*, 2015). These functions enable the EPS matrix to act as a biological shield that protects against harmful agents being absorbed into the pores of the growing cells which may otherwise hinder growth, whilst also promoting availability of beneficial substrates which can be essential to the growth of the community (Wolfaardt *et al.*, 1994). In fact, biofilm bacteria can express 1,000 times more resistance to antibiotics compared to planktonic cells, however the mechanism responsible for this is within the matrix itself, rather than the matrix acting as a barrier, as it has been shown that antibiotics quickly penetrate biofilms (Gilbert *et al.*, 1997; Stewart *et al.*, 2009).

There is a relationship between polysaccharide concentration and cohesive energy within the wastewater biofilm, which is stronger towards the base of the biofilm (Ahimou *et al.*, 2007). Protein concentration, on the other hand, does not seem to affect cohesive energy, indicating the importance of the EPS polysaccharide fraction in the structural integrity of biofilms. Bacteria with a strong affinity for EPS production are very important for biofilm formation, they act as 'colonisers' and provide a primary layer for subsequent colonisation (Pang *et al.*, 2005), in fact, the absence of EPS leads to less deposition of bacteria on surfaces in all cell types (Long *et al.*, 2009). The dead cells of the initial surface colonisers and the respective EPS that they secreted form the innermost layer of the biofilm on the biofilm-substrata interface (Herzberg & Elimelech, 2007; Khan *et al.*, 2011a) which can act as substratum in its own right and can be further colonised by other organisms (Bereschenko *et al.*, 2010). Other microbes known as 'bridger' organisms are also important for biofilm development as they are able to co-aggregate and form links with other microbes in the biofilm, an example of one such microbe in an aquatic biofilm is *Micrococcus luteus* (Buswell *et al.*, 1997)

In *Staphylococcus* biofilms, fluorescent staining has revealed that proteins also play an important role in the matrix by mediating aggregation via interactions with cells or EPS components (Schaeffer *et al.*, 2015). Recalcitrant, strong amyloid fibers contribute to the stability of the biofilm, and overexpression of these proteins increases biofilm stiffness significantly (Zeng *et al.*, 2015) and may also be required for attachment of biofilms to solid substrata (Cegelski, 2015). Amyloid proteins were found to be the most prominent

component, with cellulose being the only other major component, in an intact bacterial biofilm using nuclear magnetic resonance imaging (McCrate *et al.*, 2013).

EPS can also offer protection from metals, cations, toxins, UV radiation, pH shifts, osmotic shock and desiccation (Flemming & Wingender, 2010). Furthermore, accumulation of contaminants such as steroids and alkylphenols has been observed in biofilms when EPS was produced (Writer *et al.*, 2011), thereby reducing the concentration in the wastewater. Additionally, a range of enzymes can be secreted into the EPS matrix such as cellulase (Ko *et al.*, 2015) and hydrolase (Ju *et al.*, 2015), providing extra capacity to degrade undesirable compounds in wastewater that would either not be present or washed away quickly in suspended systems. Another benefit is that pathogens such as *E. coli* are removed in RBC biofilm systems, via a mechanism of adsorption onto the matrix or by predation by higher life forms (Tawfik *et al.*, 2004).

Whilst there are 'valleys' running in between individual microcolony clusters and streamers which allows for convection of bulk liquid around the biofilm, the EPS matrix also allows the formation of highly permeable internal interstitial channels and pores which act as a primitive circulatory system and enables substrate availability deep within the biofilm (Stoodley *et al.*, 1994; Zahid & Ganczarczyk, 1994; Sutherland, 2001). Due to these channels and the ability of EPS to present friction force, EPS is thought to be the major contributor to hydraulic resistance throughout the biofilm, whereas cells and inorganic compounds have a negligible effect (Stewart, 2012; Dreszer *et al.*, 2013; Billings *et al.*, 2015; Chomiak *et al.*, 2014). Therefore, biofilms with an EPS matrix can confer both lower and higher hydraulic resistance compared to microbial communities without EPS (McDonogh *et al.*, 1994; Dreszer *et al.*, 2013). However, the extent to which it does this and how the structure of EPS carries this out is an interesting area of research that needs to be explored (Neu & Lawrence, 2015).

2.2.1.4 Maturation phase

Following attachment and initial development of the biofilm, characterised by rapid erection of an EPS matrix, the maturation stage follows. It should be noted that in wastewater environments bacteria only initiate the process of biofilm development, in a maturing biofilm, large varieties of external multicellular organisms also join and contribute to the biofilm.

In this stage, the microbial cells are reproducing regularly and maturing on the surface of the biofilm, which can now grow thicker as well as spread over a larger surface area. Naturally, some biofilms will remain just few cells layer thick such as dental plaque while other biofilms will grow to be several millimetres thick. RBC biofilms treating domestic wastewater have been reported to be between 0.5 and 2 mm thick (De la Rosa & Yu, 2005), but the author's personal experience is that they can be considerably thicker than this, particularly if the RBC is overloaded. In any case, the biofilm grows and matures in accordance to the water characteristics, environmental, biochemical and hydrodynamic conditions as well as the availability of nutrients (Arvin & Harremoes, 1990; Davey & O'Toole, 2000; Martin-Cereceda *et al,* 2001; Cortez *et al.,* 2008; Xu *et al.,* 2010; Hassard *et al.,* 2015). With *E. coli* biofilms, the EPS matrix becomes denser with age which reduces diffusion of particles within biofilms, this is pronounced with large charged particles which diffuse more slowly than small neutral particles (Birjiniuk *et al.,* 2014).

2.2.1.5 Detachment phase

After maturation is reached, the final stage takes place where the detachment of the top layers of the biofilm occurs due to a combination of environmental, hydrodynamic and shear forces, which will be discussed in subsequent sections. As the cells extend upward in an RBC biofilm, there is a much stronger hydrodynamic force associated with the flow of wastewater over the surface of the biofilm. When the force becomes too strong, fragments brake off the biofilm and detached cells are thus released back into the aqueous environment. The rate of detachment is a balancing act between the strength of forces that cause detachment and the opposing adhesive and cohesive forces within the biofilm (Stoodley *et al.,* 2002). Detachment and dispersal of biofilm cells leads to planktonic cells migrating and offers the possibility of another biofilm forming elsewhere. Alternatively and rather uniquely, in RBCs, detachment of biofilm may confer some benefits to treatment. Due to constant rotation of the disks, RBCs naturally slough off biofilm into the bulk fluid, which is retained within the reactor and with that biofilm comes bacteria, other biomass and extracellular enzymes, this retained biomass may integrate back into the biofilm and enhance treatment (Confer & Logan, 1998).

2.2.2 Wastewater Biofilm Composition

Previous studies have demonstrated that mature biofilms in RBC systems is predominately constituted of water (95 %), with the total mass being made up of volatile solids (up to 74 %) and a biotic component (up to 15 %) (Ouyang, 1980; Donlan & Costerton, 2000). The biotic component is distributed throughout a number of defined horizontal layers. Confocal scanning microscopy reveals in real-time the characteristics of the outer layer in active aquatic biofilms (Figure 2), which is heterogeneous and complex. This layer is subjected to erosion due to shear forces, whilst the inner layers are sheltered from erosion.

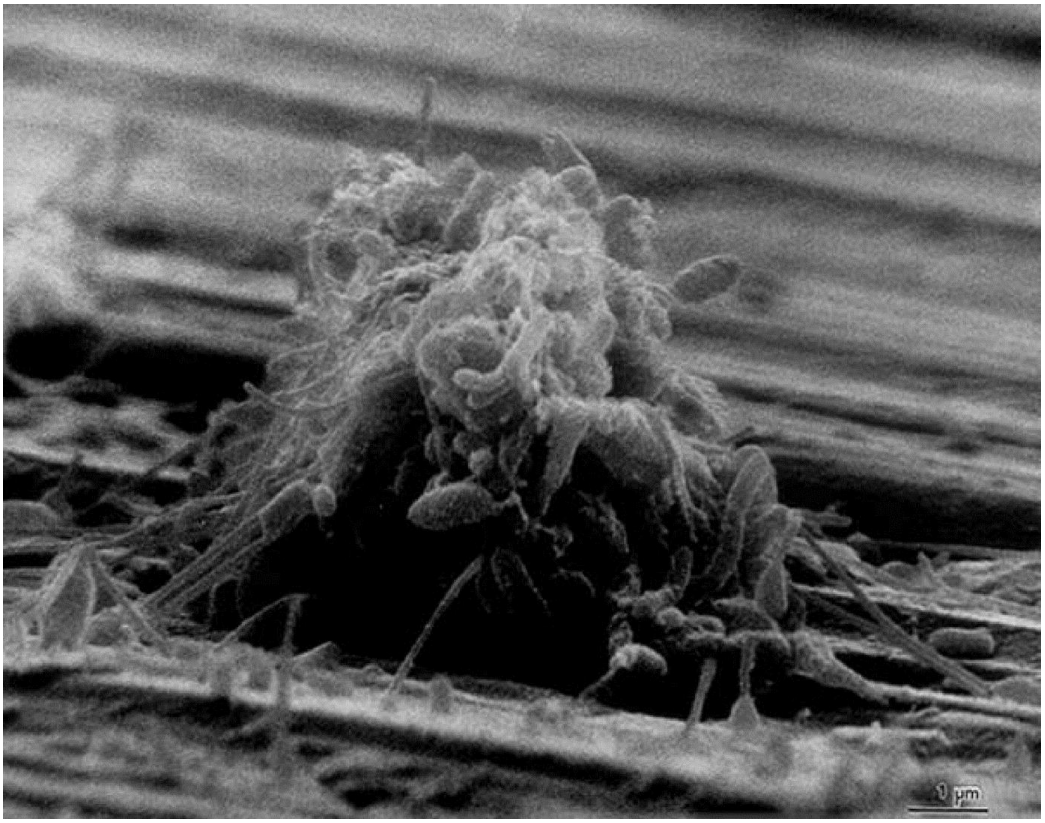

Figure 2. Scanning electron micrograph of a biofilm on a metal surface from an industrial water system. (Donlan & Costerton, 2002).

Due to the layering, gradients within RBC biofilms in terms of substrates, oxygen and other compounds, lead to anoxic, anaerobic and aerobic layers which encourage a range of metabolically differing microbes and therefore very dynamic removal regimes (Dutta *et al.*, 2007). Substrate heterogeneity through various layers enables a diverse microbial consortium with various metabolic activities, to thrive in a collaborative community,

compared with suspended systems (Stewart & Franklin, 2008; Lu & Chandran, 2010). These biofilm layers encourage regions of certain microbial groups, for instance there is a dynamic AOB consortium present throughout the layers due to reduced substrate availability as the depth of biofilm increases (Truu *et al.,* 2005). Diffusion rate of substrates and oxygen through the biofilm layers is one of the main driving factors controlling microbial populations and spatial distribution. Moving from the surface to the base of the biofilm, the diffusion rate decreases due to biofilm density, mineral deposits and mass driving force decreasing; consequently, fast growing organisms are found in the outer layer, whereas organisms with a low maximum specific growth rate reside in the base layer, the nearer the microbes to the substrata, the lower the substrate redox potential (Zeevalkink *et al.,* 1979; Okabe *et al.,* 1996; Okabe *et al.,* 1999). The deeper layers of an RBC biofilm contain 3 to 17 times lower bacterial cell density than the upper layers, the active fraction of microbes is 35 +/- 13% in the outermost layer and 15 +/- 4% in the deepest layer (Okabe *et al.,* 1996).

The effect of reduced substrate availability throughout the layers can be visualised by the use of fluorescence *in situ* hybridisation (FISH), which shows that in a membrane aerated biofilm, ammonia oxidising bacteria (AOB) and heterotrophic bacteria reside near the biofilm surface at the interface with the bulk liquid, while only AOB reside at the intermediate layer and a combination of AOB and nitrite oxidising bacteria (NOB) reside at the bottom of the biofilm near the interface with the substrata (Downing & Nerenberg, 2008). RBC biofilms often have an anaerobic layer at the very bottom, where nitrifying anammox bacteria (NAB) can be found, they exist there due to their low growth rate and requirement for negligible oxygen (Egli *et al.,* 2001). In biofilter biofilms it was found that the bacterial consortia within the middle and lower layers remained relatively constant over time, however the upper layer was very dynamic (Jeong *et al.,* 2013).

The biofilm is home to greater microbial cell numbers, compared to suspended systems (Abzazou *e al.,* 2016), which may be due to the associated high cell retention time. This is a real benefit in wastewater treatment as it enables a stable community to develop in the long-term, rather than being washed away as might happen in suspended systems (Siegrist *et al.,* 1998). In a MBBR treating extremely high ammonium influent, AOB represent a large proportion of total bacterial cells (11.3 ± 17.0 %) (Abzazou *et al.,* 2016),

other studies have also noted AOB as being the most prominent microbial group in wastewater treatment biofilms (Zhang, 2009; Calderon *et al.,* 2012; Wei *et al.,* 2014).

The existence of a dynamic community of specialist microbes within the biofilm can be distinct advantage compared to suspended systems. However, it is the diversity of the key microbial groups, rather than total population diversity that is associated with enhanced overall system stability (Briones & Raskin, 2003).

2.2.3 Biofilm Regulation and Interspecies Interactions

Interactions within wastewater biofilms can influence the physiology and function of the biofilm, such as enhanced resistance and pollutant mitigation (Burmolle *et al.,* 2014). One such route of interaction and regulation is by hormone production, diffusible extra signalling molecules composed of homoserine lactones and oligopeptides produced by the cells in response to the environmental conditions. These signals allow the community to modulate their own cellular physiology and monitor their own population growth and toxin production, thereby regulating the overall biofilm entity, in a form of communication called quorum sensing (Strous *et al.,* 1999; Watnik & Kolter, 2000; Song *et al.,* 2014). The various interactions between biofilm species, included quorum sensing can be seen in Figure 3

One such quorum sensing molecule is N-acyl homoserine lactone (AHL), which is known to be important molecule for communication between Gram-negative bacteria (Zhang *et al.,* 2012). In activated sludge microbial communities, it has been shown to regulate EPS secretion and improve reactor performance (Hwang *et al.,* 2008; Shrout and Nerenberg, 2012). Quorum sensing has been shown to be pivotal in both initial cell attachment and biofilm formation, in fact when the signalling molecules reach a specific threshold biofilm formation begins (Ren *et al.,* 2013; Tan *et al.,* 2014). However, an opposing mechanism called quorum quenching also exits, where quorum sensing signaling molecules are blocked, it is thought that this situation may be responsible for underestimating the effect of quorum sensing in wastewater treatment (Song *et al.,* 2014).

Interestingly, eukaryotic organism such as leguminous plants (Mathesius *et al.,* 2013) and squid (Chun *et al.,* 2008) have been shown to respond to quorum sensing molecules and these molecules can even promote the settlement of seaweed spores (Tait *et al.,* 2005).

This indicates that quorum signaling molecules have a further role than just communication between bacteria. No reports in the literature attempt to characterise the relationship between these molecules and eukaryotic organisms such as ciliates, nematodes and rotifers in wastewater biofilms.

Multispecies wastewater biofilms are ideal locations for horizontal gene transfer and creation of new genotypes (Burmolle *et al.,* 2014). It has been suggested that frequent horizontal gene transfer enables the most selectively advantageous genes to be exchanged throughout the biofilm, thereby making the overall biofilm entity stronger (Molin & Tolker-Nielsen, 2003; Sorenson *et al.,* 2005). Due to the sophisticated nature of the microbial biofilm, it can be perceived as a constantly evolving single organism adapting to the wastewater environment.

As well as the communication just mentioned, the interactions between the bacterial species composing the biofilm allows a city-like economy to form where individuals trade their unwanted by-products with other individuals who consider these as commodities. The metabolic interactions can either be cooperative where individual microbes within a sharing community benefit from each other's existence by exchanging nutrients (syntrophic), or competitive where individual microbes compete for space and nutrients for example (antagonistic). This is an emerging area of research and therefore the understanding of how these relationships influence biofilms is limited. As illustrated previously, knowing the abundance and activity of the microbial consortia is important for the optimisation of RBC biofilms, however, equally important are the syntrophic and antagonistic interactions between the individuals.

2.2.3.1 Syntrophic interactions

Syntrophism is a phenomenon where two species are intrinsically linked due to their reliance on each other for certain substrates usually needed for metabolism. The proximity of species within the biofilm encourages this syntrophic and cooperative relationship between phenotypically versatile biofilm residents as a whole (Davey & O'Toole, 2000). For instance, in anaerobic biofilms fermenting microbes provide substrates for acetogenic bacteria, which then provide substrates to methanogens (Mnif *et al.,* 2012; Xie *et al.,* 2014; Abzazou *et al.,* 2016). Fermenting microbes can also produce substrates (organic

acids) for hydrogen producing bacteria, and this natural food web can go further to include complex predator-prey dynamics (Davey & O'Toole, 2000).

It has been suggested that in nitrifying biofilms there is a clear association between AOB and AOA, where the availability of oxygen and ammonium are high (Schramm *et al.*, 1998), a mutualistic relationship also exists between AOB and NOB where DO is higher than 1 mg L^{-1} (Wang & Li 2015). Furthermore, in AS flocs, microcolonies of AOB and NOB exhibit considerable resistance against harsh conditions (Larsen *et al.*, 2008). A clear association between AOA and anammox bacteria has also been observed in a range of environments (Erguder *et al.*, 2009). In both wastewater treatment biofilters and wetlands, a symbiotic relationship has been reported for AOB and anammox bacteria and this conclusion was reached after comparing functional gene expression from these two groups (Ji *et al.*, 2013; Zhi & Ji, 2014). Given the range of micro-environments and residence of all the nitrifying microbes mentioned above it is possible that all these groups share a syntrophic relationship in RBC biofilms and contribute to enhanced ammonium removal.

2.2.3.2 Antagonistic interactions

Antagonistic pressures derive from competition between bacteria. For example, nitrifying and heterotrophic populations within the biofilm compete for space and substrates, which can lead to deteriorating ammonium removal performance (Nogueira *et al.*, 2002). Additionally, microbes can often secrete inhibitory compounds to give themselves a competitive edge (Avendano-Herrera & Riquelme, 2007; Rypien *et al.*, 2010; Matthias *et al.*, 2013).

In a marine biofilm 41 % (equating to 9 species) of all the examined isolates exhibited antagonistic properties (Sahar *et al.*, 2015), which is comparable to the 35 – 54 % reported previously (Long & Azam, 2001). The isolates which were shown to secrete antagonistic agents included *Micrococcus*, *Enterococcus* and *Pseudomonas*, have all previously been reported to confer antagonistic properties (Awais *et al.*, 2007; Miles, 2007; and Sader *et al.*, 2014 respectively). As mentioned before, methanogens and acetogens can cooperate in a syntrophic relationship, however sulphate reducing bacteria can also compete for substrates with both of these bacteria, thereby exhibiting an antagonistic relationship (Harmsen *et al.*, 1998; Abzazou *et al.*, 2016). There is limited information available on

the effect on antagonistic relationships within wastewater biofilms, however this relationship is likely significant.

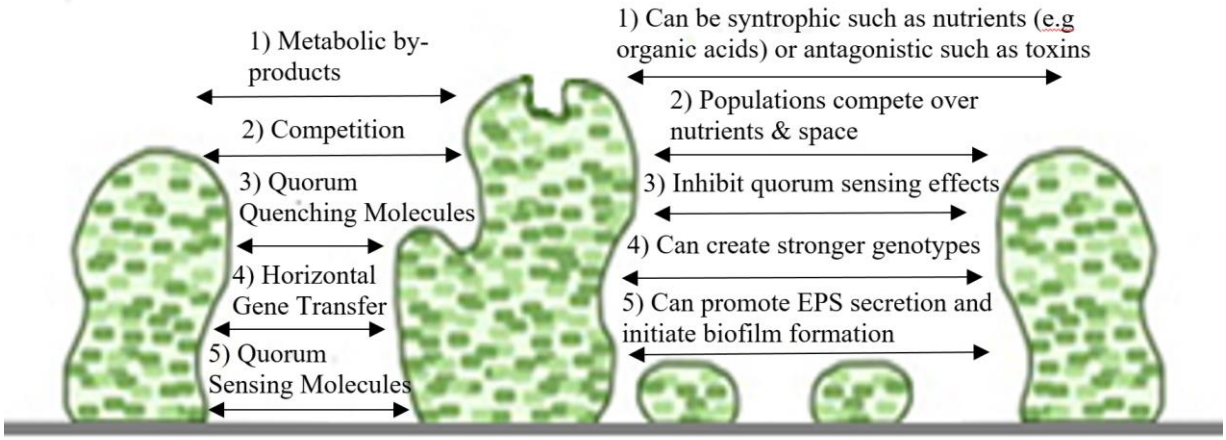

Figure 3. Summary of the interactions between microbial species, within a wastewater biofilm.

2.2.4 Role of Biofilm in Ammonium Removal

Wastewater from domestic sources generally contains around 10 to 40 mg L^{-1} of total nitrogen, most of which represents ammonia (Tchobanoglous *et al.*, 2013). One of the most important considerations in wastewater treatment is ammonia removal. Ammonia is a toxic compound which causes significant environmental damage by greatly contributing to eutrophication and deoxygenation in aquatic ecosystems. In an aqueous environment ammonia is more commonly seen as ammonium and the pathway in which ammonium is removed is termed nitrification. The transformation is mainly via biological mechanisms of the reduced forms of nitrogen to the oxidised forms, which are more benign to aquatic ecosystems. Ammonium removal in wastewater treatment normally occurs via aerobic nitrification where the succession of biological oxidation steps yield nitrite (NO_2^-) and then nitrate (NO_3^-) from ammonium (NH_4^+). The NO_3^- may then be converted to N_2 and N_2O by heterotrophic bacteria in a process called denitrification under anaerobic conditions (Grady *et al.*, 1999). The two main bacterial groups linked to the traditional aerobic route nitrification route are ammonia oxidising bacteria (AOB) and the nitrite oxidising bacteria (NOB), which are chemolithoautotrophs and are active in the presence of oxygen, they mediate the first and second step, respectively. AOB reside within the class of Gamma and/or Betaproteobacteria; examples include *Nitrosococcus*

(γ), *Nitrosospira* (β) and *Nitrosomonas* (β) (Madigan *et al.,* 2000). The Betaproteobacteria are the most prominent in freshwater (Whitby *et al.,* 1999) and biological wastewater treatment (Park *et al.,* 2002). This traditional aerobic nitrification route is typical in RBC biofilms. However, recent studies showed that nitrifying anammox bacteria (NAB) can metabolise ammonium in the presence of an anoxic environment (Lotti *et al.,* 2014). NAB are a group of aquatic microbes belonging to the phylum *Plancotomycetes* that can be found in wastewater biofilms, they consume nitrite as an electron acceptor, as opposed to oxygen (Strous *et al.,* 1999; Lotti *et al.,* 2014). As nitrite is not present in significant quantities in wastewater, nitrite must be provided by AOB or AOA via the aerobic process of nitritation in order for anammox or NOB to thrive (Joss *et al.,* 2011). These bacteria are inhibited by high DO and pH shocks, as well as excess ammonia and nitrite (Jin *et al.,* 2012).

Another alternative route to remove ammonium is known as completely autotrophic nitrogen removal over nitrite (CANON), or oxygen-limited autotrophic nitrification-denitrification (OLAND) or single reactor of high activity ammonia removal over nitrite (SHARON) (Paredes *et al.* 2007). This pathway can yield excellent nitrogen removal (89 %) (Gong *et al.,* 2008). More recently, simultaneous nitrification, anammox and denitrification, termed (SNAD) (Lan *et al.,* 2011) has been shown to yield up to 98 % ammonium removal in biofilter biofilms, without additional aeration (Wang & Li, 2015). Simultaneous nitrification and denitrification, as well as NAB have been documented in biofilm reactors (Fu *et al.,* 2010). Therefore, the SNAD process which involves AOB, NOB, NAB and denitrifying bacteria, is a likely description of the nitrogen removal process in RBC biofilms, this will be explained in more detail in subsequent sections.

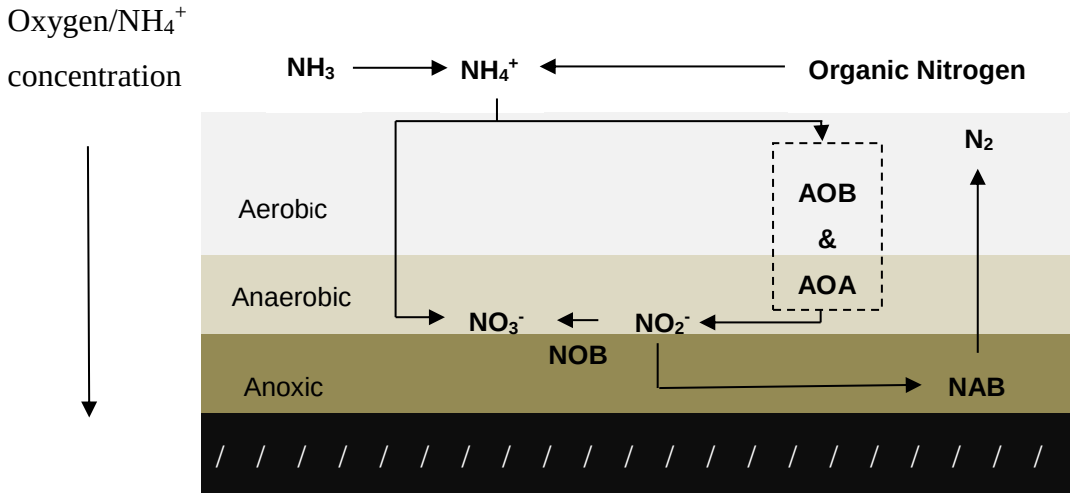

Figure 4. Ammonia removal throughout the various RBC biofilm layers

In the last decade, a new group of ammonia oxidisers termed ammonia oxidising archaea (AOA) were discovered. Isolating *Candidatus 'N. maritimus'* revealed that this organism contained all three subunits (amoA, amoB, and amoC) of ammonia monooxygenase (Konneke *et al.*, 2005). In a range of environments, the rate-limiting step in traditional nitrification lies with oxidation of ammonium (Sinha & Annachhatre, 2007; Choi & Hu *et al.*, 2008; Murphy *et al.*, 2009), which can be mediated by AOB, AOA or NAB. This can be visualised in Figure 4.

Phylogenetically, AOA are far more diverse than AOB (Urakawa *et al.*, 2008), indeed, in soils, the abundance of AOA in terms of total microbial population can range anywhere between 1 % and 5 %, whereas in AOB the range is less than 0.23 % (Leininger *et al.*, 2006). AOA populations are often larger than AOB in freshwater environments, and the nitrification activity of AOA can be affected a range of factors including DO, light, pH, particle size, nutrients (including carbon), ammonium concentration, water availability, salinity and temperature (Nicol *et al.*, 2003; Caffrey *et al.*, 2007).

It is now known that AOA have the potential to outnumber AOB and dominate the first step of nitrification in wastewater systems, especially in low pH and ammonia environments (Yan *et al.*, 2012; Erguder *et al.*, 2009; You *et al.*, 2009), partly due to their higher ammonium affinity and therefore their ability to outcompete AOB (Pan *et al.*, 2016). In fact, ammonium affinity with respect to ammonium in AOA has been reported to be significantly better than AOB, with affinities being 0.02 gN m^3 (Martens-Habben *et*

al., 2009) and 2.4 gN m^3 (Pan *et al.,* 2016), respectively. This attribute of AOA may explain why the archaeal amoA gene was 3000 times more abundant than the bacterial amoA gene in pristine soil (Leininger *et al.,* 2006). As well as higher ammonium affinity, AOA also possess higher oxygen affinity than AOB (Stahl *et al.,* 2012). AOA have been observed in municipal wastewater treatment works (AS) and have been shown to exhibit higher activity even under very low DO conditions of 0.1 mg L^{-1} (Park *et al.,* 2006; You *et al.,* 2009; Fitzgerald *et al.,* 2015). It should be noted that AOA have been found to be present in less numbers and exhibit less activity than AOB in a number of full-scale wastewater treatment works (Zhang *et al.,* 2014). Even in wastewater environments with higher populations of AOA, it has been suggested that AOB could still represent the more active fraction (Wells *et al.,* 2009). It is the authors opinion that AOA can be a significant presence in many environments, but in activated sludge systems which contains wastewater with both high ammonia and DO levels AOA are not as competitive, as explained previously. We suggest that as DO and ammonium diffuse from the upper layers to the deeper layers of the RBC biofilm, the concentrations of these substrates reduce, therefore AOB activity should be repressed in the deeper layers and AOA should be relatively more active. The role of AOA in ammonia removal in wastewater treatment remains relatively controversial.

In general, nitrification activity is principally located in the later stages of wastewater treatment post BOD removal, this is why RBCs have separate biozones in which the final biozone is predominately responsible for nitrification. Nitrification only starts when organic load is below 15 g COD/m^2day^{-1} and can only reach optimal rates below 8 g COD/m^2day^{-1} (Boller *et al.,* 1987). This is because biofilms are dominated by heterotrophic bacteria when the BOD is high and therefore they outcompete nitrifying bacteria. This is explained by the fact that heterotrophic bacteria have faster growth rates compared with slow growing autotrophic nitrifying bacteria. Thus, for combined BOD and ammonia removal, biological reactors must be designed to have a low BOD loading at the back end (Grady *et al.,* 1999).

In typical wastewater treatment systems, the dominant NOB bacteria are *Nitrobacter* and *Nitrospira* (Siripong & Rittmann, 2007; Harms *et al.,* 2003), and these are significantly influenced by DO levels. In a membrane aerated biofilm *Nitrospira* are dominant in low

DO conditions, whereas *Nitrobacter* dominate in high DO conditions, and are associated with higher nitrate oxidation activity(Schramm *et al.,* 1998). With this in mind, preferentially selecting *Nitrobacter* by increasing DO availability will enable WWTs to convert nitrite to nitrate more effectively and improve treatment. This is just one example of how altering environmental parameters of a WWT can modify the biofilm and affect wastewater treatment.

Integrated fixed film activated sludge (IFAS) is a hybrid system that combines both biofilm and suspended biomass. In comparison to the conventional AS process, nitrification is enhanced in IFAS, it has been suggested that the biofilm component of IFAS provides higher nitrification capacity (Kim *et al.,* 2010). IFAS biofilms exhibit greater abundance of anammox bacteria compared to the suspended counterpart (van der Star *et al.,* 2008) and it has been revealed that it is possible to achieve a 90 % accumulation rate of anammox bacteria in RBCs (Egli *et al.,* 2001). Furthermore, the biofilm component promotes higher diversity of nitrifiers (40 vs 16 OTUs) and enhances their activity compared to their suspended counterpart. AOB such as *Nitrosomonas* belong to the class of Betaproteobacteria, this class represents approximately 25 % of all the microbes in the IFAS reactor (Li *et al.,* 2016). The abundance of nitrifiers in the biofilm may be a result of the high cell retention time and the range of micro-niches presented in the biofilm that is not in the suspended component. This indicates that biofilm technologies represent a large potential pool of hard-working active organisms that can be tapped into by optimising the biofilm itself.

There is a whole host of microbes that contribute to ammonium removal in municipal wastewater treatment, often they are slow growing and can be sensitive to different conditions in terms of both abundance and activity (Jonsson *et al.,* 2000; Kelly *et al.,* 2004; Urakawa *et al.,* 2008). RBCs may provide all the unique micro-environments needed for AOB, AOA, NOB and NAB to thrive which can benefit treatment efficacy. However, to the authors' knowledge there is no evidence for this and no theoretical models to describe this situation. Nor is there a comprehensive understanding on the effect of microbial consortia on biofilm ammonia removal and examples in the literature do not relate to fully operational plants (Feng *et al.,* 2012; Zhang *et al.,* 2014). If addressed this gap could be of enormous interest, as the major challenge in achieving

optimal ammonia removal is ensuring the activity of AOB/AOA/NOB/NAB (among others) are sufficiently encouraged or suppressed, in order to yield a collaborative system (Winkler *et al.*, 2011).

2.3 Environmental Parameters Influencing Ammonium Removal in RBC Biofilm

In RBC systems, appropriate design and operation are essential for efficient biological wastewater treatment as the abundance and activity of the microbial consortia involved in ammonium removal (described in previous section) is dependent upon, among other parameters, DO concentration, substrate concentration, pH and temperature (Gu *et al.*, 2007). To maximise ammonium removal efficacy these microbes must be sufficiently abundant or/and active.

This section sheds light on how various parameters within the RBC reactors influences their microbial communities and their activity. The influential operating parameters that will be discussed can not only have a short-term effect on reactor performance, but also a long-term effect on the population size and diversity of the microbial consortia (Wang *et al.*, 2012). As this paper is primarily focused on ammonia removal, the role of NOB has been acknowledged thusly, however discussion on this group of microbes will be kept limited.

2.3.1 Oxygen

Early literature debated the importance of oxygen in RBC treatment performance. It was suggested that substrate was the only limiting factor to treatment performance in RBCs and oxygen was not considered as a limiting factor (Clark *et al.*, 1978). In contrast it was argued that oxygen was the key parameter and the dominant mechanism through which oxygen is transferred to the biofilm in RBCs is via contact with the air and not the bulk liquid (Hartman, 1960). This debate is seemingly ongoing, even now.

There are four routes of oxygen transfer to the biofilm; (1) oxygen being absorbed at the liquid film covering the biofilm surface whilst in contact with air, (2) direct transfer of oxygen at the air-water interface, (3) direct oxygen absorption by microbes during exposure to air and (4) from the bulk liquid to the biofilm, which can be significantly

influenced by turbulence and immersion of biofilm (Grady, 1999 (1 - 3); Patwardhan, 2003 (4)).

The first route is known as the liquid film renewal (LFR) process and this is the dominant mechanism for enabling oxygen availability in the biofilm, as there is a linear relationship between the oxygen transfer rate (OTR) and the LFR (Kim & Molof, 1982). This route has the largest current consensus. The liquid film coating on the biofilm surface is continually broken and renewed in the air/water rotational cycle due to the fluctuating film layer surface tensions (Hiras *et al.*, 2004; Chavan & Mukherji, 2008). The liquid film renewal predominantly occurs in the air phase when the force of gravity overcomes surface tension resistance, it is known as the 'falling film theory' (Zhang *et al.*, 2009). The diffusion rate of oxygen through a liquid is 10^4 times lower than through air (Bishop, 1999). So allowing the biofilm contact with air is preferable for efficient oxygen transfer. This is the case with RBCs where the biofilm boundary layer has a higher DO concentration than the bulk liquid and this is due to exposed liquid film surface and the efficient gas-liquid transfer rate (Dutta *et al.*, 2007).

Within aerobic nitrifying RBC biofilms, the microbial populations rely on oxygen to complete their metabolic activity, and so, the individual microbial niches of the biofilm are largely dependent on their competitiveness for oxygen. As a result, AOB and NOB are often found in the deeper layer of the biofilm as they are easily outcompeted by heterotrophs whom occupy the surface layers where carbon sources are plentiful (Gieseke *et al.*, 2001; Shi *et al.*, 2010). Moving deeper into the biofilm leads to reduced oxygen availability due to oxygen consumption by microbes in the upper levels and due to the Fick's law of diffusion the rate of oxygen diffusion and levels of DO are much lower than those found at the surface of the biofilm (Stewart, 2003; Hassard *et al.*, 2014). However, the surface of a mature RBC biofilm can have a very heterogeneous DO concentration profile ranging between 0 and 3.8 mg L^{-1} (depending on the individual RBC and wastewater conditions), and with increasing depth the heterogeneity decreases, but zones of oxygen ranging between 0.4 and 1 mg L^{-1} can be found as low as 750 µm within the RBC biofilm due to effective interstitial channels and pores (De la Rosa & Yu, 2005). This may partly explain the observation that the upper layers are very heterogeneous with

various cell clusters and high species diversity and deeper layers are more homogeneous, stable and compact (De la Rosa & Yu, 2005).

During the initial stages of RBC wastewater treatment oxygen availability is lower because, metabolic activity from heterotrophic bacteria is high, and oxygen is utilised to generate energy. In contrast, as the treatment progresses the amount of material capable of being metabolised decreases and dissolved oxygen levels increase. As the RBC treatment process progresses carbon sources become more limiting, oxygen availability becomes greater (> 1 mg L^{-1} DO) and ammonium availability remains high, at this point there is a clear relationship between AOB and AOA (Schramm *et al.*, 1998), as well as AOB and NOB (Wang & Li, 2015). At low DO conditions AOB have the advantage over NOB due to lower half-saturation oxygen coefficients and higher yields (Schramm *et al.*, 1998) and therefore often outcompete NOB (Sliekers *et al.*, 2005). Due to the ability to remain active at low DO levels and their high affinity for ammonia, AOA have the advantage over AOB and consequently are able to convert ammonium to nitrite for NAB in the CANON/OLAND process. However unlike NAB, AOA activity may not be affected by high DO levels, as seen in *Candidatus 'S.profunda'* (Stewart *et al.*, 2012).

Due to anoxic conditions encountered in the bottom layers of the biofilm, NAB can be prominent and have been observed in RBCs (Egli *et al.*, 2001; Zhi *et al.*, 2015). NAB can be inhibited in the presence of oxygen and have been found to be protected from DO shocks by *Nitrosomonas eutropha*-like bacteria which mop up residual oxygen, these bacteria are resident in the aerobic layers located above the NAB bacteria (Liu *et al.*, 2008).Inhibition can be reversible at O_2 concentrations at around 0.08 mg L^{-1}, whereas irreversible inhibition can occur at 1.44 mg L^{-1} (Egli *et al.*, 2001), however this has also been observed at 0.16 mg L^{-1} (Third *et al.*, 2005). As expected, it was reported that above 0.8 mg L^{-1} DO in a SBR, AOB become significantly more active than NAB (Yin *et al.*, 2016).

In membrane-aerated biofilms (MAB), DO levels at the membrane influences nitrifying bacteria and consequently ammonium removal rates. The highest ammonium flux could be achieved at 2 mg L^{-1} DO and the cell density of AOB remains relatively stable in response to altered DO, however NOB cell density is positively correlated with DO level,

with the NOB group *Nitrobacter spp.* dominating with > 2 mg L^{-1} DO and *Nitrospira spp.* dominating with < 2 mg L^{-1} DO (Downing & Nerenberg, 2008).

Oxygen availability is often stated as the most crucial design parameter in RBCs. In fact, if the rotation mechanism does not provide ample oxygen then supplemental artificial aeration is recommended (Rodgers & Zhan, 2003). The importance of oxygen in ammonium removal has been highlighted. This is particularly true for heterotrophs, AOB and NOB. Therefore it is recommended that a minimum DO level of 2 mg L^{-1} (Nowak, 2000) or 3 - 4 mg L^{-1} (Boller & Tshui, 1994) should be routinely applied in the bulk liquid of biofilm technologies such as RBC systems. In a submerged fixed bed wastewater treatment biofilm < 10 % nitrification was achieved at 1 mg L^{-1} DO, whereas 100 % was achieved at > 5 mg L^{-1} (Park *et al.*, 2006), further indicating the importance of DO.

However, regarding the specific case of RBCs, it is the authors view DO in the bulk liquid in is not as significant a parameter as is suggested in the literature. The fact that the majority of oxygen transfer to biofilm occurs during the air phase of the disk rotation, makes DO in the bulk liquid less relevant, and even with low DO nearly 90 % of total nitrogen can be removed in a RBC (Helmer & Kunst, 1998). So we would argue that a significant population of nitrifying microbes including AOA and NAB can operate effectively in low DO conditions and in the case of NAB they do not need oxygen at all. In order to complete the traditional nitrification pathway, costly artificial aeration and wasteful addition of external carbon sources are needed in a number of wastewater treatment technologies. The CANON/OLAND process has received a great deal of interest in the literature because it does not need aeration or carbon sources (Paredes *et al.* 2007), the AOA/NAB partnership may be ideal for this pathway. The RBC biofilm cannot only provide the oxygen and carbon source for the traditional nitrification pathway (assuming nitrate rich effluent is recycled to the first stage of the RBC), but also the anaerobic conditions that promote the CANON/OLAND process.

Table 1. Dissolved oxygen concentration (mg L^{-1}) found in various fixed film wastewater treatment systems

DO (mg L^{-1})	Location of Measurement	System	Comments	Reference
2.9 ~1.0	Bulk Solution 500 µm within biofilm	Rotating drum	Compressed air supplied to maintain DO	Bishop *et al* 1999
4.8 1.0 0	Bulk Solution Biofilm Surface 500 µ m within biofilm	Rotating drum	90% NH_4-N removal 35 mg L^{-1} NH_4-N influent	Bishop *et al* 2004
1.5 0 – 10	Bulk Solution Biofilm	RBC	35 mg L^{-1} NH_4-N influent 1 rpm 30cm disc 50% submersion, time in air / water phase (30s each)	Nishidome *et al* 1994
8.5 0	Bulk liquid 100 µ m within biofilm	Particle biofilm		Boessmann *et al* 2004
2.2 3.5* 5.5*[1]	Biofilm base *(w/ anoxic bulk liquid) *[1](between 0 and 2 gm^{-3} in bulk liquid)	Membrane aerated biofilm	Ammonium fluxes were 0.75, 0.1 & 1.3 gNm^{-2} day^{-1} respectively	Downing & Nerenberg 2008

2.3.2 Temperature

Temperature significantly influences nitrogen removal in biofilm reactors (Park *et al.*, 2009). A small decrease in temperature often leads to decreased removal of ammonia of an RBC biofilm (Dutta *et al.*, 2007). Similarly, small temperature increases (3 °C) can lead to increased degradation activity of polymeric organic compounds in aquatic

biofilms (Ylla *et al.,* 2014). Ammonium removal rates decrease by a factor 5 when temperature is reduced from 25-35 °C to 5 °C in completely mixed and aerated submerged fixed bed biofilm reactors (Chapanova *et al.,* 2007; Yang *et al.,* 2009).

This finding is supported by other studies that suggest nitrifying bacteria reach optimal growth at around 25 °C (Parades *et al.,* 2007) or 30 °C (Zhi & Ji, 2014). The maximum specific growth rate decreases dramatically with decreasing temperature, a decrease from 20 °C to 10 °C can reduce the growth rate of nitrifying bacteria by 65 % and AOB in particular prefer higher temperatures (Murphy & Young, 2009). Consequently, increasing temperatures typically leads to AOB outcompeting NOB in wastewater biofilms (Bougard *et al.,* 2006). Microbial abundance has been found to increase in response to small temperature increases (3 °C) in freshwater aquatic biofilms (Ylla *et al.,* 2014) and to large increases (17 °C) in saltwater aquatic biofilms (Moldoveanu, 2012). Studies on soil communities have concluded that temperature changes have a minimal impact on AOB abundance, but significant impact on AOA (Tourna *et al.,* 2008), however AOA are generally robust in temperature ranges of between 8 to 20 °C (Stahl & Torre 2012). Temperature can also affect the diversity of microbial communities, low temperature can result in reduced diversity of both AOB and AOA in freshwater aquarium filters (Urakawa *et al.,* 2008).

The previous findings state that degradation activity in biofilms is affected by temperature and that temperature significantly influences the microbial growth rate/abundance in biofilms. There may be other explanations for the increased degradation activity. For instance, temperature may influence activity of individual microbes. In terms of maximum activity, the optimal temperature for *Nitrosomonas* and *Nitrobacter* is 35 °C and 38 °C, respectively (Grunditz & Dalhammar, 2001). Additionally, high temperatures of more than 30 °C leads to greatly enhanced EPS production in *Enterobacter* (Kanmani *et al.,* 2011), *Streptococcus* (Torres *et al.,* 2012) and *Pseudomonas* (Sandhya & Ali, 2015). As explained in section 2.1.3 EPS can confer a whole range of benefits in biofilms including enhancing degradation capacity.

Under genuine conditions, it has been shown that the nitrification performance of RBCs is optimal between 15 °C and 30 °C; and starts to deteriorate at around 12 °C (US EPA, 2002; Yamamoto *et al.,* 2006). In the event an RBC reaches 5 °C it will need 2 - 2.5

times more media surface area to perform as well as it would have at 13 °C (Rodgers & Zhan, 2003).

2.3.3 pH

Unfavourable pH levels can lead to protein denaturing and destruction of enzymatic functions, whilst also disrupting intracellular catabolism as these processes are very pH sensitive (Carvajal-Arroyo *et al.*, 2014). Free ammonia concentrations in the wastewater can also be affected by pH, which can be detrimental to the biofilm organisms (Fernandez *et al.*, 2012). As described in section 2.1.3, the biofilm can act as a protective barrier against inhibitory pH levels. For instance, it has been shown that AOB and NOB in the biofilm phase continue to grow and remain active at pH levels significantly lower than what would be needed for the same cells in the planktonic phase (Allison & Prosser, 1993).

Optimal biofilm nitrification can occur within a pH range of between 6.5 and 8.3 (Heijnen *et al.*, 1998) and up to pH 9.7 (Odell *et al.* 1996). However, nitrification becomes completely inhibited outside of a pH range between 6 and 10.5 (Yin *et al.*, 2016). There is also some pH sensitivity difference between microbial groups. The optimal pH for AOB range between 7.0 and 8.5 (Villaverde *et al.*, 1997; Truu *et al.*, 2009; Liu *et al.*, 2014) , whilst for NAB it ranges between 6.7 and 8.3 (Strous *et al.*, 1999; Egli *et al.*, 2001; Yin *et al.*, 2016). Complete inhibition of NAB in biofilms occurs at approximately pH 9 (Fux *et al.*, 2004). It has also been reported that AOB are more resistant to pH shocks compared to NAB (Yin *et al.*, 2016). The optimal pH range for both AOB and NAB appears to be similar, in the neutral to slightly alkaline range.

A pH range between 6 and 7 is most favourable for EPS production and least favourable in the alkaline pH range for *Bacillus* bacteria (Dogan *et al.*, 2015). In contrast, the optimal pH for EPS production of *Enterobacter, Streptococcus* and *Pseudomonas* was found to be between 6.5 and 7 (Kilic & Donmez, 2008; Kanmani *et al.*, 2011; Torres *et al.*, 2012). As the optimal pH level for nitrifying microbes seems to be neutral to slightly alkaline, the increased production of EPS when the pH range moves into the acidic scale may be a way of the biofilm protecting itself.

Nitrification is an acid producing process which consumes approximately 8.6 mg L^{-1} of bicarbonate (HCO_3^-) per 1 mg L^{-1} of ammoniacal nitrogen (NH_4^+-N) (Gujer & Jenkins, 1974). Carbonate molecules therefore play a vital role in neutralising acid, otherwise known as alkalinity. In some biofilm reactors treating high strength ammonia influent sodium carbonate is often used to buffer wastewater systems to enhance AOB ecology and consequently nitrification in biofilm systems (Whang *et al.,* 2008). However, in the authors experience low strength domestic wastewater feeding RBCs generally lies within the optimal pH range for nitrifying bacteria and RBCs generally have ample alkalinity reserves, so pH should not pose an issue to ammonium removal in RBC biofilms.

2.3.4 Substrata

The media used in fixed film wastewater processes such as RBCs is an extremely important consideration as it influences growth and metabolic activity of microbes as well as substrate utilisation (Filip & Hattori, 1984). Thus, prudent selection of this media can enhance treatment performance and allow for higher capacity in terms of loading rates in many WWTs (Khan *et al.*, 2011). The media must provide a secure surface that promotes biofilm attachment and growth and it must allow contact between the biofilm and the wastewater. Specifically, in RBCs it must also play a part in oxygen transfer to biofilm.

RBC media has progressed from wooden slats a century ago to metal disks in the 1930s to expanded polystyrene disks in the 1950s and to plastic disks in the 1970s until present (Patwardhan, 2003). Specifically, high density polyethylene (HDPE) is the most common material, but lightweight plastic including Styrofoam can also be used (WEF & ASCE, 1998). This evolution in media selection was driven by structural attributes and cost, however, in the last 5 years there has been interest in evaluating other types of media such as, biological granules, shredded tires and coconut fibres for performance enhancement, as well as cost for example (Gullicks *et al.,* 2011).

Hydrophobicity of a surface is one of the main determinants for cell adhesion. As hydrophobicity increases, the influence of electrostatic interactions decreases and vice versa (van Loosdrecht, 1987). Cells with more hydrophobic surface characteristics have greater attachment efficacy, and surfaces with hydrophilic properties exhibit more resistance to both cell and protein attachment (Cunliffe *et al.,* 1999). However, cells with

hydrophilic surface characteristics have greater affinity for hydrophillic surfaces in terms of attachment, similarly hydrophobic cells prefer hydrophobic surfaces (van Loosdrecht *et al.*, 1987). Employing open, porous media results in less biofilm adhesion than dense media as the porous media presents many areas of water saturated hydrophilic channels which are avoided by bacteria (Knoell *et al.*, 1999). Similarly, large numbers of marine *Pseudomonas* bacteria attach to hydrophobic plastics with little surface charge such as polyethylene and polystyrene, whereas small numbers attach to hydrophilic negatively charged substrata such as glass and oxidised plastic (Fletcher & Loeb, 1979). Interestingly, positively charged surfaces have been implicated with higher cell adhesion, lower cell viability and more shear force resistance in *E. coli*, in contrast, negative surfaces result in lower adhesion, less shear resistance but higher viability (Terada *et al.*, 2012). In enamel biofilms it has been shown that hydrophobicity does not influence adhesion, however it significantly influences bacterial retention, with bacteria detaching in greater number from hydrophobic surfaces rather than hydrophilic surfaces upon introduction of high shear forces (Bos *et al.*, 2006), in clean water environments hydrophobicity may not even influence retention of bacteria (Pedersen, 1990).

Increased surface roughness provides greater surface area and enhances mass transfer coefficients by presenting surface micro-irregularities that prevent cell desorption by offering protection from shear forces and provide niches that are ideal for cell adhesion; this subsequently leads to increased biofilm colonisation and microbial accumulation (Characklis *et al.*, 1990; Terada *et al.*, 2005; Liebminger *et al.*, 2012). On the other hand, increased surface roughness results in greater protein adsorption which then leads to increasing surface hydrophilicity and therefore provides a protective aqua layer coating the substrata which can hamper the biofilm adhesion (Singh, 2011). As will be explained later increasing hydrophilicity can confer benefits for nitrification. In an aerobic mixed biofilm culture, surface roughness did not affect nitrification; however, it was inversely correlated to adhesion force (Stephenson *et al.*, 2013).

Surface roughness and hydrophobicity were the two variables shown to determine initial attachment of bacteria and subsequent biofilm formation in membranes (Myint *et al.*, 2010). Many findings confirm that in wastewater treatment membranes exhibiting high hydrophobicity, a less negative charge and high surface roughness, greater biofilm

formation and accumulation occurs (Subramani & Hoek, 2008; Myint *et al.,* 2010; Lee *et al.,* 2010). In contrast, total biomass attachment to plastic media is positively correlated with surface hydrophilicity and surface energy in aerobic wastewater treatment environment (Khan *et al.,* 2011). Similarly, aerobic heterotrophic biofilms consisting of bacterial species *Pseudomonas aeruginosa* and *Bacillus subtilis* biomass growth increased when the difference in surface energy between the biofilm and the support surface increased (Dimitrov *et al.,* 2007).

Surface chemistry can promote attachment of certain types of bacteria, for example surfaces modified with amide groups deter *Listeria* and *Escherichia* but greatly promote attachment of *Staphylococcus* and *Salmonella* (Cunliffe *et al.,* 1999). Polyvinylidene fluoride media in membrane aerated biofilms encouraged more biomass accumulation and enhanced nitrogen removal activity compared to carbon, polyethylene, polypropylene and polysulphone (Liu *et al.,* 2007). Modified glass surfaces containing CH_3-terminated groups exhibited not only greater adhesion but also enhanced *Staphylococcus* biofilm formation compared to glass with OH-terminated groups, the greater adhesion and formation was associated with more EPS production (Foka *et al.,* 2012). This shows the potential to design surfaces which select for preferential microbial communities. Rotating polyurethane disks allowed for an enhanced ammonia removal rate in an anaerobic reactor receiving domestic wastewater, compared to polystyrene disks (Tawfik & Klapwijk, 2010). This may indicate that polyurethane offers a preferential substratum for NAB. It has also been reported that nitrification rates increase with high hydrophilicity and surface energy and that AOB species *Nitrosomonas europaea* and *Nitrospira multiformis* have higher adhesion rates than the heterotroph *Escherichia coli* on high surface energy surfaces (Khan *et al.,* 2013). The wide range of studies cited in this chapter show that hydrophobicity affects biofilm attachment and accumulation differently, depending on microbial species and environmental conditions. The studies that focus on nitrification in a wastewater environment tend to agree that hydrophilicity promotes nitrification performance however the mechanism which brings this about is not yet fully understood.

2.3.5 Nutrients

Early studies showed that the thickness of a biofilm in a steady state decreases when shear force is increased and nutrient concentration is constant, however when shear is constant and nutrient concentration is increased biofilm thickness increases (Characklis, 1981 & 1990). This is true in RBC systems where biofilm thickness depends on organic loading and shearing forces (Cowan *et al.*, 1991; Griffin & Findlay, 2000). The thickness of a biofilm can react and change dramatically in less than a day, in response to sharp increases in nutrient loading, such as glucose for example (Stoodley *et al.*, 1999). In fact, RBC biofilm thickness at a COD/Nitrogen ratio of 1.5 (1070 ± 120 µm) is four times thicker than at a ratio of 0 (Okabe *et al.*, 1996).

Controlling biofilm thickness is important due to factors relating to biofilm ecology – e.g. what microbes reside in the biofilm and how abundant/active are they? In terms of biofilm ecology, the outermost layer contains a higher density and more viable microbes than the innermost layer (Rodgers & Zhan, 2003). Further to this, the nitrification rate is directly proportional to the square root of the wet biofilm mass in vertically moving biofilm systems (if DO > 6 mg L^{-1}) (Rodgers & Zhan, 2003). In terms of operation, keeping biofilm thickness to a minimum can reduce issues with clogging and material fatigue stresses (Griffin & Findlay, 2000). Controlling biofilm thickness can be achieved with the use either of supplementary air, reverse shaft rotation or by altering RBC rotational speed (Patwarden, 2003).

In the 1990s relatively little comparative data was available on the effect of nutrients on biofilm morphology, and therefore it was stated as an area of research that is particularly important (van Loosdrecht *et al.*, 1995). There is now a range of literature on the topic and this section will highlight some of key aspects in which morphology can change in response to nutrients. In aerobic fixed film systems, ammonium removal is frequently inhibited due to excess biodegradable carbon leading to heterotrophs outcompeting the nitrifying community (Guerdat *et al.*, 2011). In contrast, low biodegradable carbon promotes the growth of autotrophic and oligotrophic bacteria, and inhibits growth of heterotrophic bacteria, thereby promoting ammonium removal (Shoji *et al.*, 2008; Gullicks *et al.*, 2011). Furthermore, excess biodegradable carbon can lead to heterotrophic dominance in terms of distribution within the biofilm which can cause

internal structural changes in the matrix and alters mass transport pathways and further decreases nitrification capacity (Michaud *et al.,* 2006). In practice nitrifying autotrophs can compete effectively with heterotrophs and commonly reach maximum nitrification rates at low soluble BOD_5 levels <10 mg L^{-1} (WEF & ASCE, 1998), or 30 mg L^{-1} soluble COD in membrane bioreactors (Rodgers & Zhan, 2003). Contrary evidence suggests that nitrifiers are much more sensitive in terms of competitive capacity with heterotrophs. As soluble BOD_5 loads are increased from 0.75 to 2.1 $sBOD_5/m^2d$, there is a linear decline in nitrification from 70 to 15 % within 10 days in a nitrifying trickling filter (van den Akker *et al.,* 2010). Even in a completely carbon limited environment, nitrifying RBCs exhibited a biofilm community that consisted of 50 % nitrifying species and 50 % diverse heterotrophic community (Kindaichi *et al.,* 2004). The microbial community was largely inactive at times and mainly metabolising carbohydrates and proteins yielded from endogenous bacterial decayed within the biofilm. At a COD/N ratio of 0 in RBCs, heterotrophs and nitrifiers coexist in the upper biofilm layers; however, increasing the COD/N ratio to 1.5 leads to heterotrophs becoming more prevalent and more competitive. Therefore, nitrifiers can only be present in the deeper layers and as a result nitrification rate decreases (Okabe *et al.,* 1996). Interestingly, in an anaerobic biofilm, archaea appear to be less sensitive than bacteria to fluctuating organic loading (Mnif *et al.,* 2012; Xie *et al.,* 2014).

As mentioned earlier, the anaerobic ammonium oxidation pathway also can take place within fixed film technologies like RBC systems (Jetten *et al.,* 1997; Mulder *et al.,* 1995). In co-diffusion biofilms such as RBCs 50 % NH_4^+ removal through the anaerobic pathway can be achieved at a COD/N ratio of 2, however, further increasing the ratio past this point leads to a detrimental effect on anaerobic NH_4^+ oxidation and NH_4^+ oxidation cannot be sustained, subsequently a 90-95 % loss of biofilm occurs (Lackner *et al.,* 2007). Membrane-aerated biofilms exhibit different physiological behaviours when reacting to altered influent COD/N ratios as they are counter-diffusion as opposed to co-diffusion biofilm technologies. At a COD/N ratio of 3, EPS production, bacterial abundance as well as density was far lower than when the ratio was increased to 5 (Lin *et al.,* 2016). Reduced ammonia removal was also observed due to the increased carbon availability which contributed to the proliferation of the heterotrophic bacteria which subsequently outcompeted the nitrifying bacteria.

It is expected that the final stage of the RBC process is the most conducive stage for nitrification as this is the stage with the lowest biodegradable carbon content. However, the final stage in an RBC can have the least nitrification potential (Dutta *et al.*, 2007). This is because of substrate limitation due to effective nitrification in the previous stage and therefore senescence and decay pathways take precedence over growth and metabolism (Gujer & Boller, 1990). The density of nitrifying bacteria is thus reduced together with nitrification activity. In the event described where nitrifying bacteria are starved of ammonia, the AOB *Nitrosospira briensis* has been shown to exhibit fast recovery, reaching optimal ammonia oxidising activity within one hour of substrate addition after a two-week starvation period (Bollmann *et al.*, 2004). Other AOB such as *Nitrosomonas europea* have also been shown to recover from starvation events (Laanbroek *et al.*, 2002; Bollmann *et al.*, 2002). This is expected, as all AOB must maintain robust and resilient ammonia oxidising capabilities due to natural fluctuations in substrate levels as well as competition with other bacteria (Van Niel *et al.*, 1993; Bollmann *et al.*, 2002) and affinity for ammonia and resilience to starvation events is enhanced for AOB embedded within biofilms, compared to suspended cells. For instance, biofilm-embedded nitrifying *Nitrosomonas* bacteria almost immediately resume nitrifying in the event of a prolonged starvation event (> 40 days), the same bacteria in suspension have been shown to resume nitrifying only after a significant lag phase (up to 153 hrs) (Batchelor *et al.*, 1997; Bollman *et al.*, 2005). This makes nitrifying RBC biofilms better suited for small rural WWTs as they may receive intermittent and unreliable influent feed. The delay in recovery before reaching maximum ammonia oxidising activity is explained by a lack of NH_2OH, which the enzymatic function of ammonia monooxygenase relies upon (Hooper, 1969; Wood 1986).

The dominant AOB and NOB species will depend on the biofilm environment and in particular the substrates available to them. For instance, in WWTs receiving extremely high ammonium levels, fluorescence in situ hybridisation (FISH) revealed that NOBs were not present as they were likely inhibited by free ammonia and outcompeted by the AOB population; which made up 5.5 % of the total biofilm, *Nitrosomonas* europaea/*eutropha* made up 4.3 % and the remaining 1.2 % was *Nitrosococcus mobilis* (Gu *et al.*, 2007). *N. europaea*/*eutropha* have higher growth rates compared to other AOB and therefore they often dominate in WWTs (Okabe *et al.*, 2002; Schramm, 2003).

In response to high ammonium load both AOA and AOB have been shown to exhibit greater ammonium removal activity (Fujita *et al.,* 2010; Wang *et al.,* 2016). The question remains, does the increased activity brought about by increased substrate availability stem from population increase or increased activity per cell?

Ionic agents in the wastewater can have a large impact on biofilms. In low ionic strength media, the cell wall electronegativity increases, whereas in high ionic strength media the electronegativity reduces resulting in impaired and enhanced adhesion respectively (Hong & Brown, 2008). The addition of NaCl and $CaCl_2$ or other ionic agents can lead to a reduced negative charge on the surface of bacteria and as a result adhesion increases (Chen *et al.,* 2007), as can be seen with adhesion of *Pseudomonas* to glass (Fletcher, 1988). Multivalent cations (e.g Mg^{2+} & Ca^{2+}) are essential for the structural integrity of the EPS matrix as they are needed for the cross-links between polymers (Sobeck & Higgins, 2002), removing these cations leads to biofilm disintegration (Frolund *et al.,* 1996). Other multivalent cations (e.g Pb^{2+}, Zn^{2+}, Cu^{2+} & Ni^{2+}) can be extremely toxic to biofilms and to nitrifying bacteria in treatment systems in particular, Cu^{2+} and Ni^{2+} can inhibit nitrification with both cations being more potent inhibitors of *Nitrosomas* and *Nitrobacter,* respectively (Sirianuntapiboon & Hongsrisuwan, 2007; Sirianuntapiboon & Boonchupleing, 2009). Pb^{2+} and Ni^{2+} did not affect the system performance of RBCs in concentrations of up to 10 mg L^{-1}, however increases past this level reduced the population of nitrifiers in the biofilm (Sirianuntapiboon & Chumlaong, 2013), in contrast Ni^{2+} can inhibit nitrification at concentrations as low as 0.7 mg L^{-1} in activated sludge systems (Randall & Buth, 1984). This is because the EPS matrix has been shown to confer significant resistance to heavy metal toxicity perhaps by adsorption onto the EPS (Sirianuntapiboon & Boonchupleing, 2009). Additionally, exposure to heavy metals such as chromium may promote an increase in EPS production (Hung & Santschi, 2001; Priester *et al.,* 2006; Ozturk *et al.,* 2009), which would then further enhance heavy metal resistance. This finding is also reported *Pseudomonas* cultures where copper promoted 400 % more EPS production (Kazy *et al.,* 2002). EPS production can also be promoted by substrate shortages (Hung & Santschi, 2001).

Phosphorus has been associated with increased microbial growth and enhanced nitrogen removal (Cortez *et al.,* 2011), additionally it has been associated with enhanced biofilm

adhesion (Newell *et al.,* 2011) and biofilm formation (Monds *et al.,* 2007) in *Pseudomonas*.

The mass transfer of nutrients penetrating into the deeper regions of the biofilm will be limited when compared to the upper layer of the biofilm, therefore the biofilm interior may be characterised by slower growing organisms (Poulsen *et al,* 1993) and throughout the biofilm there will be a range of growth rates depending on the unique microenvironment. Nutrient availability is the most influential parameter regarding the microbiology of the RBC biofilm, where the mass transfer of nutrients depends on the operational regime, the structure of the biofilm, the attachment/detachment of the biofilm and the thickness of the biofilm (Wuertz *et al.,* 2004; De Clippeleir *et al.,* 2011).

2.3.6 Hydrodynamics

As previously discussed nutrients play a substantial role in influencing the wastewater biofilm and the ability to remove ammonium. The movement of such nutrients from the bulk liquid to the biofilm can be mediated by a number of fluid dynamic processes including mass transport, thermal and gravitational effects (Characklis, 1981).

A Reynolds number can be used to describe the flow conditions where a low value implies viscous forces are dominant and a smooth laminar flow is present, whereas a high value implies inertial forces are dominant and a turbulent flow is present. Turbulent flow is chaotic and characterised by eddies and other flow instabilities. It allows for good mixing of nutrients and bacteria (Characklis *et al.,* 1990). Laminar flow ensures that both nutrients and microbes maintain straight trajectory and therefore they are dictated by flow rate and remain in a more stabilised position (Lappin-Scott *et al.,* 1993). These two types of flow can significantly modify biofilm structure (Stoodley *et al.,* 1998).

Interestingly, it is possible for planktonic bacteria to initiate biofilm formation in very turbulent conditions where the presence of shear forces is equivalent to Reynolds numbers of 5,000 which means biofilm formation could occur within a human heart valve (Characklis & Marshall, 1990). Turbulent flow can also promote biofilm adhesion in an aqueous environment (Percival *et al.,* 1999), because in turbulent flow eddying currents move bacteria to within short distances of the surface and hence increase the chance of adhesion (Percival, 2011). However, turbulent flow does lead to less total bacterial

deposition on the surface, partly because the mechanism of Brownian motion deposition is redundant in these conditions (Subramani & Hoek *et al.,* 2009). Turbulent flows also result in higher biofilm density and higher NH_4 flux (Kugaprasatham *et al.,* 1992). In turbulent conditions (Reynolds number of 1192) aquatic biofilms consisting of *Pseudomonas* and *Klebsiella* are highly viscoelastic and behave in a rubbery manner, due to the excessive secretion of an exopolysaccharide matrix (Stoodley *et al.,* 1998). In these high shear turbulent flow conditions, filamentous streamers form on the top of the colony because new daughter cells are pushed downstream to the trailing edge of the biofilm by drag force. This continual deposition of cells leads to streamers being formed, which oscillate in the fluid like a tadpole swimming, as seen in real time video imaging (Lewandowski & Stoodley, 1995). The 'streamers' are implicated with increased frictional energy losses in water conduits and their rapid oscillation reduce drag and may also increase mass transfer (Stoodley *et al.,* 1998).

Pseudomonas species biofilms growing in laminar conditions (Reynolds number 120) have a greater initial rate of growth and reached a steady state quicker than biofilms in turbulent conditions (Reynolds number 3600) (Stoodley *et al.,* 1999). However, once the biofilms reach steady state, the biofilm coverage is lower in the laminar conditions. Maximal biofilm thickness occurs near the transition point between turbulent and laminar flow as it allows for biofilm accumulation without being limited by either detachment by shear force or growth induced by mass transfer (Lewandowski & Walser, 1991). This transition point has a Reynolds number value of around 1200 (Stoodley *et al.,* 1998).

By tracking particles in the RBC, confocal scanning light microscopy can confirm that the flow velocity of the bulk liquid is proportional to the flow velocity within the biofilm itself; this is important as at low velocity the boundary layer is parallel to the substratum whereas at high velocity the layer follows the inconsistent biofilm surface (de Beer & Stoodley, 1995) and therefore the boundary layer provides greater surface area at high velocity flow. Perhaps because of this phenomenon the same authors concluded that high flow conditions can increase total mass transfer to the biofilm by up to 250% when compared to low flow conditions. There is unfortunately a lack of recent literature on the topic of how wastewater flow conditions influences ammonium removal in biofilms and so this is an area of research that may provide a greater understanding in the future.

Another important aspect is the hydraulic retention time (HRT). In RBCs, HRT is inversely proportional to organic loading rate (OLR) (Patwarden, 2003). Decreasing HRT from 0.66 to 0.18 days in an anaerobic RBC using the OLAND process results in a nitrogen removal rate reduction from 2.2 to 1.6 g N $m^{-2}d^{-1}$ (De Clippilier *et al.*, 2011).

2.4 Strategies to Optimise RBC Biofilms

A decentralised wastewater treatment network in small rural communities is encouraged, as the costs of pumping from these communities to large treatment sites is not efficient or economical (DEFRA, 2012). RBCs are ideal for this situation as they are low energy and low maintenance, however a challenge for RBCs is meeting ammonia consent limits that are becoming increasingly stringent. In order to make RBC systems more competitive and more widely utilised the performance of biofilms must be optimised. Optimisation can lead to more successful degradation of ammonia, but this is dependent upon a comprehensive understanding of how the microbial system is influenced. The ability to take advantage of this with creative engineering solutions will add significant value and potentially a stepwise improvement to wastewater treatment technologies. In contrast, a partial understanding of the microbiologically mediated treatment process will limit our capability to control and truly optimise the desired biodegradation processes within a biofilm based system, and may in fact focus operational management efforts in erroneous directions, therefore wasting time and capital.

Our ability to optimise the treatment process lies with operational parameters such as temperature or pH and their influence on biofilm thickness, species distribution etc. This final section will propose the optimal attributes of an RBC biofilm for ammonium removal and based on the findings in section 2 we will suggest operational changes to promote this optimised biofilm. Two strategies can be used to bring about this optimisation – (1) biostimulation, in which altering operational parameters promotes the abundance/activity of microbes already resident in the bacteria or selects a new preferential microbial consortia and (2) bioaugmentation, where the biofilm is supplemented with exogenous microbes and operational parameters are altered to specifically retain these microbes.

2.4.1 Biostimulation Strategies

By altering operational parameters within the RBC, the biofilm can be improved in terms of ammonium removal. This was discussed in section 3 and a summary of the key facts is presented in Table 2.

From conception, accumulation of biofilm in terms of thickness and surface coverage occurs as a sigmoidal progression and eventually reaches a steady state (Stoodley, 1999). Controlling biofilm thickness is important as if it is too thick, it will present a significant barrier to mass transfer (Hassard *et al.*, 2014; Zahid *et al.*, 1994). However, if the biofilm is too thin then the potential cost is the loss of the microbial population abundance and potentially a smaller active microbial consortium to mediate the treatment process. Thus more research needs to be carried out to shed light on the optimal accumulation of biomass in RBC biofilms. Acting on this research will be simple as measuring biofilm accumulation is easily done with simple scales and controlling biofilm thickness can be achieved with the use of either a reverse shaft rotation or supplementary air or altering RBC rotational speed (Patwarden, 2003).

Table 2. Summary of review findings in terms of providing optimal operational conditions for ammonia removal in RBCs

Parameter	Optimal Conditions	Mechanism to Alter Parameter	Comments
Oxygen	2 – 5 mg L^{-1} in the bulk liquid	Rotational speed/supplemental aeration	DO in the bulk liquid is not as important to nitrifiers as is often stated. The major oxygen transfer route is via the air phase rather than bulk liquid. Furthermore, a nitrifying microbial consortia that can operate in very low DO conditions has been shown to be effective in RBCs. Aeration is important reduce the BOD load and ensure nitrifiers can compete with heterotrophs
Temperature	25 °C; with ammonium removal efficacy significantly deteriorating below 12 °C	N/A	Temperature is not only one of the main limiting factors in nitrification, high temperatures can lead to AOB/AOA becoming relatively more competitive than NOB resulting in higher ammonium removal
pH	7 – 8.5	Addition of carbonate	In reality, the pH within RBCs treating domestic wastewater lies comfortably within the optimal range
Substrata	High hydrophilicity and surface roughness	Disk material	Surface roughness provides more area for active biofilm accumulation and may also increase hydrophilicity. Hydrophilicity may preferentially select for nitrifying bacteria rather than heterotrophs
Nutrients	A significant proportion of biodegradable carbon must be removed before the nitrification step	N/A	In reality, the composition of the wastewater influent is very variable and cannot easily be altered.

Hydrodynamics	High HRT and high velocity turbulent flow	Reactor design	These conditions are conducive for optimal ammonium removal, however it would be difficult to design a reactor with all these flow attributes.

Biofilm accumulation or biofilm thickness can be summarised by the following equation:

Accumulation rate = Attachment rate + Growth rate – Detachment rate/Biomass decay (Kroukamp & Wolfaardt, 2009).

The latter two options would also introduce more oxygen into the reactor, which has been stated as very important in many of the studies cited in this review. This should theoretically improve ammonia removal in RBCs, however this parameter is not as significant as is often stated because the biofilm derives most of its oxygen through contact with air and this is always constant. Furthermore, anammox bacteria (NAB) have been shown to be effective in oxygen limited RBCs and the anoxic layers deep within the biofilm provide a perfect niche for them. Utilising NAB to metabolise nitrite to nitrogen dioxide gas via the CANON/OLAND route, rather than utilising NOB via the traditional aerobic nitrification route has the advantage of significantly reduced sludge generation and aeration costs (Kartal *et al.,* 2010) and therefore operational cost savings of 90 % could be achieved by employing NAB (Jetten *et al.,* 2001). In a high carbon environment, it is likely that the majority of the carbon will be sequestered by bacteria existing in the layers above the NAB, this supplies a further potential benefit – less potential to be inhibited by excess carbon. Normally it is not viable to employ both AOB and NAB as low DO may make AOB redundant, whereas high DO may make NAB redundant and promote NOB growth, whilst AOA can maintain high activity at very low DO levels and at medium DO levels (Pan *et al.,* 2016). Furthermore, without NAB or NOB to convert the nitrite produced by AOB and AOA, high nitrite concentration may inhibit AOB and AOA (Pan *et al.,* 2016). Some studies have highlighted the importance of all these bacteria in nitrifying biofilm wastewater systems. As discussed, various studies have investigated the activity and abundance of different groups of nitrifiers, however it is difficult to calculate the relative contributions of these microbes to ammonia removal in practice (Taylor *et al.,* 2012), as abundance of these groups may change throughout the

treatment process and individual microbes may exhibit varying activity levels. It is difficult to measure this accurately and combine the data to produce accurate conclusions.

RBC reactor design can feasibly manipulate the operational parameters mentioned in this review. Reactor configuration (completely mixed, plug flow, stirred) can determine nutrient concentrations around and in the biofilm. Reactor hydraulics can be altered to manipulate particulate matter. Biofilm sloughing and other methods can be used to affect growth rate of biofilm and distribution of species as well as thickness and density. Oxygen transfer can be controlled to some extent by altering rotational speed in RBCs.

As mentioned before temperature is another important factor in RBC treatment performance. One simple design modification that would enable a significant process improvement, particularly in northern countries where winter temperatures can be harsh is, a more insulatory cover. This may have an additional unperceived benefit if the cover was able to allow light to penetrate to the biofilm, this may enable phototrophs to grow which are associated with a range of potential benefits including the enhancement of biofilm attachment via the high expression of specific matrix substances (Roeselers *et al.*, 2008).

Although this review has stated that the type of wastewater flow has a significant influence on the biofilm, in practice this is not something that is easily manipulated in small WWTs, as often the influent flow into the sites is intermittent and unreliable. An effort should be made in the design process to maximise the hydraulic retention time as this will enable a longer contact time between the biofilm and the wastewater. As explained previously, high, turbulent flow may enhance biofilm attachment and development and so because of this it is suggested that there is one exception to the high HRT rule. During start-up of a new biofilm reactor, a low HRT significantly enhances biofilm growth on the substrata (Cresson *et al.*, 2008) and could therefore reduce the acclimatisation period needed before the biofilm reaches maturity and can perform robust wastewater treatment.

Section 3.5 outlined how influential nutrients can be to ammonium removal in RBC biofilms, however, nutrients is another parameter that is not easily manipulated due to it being an integral part of influent flow characteristics and the influent flow is determined by the wastewater pipe network and the wastewater being discharged into it. An

important consideration is the removal of carbon before the majority of ammonium removal takes place. If there is not sufficient removal of biodegradable carbon, ammonium removal efficacy will deteriorate drastically as heterotrophs will have a significant competitive advantage of nitrifying bacteria (Okabe *et al.,* 1996; van den Akker *et al.,* 2010; Lin *et al.,* 2016). Correct design of the RBC which separates the heterotrophic stage from the nitrifying stage will ensure this does not happen.

2.4.2 Bioaugmentation Strategies

Bioaugmentation can involve a range of organisms. For instance, predatory protozoa have been used in waste gas treatment to limit biofilm accumulation (decrease in biofilm mass of 0.13 g dry biomass/g toluene) without any loss in biofilm activity, with respect to toluene degradation (Cox & Deshusses, 1998), furthermore bacteria phage can also be used to control biofilm accumulation (Sutherland, 2001). Bioaugmentation of the biofilm has also been achieved in lab-scale RBC systems by supplementing a mature biofilm with nitrifying bacteria (4×10^5 cells.cm^{-2}.hr^{-1}) for 3 days. The added nitrifying population was retained and proliferated which meant NH_4^+-N removal efficacy was enhanced possibly through the mechanism of increasing cell population in the outer layer of the biofilm (Satoh *et al.,* 2003). It is known that adhesion of the initial inoculum community in biofilm reactors has a significant impact on microbial community of the mature biofilm (Habouzit *et al.,* 2004; Zhang *et al.,* 2009) and so this bioaugmentation strategy may have an even greater effect if introduced in the early stages of reactor start-up.

Rather than supplementing reactors with organisms to produce a significant change in biofilm characteristics, supplementation of their signalling molecules such as AHL and quinolone can be used. The addition of 100 nM quinolone enhanced bacterial attachment and resulted in a 95 % increase in mass in microbial fuel cells (Monzon *et al.,* 2016). Furthermore, the addition of AHL can enhance EPS production (Yang *et al.,* 2016), and can increase activity (De Clippeleir *et al.,* 2011; Tang *et al.,* 2015) and biomass (Gamage *et al.,* 2011) in a nitrifying biofilm. Introducing just 5 nM AHL to a mature nitrifying wastewater biofilm significantly increased AOB population, overall microbial activity and nitrification (+ 11 %), and the authors suggest this change was irreversible, however over-application (> 50 Nm) can reduce biofilm activity (Hu *et al.,* 2016). So, the approach of introducing these molecules to enhance treatment performance is promising and

feasible, but it must be executed carefully and we suggest further research should be carried out on this recently highlighted area beforehand (Hu *et al.*, 2016). Also as mentioned in Section 2, quorum quenching molecules may have similar effects, however this is another recent area of research which must be investigated further.

2.5 Conclusion

There is potential for enhancing ammonium removal in RBCs by altering the design to influence operational parameters and enhance the biofilm via biostimulation. Based on current knowledge regarding how these parameters influence the biofilm, this is a simple and relatively fast way to improve the efficacy of wastewater treatment. With real time molecular techniques becoming faster, simpler and cheaper, monitoring the microbial consortia in the biofilm would increase this potential for wastewater treatment improvement by adjusting operational parameters. Bioaugmentation is also a promising approach for the future, however this is not as currently feasible and may require significant research for it to be meaningful in fully operational WWTs

Acknowledgements:

This work was co-funded by the Natural Environment Research Council (NERC) (Grant reference NE/I019367/1) and Severn Trent Water (STW).

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CHAPTER 3

PERFORMANCE ROBUSTNESS ANALYSIS

3 ASSESSING THE ROBUSTNESS OF SMALL WASTEWATER TREATMENT SYSTEMS PERFORMANCE

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Abstract

The assessment of wastewater treatment systems (WWTs) has gained interest in recent years. However, most of the studies have been focused to date on the economic and environmental performance rather than on the ability of the WWTs to cope with changing conditions. In this paper, an innovative robustness performance index (RPI) is proposed to assess the performance of WWTs containing rotating biological contactors (RBCs). A database of 10 years' worth operational data across 121 WWTs was used to extract effluent ammonium concentration data as well as key asset information and population equivalent design data. WWTs were grouped according to effluent consent level in intervals of 5 mg L^{-1}. RPI scores were then calculated based on WWT current consent and theoretical altered consents. Results showed most of the RBCs in small WWTs performed well with ammonia effluent consents ranging between 10 and 20 mg L^{-1} and 50 % of the WWTs do not lose significant robustness upon a consent reduction of 5 mg L^{-1}. Results also suggested that unless RBCs need to be repaired, age does not have an effect on robustness and that no one attribute makes a more significant contribution to robustness than another.

Keywords: Asset Management, Robustness, Rotating Biological Contactor, Nitrification, Wastewater Treatment

3.1 Introduction

Wastewater treatment assets are key for protecting water resources and for allowing sustainable access to water and sanitation services. However, an expanding population together with urbanisation are leading to an ongoing increase in the amount of wastewater that must be treated, and therefore there is a risk to treatment networks becoming saturated which in turn gives rise to the risk of polluted water being discharged directly into the natural environment. At the same time, aging and sometimes disintegration of the existing infrastructure, environmental sensitivity, and the impact of climate change has increased the pressure on wastewater assets (Arnell, 2002; Dessai & Hulme, 2007). Thus, the industry is undergoing significant changes and wastewater treatment techniques are changing so as to adapt to these new challenges. The industry is now turning the treatment of wastewater into a cutting-edge industry which recognises that sustainable and efficient use of water resources is absolutely crucial. New tools that can support this transition will have a great benefit.

Meeting these challenges is made increasingly more onerous as UK water companies are continually expected to comply with tighter regulations whilst minimising total expenditure. This is to ensure that water bodies are given ever increasing protection from environmental pollution, so that customers pay lower bills and that stakeholders receive reasonable returns on their investments. Regulations such as the Waste Water Framework Directive 91/271/EEC (WFD), the Industrial Emissions Directive 2010/75/EU (IED) and the Climate Change Act (2008) mean that tighter environmental quality standards (EQS) must be achieved.

WWTs need to maintain compliant and acceptable EQS, whilst at the same time minimising operating (OPEX) and capital (CAPEX) expenditures. This crucial balancing act between environmental performance and cost saving is central to the management of all WWTs, particularly for small WWTs. In the UK these are defined as having a capacity $\leq$ 2000 population equivalent [PE]; 79% of WWTs are categorised as small, which favours employing low-energy, low-maintenance technologies such as trickling filters and rotating biological contactors (RBCs) (DEFRA, 2012). The small WWTs are overwhelmingly situated in rural communities within a decentralised system which means that it is advantageous for these small WWTs to operate robustly with nominal resources.

Furthermore, allocating large operational resource to these sites cannot be justified as resources can be utilised more cost-efficiently at large WWT systems. Instead of the conventional approach of directing large investment towards replacing existing assets with energy intensive activated sludge units or submerged aerated filters, a more desirable approach in the face of current and future challenges would be choosing to introduce or retrofit low-energy, low-maintenance units (Brookes, 2013), such as RBCs. This approach is advantageous to a sustainable, long-term strategy for wastewater treatment at small WWTs.

RBCs are capable of achieving low effluent sanitary consents for biological oxygen demand, total suspended solids and ammonia (Hassard *et al.*, 2015). They are therefore a competitive secondary treatment asset capable of meeting the challenge of enhanced treatment in a more resource efficient and carbon friendly manner at small WWT systems. However, standards are becoming stricter, for example, with ammonium (NH_4^+-N) effluent consents at some small WWTs dropping below 5 mg L^{-1} and even to 0.5 mg L^{-1} where sites are located in lowland river locations and the surrounding ecosystem is sensitive (Pearce, 2013; DEFRA, 2014). This is putting pressure on WWT performance and the extra capacity to robustly achieve such effluent standards is often needed.

Recently it was suggested that approximately 25% of all water body impairments are due to nutrient-related causes including very often nitrogen (Nourmohammadi *et al.*, 2013). Therefore, it is important to identify WWTs which experience difficulties in consistently meeting current EQS and similarly it is important to identify how nitrification performance robustness alters with changing environmental and operational parameters. Gaining such information will enable WWTs performance to be assessed and reviewed in terms of robustness, which will reveal whether assets can meet future EQS and whether investment, in order to upgrade and/or retrofit assets is necessary. Here the term robustness is used to define a WWT that is insensitive to external variables such as loading rate and flooding and can therefore handle changes well (Zakarian *et al.*, 2007). Robustness is therefore an important parameter for considerations in a future world where growth is a challenge and the WWT system model is decentralised, as previously mentioned.

Currently the main approach used at STW to help engineers identify which sites need attention is based on a traffic light system known as the 'golden measures'. Sites performing as expected are those where effluent samples taken on a weekly basis contain < 25 % of the stated consent concentration (green light); sites highlighted for investigation are those where effluent samples contain ≥ 25% of the stated consent concentration (amber light); and sites highlighted for immediate attention are those where effluent samples contain ≥ 50 % of the stated consent concentration (red light). While this is the main tool used to assess WWTs robustness at one water utility, further complementary tools would provide a multi-dimensional holistic approach and would provide great benefit.

There have been several attempts to measure robustness using indices (Huck *et al.,* 2002; Hurst *et al.,* 2004; Li *et al.,* 2004; Li *et al.,* 2008). Among them, the turbidity robustness index (TRI) (Hartshorn *et al.,* 2014) is the most recent and sophisticated attempt to do this. This index was implemented in the water treatment industry to assess the performance of filters in terms of effluent turbidity. It is a simple model that requires basic information on effluent quality and EQS and can be applied to many data points over a long period of time. The index is weighted in such a way that it takes less variance into account the more often the consent is achieved and more significance is placed on exceeding the consent than achieving the consent. The TRI can be used to assess the robustness of treatment performance in relation to EQS and because the TRI is a dimensionless scoring system it can therefore be used within a wide range of applications and to compare a variety of sites. These attributes make this index an ideal tool to assess performance robustness, which can be used to support asset management decisions.

In the present study, the ability to review and analyse 10 years of effluent quality data related to 255 small RBC-containing WWTs in the UK presents a unique opportunity to develop a tool adapted from the TRI that is capable of quantifying WWT performance robustness. This tool has been termed the robustness performance index (RPI). Subsequently, by utilising the RPI data, the influence of WWT design parameters on robustness can be investigated. A high level of robustness means that site managers can have confidence in the level of WWT performance and this can enable resources to be freed up and directed elsewhere. Hence in this study, we investigated how performance

robustness changed as a result of altered consent level and how far consents associated with a specific WWT system can be tightened before robustness is significantly affected. In other words, WWTs that may not robustly achieve future consents can be identified and investment can be efficiently targeted towards these WWTs before the consent shift occurs. In the same way, the relative performance of WWTs can be revealed at current consent levels, thereby highlighting the least robust WWTs and enabling investment to be targeted at the ones that most need additional resource and even diverted from the most robust WWTs, allowing companies to get the most out of their assets. Further to this, we identified integral attributes such as disk size and other operational parameters that influence robustness profile, thereby identifying factors that enhance or weaken robustness performance. Identifying factors which enhance robustness may enable asset design to be improved and tighter EQS to be achieved.

3.2 Methodology

3.2.1 RBC Database Mining and Selection of Attributes

Severn Trent Water (STW) operates 325 RBC containing WWTs, designed to treat a range from 3,100 PE down to only 5 PE. Ammoniacal nitrogen (NH_4^+-N) effluent concentrations data were available for 255 RBC-WWTs on the internal STW database (QuisLite). The data recorded in the QuisLite database were determined from single effluent spot samples using the standard methods for the examination of wastewater (APHA, 2005). The frequency and total amount of samples taken varied from site to site, with monthly sample collection being common. From the 255 WWTs, 127 WWTs were chosen for analysis based on the following criteria: (i) ammonium effluent consents enforced, (ii) at least 90 data points are available and (iii) WWT configuration had not been altered in the period 2004 – 2014 (no additional WWT assets were introduced and original assets were not upgraded). Key asset and site information including age, manufacturer (9 commercial RBC suppliers), treatment process type (integrated or segregated), total current population equivalent (PE), geographical location (11 types of location, according to the Environmental Agency), were also collated for each RBC WWT system to supplement and support the RPI analysis. By associating specific attributes of individual WWTs with RPI score, the relationship between certain attributes and WWT robustness, or a lack thereof, can be revealed. In the case of asset age, a sub-

group of 81 WWT were identified which had not been altered during the WWTs lifetime, the RPI score for these WWTs were then compared to assess the effect of age on robustness. Similarly, the effect of the other attributes mentioned above on robustness was investigated by comparing the 25 most robust and 25 least robust WWT using F-tests to first test the variance of the two groups, followed by one-way ANOVA analysis to assess any significant difference between the groups. This was a simple approach to confirm whether a large difference in RPI score and therefore robustness resulted from a standout attributes or indeed a range of attributes that these WWTs possessed. Further to this, additional comments from process advisors were recorded such as if WWTs had mechanical issues or *Beggiatoa* infections. Influent characteristic data were rarely accessible; hence loading rate for individual sites could not be determined. Population equivalent (PE) was instead used to indicate loading rate.

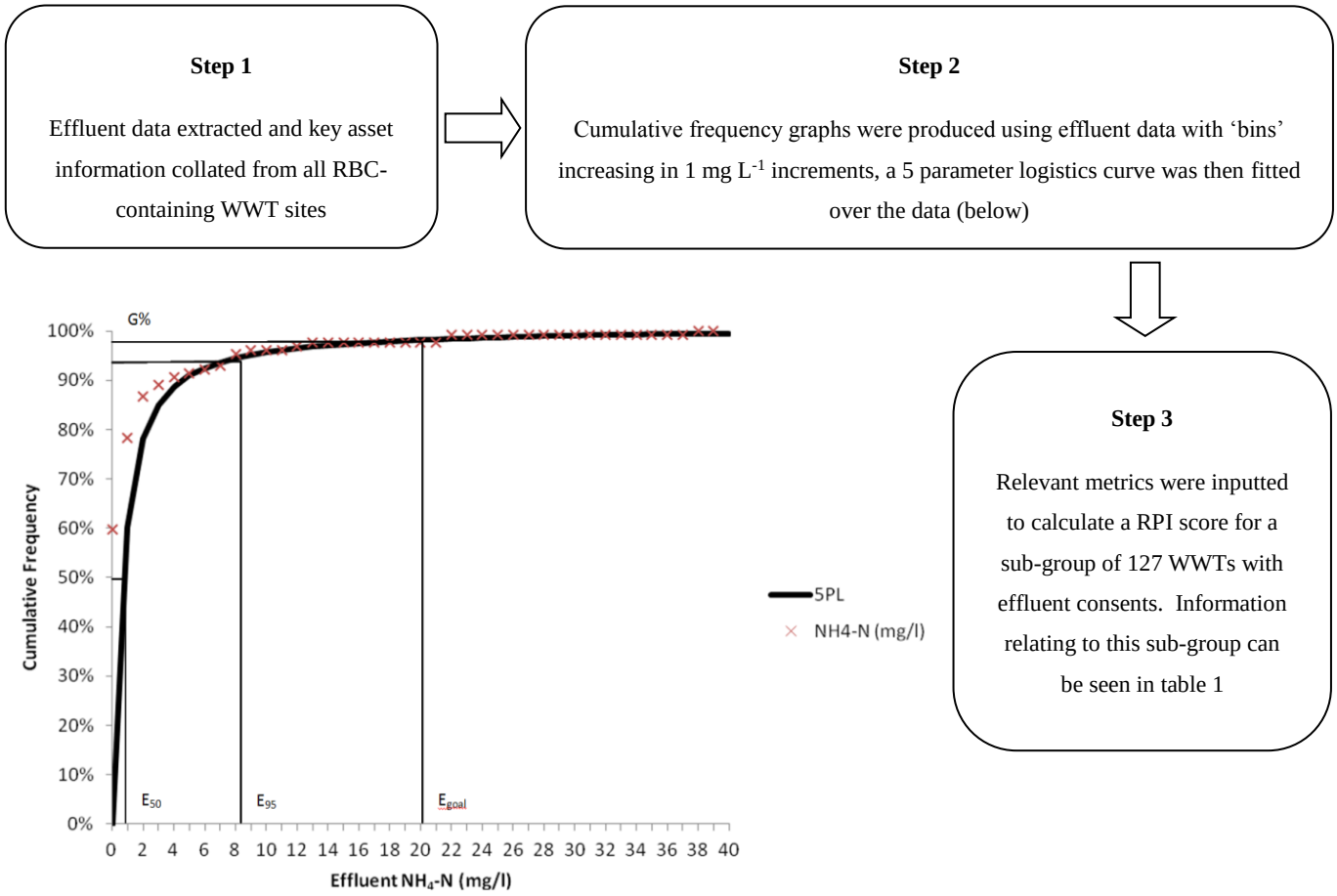

Figure 1. Flow chart summarising the approach used to calculate RPI score where the percentage of time below consent level or goal = G%, 95th percentile effluent ammonium concentration = E_{95}, percentile effluent ammonium concentration = $E_{50,}$ the effluent consent or goal level = E_{goal}.

An overview of the data mining approach is shown in Figure 1. For each WWT, NH_4^+-N concentration data were extracted and subdivided in 50 incremental concentrations groups (e.g. 0-0.99 mg L^{-1}, 1-1.99 mg L^{-1}, and so forth up to 50 mg L^{-1}). These concentration groups were chosen as increments of 1 mg/l allows for all enforced ammonium effluent EQS to be considered, furthermore it allows for good resolution in terms of data.

3.2.2 Cumulative Frequency of Effluent NH_4^+-N Samples

The cumulative frequency of effluent NH_4^+-N samples falling into each concentration group was then calculated using a 5 parameter logistics (5PL) regression approach (Equation 1; Hartshorn *et al.*, 2014) using MasterPlex Readerfit 2010 (Miraibio, CA,

USA). The cumulative frequency in this context represented the percentage of time that the WWT achieved at or below a certain effluent NH_4^+-N concentration.

$$y = a + \frac{d}{\left(1+\left(\frac{x}{c}\right)^{b}\right)^{e}}$$

Equation 1

where a is the minimum asymptote, b is the gradient of the steep part of the curve, c is the value at the inflection point, d is the maximum asymptote and e is an asymmetry factor. This was repeated for all the 127 RBC WWT systems.

3.2.3 Robustness Performance Index (RPI) Determination

From the cumulative frequency regression curves, the following key indicators were determined: (1) the percentage of time below consent level or goal (G%), (2) 95th percentile effluent ammonium concentration (E_{95}), (3) percentile effluent ammonium concentration (E_{50}) (4) the effluent consent or goal level (E_{goal}) (Figure 1). These parameters were then inputed in Equation 2 to calculate the RPI

$$RPI = \left[\left(1-\frac{G\%}{100}\right)*\frac{E_{95}}{E_{50}}\right]+\left[\frac{E_{50}}{E_{goal}}*\frac{G\%}{100}\right]$$

Equation 2

A low RPI score corresponds to a high robustness in terms of nitrification performance, the closer the score to 0, the more robust the WWT is.

Golden measures is a simple tool which helps managers assess the performance of their WWTs by assigning the effluent concentration data as either ‘green, ‘amber’ or ‘red’ and subsequently justify where they direct resources and investment.

The RPI tool was then compared, by using golden measures as a benchmark. The 5 most robust, the 5 least robust and 5 median WWTs were chosen, for the benchmark comparison. For calculating golden measures, the number of times each data point fell within the green, amber and red zone was determined, likewise the total percentage of data points within each zone was calculated. The green zone is defined as < 25 % of the effluent consent, amber zone as ≥ 25 % of the consent and red zone as ≥ 50 % of the consent. The red golden measures count (%) was then compared to the RPI score.

3.2.4 Cluster Analysis and Benchmarking against the Golden Measures

The RPI for each WWT was then analysed using a two-step cluster analysis to identify 'at risk,' 'watch out,' and 'safe' zones, similar to the 3 groups of the golden measures. Cluster analysis of the RPI score and PE per RBC variables was performed using version 22 of the statistical software package SPSS (Portsmouth, UK), each WWT was assigned a cluster group according to the analysis. Then the 5 most robust, the 5 least robust and 5 median WWTs were selected for comparison to the golden measures. Golden measures is a simple tool which helps managers to assess WWTs by identifying their performance is either green, amber or red. If the performance falls within the green category no further action is taken; amber performance must be monitored and immediate corrective action must be taken in the event of red performance. The performance categories are determined by the number of times effluent NH_4^+-N concentration data fall into the green, amber and red zone. In this paper, the golden measure tool was benchmarked against the RPI tool by comparing the red, amber and green component of golden measures (in terms of % of total data points) against RPI score.

3.2.5 RPI Tool Interface Development

In order to quantify RPI scores an excel calculator tool was developed. The RPI tool was developed in the well-known Microsoft Excel 2010 software in order for it to be easy to use and accessible to the widest range of audience. Users can input basic information about a site in addition with routinely recorded data, then follow the simple instructions to calculate RPI scores for a given WWT. This can be viewed in Appendix I

3.3 Results

3.3.1 RPI Score vs Golden Measures

Effluent NH_4^+-N concentration data between 2004 and 2014 were extracted for the 127 RBC-WWTs selected, ranging from 90 data points per WWT to 300 data points. The breakdown of the 127 WWTs serving various population equivalent groups is shown in Figure 2.

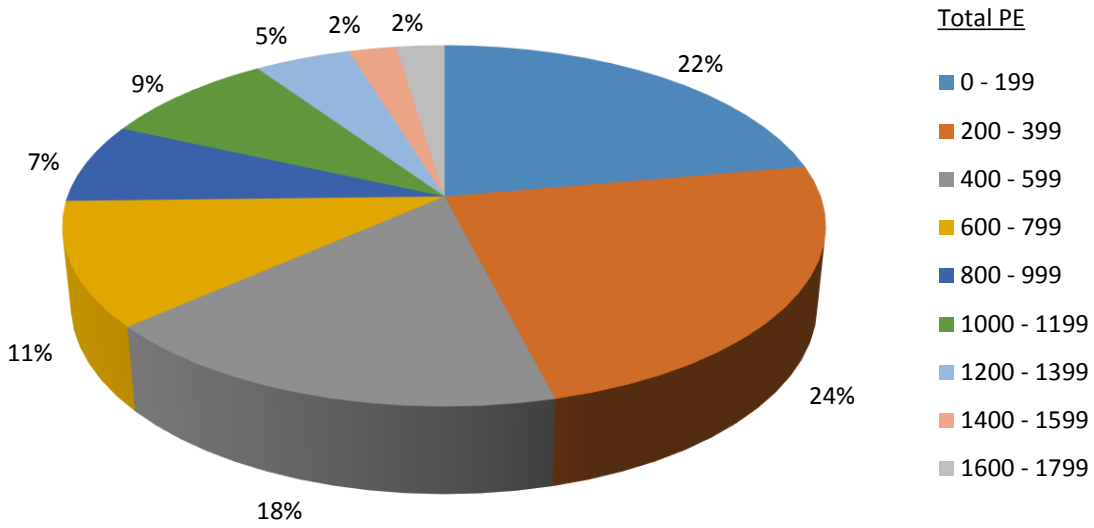

Figure 2. A breakdown of WWTs (%) serving population equivalent groups in increments of 199.

The RBC-WWTs were then grouped according to the NH_4^+-N effluent consents enforced at the site (Table 1). Population equivalent (PE) per RBC was used in this case to represent site loading as access to influent data was limited and total current population equivalent was readily available for each site. In this analysis it was assumed that WWT PE is correlated with loading, as 1 PE is defined as the equivalent of a five-day biological oxygen demand (BOD_5) of 60 g per day (as per WFD 91/271/EEC).

Table 1. Breakdown of WWTs based on PE metrics for the 4 consent groups

WWT Consent Group ($mg\ L^{-1}$)	Total WWT Count	PE Range	PE Average
5	47	20 – 1721	502
10	38	57 – 1615	662
15	32	47 – 1511	581
20	10	64 - 1210	207

The two step cluster analysis revealed a total of 3 cluster groups, with the red zone defined as > 0.5 and termed 'at risk', amber as $0.25 < x > 0.5$ and termed 'watch out' and green as < 0.25 and termed 'safe.' Following cluster analysis 12 WWTs were defined as green (mean RPI = 0.68), 12 were defined as amber (mean RPI = 0.35) and 97 were defined as red (mean RPI = 0.08) sites, respectively (Figure 3). It is clear that the 5 mg L^{-1} consent group is associated with higher RPI scores and therefore lower robustness, as a result they dominate the 'watch out' and 'risk' group. (Figure 4). Further exploration of this can be seen later in the paper (Figure 4). The data shows that increased loading per RBC does not significantly alter RPI score and therefore robustness, as there was no clear trend.

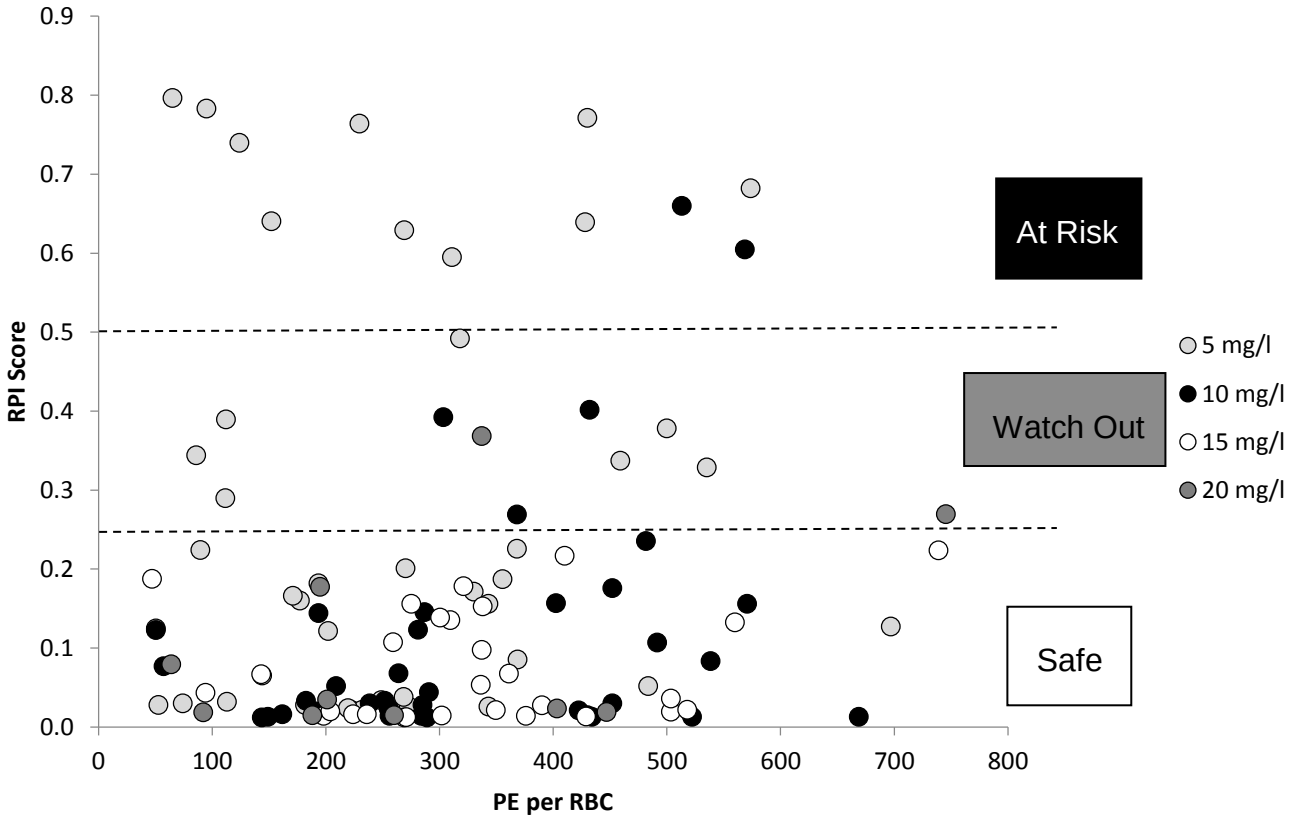

Figure 3. RPI scores (95%ile) for individual WWT system with a current NH_4^+-N effluent consents of 5, 10, 15 and 20 mg L^{-1}, as a function of population equivalent per RBC. (n=121).

Table 2. A breakdown of 15 WWTs showing their respective RPI scores, 'Golden Measures' count and incidences of effluent 'zone' occurrences.

			GOLDEN MEASURES COUNT (%)		
WWT	Effluent consent ($mg\ L^{-1}$)	RPI score	Red	Amber	Green
1.	10	0.01	3	1	96
2.	10	0.01	2	1	97
3.	15	0.01	1	3	96
4.	15	0.01	1	2	97
5.	10	0.01	0	0	100
6.	20	0.08	1	0	99
7.	10	0.08	1	2	97
8.	15	0.1	1	4	95
9.	10	0.11	1	4	95
10.	15	0.11	2	4	94
11.	5	0.74	22	21	57
12.	5	0.76	15	3	82
13.	5	0.77	20	14	66
14.	5	0.78	21	8	71
15.	5	0.80	20	7	73

Table 2 illustrates how RPI score, golden measures score and effluent quality performance differs between different robustness zones (WWTs 1 – 5 = green zone, WWTs 6 – 10 = amber zone and WWTs 11 – 15 = the red zone, in terms of RPI score). Golden measures scores appeared similar for the WWTs belonging to the 'safe' and 'watch out' group. Unusually, in the case of WWT 1 and 2 the red zone golden measures score was higher compared to the majority of 'watch out' WWTs (Table 2), the RPI scores differed significantly. RPI scores were 8 to 11 times higher in the 'watch out' group compared to 'safe' group, this reflects the fact that members of the 'watch out' group had higher ammonium effluent concentration compared to their consents (data not shown) and therefore the robustness of the treatment performance was significantly impacted. Although no strong correlation was found between RPI score and the amber golden

measures count, the correlation between RPI score and red/green golden measure counts for the 15 WWTs selected for Table 2 was strong (R^2 = 0.92 and 0.86 respectively). This shows that RPI may be able to complement the golden measures tool effectively.

With the strong relationship between RPI score and golden measures in mind, a similar green, amber, red 'traffic light' system in the golden measures tool was devised for the RPI tool. The three zones were termed 'safe,' 'watch out,' and 'at risk' respectively for the RPI scores (Figure 3).

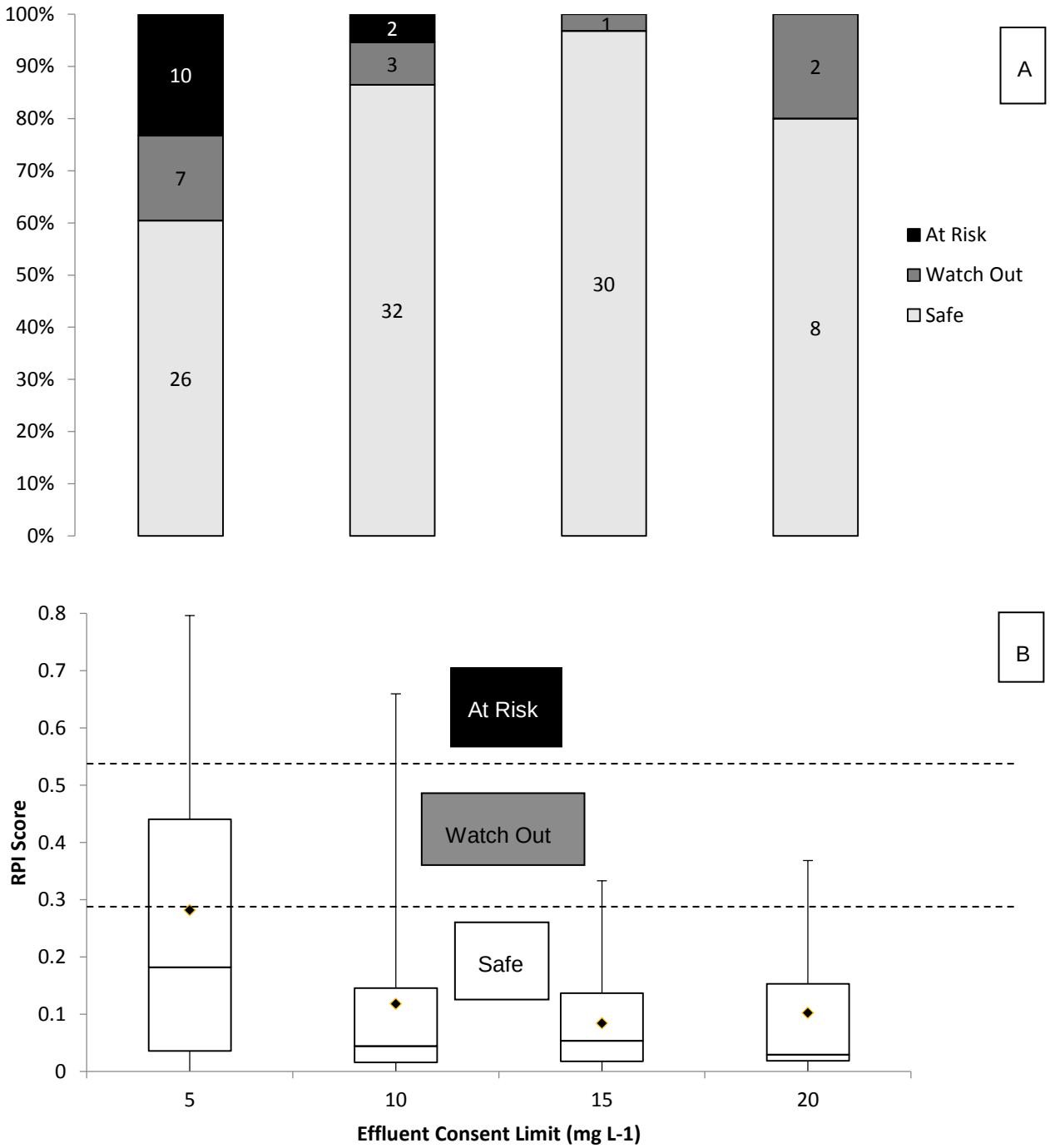

Figure 4. **(A)** Proportion and absolute number of all WWTs identified in 'at risk (RPI > 0.5),' 'watch out' (RPI > 0.25), and 'safe' (RPI < 0.25) zones (n = 121). Light grey - safe zone; dark grey – watch out; black – at risk. **(B)** RPI scores for WWTs in each NH_4-N effluent consent group (5 mg L^{-1} [n = 43]; 10 mg L^{-1} [n = 37]; 15 mg L^{-1} [n = 31]; 20 mg L^{-1} [n = 10]. The diamond represents the mean RPI score for each group. The top whisker indicates the upper quartile whilst the bottom whisker indicates the lower quartile and the box represents the second and third quartile.

Figure 4(A) shows the proportion (%) of WWTs belonging to the 4 consent groups that fall within each RPI zone. It is clear that the 5 mg L^{-1} consent group is associated with lower robustness, as a result they dominate the 'at risk' zone by both total number of

WWTs and proportion of WWTs. In fact, there are 5 times as many 'at risk' WWTs with a 5 mg L^{-1} consent, compared to the 10 mg L^{-1} which is the only other group that contains 'at risk' WWTs. All consent groups contain WWTs that fall within the 'watch out' zone, with the total number and proportion of 'watch out' WWTs decreasing as we move up the consent groups from 5 mg L^{-1}, however a small increase in 'watch out' WWTs within the 20 mg L^{-1} consent group can be observed, it should be noted that the sample size of this group is significantly smaller (n = 10) compared to the other groups (n = >30). There is a marked difference in 'safe' WWTs between the consent groups, with 60 % of WWTs with 5 mg L^{-1} consents being identified as safe and the associated proportions for 10, 15 and 20 mg L^{-1} being 86 %, 97 % and 80 % respectively. Therefore 90 % of all WWTs with a consent group of 10, 15 or 20 mg L^{-1} fall into the 'safe' zone. Assigning a consent of 15 or 20 mg L^{-1} to RBC WWTs assessed in this paper appears to be associated with little risk as all WWTs were either classified as 'safe', or 'watch out.' According the Figure 4(B) the mean RPI score for the 5 mg L^{-1} consent group was 0.27, compared to 0.12, 0.08 and 0.1 for the consent groups 10, 15 and 20 mg L^{-1} respectively. Not only does the 5 mg L^{-1} group exhibit a higher average score but also a larger range of 0.02 – 0.8, compared to 0.01 – 0.66, 0.01 – 0.33 and 0.02 – 0.37 for the consent groups 10, 15 and 20 mg L^{-1} respectively. According to these results the RBC sites within the 5 mg L^{-1} consent group are significantly less robust than RBC sites within the higher consent groups (t-test; $p < 0.05$) indicating that RBCs are more appropriate for effluent consent limits of 10 mg L^{-1} and higher.

3.3.2 Significant WWT Attributes

The 25 most robust sites and the 25 least robust sites were highlighted in order to confirm whether certain attributes contributed to their robustness. The data group consisting of 50 WWTs with a RPI score ranging from 0.01 to 0.8 (most robust and least robust respectively) and a mean score of 0.27, the mean and range of RPI differs considerably between the two groups (Table 3). After performing an F-test to assess variances among the 2 data sets, t-tests confirmed that there was no significant difference between the least and most robust sites in terms of total PE, PE loading, settlement tank volume, RBC age ($p < 0.05$).

Table 3. The range and average RPI and PE relating to the 25 most and least robust WWTs.

	Mean RPI	RPI Range	Mean PE	PE Range
Most Robust WWTs	0.01	0.01 – 0.02	492	92 - 1511
Least Robust WWTs	0.52	0.27 – 0.8	708	65 - 1721

An important problem for RBCs is infestation by a filamentous bacterium, *Beggiatoa*. *Beggiatoa* is a genus of sulphur-oxidising filamentous bacteria that colonises wastewater biofilms, particularly in RBCs. A *Beggiatoa* infestation can be seen as it will turn the biofilm a white/grey colour, their predominance can reduce the treatment performance of RBCs as they compete with heterotrophs and nitrifiers for space and oxygen. After making a comparison of all WWTs analysed against WWTs reported to be infested with *Beggiatoa*, results show that *Beggiatoa* presence does not significantly affect RPI score and therefore robustness (t-test, $p < 0.05$).

Figure 5 illustrates the effect of asset age on robustness. There is no relationship between asset age and RPI score. Unfortunately, there is limited or no data available for some asset ages, in particular newer RBCs with an age of less than 10 years. RPI score and robustness in terms of nitrification performance does not suffer after 20 years of operation in the WWT analysed, this indicates that the lifespan of an RBC may often exceed 20 years which is often seen as standard in industry.

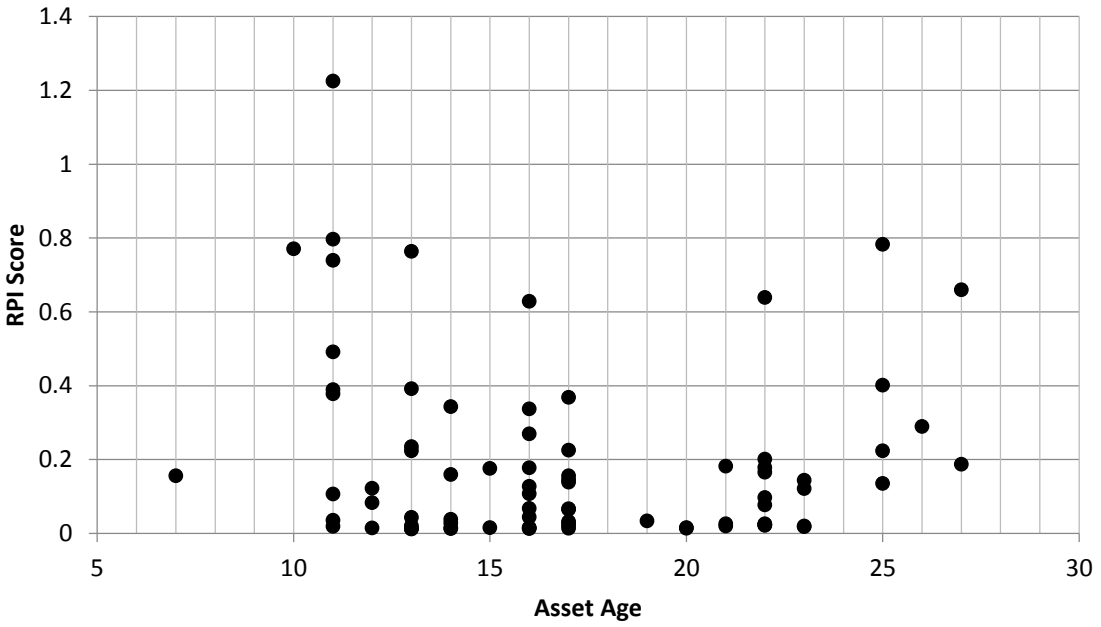

Figure 5. The RPI score of WWT systems containing RBC assets which have neither been upgraded nor repaired during their life span (n = 81).

3.3.3 Effect of Altering Theoretical Consent on RPI Score

Figure 6 illustrates how RPI score changes at different theoretical consent levels. RPI scores were calculated based on theoretical consents increasing or decreasing in increments of 1 mg L^{-1} from the current existing consent level, for all WWTs. As expected enforcing a theoretical tighter consent in the majority of WWTs leads to an increase in RPI score and hence decreased robustness. In fact, reducing the consent by 10 mg L^{-1} results in the average RPI score increasing by 215 %, similarly when the consent is reduced by 5 mg L^{-1} the average RPI score increases by 49 %.

However, many WWTs with NH_4^+-N effluent consents of 15 and 20 mg L^{-1} could cope with a significant consent reduction without losing significant robustness. Within each group, more than 50 % of WWTs with a current NH_4^+-N consent of 10, 15 or 20 mg L^{-1} will perform robustly (in the 'safe' zone) in the event of a NH_4^+-N consent reduction of 5 mg L^{-1}. This indicates that many RBC sites with the higher range of effluent consents are designed with excess process capacity and that STW could reduce the consent at many rural RBC WWTs with low risk to process compliance.

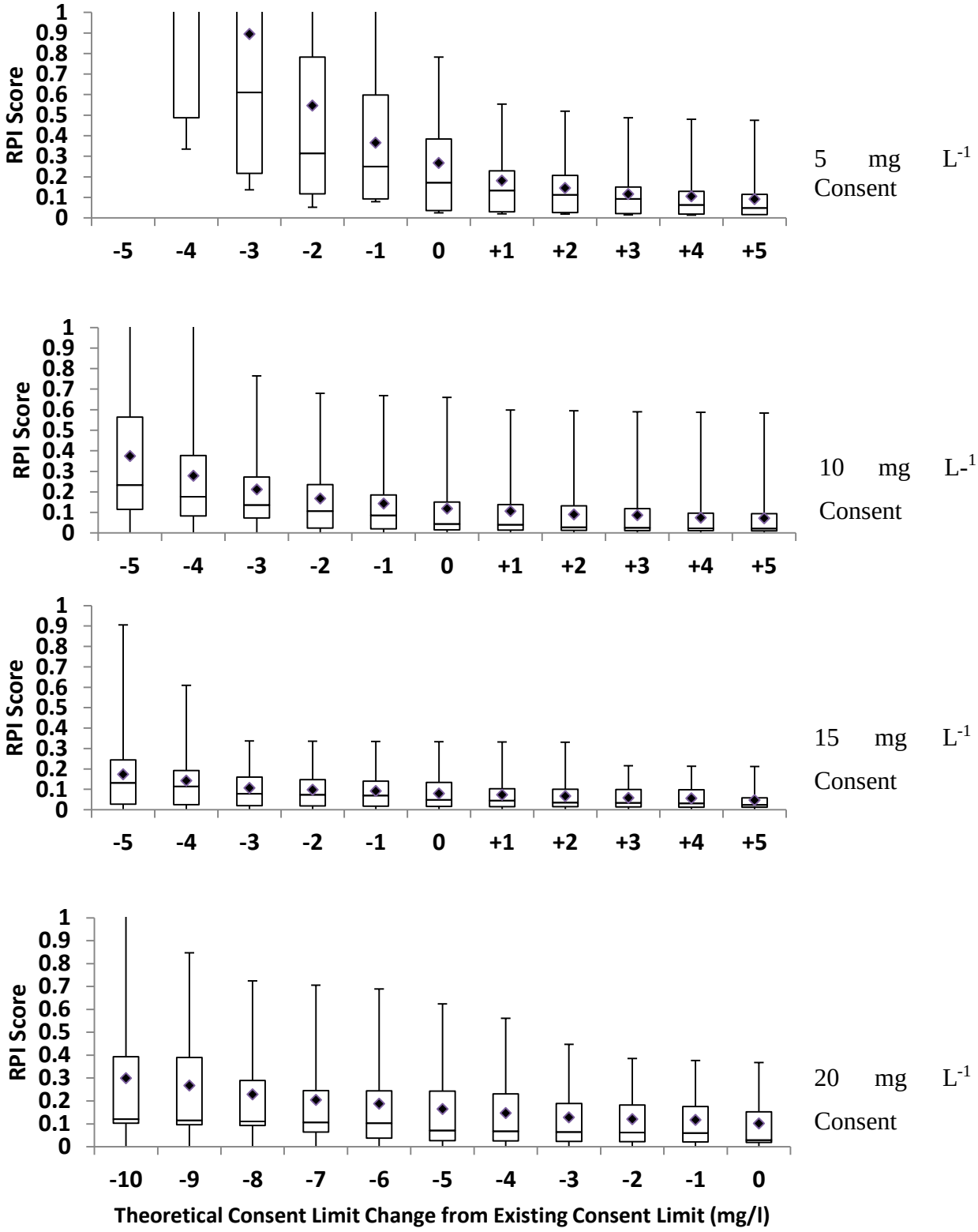

Figure 6: RPI scores for WWTs as a result of consents theoretically shifting from their current consents (marked as 0) in increments of 1 mg/l. Current WWT NH_4-N effluent consent groups of: 5 mg/l (n = 43); 10 mg/l (n = 37); 15 mg/l (n = 31); 20 mg/l (n = 10). Where the two interconnected boxes represent the 2nd and 3rd quartile, the positive and negative error bars represent 4th and 1st quartile, whilst the diamond represents the mean average.

3.4 Discussion

This study presents a promising assessment tool that has been trialled using operational industry data, it can easily and rapidly quantify WWT robustness from effluent and asset data and therefore has great practical application. By assigning a numerical robustness score to the WWT it becomes far simpler to determine which assets to prioritise with respect to investment, both at current consent levels and pre-emptively in the case of tightening future consent limits. This understanding will supplement asset investment strategy and enable asset managers to make informed decisions on where to concentrate resources in order to maximise efficiency, thereby contributing to the development of a more effective wastewater treatment system. Implementing policies to ensure every WWT conforms to a minimum robustness standard may help companies to mitigate risk and accommodate a certain level of population growth. Although this study could not identify inherent attributes associated with robustness, or lack of robustness, building upon this study may do so in the future and this will enable process design engineers to optimise design manuals and improve performance of future assets. For example, the data suggested that as PE loading onto each RBC increases, there is no trend towards robustness increasing, this indicates that RBCs maintain their robustness at various PE levels and therefore RBC design should not be restricted to a particular range within the range of PEs studied in this paper. This sort of information is valuable to WWT design engineers. Asset age did not influence RPI score which may be new information to engineers and can inform on their future design. This assessment was made on WWTs that had not upgraded or repaired the resident RBCs, so it can be further postulated that as long as the mechanical components are in working order the biological component (the biofilm) will continue to treat in a consistent manner. It was revealed that many RBC WWTs could reduce their associated effluent consent without any lack of robustness, illustrating that valuable excess process capacity exists in many sites, furthermore robustness suffers dramatically when consents are reduced to 5 mg L^{-1}. This finding was expected as treating wastewater to a standard of 5 mg L^{-1} is much more difficult compared to higher consent levels and with a low consent of 5 mg L^{-1}, there is less room for error.

Currently in the form of a Microsoft Excel spreadsheet where only effluent data and current consent or consent target needs to be entered, the tool can be used by site managers or operatives with little scientific background. The vision for this in the near future is to

convert the Excel system to an application that can be used on a phone or laptop that will rapidly calculate a robustness score from effluent data and further transform that score into a simple message that will directly illustrate the status of a WWT, in terms of performance robustness. This will ensure both capital and operating expenditure are directed more effectively, consequently making the water industry more efficient. The potential issues with this tool are that it requires accurate data related to the site and numerous effluent quality data points which are often not available, furthermore the tool is testing the robustness of a WWT system not the assets themselves, but because each RBC site follows the same basic structure and the design of RBC and reed beds are consistent we were able to make inferences about the RBC assets in this study.

3.5 Conclusion

The proposed approach using RPI can be a useful aid in asset management by providing a more efficient treatment system and allow investment to be allocated more confidently. Additionally, by quantifying the WWTs robustness and by establishing key attributes of a WWT it is possible to understand how the key operational and environmental parameters influence treatment performance. A rational and transparent asset management strategy can then be formulated to address treatment performance by altering key parameters.

1) The Robustness Performance Index can be used in conjunction with other tools to calculate how robust a wastewater treatment works performance is, in particular it can highlight sites lacking in robustness

2) RBCs are better employed at works where the ammonia effluent consent is 10 mg L^{-1} and above

3) Approximately 50% of the works at Severn Trent Water which must comply to 10, 15 or 20 mg L^{-1} ammonia consents could perform just as robustly upon a consent reduction of 5 mg L^{-1}

Acknowledgements:

This work was co-funded by the Natural Environment Research Council (NERC) (Grant reference NE/I019367/1) and Severn Trent Water (STW). Special thanks to STW for giving me access to their internal databases which allowed this study to happen.

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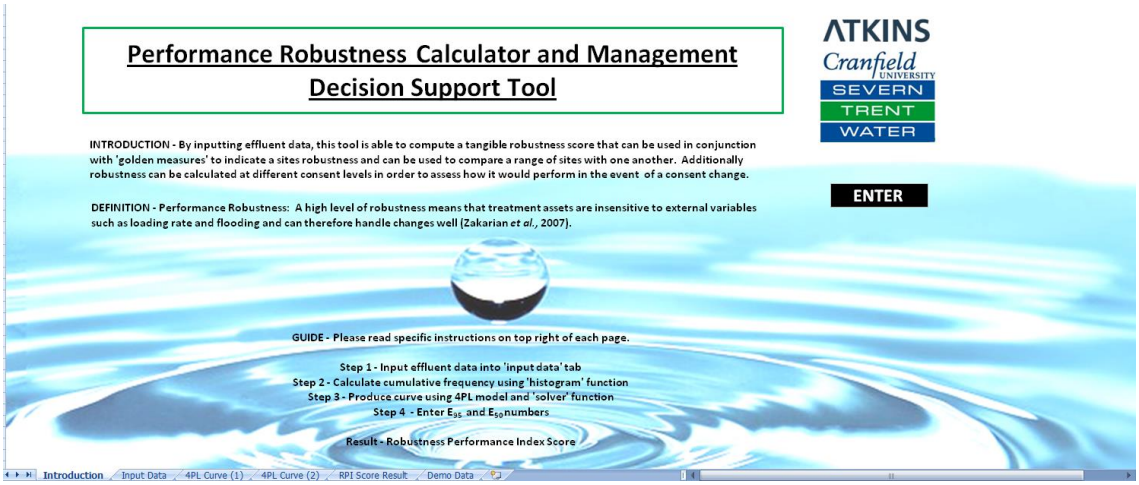
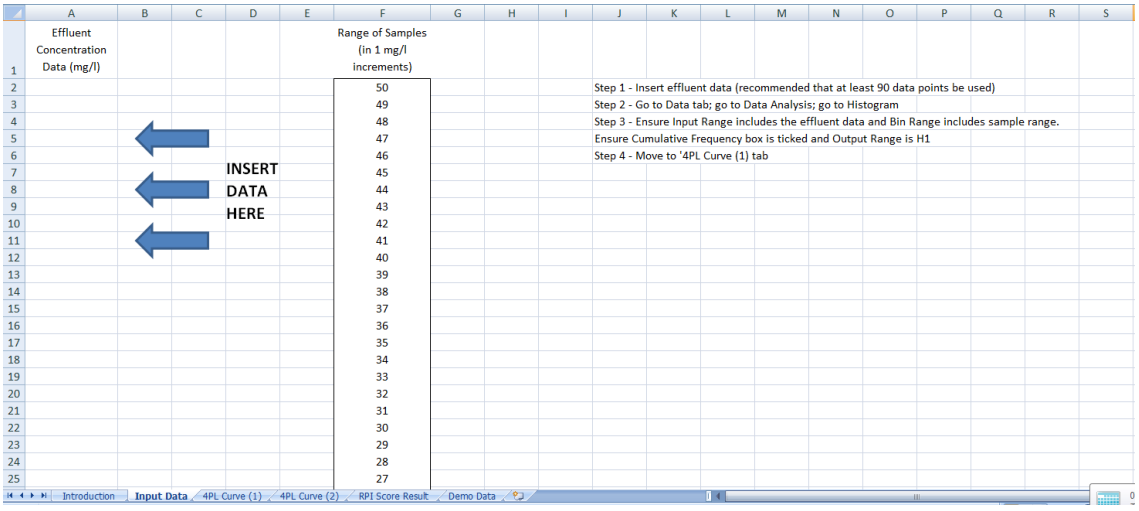
3.7 Appendix I

Images below illustrate the process used to calculate RPI score using the RPI calculator tool





Bin	CF	4PL	Squared Difference			Constants																
0	0.00	0.00	0.00																			
1	0.00	100.00	10000.00			1	A	Minimum asymptote														
2	0.00	100.00	10000.00			1	B	Gradient in middle of curve														
3	0.00	100.00	10000.00			1	C	Point of inflection														
4	0.00	100.00	10000.00			1	D	Maximum asymptote														
5	0.00	100.00	10000.00																			
6	0.00	100.00	10000.00																			
7	0.00	100.00	10000.00			500000		Sum of Squares														
8	0.00	100.00	10000.00																			
9	0.00	100.00	10000.00																			
10	0.00	100.00	10000.00																			
11	0.00	100.00	10000.00																			
12	0.00	100.00	10000.00																			
13	0.00	100.00	10000.00																			
14	0.00	100.00	10000.00																			
15	0.00	100.00	10000.00																			
16	0.00	100.00	10000.00																			
17	0.00	100.00	10000.00																			
18	0.00	100.00	10000.00																			
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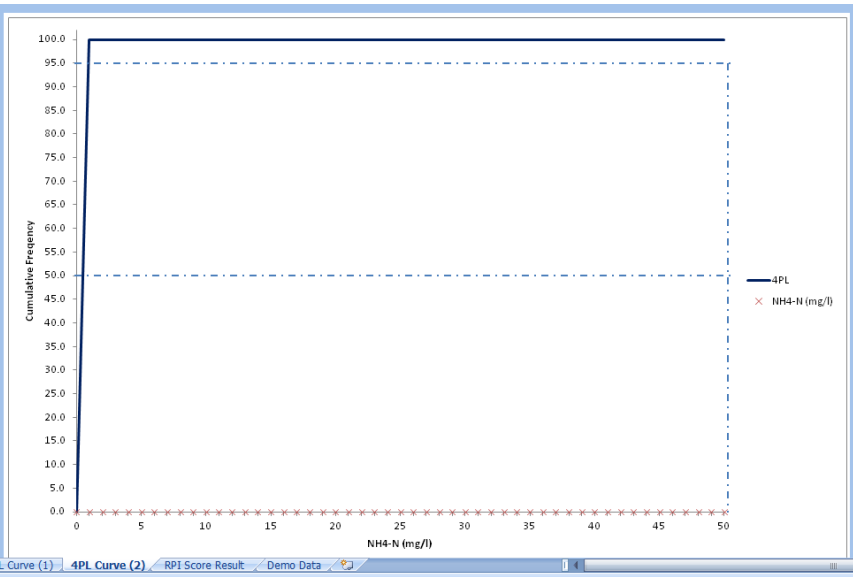
25	0.00	100.00	10000.00																			
26	0.00	100.00	10000.00																			
27	0.00	100.00	10000.00																			
28	0.00	100.00	10000.00																			
29	0.00	100.00	10000.00																			

Step 1 - Go to Data tab; go to Solver function
Step 2 - Set Solver function to equal min for target cell G9 (Sum of Squares) by changing cells G3:G6 (Constants A-D)
Step 3 - Press Solve and OK
Step 4 - Move to 'RPI Score' tab

Introduction | Input Data | 4PL Curve (1) | 4PL Curve (2) | RPI Score Result | Demo Data



Introduction | Input Data | 4PL Curve (1) | 4PL Curve (2) | RPI Score Result | Demo Data

$$RPI_{95} = \left[\left(1 - \frac{G\%}{100}\right) * \frac{E_{95}}{E_{50}}\right] + \left[\frac{E_{50}}{E_{goal}} * \frac{G\%}{100}\right]$$

Step 1 - Look at graph in '4PL Curve (2)' tab and ascertain 50th & 95th percentile values
Step 2 - Enter E_{50} and E_{95} values in boxes highlighted
Step 3 - Identify RPI score at the current T_{goal} and assess which status zone it corresponds to. Take the appropriate action
Step 4 - Identify the lowest T_{goal} value which has a RPI score of less than 0.28. This is the lowest effluent consent at which the site could still perform robustly

T_{goal}	G%	E_{95}	E_{50}	RPI SCORE
1	100			#DIV/0!
2	100			#DIV/0!
3	100			#DIV/0!
4	100			#DIV/0!
5	100			#DIV/0!
6	100			#DIV/0!
7	100			#DIV/0!
8	100			#DIV/0!
9	100			#DIV/0!
10	100			#DIV/0!
11	100			#DIV/0!
12	100			#DIV/0!
13	100			#DIV/0!
14	100			#DIV/0!
15	100			#DIV/0!
16	100			#DIV/0!
17	100			#DIV/0!
18	100			#DIV/0!
19	100			#DIV/0!
20	100			#DIV/0!

RPI SCORE	STATUS	ACTION
0 - 0.25	SAFE	NO ACTION NEEDED
0.25 - 0.5	WATCH OUT	MONITOR
0.5+	AT RISK	MANAGE

E_{50} 50th Percentile - Effluent Concentration Data
E_{95} 95th Percentile - Effluent Concentration Data

Introduction | Input Data | 4PL Curve (1) | 4PL Curve (2) | RPI Score Result | Demo Data

CHAPTER 4

ROTATIONAL SPEED

4 EFFECT OF ROTATIONAL SPEED ON AMMONIUM REMOVAL AND ENERGY CONSUMPTION IN ROTATING BIOLOGICAL CONTACTORS

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Abstract

Rotating biological contactors (RBC) are widely employed as a biological secondary wastewater treatment process due to their relatively low-cost, low-energy requirement and maintenance. They are particularly suitable for small wastewater treatment works and provide a viable treatment option as a nitrification application. However, tightening effluent quality standards, in addition to growing population means that optimising the operating efficiency of the RBC treatment process will be required to meet future challenges. One possible route to increased operating efficiency is altering the rotational speed of the disks. Disk rotation is the main mechanism by which oxygen is made available to the microbial biomass, which is carried on the disks and is responsible for the reduction of pollutants. Thus in this study, the effect of altering rotational disk speed on two operational small wastewater treatment sites, each containing two parallel RBCs was investigated. Specifically, the effect of either increasing or decreasing rotational speed on both nitrification performance and energy consumption was assessed over 12 month operating period. Decreasing the speed from 1.0 rpm to 0.7 rpm did reduce energy consumption, but only by a negligible 0.08 % and therefore operating cost was not significantly affected, in fact this only equated to a 7.3 kWh per year saving. Further to this, nitrification performance in terms of effluent NH_4^+-N concentration was not affected by an increase in rotational speed of 1.1 rpm to 1.3 rpm, but the change did appear to reduce ammonium spikes and allow for higher total removal of NH_4^+-N load. An interesting finding was that increasing rotational speed of the shafts can enhance biomass

characteristics, an example being reducing *Beggiatoa* proliferation which is known to cause significant RBC treatment capacity reduction.

Keywords: Rotating biological contactor (RBC); rotational speed; nitrification; oxygen; full-scale

4.1 Introduction

When discharged into a water body, some pollutants found in wastewater can adversely affect the aquatic ecosystem. For example, dissolved organic carbon, nitrogen and phosphates can cause, among other things, eutrophication in receiving waters. The European Union Urban Waste Water Treatment Directive 91/271/EEC dictates that European Union member states must enforce their own effluent quality standards (EQS), otherwise known as effluent consents for these pollutants. While this directive currently applies only to wastewater treatment works (WWTs) with a capacity > 10,000 population equivalent (PE), EQS are however often enforced at small treatment works (< 2,000 PE). Another key piece of European Union legislation – the Water Framework Directive (WFD) 2000/60/EC, concerns achieving good river basin management. Of the 50,000 km of rivers in England and Wales around 90% met the objectives for WFD, which means that up to 2000 km of rivers may require tighter EQS (UKTAG, 2013).

Ammonia must be an important consideration in enforcing these EQS, as due to its toxic impacts on fish and macro-invertebrates it is potentially hazardous to the environment. As wastewater effluent from treatment works is a major source of ammonia in rivers, ammonia effluent standards are commonly enforced and it is likely existing consents will be tightened, due to increasing legislator pressure. It is therefore crucial that ammonia in wastewater can be treated effectively before effluent is discharged.

Biological systems are utilised to efficiently degrade ammonia, as well as complex organic compounds such as carbohydrates, proteins and fats into simple molecules such as sugars, amino acids and fatty acids. Specifically, microbial cells consume these compounds to feed internal metabolic reactions, often via preferential aerobic respiration pathways, this is why oxygen is often crucial for rapid and efficient degradation of wastewater pollutants (Downing & Nerenberg, 2008). This metabolism enables them to fuel processes essential to their survival including growth and reproduction. Whilst this concept is simple, controlling the biological treatment process is very complex, because of the large number of variables that can affect the microbes.

These variables include constantly changing physicochemical properties of the wastewater passing into the WWT, for example, the influent can show variations in flow rate and chemical composition as well as in pH, and temperature (Tchobanoglous *et al.*, 2013). Further to this, many municipal plants also have to cope with surge flows following storms and recalcitrant chemicals that in the worst case, can inhibit the functioning of the bacterial community and produce a toxic shock that kills the bacteria. When this happens the plant may pass untreated effluent directly to the environment, until new bacterial biomass is reintroduced and replenished (Tchobanoglous *et al.* 2013). Therefore, the removal capacity of the biological community in all WWTs is heavily dependent upon the provision of a conducive environment in which it can thrive; therefore, it is the authors' view that improvement of the treatment process requires optimising the environment in which it resides.

Microbes found in traditional suspended treatment systems such as activated sludge plants (AS) are significantly influenced by the described variables, however other technologies such as the rotating biological contactor (RBC) and trickling filter are based on a fixed film or biofilm which is more resistant to external variables. The variables have less effect on a fixed film treatment system, as in this case the complex and dynamic microbial community adhere to one another and to a solid substratum or media (Husham *et al.,* 2012) and are housed within an extracellular polymeric substance (EPS) matrix. This arrangement means individual cells are anchored and more protected compared to their suspended counterpart, thus there are many advantages to biofilm fixed systems when compared to suspended systems such as activated sludge (Tawfik *et al.,* 2006). Biofilms can withstand increased organic loads whilst exhibiting resilience to traumatic loadings (Najafpour *et al.,* 2006). Other benefits include lower sludge production, rapid recuperation from starvation events and higher substrate affinity (Batchelor *et al.,* 1997).

In comparison with conventional activated sludge systems, the cost of operating RBCs are approximately 50% lower (Johnson, 2011) and RBCs require about 20 to 30% less energy compared to a similar small works technology – the trickling filter system (Rodgers & Zhan, 2003). This energy efficiency in combination with less land being required contributes to total annual operating costs which are approximately 35% lower compared to trickling filters (Upton *et al.,* 1995). Furthermore, RBCs do not require

aeration via blowers. This is a significant benefit as aeration is responsible for more power consumption than any other activity in WWTs (Krause *et al.*,2003) and accounts for approximately 55% of the total wastewater treatment budget (Ainger *et al.,* 2009). Therefore, RBCs are often associated with reduced energy consumption.

These advantages generally make fixed film technologies such as RBCs attractive candidates for wastewater treatment at small WWTs. RBCs are an example of a common and effective fixed biofilm treatment technology for small works. In a RBC a number of disks are mounted on a horizontal shaft, the disks are partially submerged and rotated in a trough containing wastewater. The surface of the disks provides an effective location for a biofilm to establish. Rotation of these disks creates a head difference resulting in convective water/air exchange, rotation exposes the biofilm to air and turbulence promotes oxygen transfer to the bulk liquid (Hassard *et al.,* 2014). It was previously suggested that transfer of oxygen into the biofilm occurred by two potential pathways: (1) a direct mechanism from air to microbes when the disk is exposed to air (Kim & Molof, 1982); (2) oxygen consumption by the microbes within the biofilm induces diffusion of oxygen into the liquid film layer surrounding the biofilm (Zeevalkink *et al.,* 1979).

When influents contain large amount of nitrogenous matter, it will need to be converted into more benign compounds by nitrifying bacteria established within the biofilm, in a process called nitrification. Nitrifying bacteria are autotrophs, requiring only inorganic chemicals as the starting point for their energy metabolism and growth. Thus ammonia is oxidised to provide the energy required for growth. With normal domestic wastewater loading the contribution of nitrifiers to the total bacterial biomass is small, as they are easily outcompeted by other faster-growing heterotrophic bacteria (Okabe *et al.,* 1996). This is why organic matter must be eliminated upstream of the ammonia nitrogen removal stage. However, in the presence of carbon limited load the nitrifying population proportion, composing of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) increases to approximately 50% (Kindaichi *et al.,* 2004). The AOB group oxidises ammonia to form nitrite, the most abundant AOB genus in RBC biofilms is *Nitrosomonas* (Egli *et al.,* 2003), but there are also others present. The NOB group oxidises nitrite to nitrate and are dominated by *Nitrospira* (Harms *et al.,* 2003). Although,

it was revealed that AOB do not necessarily require oxygen (Strous *et al.,* 1999), in aerobic fixed film reactors such as RBCs, for the optimal removal of ammonia nitrifying bacteria require oxygenated conditions. In fact, for the process to occur effectively the dissolved oxygen (DO) concentration must be ≥ 2 mg/L (Nowak, 2000).

It is now well known that for oxygen to reach the nitrifying bacteria it must first pass from the bulk liquid or air phase into the boundary layer of the biofilm and then into the biofilm layer and finally it must diffuse into the biofilm layer where the microbial consortia are housed (Hassard *et al.,* 2014). The diffusion through the various layers of the biofilm and the consumption of oxygen within the biofilm lead to enhanced concentration gradients and ensure that oxygen transfer rate (OTR) is high within RBC biofilms (Kim & Molof, 1982).

Increasing rotational speed of the disks is expected to lead to an increase in dissolved oxygen available to the biofilm (situated on the disks) as there is a positive pseudo-linear relationship between tip speed of the disk and OTR at laboratory scale (see Table 1; Rittmann *et al.,* 1983; Di Palma & Verdone, 2009). Thus increasing rotational speed can lead to more dissolved oxygen in the bulk liquid phase which is available to the biofilm and increase the frequency at which the biofilm is exposed to oxygen in the air, resulting in enhanced rate of nutrient removal, with particular improvement in nitrification (Israni *et al.,* 2002). In contrast to the view of rotational speed being positively correlated with oxygen availability via the mechanism of increased DO, it was recently shown that the vast majority of oxygen uptake (up to 85%) by nitrifying bacteria occurs in the air-phase of the RBC process (Courtens *et al.,* 2014). Thus the time spent by the biofilm being exposed to air is of greater importance than the dissolved oxygen content of the bulk liquid.

Although the biofilm undertakes the majority of treatment, it is thought that suspended biomass within the bulk liquid significantly contributes to nutrient removal, accounting for 4 - 10 % of the overall treatment (Edeline, 1993). Thus increasing the rotational speed of the disk can be a way to provide increased oxygen to both suspended microbes and fixed biofilm. An additional effect besides increasing oxygen availability can be improving mass transfer of nutrients to the biofilm by increasing turbulence and mixing of the bulk liquid (Chavan *et al,* 2008), which increases ammonium flux and biofilm

density (Kugaprasatham *et al.,* 1992). Finally, increasing the rotational speed, leads to shear forces at the surface of the biofilm and consequently erosion may occur resulting in reduced thickness (Mohle & Langemann, 2007), which poses less of a barrier to mass transfer which would encourage enhanced performance (Hassard *et al.*, 2014).

A potential disadvantage to increasing rotational speed is power consumption of the RBC units, it was shown that at pilot-scale this was proportional to the square of rotations per minute (Watanabe *et al.,* 1993). Therefore, decreasing the rotational speed may offer the advantage of lower operational cost because of decreased workload by the motor and hence reduced energy consumption (Ramsay *et al.,* 2006). This could however have the opposite effect on oxygen availability and nutrient mass transfer and lead to decreased treatment potential.

Severn Trent Water (STW), a large UK water company, uses RBCs as a biological treatment technology in approximately one third of all their WWTs, which equates to circa 320 sites. Alternating the rotational speed of RBCs may be an uncomplicated way to optimise the process and improve performance. Thus, in this study, the effect of altering rotational shafts speed on two small operational wastewater treatment sites (798 and 1005 PE), each containing two parallel RBCs (3.0 and 3.8 m disk diameter), was investigated over a 12-month operating period. Specifically, the effect of increasing RBC rotational speed on nitrification performance and the effect of decreasing RBC rotational speed on energy consumption were evaluated. To the best of the authors' knowledge this is the first comprehensive study conducted at a fully operational industry scale.

Table 1. Comparison of previous studies investigating the effect of rotational speed on RBC treatment performance

Conditions		Loading Rate		Design Parameters		Disk Information		Performance	Comments	Reference
Scale	**Type of Wastewater**	**Hydraulic Loading ($m^3/m^2/d$)**	**Organic Loading ($g/m^2/d$)**	**Media**	**Submergence (%)**	**Diameter (cm)**	**Rotational Speed (rpm)**	**Organic Removal (g/m^3)**		
Pilot-scale	Industrial wastewater from slaughterhouse	0.01-0.02	0.01-0.02 (sBOD)	Polystyrene disks	37	50	8-11	N/A	No significant effect on performance was observed	Torkian *et al.*, 2003
Lab-scale	Synthetic phenolic wastewater	5620.8	0.08 (phenol)	Stainless steel disks with cloth covering	50	9	40-150	40 rpm: 0.31 phenol removed 150 rpm: 1.14 phenol removed		Israni *et al.*, 2002
	Bakers yeast wastewater	N/A	210 (COD)	Propylene pall rings	40	2.5	15-17	15 rpm: 77% COD removed 17 rpm: 78% removed		Nahid *et al.*, 2001
	Food cannery wastewater	N/A	22.13 (sCOD)	Clear plastic disks	N/A	35	3-11	3 rpm: 62.7% sCOD removed		Najafpour *et al.*, 2006

4.2 Materials and Methods

4.2.1 WWT Details

The effect of increasing RBC rotational speed was investigated at WWT 1 and the effect of reducing RBC rotational speed was investigated at WWT 2. Both sites are located in the Midlands area within the catchment area of Severn Trent Water (STW). Each site contains two identical integral RBCs – RBC 1 and RBC 2 (Table 2), with the speed of RBC 1 remaining unchanged and RBC 2 being altered. ACS550 adjustable speed low voltage drives (ABB, Coventry, UK) were fitted at each site, the rotational speed was altered by modifying the frequency inverter. The ACS550 drives were also used to monitor the number of kilowatt hours (kWh) consumed by each RBC motor on a weekly basis.

In addition to WWT 1 and WWT 2, data from a further WWT (WWT 3) was considered in this study. The rotational speed of the single RBC at WWT 3 was increased from the standard of 1 rotation per minute (rpm) to 1.3 rpm in September 2011 during a previous trial for 48 months. WWT 1, 2 and 3 had no associated ammonia effluent consents, which was one of the key reasons for choosing these sites for the trials.

Data available on an internal database (QuisLite) within the STW server, was used to extract effluent quality data relating to WWT 1, WWT 2 and 3 over a period of 8 years. The database contained records relating to final effluent spot samples that were taken by site operatives and then analysed by analytical technicians. This data was used to support data collected during the full-scale trials.

Table 2. Key attributes of WWTs

	RBC 1 (rpm)	RBC 2 (rpm)	RBC Diameter (m)	Population Equivalent	Disk Area per PE (m^2)	BOD_5 Consent ($mg\ L^{-1}$)	SS Consent ($mg\ L^{-1}$)
WWT 1	1.1	From 1.0 to 1.3	3.0	798	9.54	25	35
WWT 2	1.0	From 1.0 to 0.7	3.8	1005	6.15	25	45
WWT 3	From 1.0 to 1.3	N/A	3.0	357	N/A	-	-

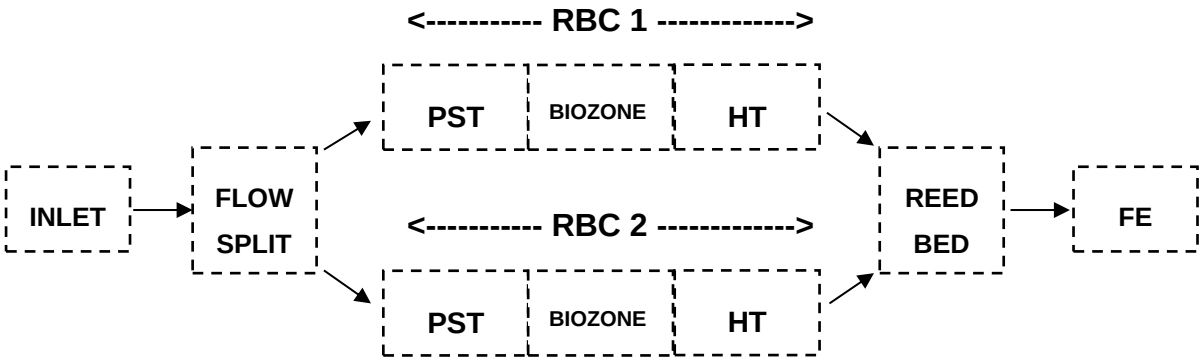

Figure 1. Site layout at both WWT 1 and WWT 2.

The following terms will be used to describe the individual RBCs:

At WWT 1, RBC 1 = **RBC 1 (1)**

RBC 2 = **RBC 2 (1)**

At WWT 2, RBC 1 = **RBC 1 (2)**

RBC 2 = **RBC 2 (2)**

At WWT 3, RBC 1 = **RBC 1 (3)**

4.2.2 Sampling and Analysis of WWT 1 & 2

Sampling was conducted from September 2012 to September 2013, mostly on a weekly basis at both WWTs. Duplicate spot samples of 500 ml were taken and combined to give a total sample volume of 1 L. Wastewater samples were taken at: the flow split just after the inlet, primary sedimentation tanks (PST), RBC biozone (between the first and second disk), humus tanks (HT) and from the effluent (E) between the HT and tertiary treatment (in this case horizontal flow reed beds, as seen in Figure 1). However, access to the PST in RBC 2 (1) was restricted and therefore sampling here was omitted. The biozone consisted of 3 disk packs at both WWTs. Composite samples of 500 ml were collected at the same points every hour using a Teledyne ISCO 3700 autosampler (Lincoln, NE, USA) over a 24-hour period and stored at 4°C until analysis.

Biofilm samples were taken weekly over a 2-month period between August and October from the disks whilst the RBC was temporarily stationary. A 36 cm^2 circular area of biofilm was scraped from the disk using a scalpel, and the weight of the wet sample was

noted before additional analysis was carried out. Access hatches at WWT 1 and 2 allowed for samples to be taken between the second and third disk pack within the biozone and so biofilm samples were taken from the front of the third biozone disk.

All wastewater and biofilm samples were stored in an insulated box with ice packs during transportation to the laboratory before analysis. Where same day analysis could not occur, samples were stored at 4 °C overnight and then analysed the next day, once allowed to reach room temperature. All biofilm samples were subjected to analysis within 3 hours and left slightly exposed to the air to ensure aerobic conditions were maintained.

Hach-Lange (Salford, UK) probes in conjunction with a Hach-Lange HQ30d meter were used to take real time measurements in the same locations in which spot samples were taken. Temperature (°C) and dissolved oxygen (DO) measurements (mg L^{-1}) were conducted using a LDO101 probe and pH with a PHC101 probe.

Standard methods for the examination of wastewater (APHA, 2005) were followed to determine total (TSS), volatile suspended solids (VSS), biological oxygen demand (BOD_5) and carbonaceous biological oxygen demand ($cBOD_5$). Three-piece filtration apparatus and glass microfiber filter papers of 70 mm diameter and 1.2 µm pore size (Fisher Scientific, Loughborough, UK) in a Büchner funnel set-up was used to measure TSS/VSS, whilst carbonaceous BOD_5 was measured with the addition of 2 mg L^{-1} allylthiourea nitrification inhibitor (Hach-Lange) before the incubation. Chemical oxygen demand (COD), ammoniacal nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3-N) were measured using colourmetric test kits (Hach-Lange LCL400, LCK303 & LCK339, respectively). A DR3900 spectrophotometer (Hach-Lange) was used to quantify the levels of compounds in the samples, triplicate readings were taken and an average recorded.

4.2.3 Flow Measurement

The flow into individual RBCs was measured in 15 minute intervals over the period of the trial using 2150 area velocity flow modules (ISCO, Lincoln, UK). Probes were placed in the channels between the flow split at the inlet and the PST at the front of the RBC (Figure 1); flowlink 5.1 software was used to extract the flow data. The probe data was used in conjunction with quality assurance measurements. The quality assurance

involved taking 4 weekly manual measurements over a month period of the wastewater level within the inlet channel, using a ruler in addition to a spirit rule which ensured the ruler placement was consistent in both RBC inlet channels.

4.2.4 Microscope Analysis

Biofilm samples (3 g) were homogenised using an Ultra Turrex 8 homogeniser (Sigma-Aldrich, Dorset, UK). Homogenate (20 µl) was placed on a 76 x 26 mm microscope slide (Menzel-Glaser, Braunschweig, Germany), a 22 x 40 mm cover slip was then placed on top of the homogenate, ensuring that no air bubbles were trapped. The homogenate was scanned under x20 magnification using an OP-DB4B stereo light microscope (OPTEX, Melbourne, Australia), microscopic protozoa and animal species within the homogenate sample were recorded.

4.2.5 Nitrification Assay

The adapted synthetic media (OECD, 2001) included constituents that mimicked domestic wastewater and allows biological growth (Grady *et al.*, 1999); these are compiled in Table 3. This was made up without the NH_4Cl component and diluted with deionised water to a volume of 1 L. This was mixed thoroughly using a magnetic stirrer. Biofilm samples (wet weight - 3 g) were collected from RBCs 1 (1), 2(1), 1(2) and 2(2) and within 3 hours they were placed into a beaker containing 500 ml of synthetic media and left for 2 hours to acclimatise at room temperature (Wunderlin *et al.*, 2012). During this period the media was aerated using an air stone diffuser connected to a 4W HD air pump (Hidom, Shenzen, China) with a capacity of 0.12 m^3 hr^{-1}. Dissolved oxygen concentration was maintained above 5 mg L^{-1} and was therefore not limiting for nitrification (Nowak, 2000).

Table 3. Synthetic sewage media recipe. Constituents were sourced from Fisher Scientific (Loughborough, UK).

Formula	(g L^{-1})
$MgSO_4$. $7H_2O$	0.033
NaCl	0.010
$NaHCO_3$	0.243
$NaCO_3$	0.162
$Na_2HPO_4.12H_2O$	0.094
$CaCl_2.2H_2O$	0.006
KCl	0.005
$CuSO_4.7H_2O$	0.0003
$ZnSO_4.H_2O$	0.0003
$MnSO_4.H_20$	0.0002
$FeCl_3.6H_20$	0.0003
$C_6H_{12}O_6$	0.05
NH_4Cl	0.02

After the acclimatisation period the NH_4Cl component was added to the synthetic media and the NH_4^+-N concentration was measured immediately. Every 2 hours, 5 ml samples were taken, and filtered using Millipore 33 mm, 0.45 µm syringes (Fisher Scientific, Loughborough, UK); total nitrogen (TN), COD and NH_4^+-N were measured using the appropriate Hach-Lange test kits. These samples were continually taken until at least five data points had been recorded, ammonium levels had depleted to 70 % of the original concentration and three samples had shown a continual, consistent fall in NH_4^+-N concentration. To assess whether any gaseous nitrogen species were lost during the experiment, TN was measured at the start and end of the experiment. A total of 3 nitrification assays were performed for each RBC – RBC 1 (1), RBC 2 (1), RBC 1 (2), and RBC 2 (2). In each case the biofilm samples were taken from the rear disk of the RBC.

4.3 Results

4.3.1 Flow and Load

Rag build up on the flow probes at WWT 1 meant that the real-time flow measurement data was unreliable and could not be used with confidence. However, this data was used in conjunction with physical on-site measurement and on-line flow measurement data to estimate flow split and calculate flow rate, respectively. According to the probes the flow split at WWT 1 was approximately 30/70 for RBC 1 (1) and RBC 2 (1), respectively. The total mean flow through the WWT based on MCERTS data, extracted from the STW servers was 295 m^3/d and therefore the flow to RBC 1 (1) was 89 m^3/d and RBC 2 (1) was 206 m^3/d.

Table 4. Mean loading (g/m^2/d) and associated standard deviation of various parameters into the treatment process at WWT 1 & 2, in terms of RBC disk area.

	RBC 1 (1)	**RBC 2 (1)**	**RBC 1 & 2 (2)**
$cBOD_5$	1.3 ± 0.9	3.8 ± 2.6	5.5 ± 1.3
NH_4-N	0.3 ± 0.1	1 ± 0.3	1.2 ± 0.3
COD	6.4 ± 3.3	18.2 ± 9.4	17.3 ± 3
TSS	2.2 ± 1.3	6.4 ± 3.6	7 ± 0.8

At WWT 2 the flow probes were unable to record any data due to the conditions on site being inappropriate – the effluent discharge pipe being of insufficient length to allow for laminar flow. However, the mean total inlet flow was 457 m^3/d, therefore assuming an equal flow split to the RBC the mean loading onto the front disk of the RBC treatment process was as illustrated above table 4.

4.3.2 Ammonium and Dissolved Oxygen Concentration

At WWT 1 as well as WWT 2, the altered rotational speed did not lead to a significant difference in NH_4^+-N removal according to a paired two-tailed t-test (Figure 3; p value > 0.05). As illustrated in Figure 2 there was not a significant difference in DO concentration between the two RBCs at any sampling point, however increasing the rpm did lead to less variance observed. The DO concentration rarely dropped below 2 mg L^{-1}, the value at which oxygen becomes a limiting factor to nitrification (Nowak, 2000), this

is undoubtedly a contributing factor as to why there was no significant difference in ammonium concentration observed between RBC 1 (1) and RBC 2 (1). At WWT 2 performance in terms of ammonium removal remained unchanged and unexpectedly DO concentration in the bulk liquid was not significantly altered at the biozone stage or effluent stage when comparing RBC 1 (2) and RBC 2 (2).

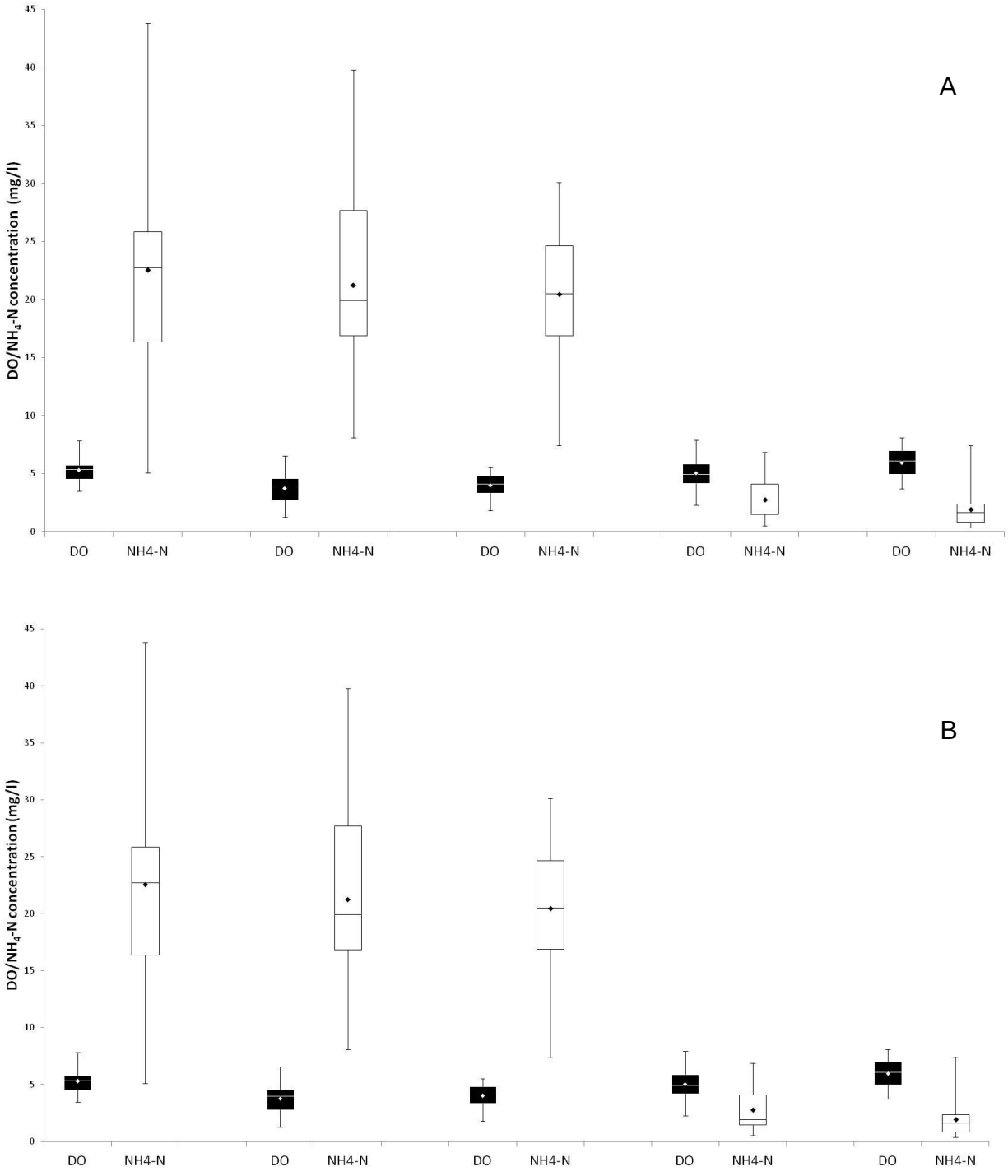

Figure 2. Dissolved oxygen (n = 32) and ammonium concentrations (n = 16) (mg L^{-1}). A: RBC 1 (1) and RBC 2 (1); B: RBC 1 (2) and RBC 2 (2). Diamonds represents the mean. The top whisker indicates the upper quartile whilst the bottom whisker indicates the lower quartile and the box represents the second and third quartile).

4.3.3 Comparison of Effluent Data from QUISLITE Before and After Speed Change

Figure 3 shows the effect of increasing (black and grey markers) or decreasing the speed (white markers) of an RBC on the final effluent ammonium concentration at WWTs 1, 2 & 3. All RBCs were designed to nitrify but they did not however have ammonia effluent consents. It suggests that increasing the speed of an RBC results in reduced ammonia spikes in the final effluent. However, a paired two-tailed t-test suggests that an increase in speed does not significantly affect ammonium concentration at either WWT (p value > 0.05).

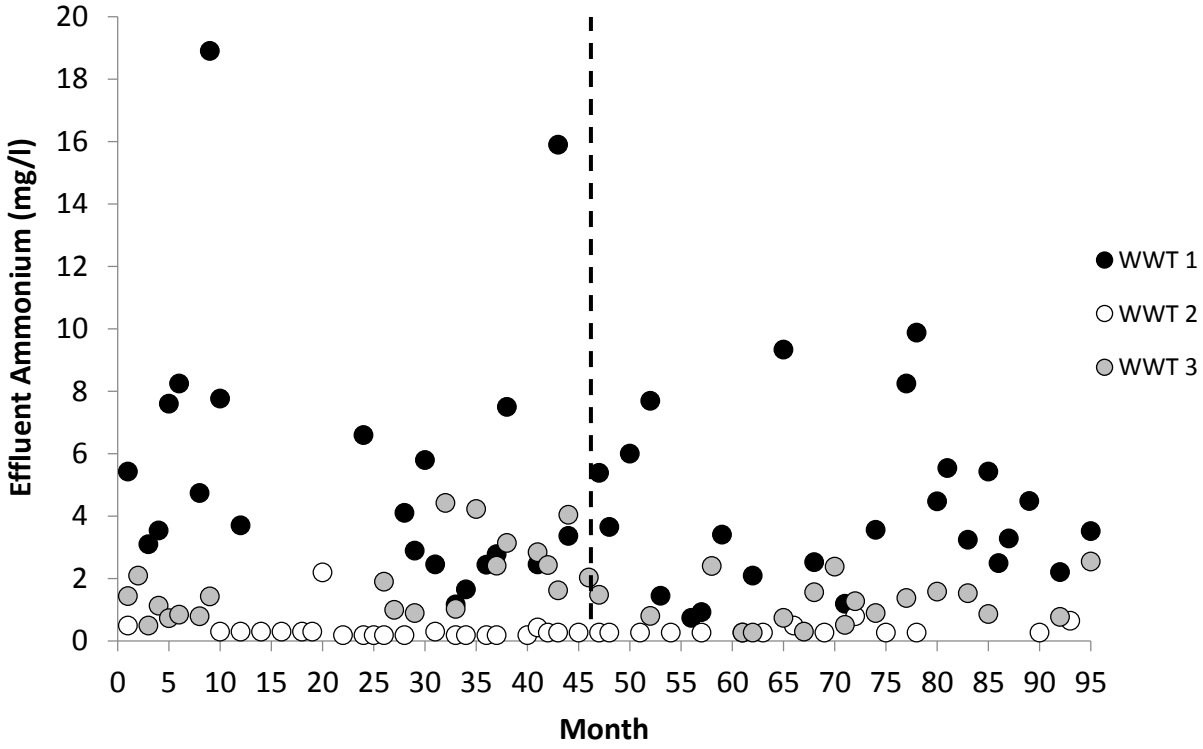

Figure 3. QUISLITE data showing ammonium concentrations in the final effluent of WWT 1, WWT 2 (containing 2 RBCs) and WWT 3 (containing 1 RBC) (n = 175). (The vertical black line represents an increase in rotational speed of RBC 2 (1) and RBC 1 (3) and a decrease in speed of RBC 1 (2). At WWT 1 the rotational speed of a RBC was increased from 1.1 rpm to 1.3 rpm, at WWT 2 the rotational speed of a RBC was decreased from 1.0 rpm to 0.7 rpm and at WWT 3 the rotational speed of a RBC was increased from 1.0 rpm to 1.3 rpm.

Prior to the speed change, WWT 1 experienced two high > 10 mg L^{-1} ammonia effluent spikes, whereas post speed change effluent concentrations were never greater than 10 mg L^{-1}. At WWT 3 there were three effluent spikes at 4 mg L^{-1}, but upon the speed increase effluent concentrations did not breach 3 mg L^{-1}. This effect is evident in Figure 3,

however no statistical difference between the pre and post speed change data for WWT 1 and WWT 3 was found. Likewise, no statistical difference was found between the pre and post speed change data for WWT 2 where the speed of an RBC was decreased, in this instance the final effluent quality was exceptionally good for the whole 8-year period and so this WWT clearly has excess treatment capacity.

4.3.4 Ammonium Removal

The ammonium loading throughout the integral RBC process varied considerably due to the uneven flow split at WWT 1, with an estimated flow split of 30/70. With this flow split the increased rotational speed did not lead to a significant improvement in NH_4^+-N removal in terms of effluent concentration, according to a paired two-tailed t-test (Figure 4; p value > 0.05). However, it is evident from Figure 4 that the treatment process removal efficiency in terms of total load removed was clearly enhanced with the increased RBC rotational speed of RBC 2 (1). Whereas at WWT 2 the total load and percentage of load removed was similar for RBC 1 (2) and RBC 2 (2) at all stages, indicating that reducing the rotational speed had no impact on performance.

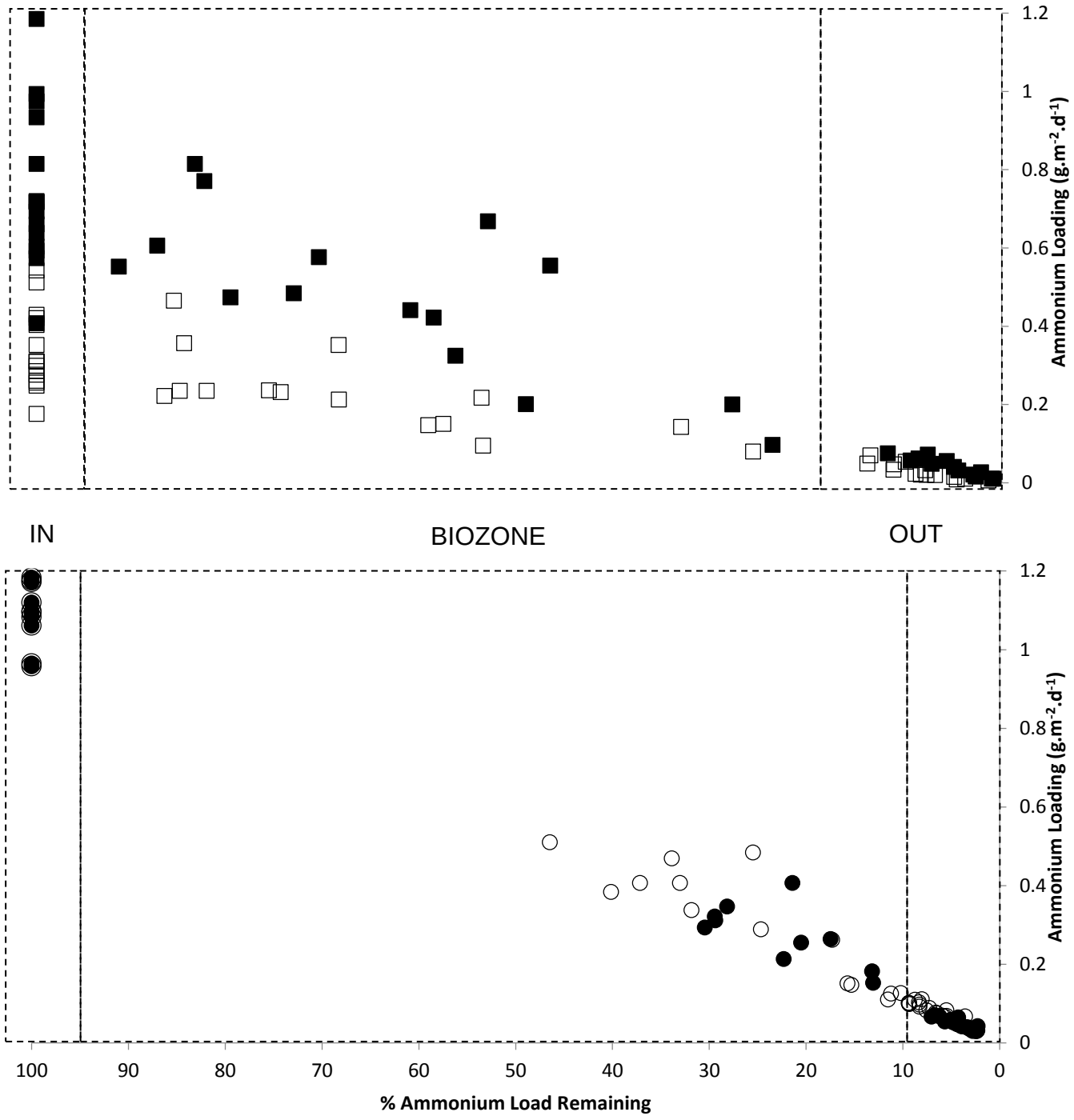

Figure 4. The ammoniacal nitrogen load through the treatment process at RBC 1 (1) [clear square], RBC 2 (1) [black square], RBC 1 (2) [clear circle] and RBC 2 (2) [black circle] and the corresponding load remaining after passing from the influent to the biozone to the effluent for each RBC (n = 16). (IN = Influent; OUT = Effluent)

Table 5. Ammonium load ($g.m^{-2}.d^{-1}$) through the RBC process from the influent to the biozone, to the effluent at RBC 1 (1), RBC 2 (1), RBC 1 (2) and RBC 2 (2).

		RBC 1 (1)	RBC 2 (1)	RBC 1 (2)	RBC 2 (2)
INFLUENT	Average	0.26	0.61	1.22	1.22
	SD	0.11	0.27	0.23	0.23
BIOZONE	Average	0.25	0.55	0.27	0.19
	SD	0.1	0.17	0.16	0.13
EFFLUENT	Average	0.03	0.05	0.09	0.05
	SD	0.02	0.05	0.02	0.01

4.3.5 Biofilm Analysis

Microscope analysis revealed that the RBC 1 (1) biofilm was absolutely dominated by the motile, filamentous bacterium *Beggiatoa* and consequently other species were rarely observed, however RBC 1 (1) contained more nematodes specimens than RBC 2 (1). Biofilm samples from RBC 2 (1) as well as biofilm samples from both RBCs at WWT 2 consisted of diverse ecosystems containing ciliates, amoebas, nematodes and rotifers.

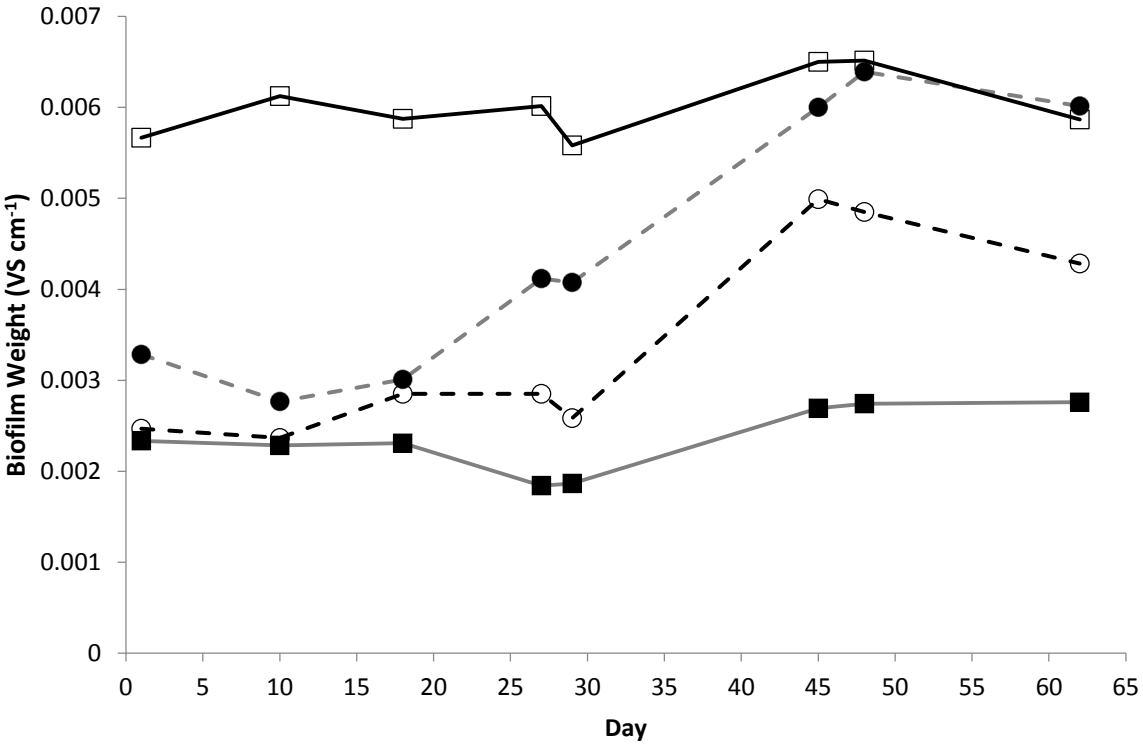

Figure 5. The biofilm weight expressed as volatile solids (g) per centimetre of disk over a period of 3 months from the rear disk of RBC 1 (1) [solid black]; RBC 2 (1) [solid grey]; RBC 1 (2) [dotted black]; RBC 2 (2) [dotted grey].

8 biomass measurements over a period of 3 months from 20th August until 21st October, showed that on average (mean) RBC 1 (1) amassed 156 % more wet weight than RBC 2 (1). Dry weight analysis revealed that the on average (mean) the biofilm established on RBC 1 (1) weighed 0.006 ± 0.0004 VS cm^{-1} whilst RBC 2 (1) biomass weighed 0.0024 ± 0.0004 VS cm^{-1}.

RBC 2 (2) amassed on average (mean) 31 % more wet weight than RBC 1 (2) and dry weight measurements revealed that the biofilm established on RBC 1 (2) weighed 0.0034 ± 0.0011 VS g^{-1} and 0.0045 ± 0.0015 VS g^{-1} for RBC 2 (2).

4.3.6 Nitrification Assay

Table 6. Average ammonium removal rate of triplicate biofilm samples from RBCs 1(1), 2(1), 1(2) and 2(2) in terms of grams of ammonium removed per centimetre or gram per hour.

RBC No.	Average Ammonium Removal Rate	
	g NH_4-N. cm biofilm. hr^{-1}	**g NH_4-N. g biofilm. hr^{-1}**
RBC 1 (1)	0.008 ± 0.001	0.01 ± 0.015
RBC 2 (1)	0.01 ± 0.002	0.12 ± 0.023
RBC 1 (2)	0.007 ± 0.001	0.094 ± 0.015
RBC 2 (2)	0.008 ± 0.002	0.1 ± 0.023

Three nitrification batch tests were performed for samples collected for each RBC in October, corresponding to day 45, 48 and 62 in Figure 5. Table 6 illustrates the average ammonium removal rate for these three samples over the period of batch tests, Figure 6 reveals the removal rate for individual samples. The ammonium removal rate in terms of mass or area is similar for RBCs 1(1), 1(2) and 2(2), whereas RBC 2(1) demonstrates a significantly enhanced ammonium removal rate.

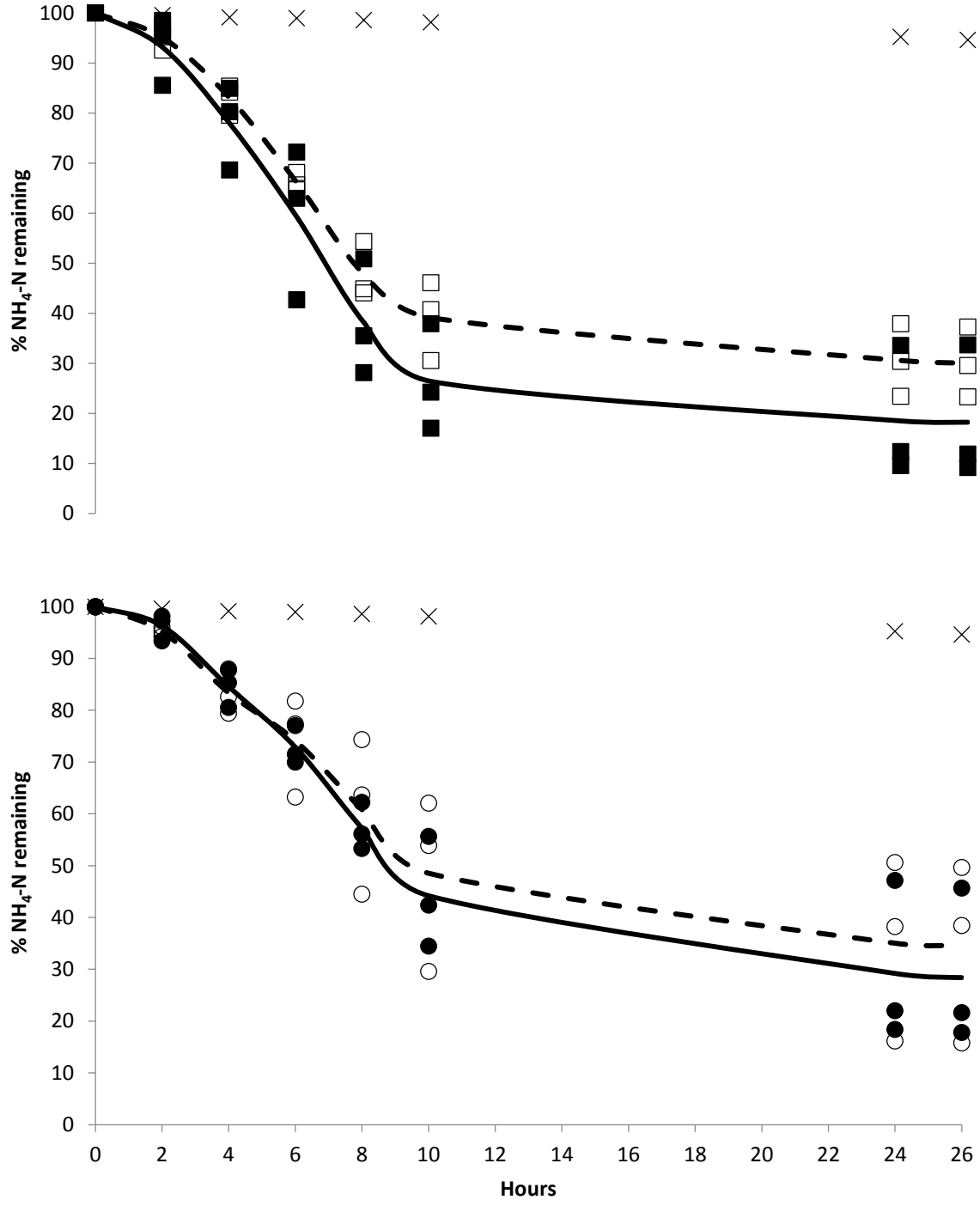

Figure 6. The percentage NH_4^+-N remaining during the nitrification batch tests (n = 3) at RBC 1 (1) [clear square], RBC 2 (1) [black square], RBC 1 (2) [clear circle] and RBC 2 (2) [black circle] over a period of 26 hours for each RBC (n = 3). Dotted (RBC 1) and full (RBC 2) lines represent the average percentage NH_4-N remaining for WWT 1 & 2 biofilm samples. Crosses represent control samples with synthetic media only and no biofilm sample.

4.3.7 Energy/Capital Consumption

Reducing the rotational speed did not result in significant operating cost or energy saving (Table 7), equating to only a 0.08% reduction in energy consumption. This finding is likely due to increased biomass establishment on the disks, which was promoted by less sloughing force present during a slower rotational speed. The increase in biomass therefore meant more work had to be done by the motor and therefore more energy was consumed.

Table 7. Comparison of energy, financial and carbon cost for altered RBC speeds.

	RBC 1 (1)	RBC 2 (1)	Annual cost from increasing rotational speed	RBC 1 (2)	RBC 2 (2)	Annual saving from reducing rotational speed
Speed (rpm)	1.1	1.3		1	0.7	
Energy Consumption (kWh/year)	8690.7	8760	**69.4**	8975.4	8968.1	**7.3**
Cost (£/year)	782	788.3	**6.3**	807.9	807.2	**0.8**
Carbon Cost ($kgCO_2$/year[1])	3871.5	3902.4	**30.9**	3998.3	3995.1	**3.3**

[1]Based on consumption of electricity from national grid (Carbon Trust, 2013).

4.3.8 *Beggiatoa* Mitigation

Increasing RBC speed seems to have improved appearance and reduced the thickness of biofilm as described in section 3.5, as well as *Beggiatoa* proliferation as seen in Figure 7.

(A) (B)

Figure 7. Image A shows the biofilm appearance on RBC 1 (1), image B shows the biofilm on RBC 2 (1).

The RBC operating with a higher rotational speed of 1.3 rpm (B) seemed to promote a more optimal biofilm, characterised with the biofilm being thinner and dark grey/brown. The RBC operating with a slower rotational speed of 1.1 rpm (A) on the other hand had consistent and severe *Beggiatoa* infection, which resulted in odour issues and potentially impacted on treatment performance.

4.4 Discussion

The accurate measurement of flow rate into individual RBCs was important for this study, as the experimental set-up was designed so that a control RBC and test RBC could be compared on a like-for-like basis. Unfortunately, the flow meters could not facilitate accurate flow measurements, consequently flow and load data was compromised. This is because the only convenient locations in which the probes could be placed were not suitable as there was either too much rag in the influent (WWT 1) or the length of pipe preceding the probe was too short to allow laminar flow (WWT 2) which the probes must operate in. This kind of challenge is characteristic of full-scale studies. Although it was difficult to compare the test and control RBCs on a like-for-like basis, the best attempt was made.

Based on the flow data, loading data shown in figure 4 suggest that increasing the speed of RBC 2 (1) to 1.3 rpm from 1.1 rpm did increase total NH_4^+-N removal but this did not lead to a significant decrease in effluent NH_4^+-N concentration. Therefore, increasing the

rotational speed may be a method to maintain effluent quality compliance in terms of NH_4^+-N removal, a statement supported by Figure 3 where the data suggests that increasing the speed of an RBC leads to less severe effluent spikes, which therefore leads to a more resilient process and ultimately a more compliant WWT. However, in the case of WWT 1 increasing the speed does not seem to significantly reduce NH_4^+-N effluent concentration and so this approach may not be suitable as a method to enhance effluent quality, as opposed to maintaining it.

Similarly decreasing the speed of RBC 2 (2) resulted in no change in effluent quality, but it also did not alter the total amount of NH_4^+-N removal. Altering the speed of RBCs within a range of 0.7 – 1.3 rpm at the WWTs this paper focused on did not make a significant difference to DO and NH_4^+-N concentration at any stage throughout the RBC.

This conclusion for WWT 1 was initially unexpected as with other similar technologies such as the moving bed biological reactor, increasing reactor DO concentration strongly correlates with increasing nitrification rate (Gapes & Keller, 2009) and the increased turbulence associated with a higher RBC rotational speed yielded a performance enhancement effect at lab-scale (Di Palma & Verdone, 2009). The altered rotational speed influenced the appearance and thickness of the biofilm, towards the end of the trial as described in section 3.5. RBC 1 (1) which acted as a control had a severe *Beggiatoa* infection throughout the trial (Figure 7), consequently the biofilm was thick, white and odourous. With the majority of the load appearing to go through RBC 2 (1), one would expect *Beggiatoa* to be present here too. It has been reported that aeration decreases *Beggiatoa* population in lab-scale continuous flow biofilms (Chung & Strom, 1997) and although the quicker rotational did not lead to higher DO concentration, it seemed to remedy the issue. *Beggiatoa* proliferate in high organic and low DO conditions, which can then lead to negative implications in RBC performance (Kinner *et al.*, 1985), an example being the formation of a thicker biofilm, which reduces the surface area and limits oxygen diffusion into the biofilm (Metcalf & Eddy, 1991) which negatively impacts on performance efficacy. Therefore, this may prove to be an important finding.

A thinner biofilm was observed on RBC 2 (1), the faster of the two. A thinner biofilm presents a larger surface area thereby increasing oxygen diffusion and treatment efficacy. Higher rotational speed is also likely to improve the mixing of compounds in the bulk

solution, which can enhance treatment efficacy (Chavan *et al.*, 2008). It is likely that the reason the enhanced mixing and optimal biofilm structure did not influence treatment performance improvement is because the DO at WWT 1 rarely dropped below 2 mg L^{-1} and therefore the rate of substrate removal was never limited. Ammonia oxidising bacteria (AOB) in particular can thrive in an environment of around 2 mg L^{-1} DO. Nitrite oxidising bacteria (NOB) such as *Nitrobacter* have a greater oxygen demand and hence will be the first nitrifying bacteria to respond to changing oxygen conditions, a result verified in a membrane-aerated reactor (Downing & Nerenburg, 2008). Nitrate levels did not significantly differ between the test and control RBCs which indicates NOB were not influenced by altered oxygen levels brought about by a change in rotational speed.

The study revealed that altering the RBC speed within the 0.7 - 1.3 rpm range does not significantly influence energy consumption and therefore operating cost. The RBC with higher rotational speed at WWT 2 tended to have a smaller amount of biomass established on it, whereas the RBC rotating slower tended to promote more biomass, with approximately 24% more wet weight. It is likely that this is reason why no significant energy savings or costs were seen – a dynamic system developed whereby the increase in energy consumption by the motor in order to accelerate the disk was compensated by the decrease in energy consumption needed to rotate a lighter load on the shaft and vice versa.

Reports in the literature suggest that full-scale RBCs typically operate at between 1 - 10 rpm (Mathure & Patwardhan, 2005; Di Palma & Verdone, 2009) and that the peripheral speed of the RBC disk should not exceed 0.3 m/s (Henze *et al.*, 1995). In order to minimise operating costs, the lowest rotational speed should be employed, for this purpose 0.7 - 2.0 rpm is common (Mba *et al.*, 1999). This is a wide range of speeds to operate RBCs within, the standard for the RBCs considered in this study was 1 rpm and it appears that this is already an optimal speed. This is an important finding as water companies can be confident of maintaining their current RBC operating costs if they need to reduce or increase the rotational speed. Reducing the rotational speed in an under loaded site may allow for a longer life span for the RBC, whereas increasing the speed may be necessary to increase consent compliance. It remains to be seen whether energy consumption is significantly affected beyond the speeds that were trialled in this study.

In conclusion altering rotational speed of an RBC surprising may have a negligible effect on energy and capital cost, as well as DO and NH_4^+-N concentration throughout the RBC process. On the other hand, it appears that increasing rotational speed provides extra capacity to WWTs in the sense that in total more NH_4^+-N can be removed and an unforeseen benefit may be as a potential remedy for *Beggiatoa* proliferation which can be a major issue for RBCs. This contribution to knowledge can assist in ensuring the challenges of future tightening consents and increased population growth can be met through a cost-effective technology.

A repeat of this study at a WWT deprived of ample DO and with appropriate conditions for flow measurement would no doubt yield more useful data and allow conclusions to be made with more confidence.

Acknowledgments:

This work was funded by Severn Trent Water (STW) in conjunction with the Natural Environment Research Council (NERC – Grant reference 1403561). Special thanks to STW for giving me access to their internal databases which allowed this study to happen.

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CHAPTER 5

AERATION

5 THE IMPACT OF ARTIFICAL AERATION ON AMMONIUM REMOVAL IN ROTATING BIOLOGICAL CONTACTORS

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Abstract

Rotating biological contactors (RBC) are widely employed as a biological secondary wastewater treatment process due to their relatively low-cost, low-energy requirement and maintenance. They are particularly suitable for small wastewater treatment works and provide a viable treatment option as a nitrification application. However, growing population means that RBCs can often become overloaded and oxygen limited. One possible route to increasing oxygen availability is altering the rotational speed of the disks, although this may incur significant costs, extra time for adaptation and increasing mechanical stress on the infrastructure. Artificial aeration of the biozone could be a fast and cost effective alternative which to date has not been tested in a fully operational RBC. Thus, a three phase approach was used in this study to assess the efficacy of artificial aeration on ammonium removal, with each phase lasting 8 weeks. In phase I, the RBC was operated in overloaded oxygen limited conditions (< 1 mg L^{-1} dissolved oxygen). Artificial aeration equating to approximately 2 mg L^{-1} O_2 in the biozone bulk liquid was then applied in phase II incremented to 4 mg L^{-1} O_2 in phase III. Artifical aeration was found to significantly enhance ammonium removal in overloaded, oxygen-limited conditions. Phase III led to a maximum ammonium load removal of 70 % when previously the RBC removed less than 20 %. Interestingly, aeration was also found to decrease biofilm accumulation (mass) by an average of 40 %. This 'plug and play' tool can provide increased treatment capacity with simple solution, rather than adding expensive new assets which is often preferable, to minimise costs.

Keywords: Artificial Aeration, Rotating Biological Contactor, Nitrification, Wastewater Treatment, Pilot-Scale

5.1 Introduction

It is important to have robust and consistent ammonia removal during wastewater treatment as it can cause significant environmental damage by greatly contributing to eutrophication and deoxygenation in aquatic ecosystems (Rockstrom *et al.*, 2009). In an aqueous environment, ammonia is more commonly seen as ammonium and the pathway in which ammonium is removed is termed nitrification. Nitrification is the biological oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and then nitrate (NO_3^-), or more generally, it is the transformation of the reduced forms of nitrogen to the oxidised forms via biological mechanisms. Traditionally the two main groups of microbes linked to nitrification in wastewater treatment were thought to be ammonia oxidising bacteria (AOB) and the nitrite oxidising bacteria (NOB). However, recently a third group, the ammonia oxidising archaea (AOA), has been identified as an important group that even has the potential to dominate nitrification in wastewater systems (You *et al.*, 2009). In nitrifying biofilms there is a clear association between these groups, which are both located in the upper 150 µm layer of the biofilm surface where availability of oxygen and ammonium are high (Schramm *et al.*, 1998). Oxygen supply has been commonly reported as a limiting factor to ammonium removal efficiency (Tchobanoglous *et al.*, 2013). In the event of oxygen limited conditions the AOB/AOA consortium responsible for removing ammonium will suffer, therefore directing supplemental air supply towards them may increase process efficacy.

Rotating biological contactors (RBCs) can be used to perform nitrification with a range of influent characteristics, and have the potential to yield effluent quality similar to that of activated sludge (AS) plants (Cortez *et al.*, 2008; Tchobanoglous *et al.*, 2013). One of the main benefits of RBCs compared to AS or membrane technologies is that aeration is inherently supplied to the system via the rotation of the disks and therefore there is generally no need for costly artificial aeration. However, if an RBC is overloaded then it may exhibit lower than optimal oxygen availability. Excessive nutrients will result in microbes utilising considerable amounts of oxygen whilst metabolising the excess substrates. In this case, it is our opinion that three routes can be used to increase oxygen availability to optimal levels including: 1) increasing the rotational speed of the disks (Di Palma & Verdone, 2009); 2) increasing the emptying frequency to reduce sludge build

up, and 3) introducing artificial aeration to the system, as is seen in ASP and membrane technologies. From the authors experience the first option would require a costly variable speed drive installation and the second option requires frequent tanker visits to sites which are often in rural locations and difficult to access. Therefore, the latter option may require less expenditure and maintenance than the other two options. It could further provide a swift option to optimise oxygen availability in overloaded RBCs and therefore sustain efficient and robust ammonia removal. To do so, the aeration must however be located strategically.

Generally, nitrification activity is located in the later stages of the RBC wastewater treatment. This is because in the presence of high BOD, as is seen at the first stage of secondary wastewater treatment, biofilms are dominated by heterotrophic bacteria which can outcompete nitrifying bacteria (Nogueira *et al.,* 2002). Heterotrophic bacteria have higher yield growth rates compared to the slow growing autotrophic nitrifying bacteria and so for nitrification to occur the reactor must be designed to have low BOD loading and high oxygen levels at the back end to enable nitrifiers to compete (Grady *et al.,* 1999). In fact, nitrification only starts when organic load is below 15 g COD/m^2day^{-1} and can only reach optimal rates below 8 g COD/m^2day^{-1} (Boller *et al.,* 1987). Consequently, correct design and operation are essential for efficient wastewater treatment in biofilm systems, especially for nitrification in RBCs (Hoccheimer & Wheaton, 1998). RBC design often includes staging, this acts as a barrier for wastewater chemistry and encourages a reduction in biodegradable substrates sufficient to allow nitrifiers to establish. RBCs commonly have two or three stages and nitrification will occur after the first stage.

In RBCs designed for extra capacity, nitrification can be achieved completely during the second biozone, leaving the third and final biozone with a much lower density of microbes and minimal nitrification potential due to limitation of substrates (Dutta *et al.,* 2007). However, incorrectly designed RBCs can have a tendency to become overloaded and be in a situation where dissolved oxygen levels are too low for nitrification to occur in the final biozone stage, this is because activity of heterotrophic bacteria requires large quantities of oxygen in order to metabolise large quantities of substrate. Therefore,

aeration should be strategically located in the final biozone stage in these cases, assuming that BOD levels have been sufficiently reduced beforehand.

The biofilm attains oxygen through contact with dissolved oxygen in the bulk liquid (Patwardhan, 2003) and with gaseous oxygen when the biofilm is exposed to air (Grady, 1999). Contact during the air phase is the dominant mechanism which is known as 'falling film' (Hiras *et al.,* 2004; Chavan & Mukherji, 2008; Zhang *et al.,* 2009). The mechanism of attaining oxygen through the bulk liquid is also important. Even in membrane-aerated biofilms (MAB), where sufficient oxygen is supplied to the biofioms through aeration of the membrane, additional aeration of the bulk liquid significantly enhances ammonium flux, therefore bulk liquid oxygen levels are indeed very important (Downing & Nerenberg, 2008) More than 2 mg L^{-1} of dissolved oxygen in the bulk liquid is considered necessary for effective nitrification in RBC biofilm systems, but generally a minimum level of 3-4 mg L^{-1} DO is routinely adhered to (Boller & Tshui, 1994). Aeration is so vital to RBCs that it is often stated as the most crucial design parameter, if the rotation mechanism does not provide ample oxygen then supplemental artificial aeration is recommended (Rodgers & Zhan, 2003). Aeration of the bulk liquid should increase oxygen availability to the biofilm via two mechanisms – providing more dissolved oxygen in the bulk liquid and introducing fresh relatively oxygen rich air into the enclosed RBC space containing stale air consisting of greater methane and carbon dioxide levels. With aeration of the bulk liquid, the RBC will be similar to an integrated fixed film activated sludge (IFAS) process, which requires a higher level of DO (approx 6 mg L^{-1}) compared to RBC in order to fully nitrify (Liu *et al.,* 2008; Randall & Sen, 1996). The aerated hybrid RBC in this study should require less DO and therefore less aeration as the biofilm is consistently and frequently exposed to air, this is not the case in IFAS where the biofilm is constantly submerged.

In view of the above, the study will investigate the relationship between oxygen availability in the RBC bulk liquid, and the biofilms ability to nitrify. This will be done by manipulating the levels of dissolved oxygen. We will therefore demonstrate that the introduction of sufficient levels of oxygen can enable enhanced ammonium removal in an overloaded non-nitrifying system and that AOM can recover activity after periods of inactivity.

5.2 Materials and Methods

5.2.1 RBC Set-Up

A fully operational pilot-scale RBC was installed adjacent to a medium sized wastewater treatment plant (capacity: 100,000 PE) that treats raw domestic and trade wastewater. The pilot RBC consists of a primary settlement tank (PST), 2 biozones and a final settlement tank (FST) (Figure 1). The 2-stage integral RBC was designed to treat 50 PE and was intermittently fed with screened and de-gritted influent from the inlet of the WWT. The polypropylene disks in the biozones were 40 % submerged and the rotational speed was 2.4 rpm. The disk diameter was 1.6 m which gave a total surface area of 800 m^2. The density of the disks was increased from 150 kg/m^3 to 225 kg/m^3 from the first to the second biozone. The total bulk liquid capacity of the biozone, PST and FST was 2.5 m^3, 9 m^3 and 1 m^3 respectively. The RBC was initially operated at 13 m^3/day, following this, load was increased every four weeks in intervals of 6 m^3/day until a flow rate of 38 m^3/day was attained. At this flow rate oxygen availability was limited, conditions were anaerobic and nitrification was inhibited. This is termed the acclimatisation phase. This phase allowed biofilm to gradually accumulate and reach a steady state before the effect of aeration on performance was tested.

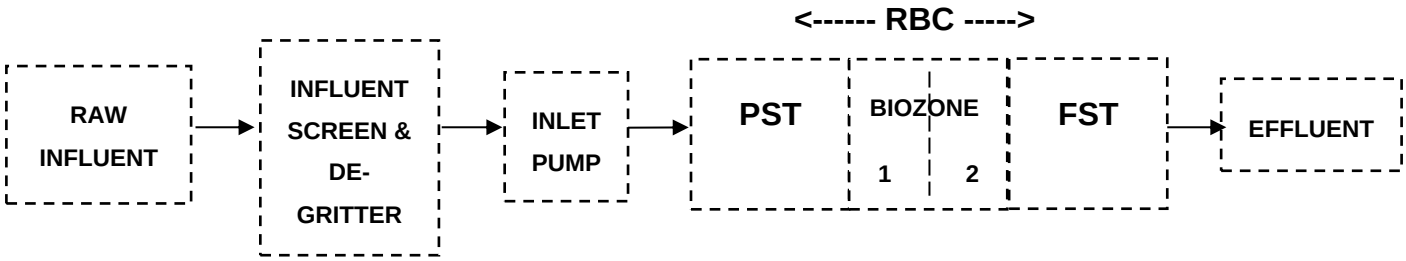

Figure 1. The flow layout for the pilot RBC aeration experiment.

The performance of the RBC was assessed during the three following phases, with each one lasting 8 weeks:

Phase I – Control phase where the pilot RBC environment is oxygen limited (<1 mg L^{-1} DO)

Phase II – Test phase where the pilot RBC environment has sufficient oxygen availability (~2 mg L^{-1} DO)

Phase III – Test phase where the pilot RBC environment has excessive oxygen availability (~4 mg L^{-1} DO)

The air supply in the biozone 2 was provided using an air pump (Blagdon KA 50, Ireland) designed for a large pond with a capacity of 150 litres per minute connected to a pond-grade 180 mm HDPE pipework and 2 pond-grade 3-inch diameter circular ceramic air stones. The pump was in constant operation and was calibrated so that the DO concentration consistently was 2 ± 0.5 mg L^{-1} DO for phase II and 4 ± 0.5 mg L^{-1} DO for phase III, respectively. Air flow and pressure was regulated using adjustable valves on the pump itself.

5.2.2 Sampling and Analysis

Duplicate spot samples of 500 ml were taken on a weekly basis from November 2015 to February 2016. Wastewater samples were taken at: the inlet pump (influent or IN), the PST, RBC biozone (between the first and second biozone), FST and from the effluent (E) (See Figure 1).

Biofilm samples were taken from the disks whilst the RBC was temporarily stationary on a weekly basis. A 36 cm^2 circular area of biofilm was scraped from the disk using a sterile scalpel, and the weight of the wet sample was noted before additional analysis was carried out. Numerous access hatches enabled samples to be taken at the end of the first biozone and the end of the second biozone.

All wastewater and biofilm samples were stored in an insulated box with ice packs during transportation to the laboratory before analysis. Where same day analysis could not occur, samples were stored at 4 °C overnight and then analysed the next day, once allowed to reach room temperature.

All biofilm samples were analysed for VSS within 3 hours and left slightly exposed to the air to ensure aerobic conditions were maintained. VSS data was used to calculate biofilm mass in terms of mg cm^{-2}.

Standard methods for the examination of wastewater (APHA, 2005) were followed to determine total (TSS), volatile suspended solids (VSS), biological oxygen demand (BOD_5) and carbonaceous biological oxygen demand ($cBOD_5$). Three-piece filtration

apparatus and glass microfiber filter papers of 70 mm diameter and 1.2 µm pore size (Fisher Scientific, Loughborough, UK) in a Büchner funnel set-up was used to measure TSS/VSS, whilst carbonaceous BOD_5 was measured with the addition of 2 mg L^{-1} allylthiourea nitrification inhibitor (Hach-Lange) before the incubation. Chemical oxygen demand (COD), ammoniacal nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3-N) were measured using colourmetric test kits (Hach-Lange LCL400, LCK303 & LCK339, respectively). The standard parameters were measured in duplicate and an average reading taken from these. Hach-Lange (Salford, UK) probes in conjunction with a Hach-Lange HQ30d meter were used to take real time measurements in the same locations in which spot samples were taken. Temperature (°C) and dissolved oxygen (DO) measurements (mg L^{-1}) were conducted using a LDO101 probe and pH with a PHC101 probe. A DR3900 spectrophotometer (Hach-Lange) was used to quantify the levels of compounds in the samples, triplicate readings were taken and an average recorded.

5.2.3 Statistical Analysis

Statistical analysis was performed using version 22 of the statistical software package SPSS (Portsmouth, UK). After testing the assumption of homogeneity of variances using 'Levene's test' [$F(2,19) = 2.5$, $p = 0.109$)], one-way ANOVA was carried out to test the null hypothesis of whether total ammonium load removal was different in phase, I ($n = 7$), II ($n = 7$) and III ($n = 8$).

5.3 Results

From the initial start-up, load into the RBC system was gradually increased from 13 to 38 m^3/day in increments of 6.4 m^3/day. This period lasted 9 months before the 3 experimental phases of the aeration were initiated (Table 1). The RBC received a low flow of rate of 13 m^3/day during the first two months of operation. In this period significant fluctuation in biofilm accumulation within both the first biozone and second biozone was noticeable (Figure 2). Biofilm growth was detected after 10 days of operating the RBC in the rear biozone and 3 weeks later biofilm accumulation peaked in both biozones. Then, the biofilm accumulation decreased until steady-state was reached 3 months after start-up (Figure 2).

Table 1. The flow regime over the whole experimental period

	ACCLIMATISATION PERIOD				PHASE I	PHASE II	PHASE III
FLOW RATE (m^3/day)	13	19	27	32	38	38	38
DATE	4/11 – 14/01	29/01 – 25/03	01/04 – 17/06	27/06 – 09/09	21/09 – 28/10	16/11 – 04/01	08/01 – 19/02

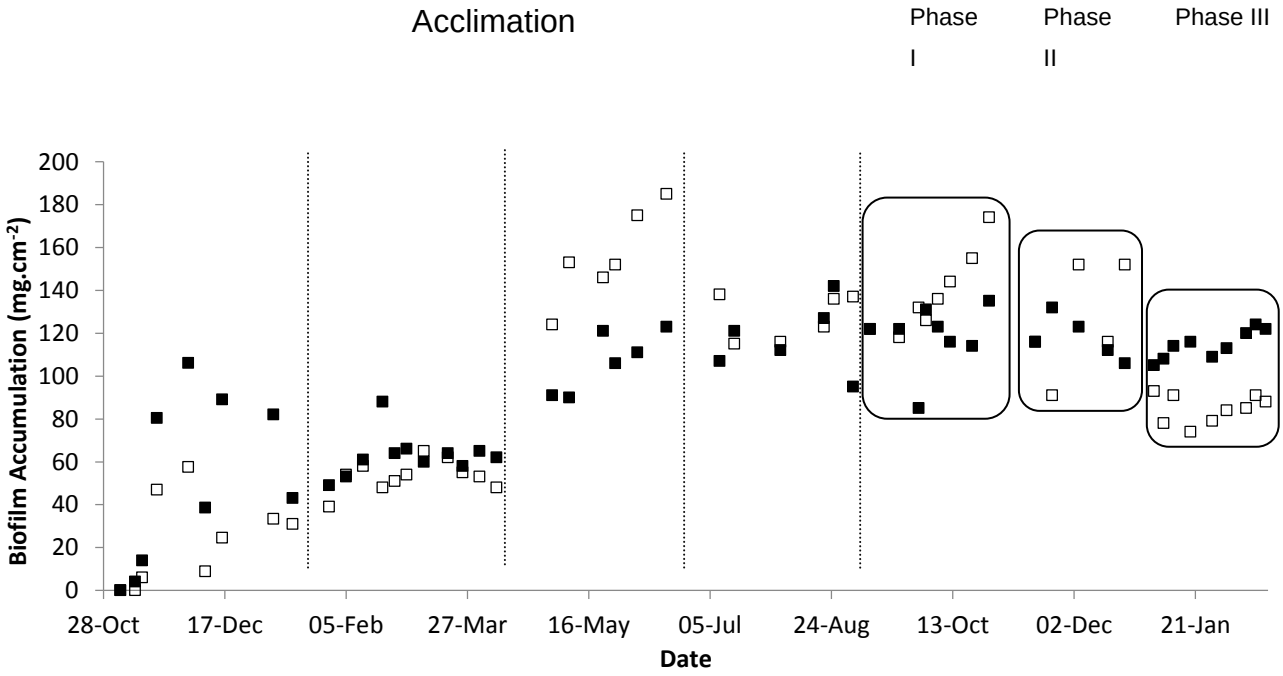

Figure 2. Biofilm accumulation ($mg.cm^{-2}$) over a period of 13 months at the rear of the first biozone (black squares) and the second biozone (clear squares). Vertical dotted lines represent when the flow rate was increased into the system. Moving from left to right, the flow rate per day was increased from 13 m^3, to 19 m^3 to 27 m^3 to 32 m^3 to 38 m^3. At 38 m^3/day flow rate, the 3 aeration experiment phases begun (highlighted from the final vertical line).

The consistent biofilm mass was maintained at approximately 55 $mg.cm^{-2}$ for the entirety of period in which the RBC was operated at a 19 m^3 flow rate, however the rear biozone tended to have a higher mass of biofilm. At a flow rate of 27 m^3 the biofilm mass increased rapidly, with the peak mass in the rear biozone reaching 185 $mg.cm^{-2}$. Following this the biofilm mass in the second biozone fluctuated significantly, however the biofilm mass in the first biozone plateaued.

There were 4 occasions where the RBC was emptied or 'desludged,' (12th Feb, 27th May, 9th Sep, 20th Dec). However, according to Figure 2, the biofilm mass did not appear to be influenced, nor was it influenced by the build-up of sludge prior to the desludging.

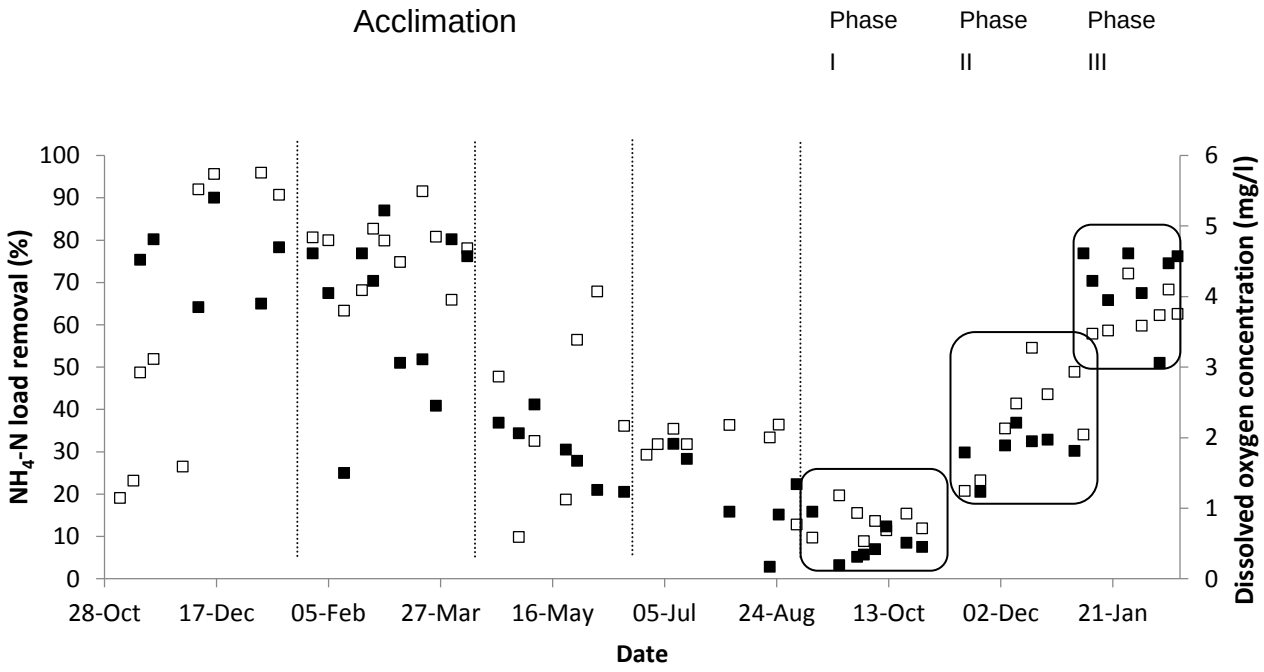

Figure 3. The influence of dissolved oxygen [black squares] in the second biozone on total ammoniacal nitrogen load removal (%) [clear squares]. Vertical dotted lines represent when the flow rate was increased into the system. Moving from left to right, the flow rate per day was increased from 13 m^3, to 19 m^3 to 27 m^3 to 32 m^3 to 38 m^3. At 38 m^3/day flow rate, the 3 aeration experiment phases begun.

The ammoniacal nitrogen removal capacity of the RBC was poor until one month after the start-up of the plant, then removal of ammoniacal nitrogen load consistently reached 90 %. Following this, the period of 7 months where flow rate was increased from 13 to 32 m^3/day resulted in the overloading of the RBC with typical DO concentration in the bulk liquid < 2 mg L^{-1}. Increasing the flow rate further to 38 m^3/day meant that DO did not exceed 1 mg L^{-1} and ammoniacal nitrogen load removal did not exceed 25%, as seen in phase I.

Aerating the rear of the biozone from the 16th November resulted in immediate improvements in DO levels and enhanced ammoniacal nitrogen load removal. Further aeration from 2 to 4 mg L^{-1} DO increased ammoniacal nitrogen removal capacity, as seen from phase II to phase III in Figure 3. From phase I to phase II and then from phase II to phase III, the average ammoniacal nitrogen load removal improved from 14 ± 3 % to 38

± 13 % and then to 59 ± 11 %, respectively. This illustrates that increasing dissolved oxygen in the bulk liquid of the biozone has the effect enhancing ammoniacal nitrogen load removal in significantly overloaded conditions where DO is inherently very low. Although removal was significantly enhanced in phase III, the effluent NH_4^+-N concentration was still relatively high, ranging from 9.8 to 19.3 NH_4^+-N mg /l. Artificial aeration in the biozone, not only influenced dissolved oxygen concentration levels in the bulk liquid of the biozone itself, but also in the effluent, as seen in Figure 4. Artificial aeration had a clear and intended effect of increasing DO, almost doubling the DO concentration in phase II, compared to phase I, DO then doubled again in phase III.

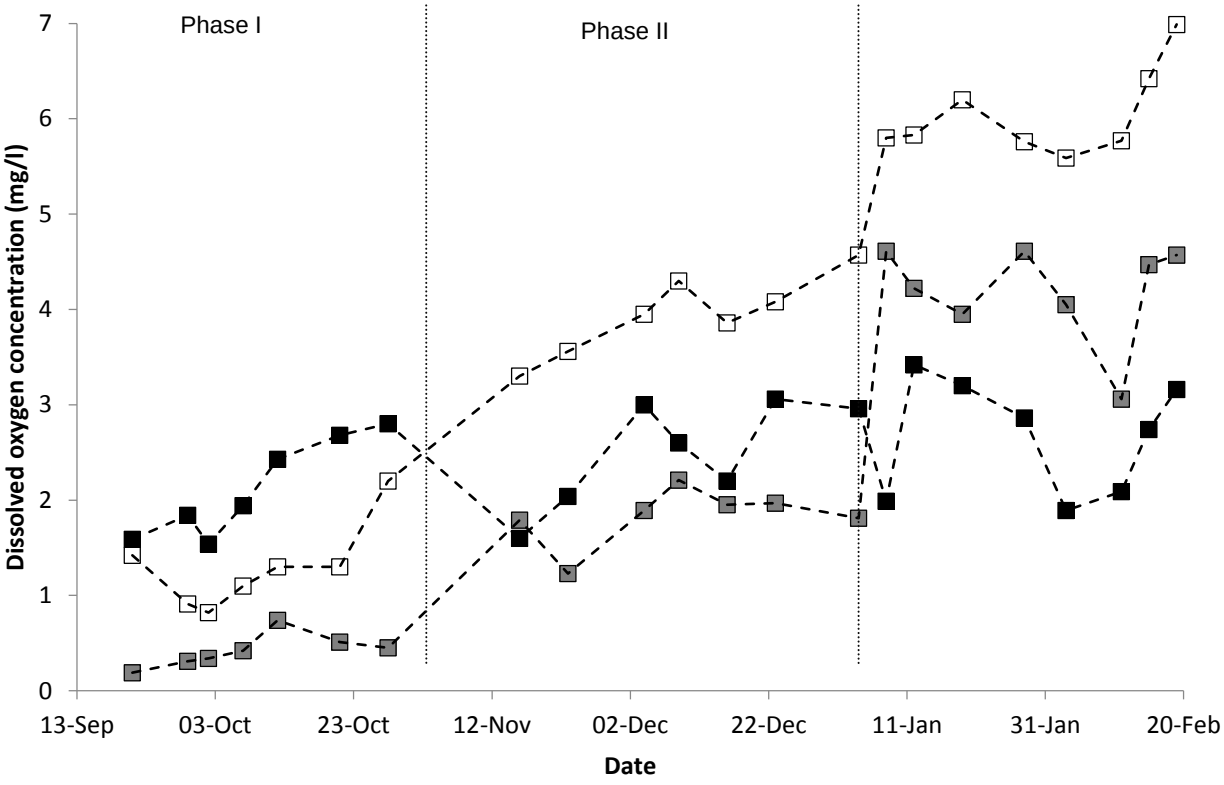

Figure 4. Average dissolved oxygen levels in the influent (black square), biozone (grey square) and effluent (clear square), during the 3 phases of the aeration experiment, moving from no artificial aeration on the left to full supplementary artificial aeration.

As flow rate and load (Table 1) remained largely constant, the most significant variable during the duration of each phase was dissolved oxygen. The positive effect of the artificial aeration on the DO of the bulk liquid in the biozone and the effluent is shown in Figure 4. After phase I, DO concentration in the biozone bulk liquid increased by 62 %, from 1.1 ± 0.6 to 1.9 ± 1.4 mg L^{-1}. After phase II DO concentration in the biozone bulk liquid increased by 138 %, from 1.9 ± 1.4 to 4.4 ± 0.6 mg L^{-1} (Table 2). This extra oxygen

availability translated to extra nitrification capacity and enhanced ammonium load removal at both higher and lower ammonium loads (Figure 5).

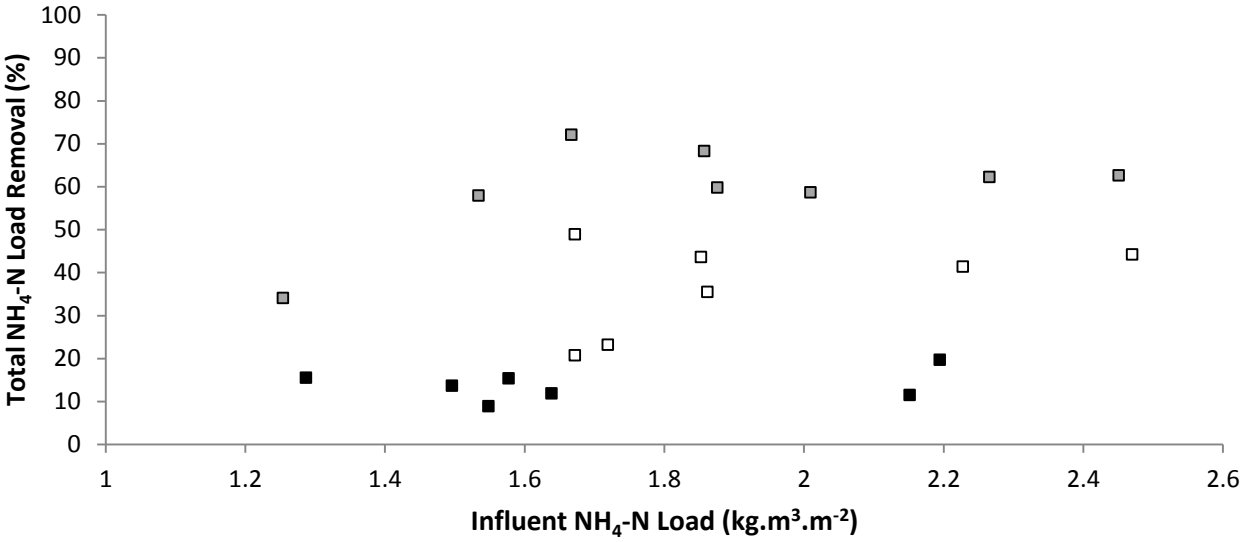

Figure 5. The effect of influent ammoniacal nitrogen load on total ammoniacal nitrogen removal during the 3 experimental phases. Phase I with no aeration (black squares), phase II with partial aeration (clear squares) and phase III with full aeration (grey squares).

The total ammoniacal nitrogen removal throughout the whole process is shown in Figure 5. In the non-aerated and DO limited phase (I), ammoniacal nitrogen load removal never exceeded 0.44 $kg.m^3.m^{-2}$ or 20 %. In this phase NH_4^+-N load removal remained fairly consistent, despite initial loading fluctuating. Aeration in phase II and III enabled much higher ammoniacal nitrogen load removal within the system. In the case of phase III, aeration enabled significantly enhanced ammoniacal nitrogen load removal at increased initial loadings, until around 1.6 $kg.m^3.m^{-2}$ where the effect of aeration diminished and ammoniacal nitrogen load removal plateaued out. ANOVA test analysis [$F(2,19) = 21.4$, $p = 0$] in conjunction with Tukey Post Hoc test analysis yielded a statistically significant result to reject the null hypothesis and proved the mean difference between all phases was significant to the 0.05 level. Therefore, aeration at approximately 2 mg L^{-1} significantly improved ammonium load removal compared to < 1 mg L^{-1} and 4 mg L^{-1} DO significantly improved ammonium load compared to 2 mg L^{-1}.

The biofilm mass in the first biozone increased by an average of 35 and 33 % in phase II and phase III, yet in the second biozone the average mass decreased by 12 and 40 %

respectively (Table 2). This indicates that aeration in the second biozone had a significant effect on biofilm accumulation.

Table 2. Summary of performance of RBC and biomass fluctuation during the 3 experimental aeration phases. Figures shown are an average over each phase, with the corresponding standard deviation figure.

		Phase I (< 1 mg L^{-1} DO)	Phase II (~ 2 mg L^{-1} DO)	Phase III (~ 4 mg L^{-1} DO)
Biofilm Mass ($mg.cm^{-2}$)	First Biozone	118 ± 17	159 ± 21	157 ± 16
	Second Biozone	140 ± 19	123 ± 26	84 ± 6
DO ($mg\ L^{-1}$)	Influent	2.7 ± 0.6	2.2 ± 0.8	2.3 ± 1.3
	Biozone	1.2 ± 0.6	1.9 ± 1.4	4.4 ± 0.6
	Effluent	3.4 ± 1.3	4.2 ± 1.4	6.4 ± 0.7
NH_4-N Load ($kg.m^3.m^{-2}$)	Influent	1.7 ± 0.3	1.9 ± 0.4	1.9 ± 0.4
	Biozone	1.6 ± 0.3	1.6 ± 0.3	1.4 ± 0.3
	Effluent	1.5 ± 0.3	1.2 ± 0.2	0.7 ± 0.2
BOD_5 Load ($kg.m^3.m^{-2}$)	Influent	7.4 ± 2.3	6.4 ± 3	6.7 ± 2.1
	Biozone	3.7 ± 0.7	3.3 ± 1.9	3.6 ± 1.1
	Effluent	1 ± 0.2	0.8 ± 0.6	0.6 ± 0.3

5.4 Discussion

Previous studies suggested that dissolved oxygen concentration must be at a minimum level of 2 mg L^{-1} for effective nitrification to occur (Odegaard *et al.*, 1994; Boller & Tshui, 1994; Downing & Nerenberg, 2008). Consequently, supplemental aeration has been recommended as an effective strategy for enhancing ammonium removal when DO is limited (Rodgers & Zhan, 2003). This study demonstrates that supplemental aeration in the second biozone can enhance DO concentrations in the bulk liquid (Figure 4) and this extra oxygen availability led to a significant increase in ammonium load removal capacity (ANOVA, $p > 0.05$) in overloaded conditions at 38 m^3. Ammonium load removal increased to up to 70% when the aeration in the RBC was 4 mg L^{-1} DO, compared

to a maximum of 20 % without the supplemental aeration and 55 % with 2 mg L^{-1} DO of supplemental aeration. This change in removal capacity occurred despite the fact that NH_4^+-N and BOD_5 load remained similar in all phases (Table 2).

Moreover, the maximum ammonium removal load occurred within a week of increasing aeration from 2 to 4 mg L^{-1}, which illustrates that artificial aeration can have a rapid impact. This finding suggests that aeration can be an effective mean to quickly remedy an overloaded or struggling biological wastewater treatment works. This is interesting data, to the authors' knowledge no similar reports have been published in the literature and this may suggest that AOM can recover ammonium oxidising activity rapidly after periods of stress. During phase III with supplemental aeration of 4 mg L^{-1} ammonium removal capacity was significantly enhanced, however effluent NH_4^+-N concentration was still relatively high, with a range of $9.8 \leq NH_4^+$-N (mg L^{-1}) ≥ 19.3. This indicates that supplemental aeration could be used to ensure consent compliance where 20 mg L^{-1} NH_4^+-N effluent consents are enforced, as is often the case at rural RBC WWTs.

For AOM activity to be significant, biodegradable compounds need to be sufficiently removed so that heterotropic microbes do not outcompete AOM (Nogueira *et al.,* 2002). Fortunately, in this trial, the RBC was effective at BOD_5 removal and artificial aeration allowed a greater proportion of BOD_5 to be removed (Table 2). This may have been a contributing factor in why AOM were more active and presumably more competitive.

An additional effect of supplemental aeration was the influence on biofilm thickness. This is important as the thicker the biofilm, the greater the barrier to mass transfer (Zahid & Ganczarczyk, 1994; Hassard *et al.,* 2015). Aeration led to a reduction in biofilm mass in the second biozone, particularly in phase III (Figure 2 & Table 2). The data shows that there was a trend of increasing biomass from phase I to phase II, supplemental aeration reversed this and it is not clear why this occurred. Possible explanations could be that the force of constant bubbling aeration applied to the biofilm resulted in detachment. As previously explained, aeration led to increased removal of BOD_5, and ammonium. An alternative explanation for why the biofilm thickness decreased may be that the reduced substrate availability restricted microbial growth, particularly fast-growing heterotrophs and therefore total microbial biomass and the abundance of their respective predators in the biofilm decreased.

This study was carried out on a full-scale RBC operating with genuine municipal wastewater. The unique opportunity came with great advantages such as the provision of data that is readily applicable to industry and therefore can be used to develop optimisation strategies, among other things. In contrast, at lab-scale, data collected is often not applicable and needs to be theoretically manipulated in order to be meaningful. However, this situation also came with disadvantages. To study the consequence of aeration effectively, the RBC had to be manipulated into an overloaded state which was induced by a large incremental increases in flow rate. This presented a problem, which should be considered if this type of study is to be conducted again. Increasing the flow rate led to a reduction in hydraulic retention time (HRT) which reduced the time that nitrifying microbes are exposed to the wastewater. Therefore, ammonium removal efficacy decreases as a result. Unfortunately, with the pilot scale set-up in this study, flow was the only way to alter loading rate. This may have resulted in data that underestimated the actual effect of aeration, had the RBC been operated at optimal HRT. A smaller set-up may have been more suitable in this regard, as nutrient concentration in the wastewater could have been manipulated by the addition/subtraction of reagents and therefore HRT could be maintained at a relatively optimal level.

RBCs are often employed at small WWTs due to their low energy and maintenance, low land requirements, low capital requirements and suitability for decentralised, rural networks (Dutta *et al.*, 2007). Any supplementary aeration tool would ideally complement these attributes. The pump used in this study was unsophisticated, low energy and cheap, compatible with 240 V mains power supply and the total set-up including pipework, air stones and pump only cost £150. At only 40 W this particular pump costs less than £1 per week to run. Therefore, the CAPEX and OPEX for this pump is negligible compared to the overall budget of WWTs and would be negligible in the face of a regulatory fine for breaching effluent consent limits, which routinely and publically exceed thousands of pounds. This study has shown that supplemental artificial aeration is a 'plug & play' strategy that can be implemented as a solution to poor nitrifying performance in overloaded RBCs with a relatively low capital, operating and energy cost, with negligible maintenance and rapid implementation.

Acknowledgements:

This work was co-funded by the Natural Environment Research Council (NERC) (Grant reference NE/I019367/1) and Severn Trent Water (STW). Special thanks to STW for giving me access to their treatment works and internal databases which allowed this study to happen.

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CHAPTER 6

AOM ABUNDANCE AND DIVERSITY

6 DIVERSITY AND ABUNDANCE OF AMMONIA OXIDISING MICROBES IN ROTATING BIOLOGICAL CONTACTORS

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Abstract

Ammonia oxidising microorganisms (AOM) play an important role in ammonia removal in rotating biological contactor (RBC) wastewater treatment systems. However, little is known on the effects of environmental factors within RBC on the population dynamics of AOM. In this study, ammonia oxidising bacteria (AOB) and archaea (AOA) community structure and abundance within microbial biofilms of seven full-scale RBC systems was examined by high-throughput sequencing of 16S rRNA gene amplicons and quantitative PCR of the ammonia monooxygenase (*amoA*) genes. Illumina Next Generation sequencing of the 16S rRNA gene showed that *Nitrosomonas* (e.g. *Nitrosomonas ureae*) and *Nitrososphaera* (e.g. *Candidatus nitrososphaera* SCA1145) were the most abundant AOB and AOA, respectively. Although this finding has been reported numerous times in WWTs, to the authors knowledge this is the first report for RBCs. This study presents evidence that whilst AOA were the dominant ammonia oxidisers in most samples, AOB were more competitive in environment of lower loading and higher oxygen availability. Such an environment is often encountered at the rear of RBC systems. In our study the mean *amoA* AOB gene copy number per gram of biofilm was two orders of magnitude higher than for AOA (1.48×10^8 AOB compared to 1.1×10^6 AOA across the seven full scale RBC treatment systems. Whereas, AOB were dominant in optimal conditions, AOA maintained 'background activity,' despite the load in which the biofilm was exposed. Further, diversity has a significant correlation with

AOA abundance. Our findings demonstrate that Next Generation sequencing provides a powerful tool to elucidate the microbial community composition (particularly the ammonia-oxidisers) in RBC biofilms. Gaining greater insights into AOM community dynamics in RBC systems will help to guide optimisation strategies for more effective ammonium removal.

Keywords: Biofilm, Rotating Biological Contactor, Nitrification, Wastewater Treatment, Ammonia Oxidising Bacteria, Ammonia Oxidising Archaea

6.1 Introduction

Nitrification influences the availability of two major nitrogen compounds (ammonium and nitrate) in nature (Bollman *et al.,* 2014). Removal of these nitrogen compounds is required in wastewater treatment systems to mitigate the toxic eutrophication process (Erisman *et al.*, 2013) which can lead to hypoxia (Rabalais *et al.*, 2010) and algal blooms (Anderson *et al.,* 2002) in receiving water body. Aerobic nitrification is a microbial mediated process that oxidises ammonium (NH_4^+) to yield nitrite (NO_2^-) which is subsequently oxidised to nitrate (NO_3^-). Ammonium oxidation is the first and often rate-limiting step of nitrification in wastewater treatment systems (WWTs) (Sinha & Annachhatre, 2007; Choi & Hu *et al.,* 2008; Murphy *et al.,* 2009). For this reason, ammonium removal is particularly important to wastewater treatment and Governments have acted on this by implementing ammonium effluent environmental quality standards (EQS) which encourage more stringent treatment of wastewater.

Originally, chemolithoautotrophic ammonia oxidising bacteria (AOB) affiliated with β- and γ-proteobacteria were thought to be the key actors for ammonia oxidation as they have been shown to possess the ammonia monooxygenase α-subunit (amoA) gene for ammonia monooxygenase (AMO), the key enzyme of nitrification (Madigan *et al.,* 2000). Since both AOB and AOA are responsible for ammonium removal in WWTs, it is important to understand which environmental factors influence AOB/AOA abundance, diversity and activity in WWT treatment technologies such as rotating biological contactors (RBCs).

In WWTs AOA have been found to dominate over AOB, especially in low ammonia environments (Yan *et al.,* 2012; Erguder *et al.,* 2009; You *et al.,* 2009). The ability of AOA to outcompete AOB is partly due to their superior ammonium affinity (Ks), reported as being 0.02 gN m^3 which is 100 lower than the values reported for AOB (Martens-Habben *et al.,* 2009; Pan *et al.,* 2016). As well as higher ammonium affinity, AOA also possess higher oxygen affinity than AOB (Stahl *et al.,* 2012). Consequently, AOA have been shown to exhibit higher activity than AOB under very low DO conditions of 0.1 mg L^{-1} in municipal WWTs (Park *et al.,* 2006; You *et al.*, 2009; Fitzgerald *et al.,* 2015). Despite the evidence that AOA can outcompete AOB in WWTs, a survey of 52 WWTs showed that AOA were only detected 8 % of the time (Mussmann *et al.*, 2011).

Furthermore, when AOA are detected in WWTs they often exhibit lower activity and lower abundance than AOB (Zhang *et al.,* 2011; Gao *et al.,* 2013). Even in wastewater environments with higher populations of AOA, it has been suggested that AOB could still represent the most active microbial group (Wells *et al.,* 2009). Thus, it remains unclear under which environmental conditions AOB and AOA will dominate and what their relative contributions to ammonia removal are in WWTs. Gaining insights into these aspects can contribute to the improvement of process efficiency in terms of ammonium removal especially for RBC which are often employed at small WWTs due to their low energy and maintenance, low land requirements, low capital requirements and suitability for decentralised rural networks (Dutta *et al.,* 2007). They are used to perform ammonium removal with a range of influent characteristics and have the potential to yield effluent quality similar to that of activated sludge plants (AS) (Cortez *et al.,* 2008; Tchobanoglous *et al.,* 2013).

The biofilm carries out the bulk of wastewater treatment in RBC, itis constituted of gradients in terms of both oxygen and substrate availability, which results in oxic and anoxic layers (Stewart & Franklin, 2008; Lu & Chandran, 2010). This encourages the establishment of diverse microbial consortia with various metabolic activities that thrive in a collaborative manner compared to suspended systems (Dutta *et al.,* 2007; Stewart & Franklin, 2008; Lu & Chandran, 2010). For instance, it has been shown using fluorescence *in situ* hybridisation (FISH) that greater abundance of AOB is observed in the surface layer of aerobic biofilms where ammonia concentration is the highest, however due to heterogeneous substrate availability a dynamic AOB consortia is present throughout the layers (Truu *et al.,* 2005; Downing & Nerenberg, 2008). In contrast, AOAs superior affinity for oxygen and ammonia, suggests that AOA communities tend to occupy deeper layers within the biofilms where these two substrates are more limited due to mass transfer.

The varying affinities for ammonia may explain the different growth patterns of ammonia-oxidisers throughout the structure of the biofilm in various niches (Pan *et al.,* 2016).

Here we present a study investigating the abundance and diversity of AOB and AOA in seven full-scale RBC systems using next Generation sequencing of 16S rRNA gene

amplicons and qPCR of the AOB and AOA *amoA* genes. The RBC biofilms were investigated to reveal the specific species, the diversity and the abundance of ammonia-oxidisers within the microbial consortia. Our main objectives were to answer the following questions: (i) what is the diversity and abundance of AOB and AOA within biofilms of RBC systems? (ii) How similar are these communities between different distribution systems and environmental conditions? This approach provided useful insight into the distribution, dynamics and relative contribution of AOB and AOA in RBC systems for ammonia removal and how environmental factors can affect their dynamics and activities.

6.2 Materials and Methods

6.2.1 RBC Sampling

Biofilm samples were taken from 7 wastewater treatment works (WWTs) in the Midlands area of the United Kingdom (Table 1). Each WWT contained between 1 and 3 RBCs. Biofilm samples were taken from WWT 1 over a period of one year in two phases between November 2014 and February 2015, and August 2015 and November 2015. During this period the RBC was operated under increasing load from 12 m^3/day to 38 m^3/day. Access hatches enabled samples to be taken from the front, the middle and the rear of the RBC at WWT 1. A 36 cm^2 circular area of biofilm was scraped from the disk using a sterile spatula and stored in an insulated box with ice packs during transportation to the laboratory before analysis. Biofilm samples were taken from WWTs 2 – 7 over a period of 1 month between November 2015 and December 2015, access hatches only enabled samples to be taken from the front and/or the rear of the RBC in this case. For a summary of sample details from WWTs 1 – 7, see Table S2. The weight of the wet sample was noted prior to being stored at −80 °C.

Table 1. Attributes of the 7 full-scale WWTs from which biofilm samples were taken and analysed.

	Design Population Equivalent	DWF flow (m^3/day)	No. of RBCs	Comments
WWT 1	50	12 - 38	1	
WWT 2	438	147	1	New RBC in 2012
WWT 3	572	223	2	
WWT 4	602	230	3	
WWT 5	463	140	3	
WWT 6	167	45	1	Ongoing filamentous bacteria (*Beggiatoa)* infection
WWT 7	11	< 5	1	RBC extremely small

*DWF = Dry Weather Flow

6.2.2 DNA Isolation

Total nucleic acids were extracted from 0.3g (wet weight) biofilm samples according to Griffiths *et al.*, (2000) using the Powersoil DNA isolation kit (Mo Bio Laboratories, USA) according to the manufacturer's instructions and stored at −80 °C. Procedural blanks were included throughout..

6.2.3 qPCR of AOB and AOA *amoA* genes

qPCR for *amoA* genes of AOB and AOA were performed on a CFX96 Real-Time Detection System (Bio-Rad, USA). The *amoA* gene fragment of AOB was amplified using the primers amoA-1F (5'- GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCTCKGSAAAGCCTTCTTC-3') (Rotthauwe *et al.,* 1997). The *amoA* gene fragment of AOA was amplified using the primers CrenamoA23F (5'-ATGGTCTGGCTWAGACG-3') and CrenamoA616R (5'-GCCATACABCKRTANGTCCA-3') (Tourna *et al.,* 2008). For both AOB and AOA *amoA* genes, each 15 µl reaction mixture contained 1 µl of DNA template, 7.5 µl of 2× RedTaq Ready Mix (Sigma, UK), and a 1 µM concentration of each primer. A

dissociation curve analysis was performed at the end of each reaction to verify amplification of a single PCR product. The qPCR conditions for both AOA and AOB reactions were set as follows: initial denaturation for 3 min at 95 °C, followed by 40 cycles consisting of 95 °C for 5 s, then 60 °C for 30 s,. Negative control was performed with sterile water as the template instead of the extracted DNA to detect any contamination. The samples were quantified against the corresponding standard curve using CFX Manager version 2.0 software (Bio-Rad).

DNA standards of known quantity were created using a dilution series of previously extracted amoA clones from amplification of sediment DNA extracts (obtained from University of Essex). The target abundance for standards was calculated by assuming a molecular mass of 660 Da for double-stranded DNA using the following formula: gene abundance = 6.023×10^{23} (copies mol^{-1}) × standard concentration (g ml^{-1})/molecular mass (g mol^{-1}).

DNA standard ranging from 10^2 to 10^7 target genes ml^{-1} for AOB *amoA* and AOA *amoA*. Standards, samples, and no-template controls (NTC) were amplified in duplicate with each primer set. The standard curve correlation coefficient (R^2) and amplification efficiencies (%) for the AOB and AOA were 0.981, 95% and 0.997, 96%, respectively. If C_Q values between duplicate sample reactions exceeded 1 then data was discarded.

6.2.4 Sequencing of 16S rRNA gene amplicons

For bacterial 16S rRNA gene libraries, primers 341F (5'- CTACGGGNGGCWGCAG-3') and 805R (5' GACTACHVGGGTATCTAATCC-3') were applied (Herlemann *et al.*, 2011). For archaeal 16S rRNA gene libraries, primers A189-F pmoA (5'-GGNGACTGGGACTTCTGG -3') and A650-R pmoA (5' ACGTCCTTACCGAAGGT -3') were applied (Bourne *et al.*, 2001). Samples were PCR amplified with a Veriti 96-Well Thermal Recycler (Applied Biosystems, UK) using the following conditions: initial denaturation for 3 min at 95 °C, followed by 28 cycles consisting of 95 °C for 30 s, then 55 °C for 30 s, and 72 °C for 30 s and finally 72 °C for 5 mins. Each reaction mixture contained 1 µl of DNA template, 12.5 µl of 2× RedTaq Ready Mix (Sigma, UK), and a 5 µl of each primer (1µM). Following PCR amplification of archaea and bacteria 16S rRNA genes for each initial sample of extracted DNA, the products were cleaned up using

AMPure XP beads in conjunction with a SPRI super magnetic plate (Beckman Coulter, UK). Nextera XT identification indexs (Illumina, UK) and flow cell adapters were then applied to the 16S rRNA amplicons, with each reaction containing 5 µl of 16S rRNA amplicon product, 25 µl of 2 × RedTaq Ready Mix (Sigma, UK), and a 5 µl of each index primer. The second PCR was run under the same conditions as the first, except with 8 cycles. The PCR product was cleaned using the AMPure XP beads in conjunction with a SPRI super magnetic plate (Beckman Coulter, UK). A 1:10 dilution of PCR samples was the used to quantify the products using the PicoGreen (ThermoFisher, UK) assay and fluorescence was measured using FLUOstar Omega microplate reader (BMG, UK). The purified 16S amplicon product with attached barcoded index sequences was pyrosequenced by The Earlham Institute (formerly The Genome Analysis Centre; Norfolk, UK) using a MiSeq Reagent Kit v3 (600-cycle) on an Illumina MiSeq instrument..

6.2.5 Analysis of the sequencing data

The sequencing reads were analysed using the QIIME pipeline (Caporaso *et al.*, 2010). Sequences were quality filtered to remove any sequences below Q20, chimeras, or sequences that contained above 6 ambiguous bases or homopolymer runs. The quality filtered reads were clustered into operational taxonomic units (OTUs) using the UCLUST algorithm (Edgar, 2010) at the 0.97 level. 16S rRNA representative sequences from each OTU were assigned taxonomic identities with the RDP classifier (Wang et al 2007).

6.2.6 Statistical and phylogenetic analysis

A two-tailed Pearson's correlation test was conducted with the SPSS (version 22) statistical programme to assess the correlation between cell copy number (abundance) and Shannon-Wiener Index (diversity). Shannon-Wiener Diversity Index scores were calculated using Microsoft Excel, based on the following equation:

$$H = -\sum_{i=1}^{s} \mathrm{p}_i \ln \mathrm{p}_i$$

Where p_i is the proportion of OTUs that were matched with specific species in any given sample, *s* is the number of OTUs or species richness. As the score is based on the sum (p

× ln(p)) a sample with a diverse spread of species that are relatively evenly distribute will report a high score.

A Draftsman Plot in conjunction with Principal component analysis (PCA) was used to determine relationships between AOA and AOB communities, bacterial and archaeal *amoA* gene copy numbers and the environmental parameters (Figure S1 & S2).

6.3 Results

6.3.1 Archaeal and bacterial community diversity

Across the 7 RBC systems investigated, 62 biofilm samples were collected as summarised in Table S2. A total of 1 286 478 OTUs relating to archaeal and bacterial species were identified across all the WWTs. Of these OTUs only 97 869 (8 %) were related to archaea and 1 188 609 (92 %) were related to bacteria (Table S2). Of the archaea, *Halobacteriaceae* was the most dominant. Although they are ubiquitous among many aerobic environments, this is a surprising finding nonetheless and one that has not been reported previously in RBCs. AOA relating to *Nitrososphaeraceae* were also identified across the RBC biofilm samples (Figure 1, Figure 2a and Table S3), which represented 2 % of total archaeal families in terms of total sequences. *Candidatus nitrososphaera* SCA1145 was by far the most dominant species (92 %) followed by *Candidatus nitrososphaera* SCA 1170 and *Candidatus nitrososphaera gargensis* (Figure 1).

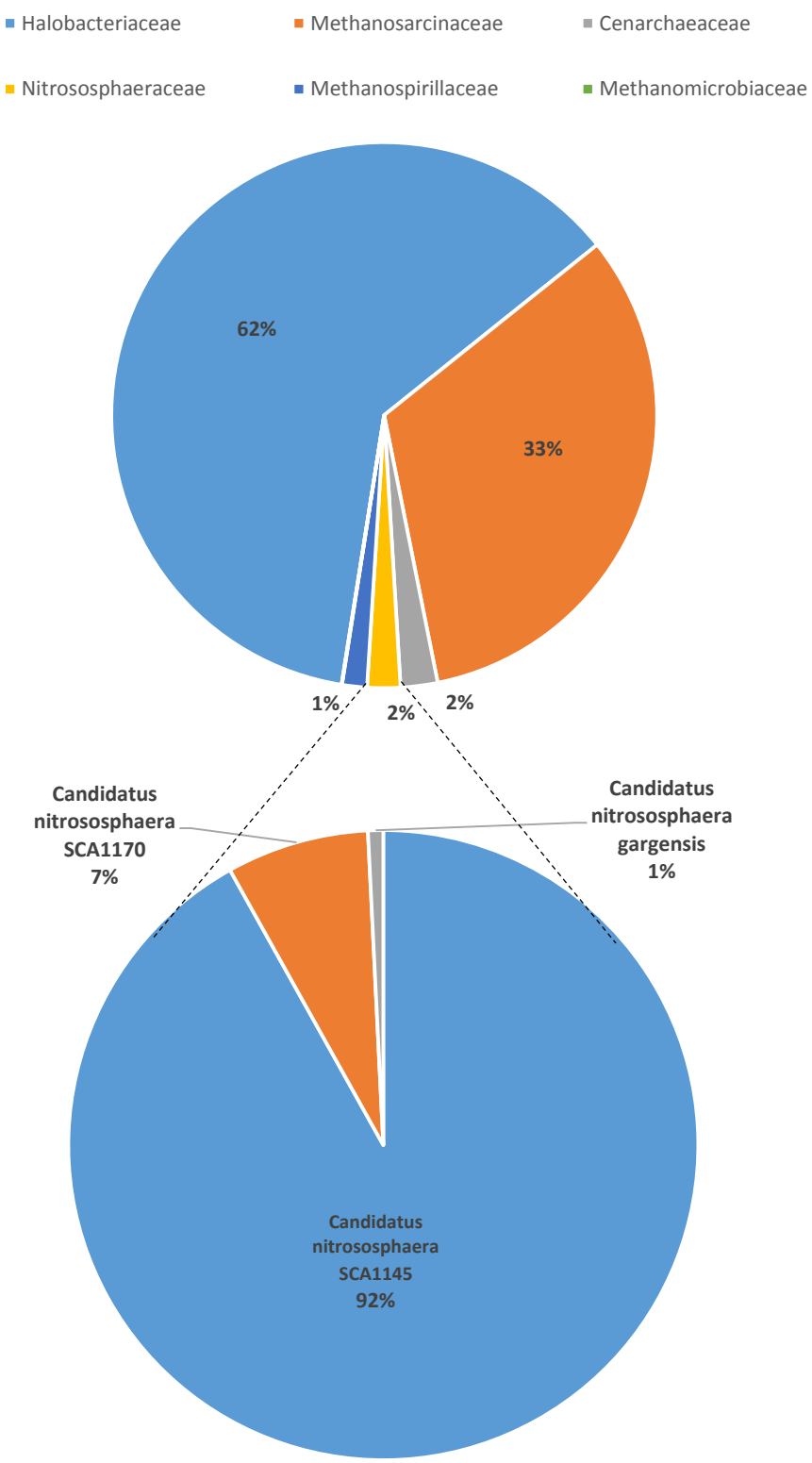

Figure 1. The relative abundance (% of total OTUs) of archaeal families and *Nitrososphaera* species across all RBC biofilm samples (n = 62).

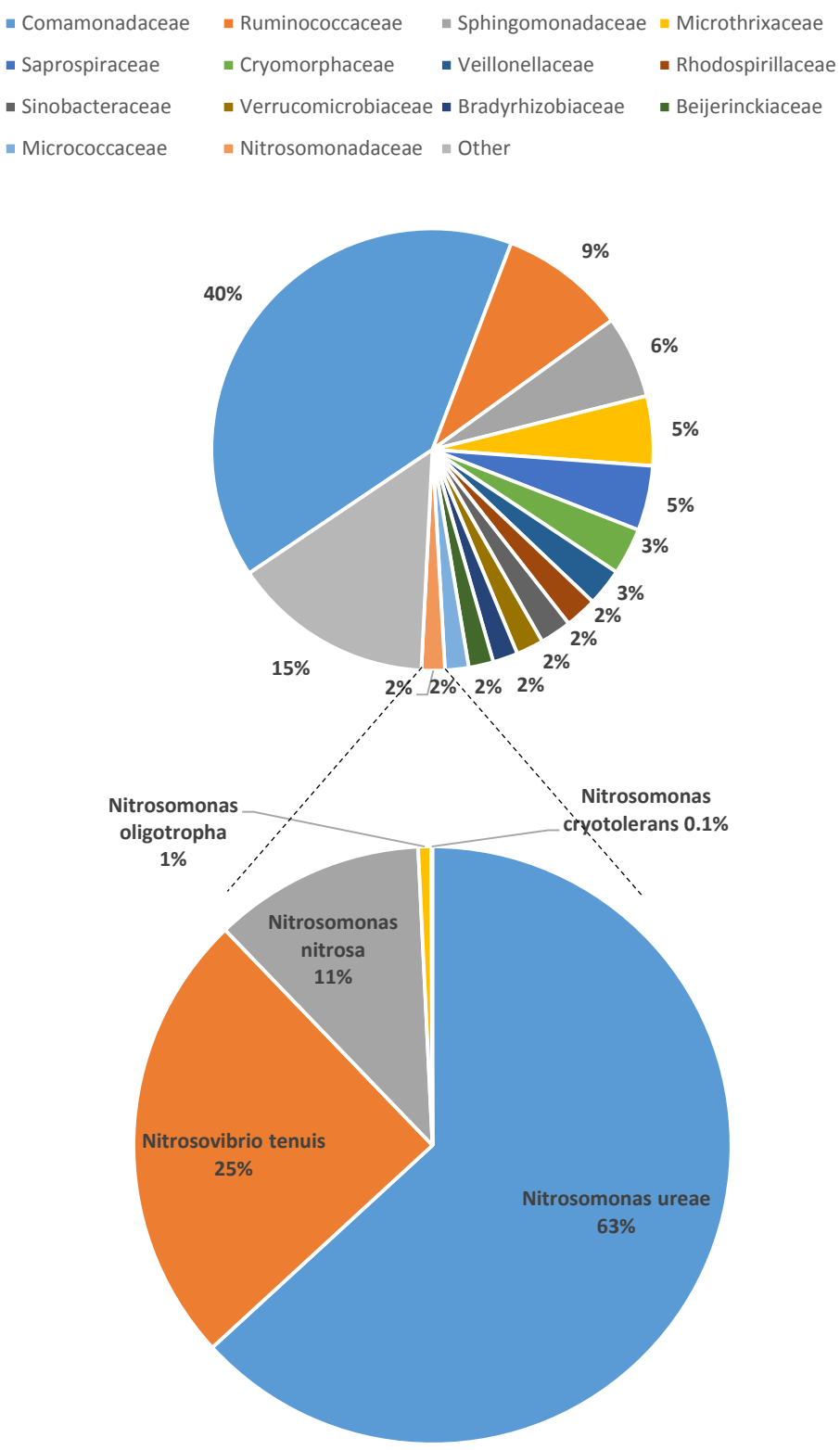

Figure 2. The relative abundance (% of total OTUs) of bacterial families and *Nitrosomonadaceae* species across all RBC biofilm samples (n = 62).

The most dominant bacterial OTUs (40%) were affiliated to the *Comamonadacea* family which consists of aerobic and rod-shaped bacteria. Among the OTUs identified, a bacterial species of interest is the potentially lethal pathogen *Bacillus anthracis*, responsible for producing the toxin anthrax, indicating the importance of pathogen mitigation in wastewater treatment. All AOB were affiliated with the *Nitrosomonadaceae* family which represented only 2% of the total bacterial OTUs (Figure 2). Among the *Nitrosomonadaceae* family, *Nitrosomonas* genus represent 75 % the AOB group and *Nitrosomonas ureae* was the most dominant (63 %) AOB species.

6.3.2 AOA and AOB community structure

The RBCs from WWT 4, 6 and 7 were dominated by AOB particularly *Nitrosomonas ureae*. Between 17 % and 98 % of OTUs are related to *Nitrosomonas ureae* in WWTs 3 – 7. In contrast, RBCs from WWT 1, 2, 3 and 5 are dominated by AOA e.g. *Candidatus nitrososphaera*. Between 97 and 99 % of OTUs are related to *Candidatus nitrososphaeara SCA* sp. in WWTs 1 and 2.

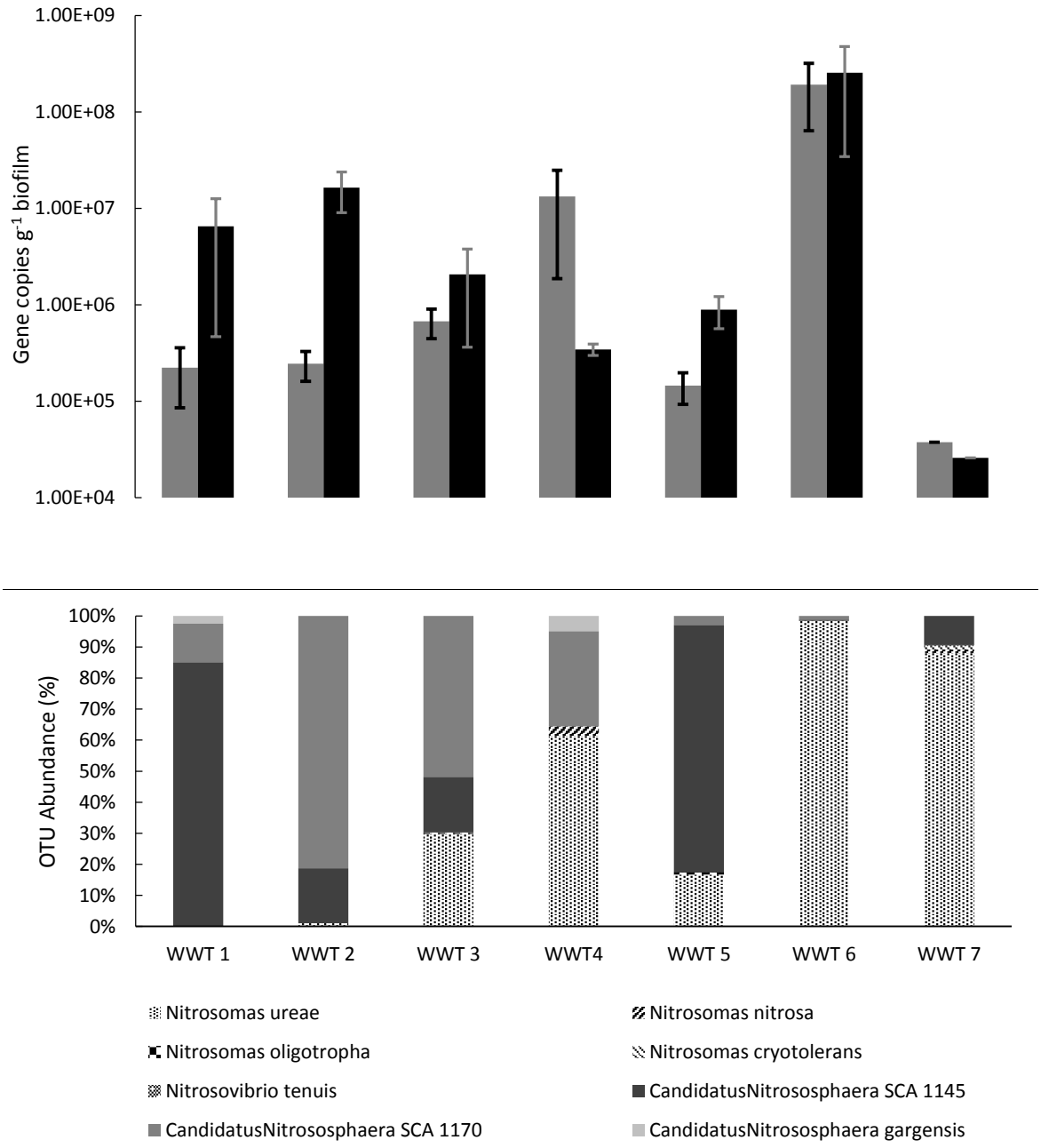

Figure 3. The *amoA* gene copies g^{-1} biofilm (mean ± SD) for AOA (grey) and AOB (black), and relative proportions of AOB for each full-scale WWT. Gene copy numbers are expressed as mean ± standard deviation.

The *amoA* gene copy number per gram of biofilm ranged from 3.8 x 10^4 in WWT 7 to 1.9 x 10^8 in WWT 6 for AOA. For AOB the gene copy number ranged from 2.6 x 10^4 to 2.6

x 10^8 in the same WWTs. WWTs 6 exhibited the highest abundance of both AOA and AOB in contrast to WWTs 7 which had the lowest abundance of both AOA and AOB. According to Table 2, WWT 4 and 6 had excellent quality effluent and also exhibited the highest abundance of AOA. On the other hand, WWT 7 had low effluent quality and exhibited the lowest abundance of both AOA and AOB.

WWTs 1 - 3 exhibit more AOA OTUs, but greater AOB gene copy number and WWTs 4 and 7 exhibit more AOB OTUs, but greater AOA gene copy number (Figure 3). Therefore, AOA OTU abundance does not appear to affect AOA gene copy number overall, and the same applies to AOB.

Table 2. Ammonium removal capacity of the 7 WWTs 10 months and 2 weeks' prior biofilm samples collection

			10 months prior to sampling			**2 weeks prior to sampling**		
	NH_4^+ effluent consent	**DWFflow (m^3/day)**	**NH_4^+ effluent mean (mg/L)**	**NH_4^+ effluent st dev**	**NH_4^+ effluent count (n)**	**NH_4^+ effluent mean (mg/L)**	**NH_4^+ effluent st dev**	**NH_4^+ effluent count (n)**
WWT 1*[1]	NONE	12 - 38	17.4	10.8	28	32.9	6.4	3
WWT 2	10	147	N/A	N/A	N/A	N/A	N/A	N/A
WWT 3	10	223	3.42	3.3	11	0.1	0	1
WWT 4	5	230	0.29	0.4	13	0.1	0	5
WWT 5	NONE	140	2.8	3.9	40	3.4	3.8	3
WWT 6	NONE	45	2.4	2.5	44	2.3	2.1	5
WWT 7	NONE	< 5	2.6	4.5	10	6	7.9	3

*DWF = Dry Weather Flow; N/A = no data available; *[1] data is prior to final sample taken, however samples were taken over a period of a year

WWTs 3 and 4 had the greatest influent flow, whilst also having good effluent quality for ten months prior to the sampling and excellent effluent quality for the two-week period

prior the sampling (particularly WWT 4; Table 2). In comparison to the other WWTs, these two WWTs exhibited the most balanced AOM distribution in terms of AOA and AOB OTU proportion (WWT 3 – AOA 30 % AOB 70 %; WWT 4 - AOA 64 % AOB 36 %). The other WWTs were almost entirely dominated by either AOA or AOB OTUs. The fact that the two WWTs with superior effluent quality had the most balanced AOM community may be coincidental, as a two-tail Pearson's correlation test confirmed that there was no significant relationship between abundance or diversity and ammonium effluent concentration across WWTs 3 - 7.

In terms of gene copy number, AOA:AOB ratios (Table S3) of 0.8 and 1.5 indicated there was similar abundances of AOA and AOB in WWTs 6 and 7. Both WWTs received low flow (5 – 45 m^3/day). In contrast, AOA quantitatively dominated over AOB in WWT 4 (AOA:AOB = 39), which received high flow (240 m^3/day).

6.3.3 AOB and AOA distribution and dynamics within the biozones of WWT 1

All RBCs in this study consisted of two biozones, which are a collection of disks separated by a baffle. Access hatches at WWT 1 enabled samples to be taken at two locations in each biozone – the front and rear of each biozone. Zone 1 being the front of the process and zone 4 being the rear. Moving from the front to the rear there is a clear difference between abundance, both in terms of OTUs identified and amoA bacterial and archaeal 16S rRNA gene copies (Figure 4). In fact, mean AOB *amoA* gene copy number steadily increases from 2.77×10^5 to 1.48×10^8 from zone 1 to zone 4. On the other hand, AOA *amoA* gene copy number per gram of biofilm was similar throughout the process, with mean gene copy numbers ranging from 4.89×10^5 to 2.39×10^6.

AOA abundance in terms of OTU number is significantly higher at all stages, representing 99, 96, 89 and 55 % for stages 1, 2, 3 and 4, respectively. A notable observation is that higher OTU number related to AOB corresponds to higher *amoA* gene copy number, this is most evident in zone 4 where AOB abundance in terms of OTU is 45 % and AOB exhibit a significantly higher bacterial 16S rRNA gene copy number compared to AOA

(Figure 4). Figure 4 further shows that zone 3 in the second biozone exhibits high abundance in terms of both OTU number and *amoA* gene copy number.

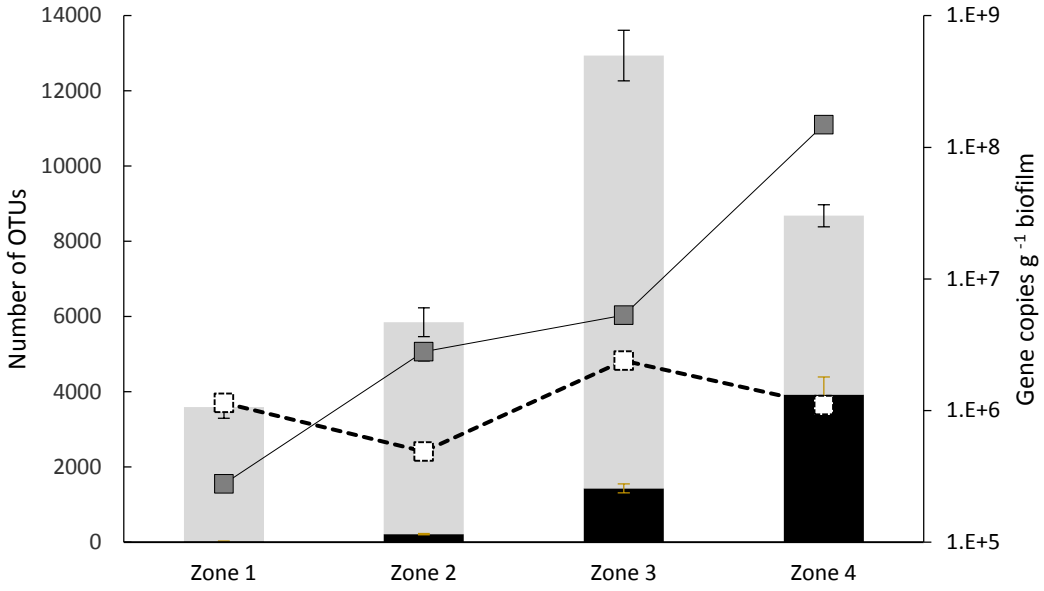

Figure 4. The relative abundance of AOB (black) and AOA (grey) affiliated OTUs and the mean *amoA* bacterial (grey square) and *amoA* archaeal (white square)16S rRNA gene abundance in the front and rear of the two biozones of the RBC WWT 1 (n = 40). Standard deviation is shown with error bars.

Figure 5 represents biofilm samples taken on a monthly basis from zone 3 over two phases which are delineated by the dashed line. The first phase corresponds to loading of 12 m^3/day, where the RBC was nitrifying well (between 4 and 20 % NH_4^+-N remaining in December and January). However, November 13th was only 1 month after the initial start-up and so the biofilm was still acclimatising and did not reach steady-state until December. The second phase instead corresponds to loading of 38 m^3/day, where the RBC was overloaded and nitrification capacity was minimal (between 34 and 85 % NH_4^+-N remaining from March to October). When the RBC was performing well during the first phase, the AOB *amoA* gene copy number per gram of biofilm was at its greatest, reaching 9.2 x 10^7 and was significantly higher than AOA *amoA* gene copy number (1 or 2 orders of magnitude). On the other hand, AOA *amoA* gene copy number remained consistent ranging between 6.1 x 10^4 and 9.8 x 10^5 gene copies per gram of biofilm throughout the two phases. When the RBC was overloaded and nitrification was limited, the AOA *amoA* gene copy number was the most abundant and could reach one order of magnitude difference higher than AOB *amoA* gene copies (Figure 5, August 2016).

In terms of OTUs identified AOA dominated in every sample, except in January. The four greatest AOB abundance occurred when the samples also exhibited the highest number of AOB OTUs. AOA and AOB OTU number were both higher in the first phase, compared to the second phase indicating that there was less AOM species present as load increased to a very high level of 38 m^3/day.

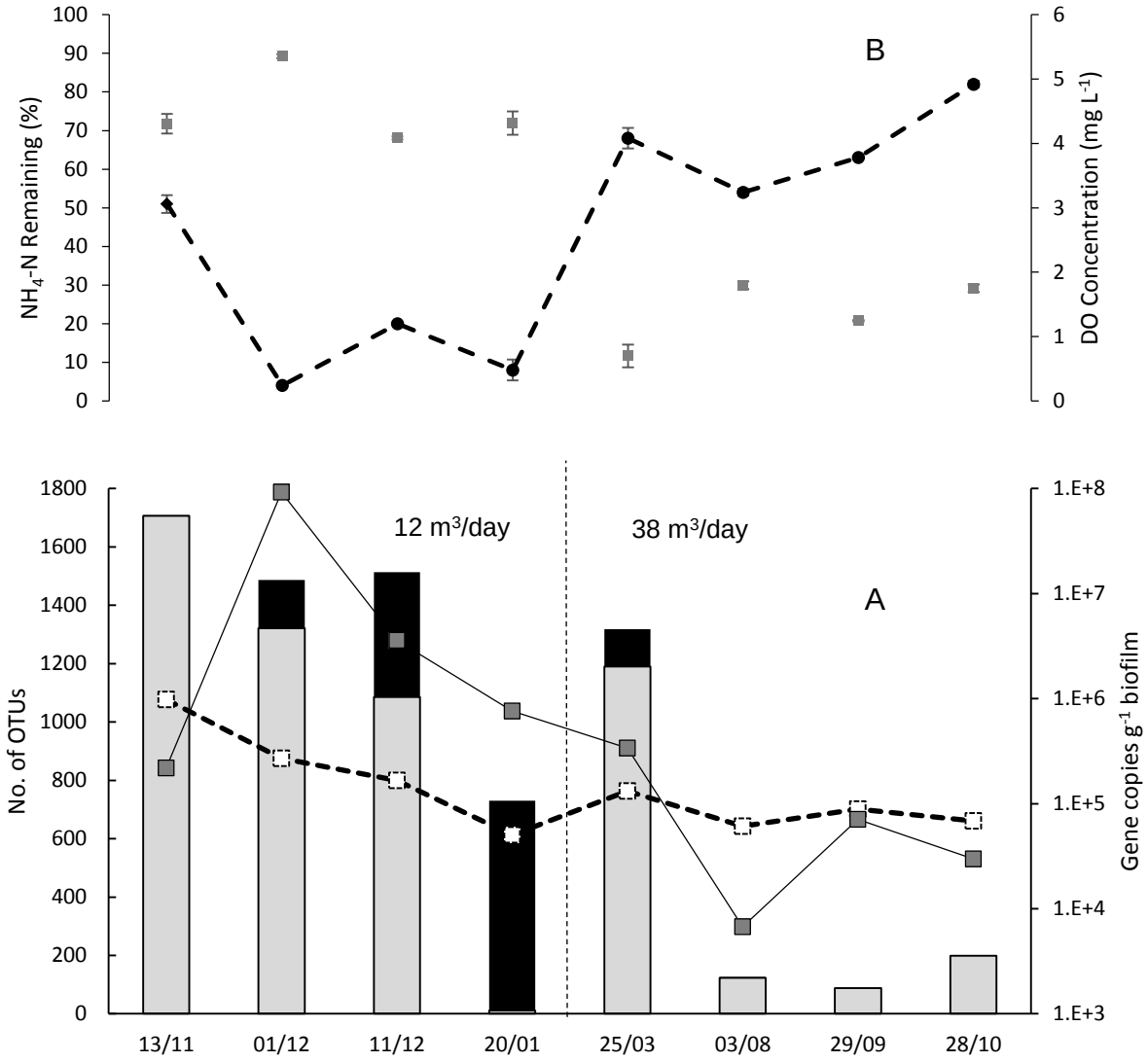

Figure 5. (a) The relative abundance of AOB (black) and AOA (grey) OTUs and the mean *amoA* bacterial (grey square) and archaeal (white square) gene abundance (copies per gram biofilm) in zone 3 of the RBC biofilm from WWT 1 over 1 year. **(b)** associated ammonium removal performance (black circle) and dissolved oxygen concentration (grey circle) in the bulk liquid (n = 8).

Overall the data indicate that bacteria dominated quantitatively over archaea and played the most significant role in WWT 1. Also, AOA and AOB OTU number were both greater in the first phase, compared to the second phase indicating that there was less AOM present as load increased to a very high level of 38 m^3/day.

OTU data related to AOA and AOB species for each biofilm sample was converted using the Shannon-Wiener (SW) index to illustrate diversity of AOA and AOB species. This was compared against *amoA* gene copies for AOA and AOB to illustrate abundance (Figure 6). However, samples which displayed no AOM activity were omitted.

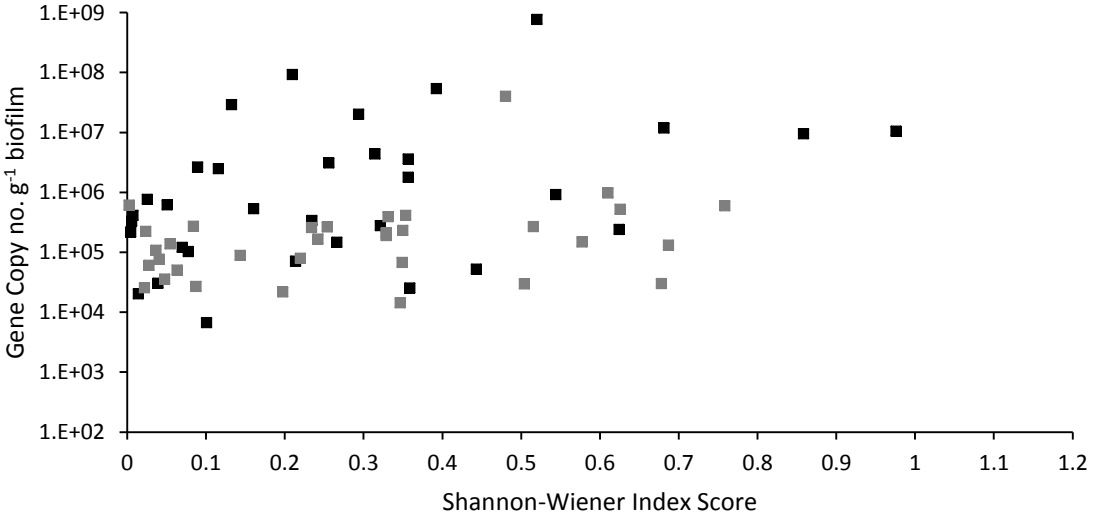

Figure 6. The relationship between ammonia-oxidiser diversity (Shannon-Weiner index score) and AOM abundance (gene copies per gram of wet biofilm) for AOA (grey) and AOB (black) n = 46.

Figure 6 shows a trend between diversity and abundance. At the lower end of the SW score there are many data points (AOA, n = 16; AOB, n = 17) that have an index score < 0.1. The gene copy number per gram of biofilm for these samples never exceeded 6.1 x 10^5 and 2.6 x 10^6 gene copies for AOA and AOB, respectively. As the SW score increases from 0.1 the variance related to gene copy number also increases, with gene copy number ranging from 1.4 x 10^4 to 4 x 10^7 for AOA and from 2.5 x 10^4 to 7.7 x 10^8 for AOB. A two-tail Pearson's correlation test confirmed a significant correlation between abundance and diversity ($r = 0.442$; $p < 0.001$) within the AOA community (Table 3). However, no

correlation was found between total AOM diversity (including AOA and AOB) and separate AOA/AOB abundance.

Table 3. Pearson correlations of AOA and AOB abundances (*amoA* gene copies per gram biofilm) and AOM diversity (Shannon Wiener Index) (n = 54). * indicates a significant correlation at the 0.01 level (2-tailed).

	AOA Abundance	AOB Abundance
AOA Diversity (SW)	0.422*	-0.19
AOB Diversity (SW)	-0.133	0.16
AOM Diversity (SW)	.213	-0.19

Further relationships between AOM diversity, AOA/AOB abundance, dissolved oxygen, ammonium effluent concentration, biofilm weight, design PE and flow were investigated using Draftman Plot and Principle Component Analysis (Figure S1 and 2), however no significant relationships could be identified.

6.4 Discussion

It is widely accepted that the *amoA* gene is involved in the process of ammonium oxidation in AOM (Tourna *et al.*, 2008) and so by quantifying the gene copies we can make assumptions about the abundance of these organisms. Such an approach has already been used to shed light on the interactions between AOM and their environment, for instance, due to their greater ammonium and oxygen affinity it is known that AOA may outcompete AOB if the conditions within the WWT are appropriate (Stahl *et al.*, 2012; Pan *et al.*, 2016).

Populations of AOM, as measured by abundance of *amoA* copy genes has been reported to correlate with increased ammonium oxidation (Bai *et al.*, 2012). However, contribution to total ammonium removal is a balance between diversity, abundance and activity of AOM and even in wastewater environments with higher populations of AOA, it has been suggested that AOB could still represent the more active group (Wells *et al.*, 2009). This study reinforced this observation, where AOA OTU abundance is far greater than AOB OTUs (<1 %) when the RBC is overloaded (WWT 1; Figure 5). However, the abundance of the *amoA* gene for AOB was higher than for AOA. This may indicate that

AOB are responsible for the increased ammonium removal activity in phase I, when oxygen availability was greater (Figure 5). AOB gene copy number fluctuates greatly depending on the environmental conditions, whereas AOA populations stay relatively similar and are more resilient to fluctuating load and oxygen availability. This finding is an interesting one and it indicates that AOA maintain a 'background activity' independent of the environmental conditions investigated in this study. As previously described, AOA have greater oxygen affinity compared to AOB and consequently AOA have been found in low DO WWTs (Park *et al.,* 2006). Therefore, AOA are able to fill oxygen limited niches within the deep layers of biofilm and maintain a stable population where there is less competition, whereas AOB must occupy the upper layers of the biofilm where oxygen is more available, however in oxygen limited competitions they can be outcompeted by heterotrophs, for example.

A similar conclusion was made in this study where AOB become dramatically more competitive at the rear of the RBC second biozone (zone 4, Figure 4) where organic carbon concentration is at its lowest and oxygen availability is at its highest. In terms of *amoA* gene copies, abundance of AOB (1.48×10^8) was significantly greater than AOA (2.39×10^6) (Figure 4). Both gene copy number and OTU number related to AOB increased steadily throughout the process, from zone 1 to zone 4. The findings from Figure 4 and 6 indicate that AOB dominated quantitatively over AOA and played the most significant role in ammonia oxidation when conditions were optimal whereas AOA populations remain stable independent of loading conditions and oxygen availability.

Most studies agree that *Nitrosomonas* are the dominant AOB players (Park & Noguera, 2004; Siripong & Rittmann, 2007; Gao *et al.,* 2014) in WWTs. It is also reported that *Nitrosospira* can be a competitive AOB actor (Sofia *et al.,* 2004). However, in this study this species was not detected. In contrast four species of *Nitrosomonas* were detected with *Nitrosomonas ureae* being the most prominent (Figure 2). It must also be noted that AOB communities varied dramatically throughout the various WWTs (Figure 3). Four clusters which fall into the groups of *Nitrososphaera, Nitrosopumilus, Nitrosotalea* and *Nitrosocaldus* have previously been recognised (Pester *et al.,* 2012). All AOA identified in this study belong to the order of *Thaumarchaeota* and the *Nitrososphaera* cluster, with *Candidatus nitrososphaera* SCA1145 being the most prominent species (Figure 2).

Previous studies also reported *Nitrososphaera* genus to be dominant in WWTs (Kayee *et al.* 2011; Limpiyakorn *et al.* 2011; Mußmann *et al.* 2011; Park *et al.* 2006; Zhang *et al.* 2011 Gao *et al.*, 2014). *Nitrososphaera* members are slow growing (Li *et al.*, 2016) and their ammonia oxidation activity (1.4 fmol $cell^{-1}$ day^{-1}) is less than other AOAs including *Candidatus nitrosoarchaeum koreensis* (2.5 fmol $cell^{-1}$ day^{-1}), *Candidatus Nitrosotenuis cloacae* (3.8 fmol $cell^{-1}$ day^{-1}), *Nitrosopumilus maritimus* (12.8 fmol $cell^{-1}$ day^{-1}). Therefore, *Nitrososphaera* members are significant beneficiaries of the protective biofilm structure where they can exploit niches of low ammonium concentration. Another AOA species identified in this study was *Candidatus nitrososphaera gargensis.* This is an interesting finding as it has been found to utilise urea as an energy source (Spang *et al.*, 2012). As well as the low ammonium and oxygen affinities (Stahl *et al.*, 2012; Martens-Habben *et al.*, 2009; Pan *et al.*, 2016), the ability to metabolise other substrates means that AOA can be more competitive than AOB in certain niches within the biofilm. This may be a contributing factor as to why AOA abundance was generally greater than AOB in terms of OTU number. AOA dominance has also been reported in domestic WWTs in China (Bai *et al.*, 2012), municipal WWTs in Thailand (Kayee *et al.*, 2011; Limpiyakorn *et al.*, 2011), industrial WWTs in Europe (Mußmann *et al.*, 2011), and a RBC treating municipal wastewater in Canada (Sauder *et al.*, 2012). In contrast, other studies found that abundance of AOB is greater than AOA, in municipal WWTs in the USA (Wells *et al.*, 2009), a lab-scale WWTs in China (Jin *et al.*, 2010), industrial WTTs in China (Bai *et al.*, 2012), full-scale WTTs in China (Gao *et al.*, 2013) and WTTs from a range of countries (Zhang *et al.*, 2011). This study reports that abundance varies significantly when comparing individual WWTs, however AOB abundance is generally greater than AOA abundance in optimal conditions where excessive load is not present and oxygen availability is sufficient. Interestingly, Pearson correlation analysis suggests that there was a significant correlation at the 0.001 level between species diversity and AOM abundance (Figure 6), but this was only the case in AOA and not AOB.

Previous studies have found that AOB *amoA* gene copy number are greater than AOA *amoA* gene copy number in WWTs, with the highest gene copy number of 9.9×10^9 gene copies per gram of biofilm reported by Gao *et al.*, (2014). In contrast, the highest copy number reported in this study was 7.7×10^8.

6.5 Conclusion

The data from this study suggests that AOB are more likely to dominate the RBC biofilm further down the treatment process, where oxygen availability generally increases. Moving through the treatment process, both AOB diversity and abundance steadily increases. In contrast, AOA dominate in conditions where oxygen availability is lower such as at the front of the RBC or in overloaded conditions. This finding suggests that AOB are the largest contributor to ammonium removal activity in optimal conditions and that AOA maintain a 'background activity' despite the load that the biofilm is exposed to. Therefore, under overloaded conditions when AOB abundance dramatically decreases, the AOA exhibit the dominant share of activity. The most dominant AOA and AOB was *Candidatus Nitrososphaera* and *Nitrosomonas ureae,* respectively. To the best of the authors' knowledge this is the first time such findings are reported in RBC wastewater treatment systems. The study also demonstrated that biofilm microbial diversity is a significant factor influencing AOA abundance, but not AOB abundance.

Acknowledgements: This work was co-funded by the Natural Environment Research Council (NERC) (Grant reference NE/I019367/1) and Severn Trent Water (STW). Special thanks to STW for giving me access to their treatment works and to Dr Whitby and Dr McKew for enabling me to carry out the molecular analysis at the University of Essex.

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Supplementary Materials

Table S1. Primer length and sequence for specific primers used in this study.

Primer	Sequence (5' – 3')	Length (bp)	Reference
amoA-1F	GGGGTTTCTACTGGTGGT	491	Rotthauwe *et al.*, 1997
amoA-2R	CCCTCKGSAAAGCCTTCTTC		
CrenamoA23F	ATGGTCTGGCTWAGACG	593	Tourna *et al.*, 2008
CrenamoA616R	GCCATCCATCTGTATGTCCA		
341F_16S_MiSeq	CCTACGGGNGGCWGCAG	400	Herlemann *et al.*, 2011
805R_16S_MiSeq	GACTACHVGGGTATCTAATCC		
A189-F pmoA	GGNGACTGGGACTTCTGG	478	Bourne *et al.*, 2001
A650-R pmoA	ACGTCCTTACCGAAGGT		

Table S2: Biofilm samples, OTU numbers and diversity index across the 7 WWTs

WWT	No of RBCs	No of samples	No of samples (front)	No of samples (rear)	Total no of Bacterial species OTUs	Total no of Archaeal species OTUs	Mean AOM Shannon Wiener Index
1	1	40	20	20	967079	54303	0.34
2	1	3	1	2	13396	2843	0.3
3	2	4	2	2	27056	2228	0.73
4	2	4	2	2	111552	20690	0.49
5	3	6	3	3	33371	15691	0.72
6	1	3	1	2	29827	1162	0.63
7	1	1	0	1	6328	952	0.54

Table S3. The total OTUs related to AOM identified in each WWT

	WWT 1	WWT 2	WWT 3	WWT4	WWT 5	WWT 6	WWT 7
Nitrosomas ureae	11	17	471	541	551	705	963
Nitrosomas nitrosa	0	0	0	27	0	0	12

Nitrosomas oligotropha	0	0	5	0	21	0	1
Nitrosomas cryotolerans	1	0	2	0	0	0	12
Nitrosovibrio tenuis	32	0	0	0	1	0	4
Total OTUs belonging to AOB	**44**	**17**	**478**	**568**	**573**	**705**	**992**
C. Nitrososphaera SCA 1145	21634	297	282	0	2630	2	102
C. Nitrososphaera SCA 1170	3190	1367	819	269	97	9	0
C. Nitrososphaera gargensis	633	0	0	44	0	0	0
Total OTUs belonging to AOA	**25457**	**1664**	**1101**	**313**	**2727**	**7**	**102**
TOTAL OTUs	**25501**	**1681**	**1579**	**2715**	**3300**	**716**	**1094**

Table S4. Ratio of AOA and AOB abundance in terms of gene copies per gram biofilm.

	WWT 1	**WWT 2**	**WWT 3**	**WWT4**	**WWT 5**	**WWT 6**	**WWT 7**
AOA:AOB Ratio	0.03	0.02	0.33	39	0.16	0.75	1.45

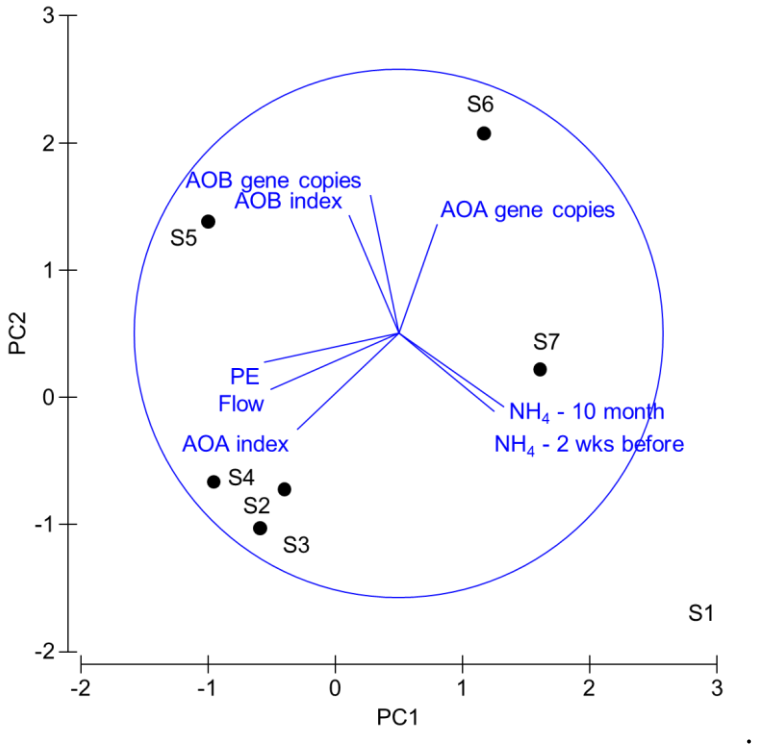

Figure S1. Principal component analysis for quantitative data presented in Table 2 and Figure 3

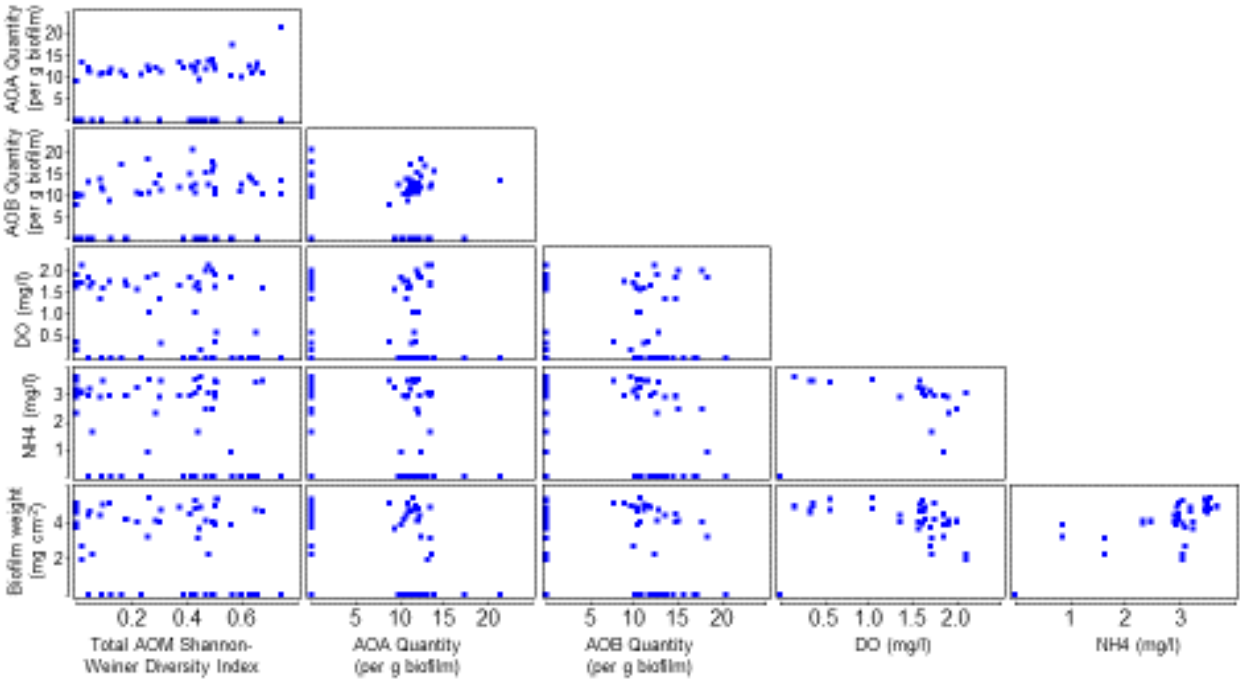

Figure S2. Draftsman plot to show the correlation between AOM diversity, AOA/AOB abundance, dissolvedoxygen, ammonium effluent concentration and biofilm weight.

CHAPTER 7

BUSINESS CASE

7 AN ECONOMIC ASSESSMENT FOR OPTIMISING NITRIFYING ROTATING BIOLOGICAL CONTACTORS

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Abstract

Using data from 3 fully operational WWTs, 3 optimisation strategies for rotating biological contactor (RBC) design are proposed. The 3 options included (A) insulation, (B) rotational speed and (C) aeration. At a life cycle cost (LCC) of £10 790 for a 200 PE RBC, the optimised RBC offers enhanced ammonium removal performance of up to 1.5 % during cold temperatures (< 13 °C) and up to 46 % during periods of significant overloading. Furthermore, it offers increased flexibility of operation and more capacity for ammonium removal. The 3 options can be retrofitted during the routine desludging of the RBCs (normally occurring every 3 months), therefore not significantly impacting on the treatment. Implementing these changes is a simple, inexpensive pathway to optimising performance and enhancing ammonium removal in RBCs at small wastewater treatment works

Keywords: Rotating Biological Contactor, Optimised Design, Ammonia Removal, Economic Assessment

7.1 Introduction

This Chapter will apply the findings from previous chapters and further data from industrial sources to propose strategies to update RBC design and operation. The feasibility of this updated RBC will be assessed using whole life costing. The main factors considered are treatment performance, financial cost and energy consumption.

RBCs offer low land requirements (they are compact), low capital requirements and suitability for decentralised, rural networks (Dutta *et al.,* 2007). They are commonly employed in WWTs receiving less than 5000 population equivalent (P.E) and typically employed in small WWTs receiving less than 2000 P.E (Tchobanoglous *et al.,* 2013; Mba *et al.*, 1999). The vast majority of WWTs in the UK (79 %) are categorised as small and favour employing low-energy, low-maintenance technologies like RBCs (DEFRA, 2012). Thus, introducing an optimised RBC design could make a great impact to the industry.

7.1.1 Typical RBC Design & Operation

The standard layout of an RBC is shown in Figure 1. Generally, primary and final settlement tank are required before and after the RBC unit, to ensure settlement of solids and sloughed biomass which enhances treatment and improves effluent quality. The disks are usually submerged to a point that allows the motor to be above the water level whilst at the same time, ensuring the majority of the disk can be in contact with the wastewater (EPA, 2002). The purpose of corrugation is to maximise the surface area of the disks which means large amounts of biofilm can accumulate on the disks. This biofilm accumulation usually takes 6 to 8 weeks to reach steady-state (EPA, 2002). As the wastewater passes through the reactor, slow rotation of the disks enables consistently intermittent contact of the biomass with air and wastewater. The biofilm attains oxygen through contact with dissolved oxygen in the bulk liquid (Patwardhan, 2003) and with gaseous oxygen when the biofilm is exposed to air (Grady, 1999). Contact during the air phase is thought to be the dominant mechanism, explained in a theory described as ‘falling film’ (Hiras *et al.,* 2004; Chavan & Mukherji, 2008; Zhang *et al.,* 2009). Upon entering the air phase, the boundary layer of the biofilm gets saturated with dissolved oxygen, which can then diffuse into the main body of the biofilm. Due to the importance of the air phase to the biofilm for the purpose of acquiring oxygen, it is recommended that the

unit be effectively ventilated. Rotation of the disks not only provides constant contact with wastewater, consistent supplies of oxygen, but also wetting to the considerable amount of biofilm, which all contribute to a stable treatment process with enhanced resilience to variable loading rates and shock loads. Within the biofilm a consortia of microbes establish, which metabolise and therefore remove substrates from the wastewater. As the name suggests, ammonia oxidising microbes (AOM) are responsible for removing ammonium, this group includes archaea and bacteria, with bacteria either being aerobic or anaerobic, all AOM can co-exist in different micro-niches within the biofilm. The surface area of the disks is mainly influenced by the width of disks or the number of disks and the greater surface area represents potential for more AOM abundance and therefore enhanced ammonium removal, assuming the correct conditions are present.

RBC design often involves staging (commonly 2 or 3 stages), from which physical barriers (baffles) which separate sections of bulk liquid and separate RBC disk packs known as 'biozones' arise. The barriers result in distinct wastewater chemistry characteristics in each stage and improve plug flow which is particularly beneficial ammonia removal as AOM are only active in certain conditions. These conditions are often only found in the later stages of the RBC process. This is because in the presence of high biodegradable matter, as is seen at the first stage of secondary wastewater treatment, biofilms are dominated by heterotrophic bacteria which can outcompete AOM in the presence of high BOD. Heterotrophic bacteria have higher yield coefficients and faster growth rates as opposed to slow growing autotrophic ammonia oxidising microbes (AOM) and so for nitrification to occur the reactor must be designed to have low BOD loading and high oxygen levels at the back end to enable AOM to compete (Grady *et al.*, 1999). Consequently, correct design and operation are essential for efficient wastewater treatment in biofilm systems, especially for nitrification in RBCs (Hoccheimer & Wheaton, 1998).

The efficacy of ammonia removal of RBCs depends upon (1) the activity and abundance of AOM and (2) substrate availability. Both of these two factors can be influenced by the structure of the biofilm, in particular its thickness. Thickness can fluctuate depending on disk rotation and wastewater characteristics, with shearing force brought about by disk

rotational speed being negatively correlated with biofilm thickness as shearing force can overcome the adhesion force of the biofilm and nutrient concentration generally having a positive correlation with thickness (Griffin & Findlay, 2000). It can also be influenced by seasonal period, excessive thickness can cause bridging of the biofilm between disks which leads to reduced surface area of the biofilm and increases mechanical loading which can be detrimental to the treatment process (Mba *et al.*, 2007). Furthermore, excessive biofilm thickness can reduce mass transfer of substrates such as ammonium and oxygen into the biofilm where the AOM community is residing (Hassard *et al.*, 2014; Zahid *et al.*, 1994) and therefore reduce ammonium removal capacity.

The whole RBC unit including the disk packs mounted on the rotating shaft are fully enclosed which mitigates odour issues. However, high long-term organic loading can lead to anaerobic conditions within the biofilm and lead to performance deterioration (EPA, 2002) and encourage undesirable microbes such as filamentous *Beggiatoa* bacteria can dominate the biofilm which may cause both odour issues. To avoid this situation care must be taken to ensure the RBC does not become overloaded. In WWTs with 2 or more RBCs, equal flow splitting is necessary to minimise the risk of RBCs receiving proportionally more load and as few RBCs as possible should be employed per WWT to minimise both costs and the risk of flow splitting not being equal.

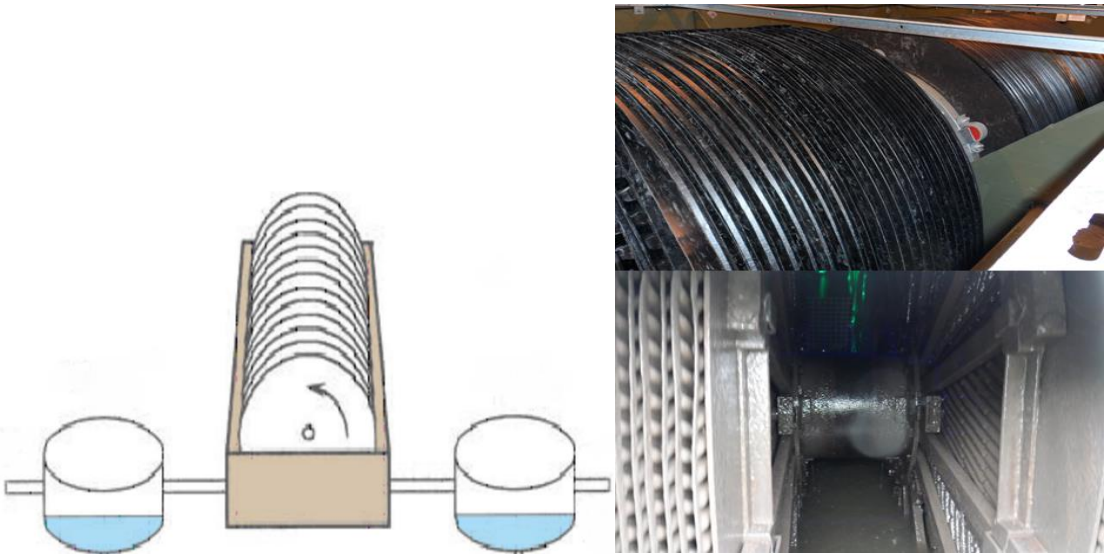

Figure 1. A typical RBC set-up with primary and final settlement tanks either side of the unit and the RBC itself consisting of two biozones, each made of a collection of plastic media disks and separated by a baffle, mounted on a horizontal shaft. The entire unit is protected with a plastic shell.

7.1.1.1 RBC Design

Disks - consisting of corrugated plastic disks, often high density plastic such as polyethylene, polypropylene or polyvinylchloride (NSFC, 2004) with a diameter of between 1.6 and 3.8 m. The disks are usually submerged about 40 % in wastewater (EPA, 2002). According to RBC commercial suppliers bridging can be avoided by requiring a maximum specific surface area of 150 m^2/m^3 for the coarse discs in the first biozone and 220 m^2/m^3 for the fine discs which follow. The design of the disc packs allows a maximum biofilm thickness of 5 mm on coarse disc packs and 3 mm on fine disc packs, with 1 mm being common (STW, 2016).

Staging – provides physical barriers separating 'biozones,' that promotes distinct wastewater chemistry characteristics in each stage and improves plug flow. Typically, between 2 and 4 stages are implemented into RBC design, with fewer stages restricting nitrification capacity. Three stages are recommended for nitrification and for ammonia consents of less than 5 mg L^{-1} four stages are essential (STW, 2016).

Shell – the shell usually made of glass reinforced plastic (GFP) mitigates odour issues and protects the RBC from adverse weather conditions including cold temperatures as below 12 °C performance deteriorates significantly (EPA, 2002).

Loading and HRT – Hydraulic retention time (HRT) of at least thirty minutes is desirable to enable sufficient contact time between wastewater and biofilm. Ammonium removal can only occur when organic loading is below 15 g COD/m^2day^{-1} and can only reach optimal rates below 8 g COD/m^2 day^{-1} (Boller *et al.*, 1987). Therefore, it is recommended that BOD_5 load into the first stage of individual RBCs should not exceed 15 g/m^2/d, with m^2 relating to disk area. Loading of 4, 2.5 and 2 g/m^2/d is recommended for ammonium EQS of 15, 10 and 5 mg L^{-1} respectively (STW, 2016).

7.1.1.2 Typical RBC operation

The major operational parameter that influences performance efficacy, cost and energy consumption in RBCs is rotational speed (Chapter 2 and 4). Rotation of the RBC is important to the process as it affects the contact time of the biofilm per rotation with wastewater/air, turbulence of the wastewater, oxygen availability and finally the sloughing force exerting on the biofilm. Rotational speed has a positive relationship with these factors. Commercial RBC manufacturers often recommend a rotational speed of between 0.5 – 0.65 revolutions per minute (rpm), however based on internal trials Severn Trent Water use a standard speed of 1 rpm as it enhances asset life as well as nitrification performance (STW, 2016). Increasing rotational speed may confer benefits, but it should be noted that power consumption is theoretically proportional to the square of the rotational speed, and therefore > 1.4 rpm may be inefficient and to use another metric, a peripheral velocity or tip speed should not exceed 0.3 m/s (STW, 2016). If the WWT is gravity fed, shaft rotation is the only significant expenditure relating to energy. Consequently, energy consumption ranging between 1 and 8 kWh/day is common which equates approximately between £25 and £205 per year. Whilst this can be 3 times more expensive than trickling filters, the capital costs for RBCs are significantly cheaper (EPA, 2002). However, the energy consumption associated with running RBCs with STW is considerably more than that reported by the EPA, nearer 20 kWh/day was reported in Chapter 4.

7.1.1.3 Typical RBC CAPEX & OPEX

The traditional RBC design is particularly appropriate for rural small WWTs in decentralised systems due to its simple design and operation, low maintenance, cost and

energy consumption. Table 1 illustrates how it compares with other competitive treatment options, based on a standard small WWT.

Table 1. Costs and energy consumption for the installation and operation of various small WWT treatment technologies including RBCs, based on a 20-year life span (according to a leading wastewater engineering company).

	£ per PE FOR RBCs (50 - 500 PE)			
	TOTEX	**CAPEX**	**ANNUAL OPEX**	**LAND AREA (m^2)**
RBC	531	291	12	60
Trickling Filter	649	369	14	600
Sequential Batch Reactor	542	222	16	70
Oxidation Ditch	671	351	16	150
Constructed Wetlands	423	243	9	650

As described previously the RBC is capable of effective ammonium removal and meeting low ammonium effluent EQS. After treatment process efficacy the next most significant consideration is cost. In terms of both capital expenditure (CAPEX) and operating expenditure (OPEX) the RBC is a very competitive choice compared to the other common treatment technologies described in Table 1, with only the wetlands being cheaper.

Compared to other common small WWT treatment technologies, OPEX for the RBC is the second lowest. Besides labour, the two largest contributors to OPEX is energy consumption by the motor and removing sludge from the settlement tanks on a 3-month basis. For an RBC with a 3 m diameter disk and 10 m^3 volume, designed for approximately 500 PE the cost of disk rotation is approximately £700 per annum and emptying the RBC is £420 (PA Wright & Sons, 2016). As well as cost, land use can also be an important factor. In comparison to other treatment technologies, RBCs are the most compact and require the amount of land which is a great advantage for many WWTs with limited space. RBCs are attractive because they are associated with both low land use and low costs. This coupled with their ability to effectively remove ammonia and yield effluent to a similar quality as activated sludge (Tchobanoglous *et al.*, 2013), as well as their other benefits such as low maintenance, low energy and limited odour issues (Hassard *et al.*, 2014) give RBCs huge potential as treatment technologies for

decentralised small WWTs. However, there is scope for improving their design and operation, particularly by increasing their capacity for ammonium removal.

7.2 Rationale for Updated RBC Design & Operation

AOM in RBCs consist of a diverse community of ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) (Chapter 6) and are the key drivers behind ammonia removal, the rate-limiting step in nitrification (Murphy *et al.*, 2009). AOM abundance and activity is key to ammonium removal and is heavily influenced by many key operational parameters within the RBC including temperature and oxygen/ammonium availability (Chapter 2). The following section will use data collected over the duration of the thesis to outline how three alterations to the design and operation of RBCs can lead to an optimised RBC capable of enhanced ammonium removal. These alterations include insulation (A), rotational speed (B) and aeration (C).

The feasibility of implementing this optimised RBC will be assessed in Severn Trent Water (STW) WWTs. STW is a major UK water company and a world leader in RBC implementation, with, approximately a third of their WWTs utilising RBCs.

7.2.1 Insulation (A)

Temperature decreases have been shown to negatively affect ammonium removal in biofilm wastewater treatment systems (Park *et al.*, 2009; Yang *et al.*, 2009) including RBCs (Dutta *et al.*, 2007). Specifically, it has been shown that RBC performance deteriorates significantly below 12 to 15 °C (US EPA, 2002; Rodgers & Zhan, 2003; Yanamoto *et al.*, 2006). At 5 °C, ammonium removal rate is reduced by 5 times compared to the optimal temperature of 25 °C (Yang *et al.*, 2009). The RBC unit will also need at least 2 times more surface area of media at 5 °C than at of 25 °C to compensate for reduced microbial activity (Rodgers & Zhan, 2003). This is because optimal growth of nitrifying bacteria occurs between 25 and 30 °C (Parades *et al.*, 2007; Zhi & Ji, 2014). In fact, the growth rate of nitrifying bacteria can be 65 % lower at 10 °C compared to 20 °C (Murphy & Young, 2009).

While these studies suggest that RBC reactor temperatures of > 20 °C will provide optimal conditions for ammonium removal, this may not be achievable in RBC WWTs situated in the UK. Wastewater from 2 RBC WWTs in the Midlands area of the UK was

analysed from February to July (Figure 2) and the maximum temperature of the wastewater in RBC units did not exceed 18 °C. The temperature duing the winter period was as low as 8 °C. These WWTs were typical rural small WWTs with PEs of 536 and 713, each containing two RBCs.

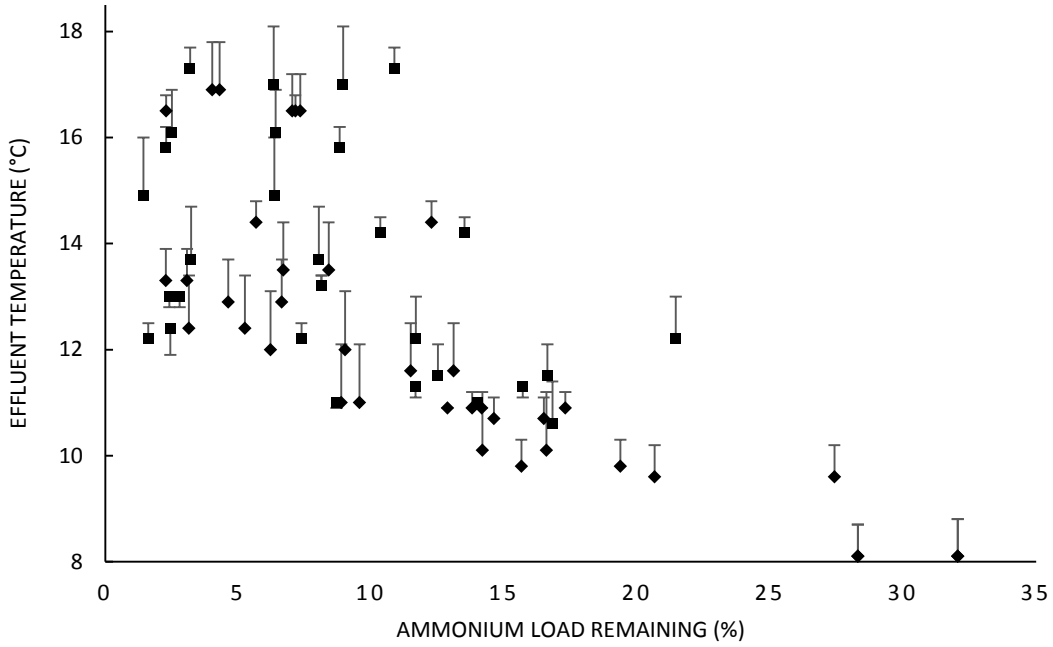

Figure 2. The relationship between temperature of the wastewater in the effluent (°C) and ammonium removal capacity in terms of % of influent ammonium load remaining in the effluent. Squares represent data from WWT 1 (n = 30) and diamonds represent data from WWT 2 (n = 38). Error bars show the difference in temperature between the influent and effluent

According to the data, ammonium removal performance does indeed start to suffer below 12 °C. However, above this point performance is more robust, with total ammonium load remaining (%) in the effluent being on average (mean ± SD) 5.9 ± 2.6 when influent temperature is more than 12 °C and it being 15.3 ± 4.2 when temperature is less than 12 °C. The error bars in Figure 2 illustrate a significant difference in temperature between the influent and effluent. In fact, an average of 0.6 (± 0.4) °C is lost through the RBC process, in other words, from the influent to the effluent.

Table 2. The temperature variation from February to March

	Feb	**March**	**April**	**May**	**June**	**July**
Ammonium remaining (%)	30.2	24.0	17.5	13.6	6.1	5.4
Average Temperature (°C)	8.1	9.6	9.8	10.9	13.1	16.6

Figure 2 suggests that ammonium removal would be enhanced if temperature within the units was maintained above 12 °C. This is particularly true in the winter months where wastewater temperature is lowest, however average temperatures of less than 12 °C have been observed as late as May (Table 2). Retaining as much heat as possible within the RBC unit can be a simple and effective strategy to enhance performance which could be achieved by installing RBCs below ground to provide considerable insulation against cold air temperatures.

Alternatively, insulation can be added to the walls of the RBC. Expanded polystyrene foam sheets can be glued to the inside of the glass reinforced plastic walls and have a thermal conductivity factor of 0.041 (λ) compared to 0.23 for 30 % glass reinforced plastic (Papadopolous, 2005). These can be used to provide increased insulation properties by a factor of 5. Polystyrene with a thickness of 25 mm represents a cost of £10 per m^2 (Impreglon, 2016). Unfortunately, the data is lacking to illustrate the effect that this would have on retaining the heat from the wastewater. However, on the assumption that this insulation can reduce the average heat loss by 50 %, from 0.5 to 0.25 °C then based on the linear relationship ($y = -0.1772x + 13.6$) between performance and wastewater temperature below 14 °C (Figure 3), performance could be increased by 1.5 % in terms of ammonium load removal. Table 2 also suggests the effect of increasing wastewater temperature significantly enhances ammonium removal performance, from April to May there was an average temperature increase of 1.1 °C which translated into a 3.9 % less ammonium remaining in the effluent.

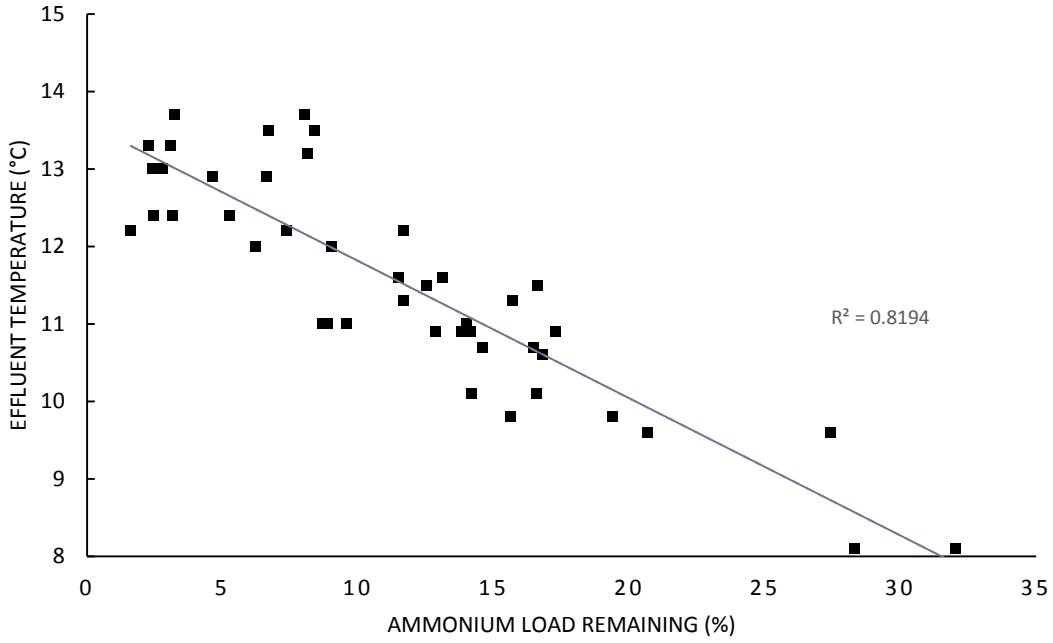

Figure 3. Relationship between wastewater temperature and treatment performance below 14 °C. n = 37 (one outlier removed at 12.2 °C). Data used are the same as in Figure 2.

7.2.2 Rotational speed (B)

The survey of WWTs containing RBCs in Chapter 3 revealed data that illustrates the relationship between RBC diameter and WWT capacity (Figure 4). According to the data, RBCs of < 2 m diameter are employed exclusively for < 500 PE, < 3 m are employed exclusively for < 1200 PE and WWTs with higher capacity may employ RBCs with a diameter of > 3 m. Clearly, the diameter of RBCs differs considerably depending on the capacity of the WWTs. This is understandable as more media surface area is required to treat greater loads.

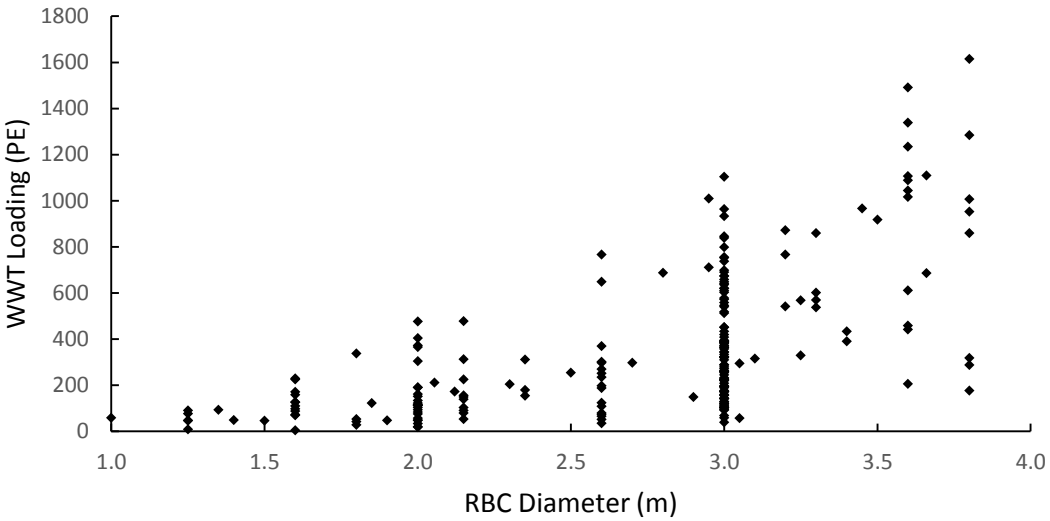

Figure 4. The diameter of RBCs in fully-operational WWTs within the Midlands area of the UK and their respective loading in terms of population equivalent (n = 245)

The design manual of Severn Trent Water states that 1 rpm is the company standard as this is conducive for desirable energy consumption, asset life span and treatment performance. However, a standard rotational speed presents an issue for the operation of RBCs. The larger the disk diameter the faster the tip velocity of the disk, which means the vast majority of RBCs in WWTs are rotating at 0.16 m/s, whereas others are rotating at 0.21 or 0.08 m/s (Table 3). This considerable differential in tip speed across the group of RBCs employed in WWTs means that treatment may not be consistent. Furthermore, presenting speed as 'rotations per minute' is common in published literature, however it is difficult to compare findings across studies as explained above. Thus, design manuals and future studies should refer to tip velocity, rather than rotations per minute.

Table 3. A description of how RBC disk diameter influences tip velocity when the disk is rotating at 1 rotation per minute. The number of WWTs with different RBC diameters is also shown.

Disk Diameter (m)	Tip Velocity (m/s)	No. of WWTs	RPM at 0.2 m/s
3.8	0.21	8 (3 %)	1
3.5	0.18	23 (10 %)	1.1
3	0.16	113 (49 %)	1.3
2.5	0.13	23 (10 %)	1.5
2	0.1	43 (19 %)	1.9
1.5	0.08	20 (9 %)	2.5
1	0.05	1 (< 1 %)	3.8

Chapter 4 demonstrated that reducing the speed from 0.2 to 0.14 m/s did not reduce energy consumption and increasing speed from 0.16 to 0.2 m/s was associated with a range of benefits without incurring further costs related to energy consumption (only (0.7 % increase). For example: (1) reducing ammonium effluent spikes, (2) increasing ammonium load removal capacity and (3) enhancing biofilm characteristics.

(1) Analysis of effluent quality for approximately 45 months before and after an RBC speed change from 0.16 to 0.2 m/s lead to a reduction in maximum ammonium effluent concentration of 44 and 43 % for 2 WWTs.

(2) Increasing speed from 0.16 to 0.2 m/s resulted in a significant increase in average ammonium load removal (paired two-tailed t-test; p value > 0.05) from 88 % to 92 %

(3) Figure 5 illustrates the difference in biofilm appearance after different speed regimes 0.16 (left) to 0.2 m/s (right).

Figure 5. The biofilm at tip speeds of 0.16 (left) to 0.2 m/s (right). Filamentous *Beggiatoa* bacteria can be seen on the slower RBC, whereas on the quicker RBC the biofilm is thinner and brown.

Based on this full-scale data, altering the speed to 0.2 m/s has been shown to be beneficial for performance without compromising energy consumption, in comparison to 0.14 and 0.16 m/s (Chapter 4). It is therefore recommended a tip speed of 0.2 m/s should be used as a standard in the design manual. This would mean the rotational speed (rpm) of the larger RBCs would remain similar, but rotational speed (rpm) of small RBCs would need to be increased accordingly. Rotational speed of new RBCs could be altered during installation and set-up with no extra CAPEX cost, however, to alter the speed of existing assets variable speed drives (VSD) would need to be installed. Rather than setting a permanent rotational speed at set-up, installing VSDs to new RBC assets would offer flexibility in operation and allow speeds to be altered if further work reveals a more optimal rotational speed. Therefore, VSDs would be required to implement the improved speed change, described in this section. The cost of this installation process per RBC is approximately £5000 and is outlined in Table 4. Although the labour itself takes 3 days, it is not necessary to isolate the RBC for any significant amount of time and therefore it does not impact on treatment.

Table 4. Cost (exc. VAT) of installing a 2.2 kW inverter ABB ACS 355 variable speed drive, according to three leading engineering companies

	Materials (£)	Labour (£)	Profit & Insurance (£)	Duration of Work (days)	Total Cost (£)
Quote 1	4168	1016	456	3	5640
Quote 2	2954	1515	566	2	5035
Quote 3	2798	2034	628	2	5460

7.2.3 Aeration (C)

Increasing the rotational speed may increase the ability of the WWT to remove greater ammonium load, or in other words increase robustness. Chapter 5 suggests that artificial aeration may similarly increase ammonium removal robustness and may be a valuable strategy for increasing WWT capacity to remove ammonium load during periods of stress. This could apply for WWTs situated in tourist locations that are inundated with visitors during certain months for example.

Artificial aeration of the biozone was shown to enhance ammonium removal in an overloaded non-nitrifying RBC (Chapter 5). Without aeration the maximum ammonium load removal that could be achieved was 20 % and each effluent sample (n = 7) contained > 20 mg L^{-1} ammoniacal nitrogen. With supplemental aeration (approximately 4 mg L^{-1} of dissolved oxygen in the bulk liquid), the maximum ammonium load removal was 72 % and the maximum concentration of ammoniacal nitrogen in effluent samples (n = 8) was 19.3. Aeration resulted in effluent concentrations of between 9.8 and 19.3 mg L^{-1} from influent ammoniacal nitrogen concentrations of between 26.4 and 51.6 mg L^{-1} and increased average ammonium removal by 46 %. This illustrates that artificial aeration can be a quick and effective remedy to overloaded WWTs that require extra capacity. In this case supplemental aeration resulted in an RBC that would comply with a 20 mg L^{-1} effluent EQS, whereas previous it would have failed in each sample. The cost of all the aeration equipment used in the trial for Chapter 5 was less than £200 and the aeration could be used as and if it is needed. The implementation of this aeration strategy will be based on the same equipment and methodology as in Chapter. The installation of the

aeration system can be carried during the routine desludging which occurs every 3 months. This event requires the power to the RBC to be isolated and leaves the RBC trough empty for the duration of the work. During this time the pipes and air stones can be fixed to the inside of the GRP trough.

Both increasing rotational speed and artificial aeration were shown to improve the characteristics of the biofilm in terms of appearance and thickness as well as decrease the prevalence of the nuisance bacteria *Beggiatoa* in Chapters 4 and 5. The mechanism responsible for enhancing ammonium removal in this case could either be the introduction of greater concentrations of oxygen to the bulk liquid or internal air space or relating to this or entirely independently the aerations effect on biofilm characteristics may have led to ammonia oxidising microbes being more competitive in the biofilm.

Updating the asset design manual to specify that all newly installed RBC should have pond grades air stones and HDPE pipework attached to the bottom of the RBC reactor that are compatible with a small air compressor would both be cheap and simple. This would enable extra capacity when necessary and potentially stop a WWT from breaching consent during unforeseen periods of overloading. A survey of 45 RBC-containing WWTs (15 identified as green, 15 identified as amber and 15 identified as red in terms of ammonium removal performance) in Chapter 3 revealed that on average 86 % of the time ammonium concentration in effluent samples contain < 25 % of the stipulated effluent EQS and 14 % the ammonium concentration exceed 25 % of the effluent EQS. It is STW policy that when effluent concentration exceeds 25 % that the WWT is investigated and reactive measures may be necessary. In other words, the average WWT is performing robustly 86 % of the time, but 14 % of the time the operators have reason to be concerned. Therefore, approximately 14 % of the time this aeration strategy could be of great use.

7.3 Implementing the Improved RBC

A summary of the three options described above for improving the RBC is provided in Table 5.

Table 5. Summary of proposed updated RBC design (based on trials from Chapter 4 & 5) vs the conventional RBC design guidelines.

	Operation					
Optimisation options	**Before**	**After**	**Performance Increase (%)**	**CAPEX (£)**	**Annual OPEX (£)**	**Annual Energy (kWh)**
Insulation (A)	N/A	Polystyrene Insulation	1.5	10 m^{-2}	N/A	N/A
Rotational Speed (B)	≤ 0.2 m/s (1 rpm)	0.2 m/s	4	5000	6	69
Aeration (C)	N/A	At ~ 4 mg L^{-1}	46	150	4	50

For reasons stated in the previous section, option A should enhance the performance of all RBCs that suffer from low (< 13 °C) wastewater temperatures, which can be common in RBC systems (Section 7.2.1). Option B will improve the performance and give more flexibility to RBC operation, whilst incurring negligible additional OPEX. Option C is associated with both negligible CAPEX and OPEX and the extra aeration capability will provide extra performance capacity for RBC which encounter overloading events. This section will evaluate whether implementing these changes would be viable.

Data collected in the RBC survey (Chapter 3) reveals a comprehensive picture of the RBC WWTs in the STW catchment area. The majority (71 %) of RBC containing WWTs are designed for less than 400 PE, with 18 % being designed for less than 800 PE and 11 % being designed for between 800 and 1800 PE (Figure 6). At less than 200 PE, WWTs exclusively contain 1 RBC, between 200 and 600 WWTs may contain 1 or 2 RBCs and for more than 600 PE, WWTs contain either 2 or 3 RBCs.

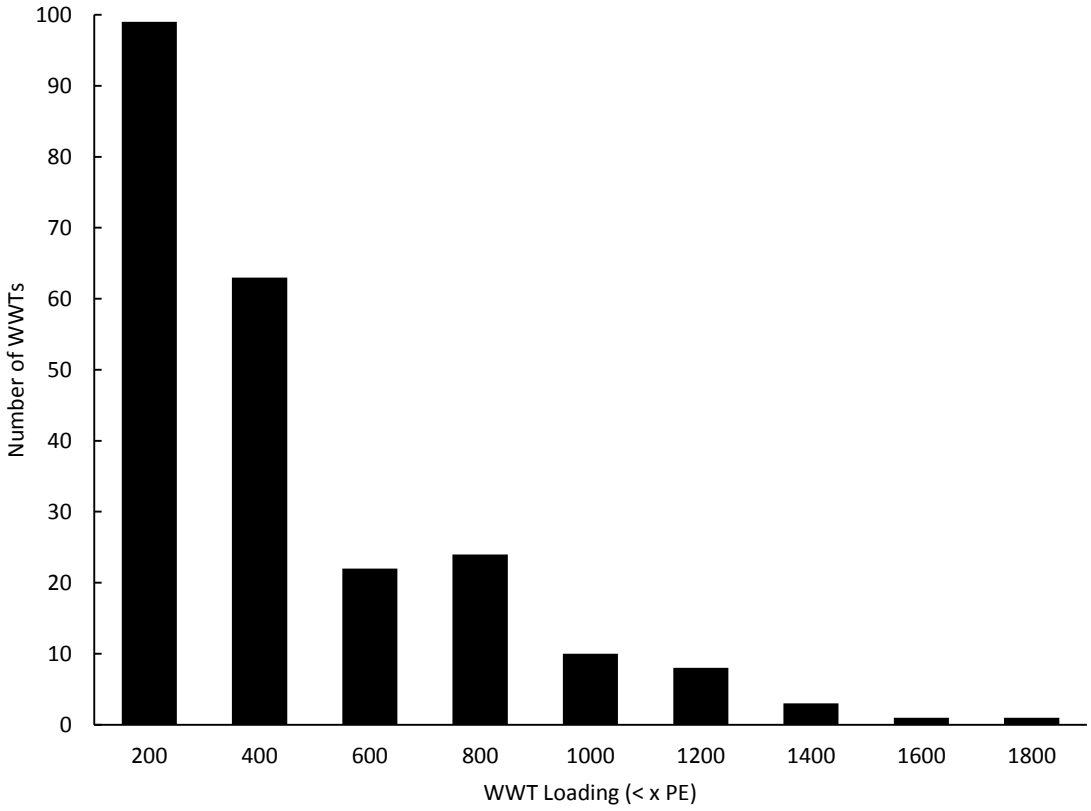

Figure 6. Breakdown of WWTs sizes according to population equivalent. n = 231

Taking into account the PE per WWT and the number of RBCs per WWT, the mean PE per RBC is 230. Therefore, based on this data the feasibility of the optimised RBC design will be assessed for an RBC designed to treat 200 PE. As it will be designed for ammonium removal, the RBC will contain 3 biozones and possess disks of approximately 3 m diameter (as this is the most common diameter and often employed for 200 PE [Figure 4]), standard settlement tanks either side of the RBC unit and a shell with the dimensions of 10 (l) x 4 (w) x 3 (h) metres.

7.3.1 Whole Life Costing

The whole life cost (WLC) was analysed using Severn Trent's whole life costing tool with a reported methodology (Newton & Reid, 2007). The assumptions were as follows: an asset life of 20 years, a CAPEX installation cost 25% of the total price, an June 2016 interest rate (consumer price index) of 0.5 % (Office for National Statistics, 2016), an inflation rate of 0.5 % (Bank of England, 2016), average electricity unit cost of 8 p.kWh^{-}

[1] for industry (GOV.UK, 2016) and a Severn Trent operator hourly rate of £26.50 (including salary, training and pension costs).

Using these values along with equipment capital and consumable / overhaul costs stated in section 2 and in previous thesis Chapters, a WLC analysis was carried out on the optimised RBC.

Table 6. Energy consumption for options A, B and C.

		Insulation	VSD	Aeration
	kW consumed	**0**	**0.008**	**0.04**
Average operating hours per day per item	Mon	0	24	24
	Tues	0	24	0
	Wed	0	24	0
	Thu	0	24	0
	Fri	0	24	0
	Sat	0	24	0
	Sun	0	24	0
Total operating hrs per week		**0**	**168**	**24**
Total kWh per week		**0**	**1.344**	**0.96**
Total kWh per year		**0**	**69.888**	**49.92**

Table 7. Maintenance and overhaul costs for the optimised RBC

Description of Work	Freq per Annum	Cost of Work (£)	Labour Hrs	Labour Cost (£)	Total Annual Cost (£)
Air pump annual service kit	1	10	1	26.5	36.5
New aeration set-up incl. pump, pipes and airstones	0.2	150	3	79.5	45.9
New insulation	0.1	1240	6	159	139.9
Total					222.3

Table 8. Total Life Cycle Costs (LCC) associated with the optimised RBC.

Asset Life (n)	20
Interest Rate (i%)	0.5
Inflation Rate (p%)	0.5
Real Discount Rate (i-p)	0
Initial Investment Costs - Civil Works (£)	0
Initial Investment Costs - M&E Equipment (£)	6390
*Above includes Installation and Commissioning Cost	
Energy Price (pence per kWh)	0.08
Annual Total Energy Consumption (kWh)	120
Annual Energy Cost (£)	9.6
Annual Maintenance & Overhaul Cost (£)	222
Sum of Annual Costs (£)	231.6
Present Value of Annual Costs (£)	4400.4
Present Value Life Cycle Cost (LCC)	10790.4

This Life Cycle Cost (LCC) analysis was only based on the improvements which have been suggested in section 2 (Options A, B & C). As such, the LCC is associated with retrofitting the improvements themselves rather than the actual RBC unit. Details relating the costs, energy consumption and land use were however set out in section 1. The LCC of £10,790 over a 20-year asset life-span represents the potential gains of enhanced performance of 1.5 % during the winter, and potentially into autumn and spring (Table 2). Furthermore, it represents up to a 46 % performance increase during overloading events which typically occur around 14 % of the time in average WWTs. If an operator observes that one of these events is affecting a WWT or is certain one will happen (such as tourists inundating a town during the summer, or a local music festival or a regular trade discharge event), then this person can just switch the air pump on to provide the

biofilm with extra oxygen availability and enhance performance in the short-term. Lastly, it represents more flexibility of operation and more robust performance. Increasing the disk rotational speed to 0.2 m/s has been shown to enhance biofilm characteristics, increase ammonium load capacity and may offer a long-term solution to ever-increasing demand for WWTs.

In the last asset management plan (AMP), STW allocated £16 million for quality and growth drivers at small WWTs. With approximately two thirds of small WWTs employing RBCs, this is an area of improvement which could make a significant impact. At the LCC of £10,790, implementing this at every RBC WWT would cost £3.5 million and contribute to enhancing effluent quality, whilst also catering for future growth.

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CHAPTER 8

CONCLUSIONS

8 CONCLUSION

8.1 Summary

Rotating biological contactors (RBCs) are currently an attractive treatment technology option for small WWTs in rural and/or decentralised system due to their low cost, low energy and low maintenance. Currently, companies are facing resource constraints (labour, finance, energy etc.), regulatory bodies are enforcing stricter environmental quality standards (EQS), and customers are demanding the lowest bills possible, therefore, industry experts have claimed effluent quality, energy and costs are the most important factors in wastewater treatment of the future (STOWA, 2010). Thus, there is great benefit in optimising the RBC to improve performance and reduce costs. In terms of wastewater treatment performance, ammonium is of particular importance as it can cause great harm to the aquatic environment if discharged.

Therefore, this PhD addresses a current issue and is of great interest to industry and society. It provides multidisciplinary approach which integrates a literature review, a performance assessment tool, experimental trials at fully operational treatment works and molecular biology tools to investigate this issue. The aim of the thesis was to investigate how various parameters within a rotating biological contactor system influences the RBC treatment process in terms of ammonium removal, with a specific focus on understanding how biofilm is affected. It is important to reveal the mechanisms of the biofilm as this is where the ammonia oxidising microorganisms (AOM), which facilitate ammonium removal, reside. Here, in particular, is where many knowledge gaps exist. By understanding how operational parameters within the RBC influence ammonium removal it is possible to use this knowledge to rationally optimise the process by altering the design or operation of the RBC. To fulfil this aim 6 objectives were set out, the following sections provide an overview of the findings and contribution to knowledge related to each objective, as well as the implications to industry and suggested further work.

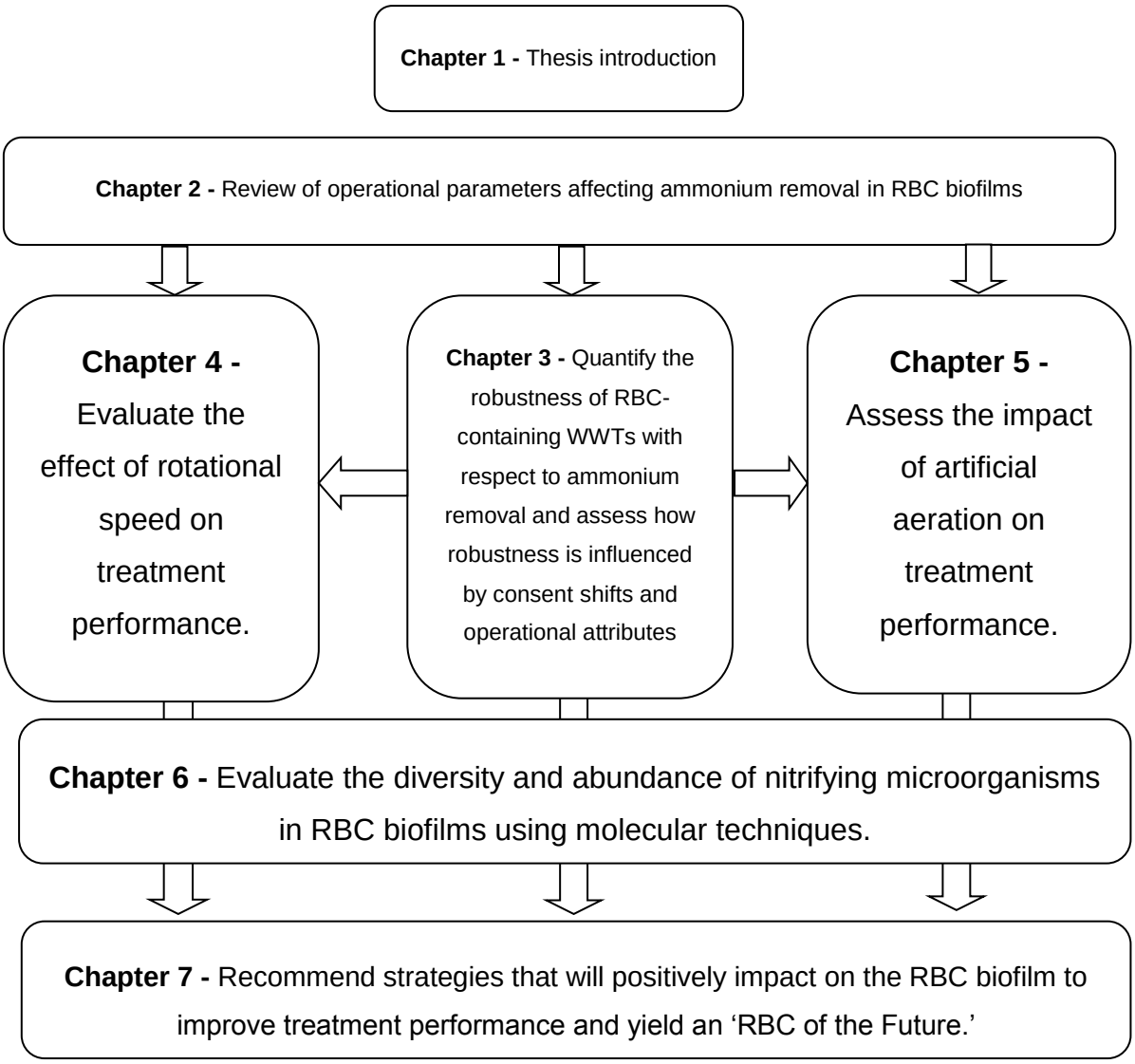

Figure 1. Reminder of thesis layout with brief Chapter descriptions.

8.2 Key Findings

Based on the literature, **Chapter 2** highlighted a number of key operational parameters that can influence ammonium removal in RBC biofilm. Two strategies to enhance the performance of the biofilm were investigated – biostimulation and bioaugmentation. Literature suggested that bioaugmentation was a promising route, however as a strategy it is still in its infancy and is currently unreliable. Biostimulation on the other hand, is better understood and there is clear evidence that it can be used to enhance ammonium

removal. It was found that altering environmental parameters such as oxygen and temperature in particular can be an impactful and simple way to manipulate RBC biofilms. Biostimulation therefore became the basis for much of the subsequent work including **Chapters 4, 5 and 7**.

A unique position with a water company presented an opportunity to assess 128 WWTs with an innovative robustness performance index using 10 years of operational data on effluent ammonium concentrations. The data suggested that RBC WWTs are well suited to ammonium effluent consents of 15 and 20 mg L^{-1}, with many retaining their current robust performance in the event of a consent reduction of 5 mg L^{-1}. A key objective of **Chapter 3** was to evaluate any relationship between attributes of WWTs and performance robustness, however, no single operational attribute could be identified as contributing significantly to robustness.

In **Chapter 4** the effect of altering disk rotational speed was assessed at 2 full-scale WWTs. The effects of altering this rotational parameter on ammonium removal and operating cost was investigated. It was previously thought that the energy consumption of RBC was proportional to the square of the rotational speed (Watanabe *et al.,* 1993) and that decreasing rotational speed would therefore reduce energy consumption (Ramsay *et al.,* 2006). For the first time, we presented data that suggests the fluctuation in biomass on the disks brought about by alterations in rotational speed mitigates the change energy consumption. Therefore, decreasing rotational speed from 1.0 rpm to 0.7 rpm did not significantly reduce energy consumption, in fact this only equated to a 7.3 kWh per year saving. Furthermore, increasing rotational speed can enhance ammonium removal capacity by enabling higher total removal of NH_4^+-N load and increased robustness. Additionally, increasing rotational speed can enhance biomass characteristics and reduce *Beggiatoa* proliferation which is known to cause significant RBC treatment capacity reduction.

It was thought that the positive effects that resulted from an increase in rotational speed were mainly brought about by increased oxygen availability. **Chapter 5** tested this further. In an overloaded, DO limited, non-nitrifying RBC unit, the introduction of artificial aeration was able to significantly improve ammonium removal, reduce biofilm

thickness and reduce *Beggiatoa* proliferation. It was shown that artificial aeration could be used as a quick, cheap and simple remedy to overloaded WWTs.

To investigate whether the microbial consortia changes as a function of operational and environmental parameters, **Chapter 6** analysed RBC biofilm from 7 fully operational RBC WWTs using qPCR and high throughput DNA sequencing. *Nitrosomonas* was the most abundant genus of AOB and *Candidatus Nitrososphaera* was the most abundant AOA. AOA represented the most abundant AOM group, but AOB were associated with the higher *amoA* gene copy number per gram of biofilm, with a mean copy number of 1.48×10^8 at the rear end of the RBC. The microbial consortia were highly dynamic and was not strongly correlated with any particular parameters.

Using data collected from **Chapters 3, 4** and **5**, three improvements to the conventional RBC were discussed. Implementing these new design/operational features would have a clear benefit in terms of ammonium removal enhancement with little additional cost. Over a 20-year asset life-span, the enhanced performance robustness is associated with a life cycle cost of £ 10 790. Implementing these changes to current RBCs would be uncomplicated and relatively fast.

8.3 Implications of Research

In the author's experience, the vast majority of research related to RBCs are lab-scale studies. This is expected and justifiable as there are many barriers to accessing full-scale and even pilot-scale RBCs. This thesis represented a unique opportunity to study various fully operational RBCs over a 3-year period and therefore provides valuable data that is applicable to fully operational RBCs. The implication of this is that many of the findings are directly transferable and beneficial to industry. For instance, the performance robustness index tool developed in Chapter 3 can be used to support asset management decisions and aid operatives in identifying where resources should be directed to maximise efficiency and minimise risk. This will benefit individual WWTs as well as the wider treatment network. The thesis also provides great insight into how RBCs should be operated to yield enhanced ammonium removal, this is addressed by Chapters 4, 5 and 7 and will contribute towards ensuring RBCs can be more robust in their compliance in

EQS. As well as providing real operational data to the academic field, this thesis also highlighted area that need attention from academia and contributed to the understanding of other areas. The areas that need investigation will be discussed in section 5. The microbial components of the RBC biofilm are now better understood – for example, the data in Chapter 6 revealed that ammonia oxidising archaea have a consistent presence in the biofilm in independent of the load received by the RBC, however ammonia oxidising bacteria are very sensitive to load and therefore increase dramatically in numbers from the front to the rear of the RBC.

8.4 Limitations of Research

Having the ability to experiment with fully operational RBCs and being able to access vast RBC-related databases was a great advantage, however, this also presented many problems. First, was the inability to control operational parameters (particularly load) which made it far more difficult to compare the control and the test samples in Chapters 4 and 5. Second, the databases contained a great wealth of information, but much of it was incorrect and out-of-date, it was very time consuming to extract the quality data, which involved collaborating with many teams. Related to this, a third limitation was the reliance on a complex chain of individuals/teams to carry out work which complicates the process. Time delays and unforeseen events are inevitable in any research project, but these are enlarged when working with fully operational assets.

8.5 Recommendation for Further Research

Observations were made during the thesis, in which the mechanism behind the phenomena could not be explained during the project time frame. It was noted that a thinner biofilm resulted from both artificial aeration and increased rotation. It was shown that both actions increased dissolved oxygen content and both actions may also increase sloughing force on the biofilm also. Which of these is the dominant mechanism responsible for yielding a thinner biofilm? This could be of great interest, because as discussed in Chapter 2, a thinner biofilm is desirable for treatment due to better mass transfer properties.

The authors would highly recommend repeating full-scale experiment on rotational speed (Chapter 4), except whilst ensuring the load to each RBC was equal (see section 4) and comparing the RBCs using the tip velocity unit rather than rotations per minute. This would enable a better comparison of control and test units. It is the author's viewpoint that understanding the micro-components of the biofilm is the most promising route towards further optimisation of RBC units and with molecular techniques becoming cheaper and more effective every year this area should be concentrated on.