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Influence of biofilm thickness and control possibilities

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New Dimensions of Moving Bed Biofilm Carriers

Influence of biofilm thickness and control possibilities

MARIA PICULELL | FACULTY OF ENGINEERING | LUND UNIVERSITY



New Dimensions of Moving Bed Biofilm Carriers

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Maria Piculell



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DOCTORAL THESIS

By due permission of the Faculty of Engineering, Lund University, Sweden,
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Professor Jürg Keller, Advanced Water Management Centre,
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Abstract <p>The moving bed biofilm reactor (MBBR) is a biological wastewater treatment process in which microorganisms grow as biofilms on suspended carriers. Conventionally, MBBRs are mainly designed and optimized based on the carrier surface area, neglecting the dynamic relationship between carrier design, reactor operation and biofilm characteristics, such as biofilm thickness and the composition of the microbial community. The purpose of this research project was to learn more about the roles of the biofilm carriers in the MBBR process, with the intention to improve process performance and develop new MBBR applications. In doing so, the MBBR performance was evaluated in several lab studies, considering different aspects such as carrier design and operational strategies. A new carrier type, the Z-carrier, was developed, with which it was possible to control the biofilm thickness in the MBBR. Hence, the Z-carrier enabled the evaluation of having different, pre-defined biofilm thicknesses in the MBBR process, something that has not previously been achievable. This thesis shows that biofilm thickness control can be used to ensure a more stable process performance as well as to avoid carrier clogging and minimize issues with biofilm scaling that may have detrimental effects on the MBBR performance. It was also shown that the microbiology in biofilms can be altered by biofilm thickness control. Based on these findings, a novel process configuration was developed, showing that successful nitrification of mainstream municipal wastewater could be achieved when combining thin, controlled biofilms with a periodic exposure of the biofilm to reject water from sludge dewatering. Finally, the role of suspended biomass in the MBBR was evaluated in relation to carrier surface area, HRT and loading rate, showing that the suspended biomass can have a considerable effect on the overall process performance, and that the design of MBBRs should not always be solely based on biofilm surface area. For future studies, the potential of using biofilm thickness as a control parameter for the MBBR should be investigated further, especially for specific microbial applications such as nitrification and anammox, and the importance of suspended biomass in the MBBR should be studied in relation to the settling characteristics of the excess sludge.</p>		
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New Dimensions of Moving Bed Biofilm Carriers

Influence of biofilm thickness and control possibilities

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Water is the main thing

Abstract

The moving bed biofilm reactor (MBBR) is a biological wastewater treatment process in which microorganisms grow as biofilms on suspended carriers. Conventionally, MBBRs are mainly designed and optimized based on the carrier surface area, neglecting the dynamic relationship between carrier design, reactor operation and biofilm characteristics, such as biofilm thickness and the composition of the microbial community. The purpose of this research project was to learn more about the roles of the biofilm carriers in the MBBR process, with the intention to improve process performance and develop new MBBR applications. In doing so, the MBBR performance was evaluated in several lab studies, considering different aspects such as carrier design and operational strategies. A new carrier type, the Z-carrier, was developed, with which it was possible to control the biofilm thickness in the MBBR. Hence, the Z-carrier enabled the evaluation of having different, pre-defined biofilm thicknesses in the MBBR process, something that has not previously been achievable. This thesis shows that biofilm thickness control can be used to ensure a more stable process performance as well as to avoid carrier clogging and minimize issues with biofilm scaling that may have detrimental effects on the MBBR performance. It was also shown that the microbiology in biofilms can be altered by biofilm thickness control. Based on these findings, a novel process configuration was developed, showing that successful nitrification of mainstream municipal wastewater could be achieved when combining thin, controlled biofilms with a periodic exposure of the biofilm to reject water from sludge dewatering. Finally, the role of suspended biomass in the MBBR was evaluated in relation to carrier surface area, HRT and loading rate, showing that the suspended biomass can have a considerable effect on the overall process performance, and that the design of MBBRs should not always be solely based on biofilm surface area. For future studies, the potential of using biofilm thickness as a control parameter for the MBBR should be investigated further, especially for specific microbial applications such as nitrification and anammox, and the importance of suspended biomass in the MBBR should be studied in relation to the settling characteristics of the excess sludge.

Mer än bara yta – nya möjligheter med biofilmskontroll i MBBR-processen

Om du någonsin har glömt din disk i diskvattnet lite för länge, har du antagligen märkt hur den gradvis täcks av en slemmig hinna. Hade du haft tillgång till ett mikroskop, skulle du ha sett att detta slem i själva verket är fullt av aktivitet. Näringsämnena som finns kvar i matresterna på din disk har nämligen blivit mat till en mängd olika mikroorganismer, och en tät matta av bakterier och mikrodjur har börjat växa i en så kallad biofilm. Biofilmer finns nästan överallt där det finns fukt och tillgång till näringsämnen, både ute i naturen och i våra hem. Till och med dina tänder är täckta av biofilm, allmänt känd som plack.

I vår vardag spolar vi ut mängder av näringsämnen med vårt avfall, och ett näringsrikt avloppsvatten bildas. Näringsämnen är livsviktiga för allt levande, men alltför höga koncentrationer på fel plats kan ha dramatiska konsekvenser, såsom algblooming och fiskdöd. Därför måste avloppsvattnet renas från näringsämnen innan det kan återföras till våra naturliga vattendrag. Precis som när bakterierna i din gamla müsliskål äter matresterna i ditt diskvatten, används ofta mikroorganismer för att rena avloppsvatten i s.k. biologisk avloppsvattenrening.

En variant av biologisk avloppsvattenrening som har utvecklats de senaste 30 åren är Moving Bed Biofilm Reactor (MBBR)-processen. En MBBR baseras faktiskt på samma sorts biofilm som växer på din disk, men för att få in så mycket bakterier som möjligt används miljontals små plastbitar, s.k. bärare, i en tank som kontinuerligt matas med avloppsvatten. Biofilmen växer på bärarna, och ju fler bärare du har i din reaktor, desto fler bakterier kan växa och desto snabbare bryts näringsämnena i vattnet ner.

Utöver den ökade kapaciteten, erbjuder bärarna en skyddad miljö för bakterierna, där de kan växa i sin egen takt utan att bli utsköljda med det genomströmmande avloppsvattnet. På grund av denna skyddande miljö kan specialiserade, långsamt växande bakterier frodas på bäraren, vilket gör MBBR-processen ideal för att reducera svårnedbrytbara ämnen i avloppsvattnet. Genom att dela upp bärarna i olika steg kan man också bygga upp en serie av olika funktioner i samma reningsverk.

Bäraren är med andra ord central i MBBR-processen, och det finns en mängd olika sorters bärare med varierande storlek och design. Vid utveckling av nya bärare har fokus främst legat på att öka bärarens yta, med målet att kunna få in ännu mer bakterier i processen. Dock försummas ofta andra aspekter, såsom hur biofilmen växer på olika sorters bärare och hur biofilmens struktur i sin tur kan påverka och påverkas av olika förutsättningar i reaktorn. Dessutom har det påvisats att bärarens utformning och storlek, samt hur mycket bärare som finns i reaktorn, kan påverka omblandning och andra viktiga faktorer i MBBR-processen, som i sin tur är kopplade till den totala energiförbrukningen på avloppsreningsverket.

I detta doktorsarbete har bärarens roll i MBBR-processen undersökts närmare, med fokus på att kunna kontrollera biofilmens tjocklek – den tredje dimensionen av biofilmen. En ny bärardesign, Z-bäraren, har utvecklats och olika experimentella studier har genomförts för att utvärdera samspelet mellan bärarens design, biofilmens egenskaper och den övergripande effektiviteten av MBBR-processen. Dessutom har betydelsen av biofilmens tjocklek utvärderats som en möjlig kontrollparameter, i syftet att kunna skräddarsy nya tillämpningar av MBBR-processen.

Det har tidigare inte varit möjligt att utvärdera effekten på MBBR-processen av biofilmens tjocklek, eftersom möjligheterna att kontrollera biofilmen har varit begränsade. Men med hjälp av den nya Z-bäraren gick det att styra biofilmens tjocklek på en helt ny nivå, vilket möjliggjorde ett antal studier där vikten av biofilmstjockleken i MBBR-processen kunde utredas. Studierna visade bl.a. att en tjockare biofilm erbjöd fler miljöer, i vilka olika sorters mikroorganismer kan växa, samt ett bättre skydd mot olika störningar av processen, medan en tunnare biofilm var mindre motståndskraftig. Det visade sig dock även att den tunna biofilmens känslighet för påverkan, i kombination med väl valda driftsstrategier, kunde användas för att styra biofilmens sammansättning och funktion.

Studien visade även på fördelarna med biofilmskontroll för att förhindra igensättning av bärare. Detta kan hända i MBBR-processen, t.ex. om näringstillgången är hög, och kan resultera i att bäraren blir ineffektiv. Slutligen studerades också biofilmens ymp-effekt, d.v.s. huruvida avskavda biofilmsfragment fortfarande kunde vara aktiva i MBBR-processen och bidra till den övergripande nedbrytningen i processen.

Överlag visar den här avhandlingen på vikten av att utvärdera MBBR-processen ur ett helhetsperspektiv, där en förståelse för bärarens och biofilmens olika roller är central för att kunna utveckla och designa framtidens reningsverk.

List of papers

This thesis is based on the following papers, listed in the order of submission. All papers are appended at the end of the thesis, and will be referred to by Roman numerals in the text.

- I. Maria Piculell, Thomas Welander, Karin Jönsson. Organic Removal Activity in Biofilm and Suspended Biomass Fractions of MBBR Systems. *Water Science and Technology*, 2014, Vol. 69 (1), pp. 55-61^{*,a}
- II. Maria Piculell, Pia Welander, Karin Jönsson, Thomas Welander. Evaluating the Effect of Biofilm Thickness on Nitrification in Moving Bed Biofilm Reactors. *Environmental Technology*, 2016, Vol. 37 (6), pp. 732-743^{*,b}
- III. Maria Piculell, Magnus Christensson, Karin Jönsson, Thomas Welander. Partial Nitrification in MBBRs for Mainstream Deammonification with Thin Biofilms and Alternating Feed Supply. *Water Science and Technology*, 2016, Vol. 73 (5), pp. 1253-1260^{*,a}
- IV. Maria Piculell, Stina Lidén, Gunilla Henningsson, Flemming Wessman, Chris Thomson, Thomas Welander, Maria Ekenberg. Minimizing Clogging and Scaling Issues in the Moving Bed Biofilm Reactor Using Biofilm Control. *Conference proceeding for WEFTEC 2015, Chicago*^{*,c}
- V. Maria Piculell, Carolina Suarez, Chunyan Li, Magnus Christensson, Frank Persson, Michael Wagner, Malte Hermansson, Karin Jönsson, Thomas Welander. The Inhibitory Effects of Reject Water on Nitrifying Populations Grown at Different Biofilm Thickness. (*Submitted*)
- VI. Maria Piculell, Pia Welander, Christian Rosén, Thomas Welander. The Importance of Biofilm Surface Area and Suspended Biomass in the MBBR. (*Manuscript*)

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My contributions

- I. I performed all lab trials and was responsible for the continuous operation of the reactors together with Pia Welander. I wrote the manuscript with input from my co-authors, and presented the study at the IWA conference *Biofilm Reactors*, in Paris (France), May 2013.
- II. I planned the study and developed the Z-carrier prototypes together with Thomas Welander. I shared the laboratory responsibilities with Pia Welander, and was responsible for data assessments and statistical evaluations. I wrote the manuscript with input from all co-authors.
- III. I planned the study together with Thomas Welander and Magnus Christensson, and performed the on-going data assessment. I was responsible for the laboratory work with the assistance of Pia Welander. I wrote the manuscript with the help from my co-authors, and presented the study at the IWA conference *Nutrient Removal and Recovery*, in Gdansk (Poland), May 2015.
- IV. I managed the research project, while the lab work and detailed planning was performed by Stina Lidén, Gunilla Henningsson and Maria Ekenberg. I was the corresponding author and presented the study at *WEFTEC*, in Chicago (USA), September 2015. I wrote the manuscript in close collaboration with Maria Ekenberg, with support from Thomas Welander.
- V. I designed the study together with Thomas Welander and Magnus Chirstensson, and performed all lab trials. I also processed the OCT-images, with help from Chunyan Li who performed the imaging. Carolina Suarez performed the FISH-analyses and microbial assessments. I wrote the manuscript with input from all co-authors.
- VI. I planned the study together with Thomas Welander. I performed the lab trials with the assistance of Pia Welander, and derived the mathematical evaluations together with Christian Rosén. Finally, I was responsible for writing the paper together with Thomas Welander.

Abbreviations

AOB	Ammonia oxidizing bacteria
AnAOB	Anaerobic ammonia oxidizing (anammox) bacteria
BOD	Biochemical oxygen demand
CLSM	Confocal laser scanning microscopy
COD	Chemical oxygen demand
DO	Dissolved oxygen
EBA	Exposed biofilm area
FA	Free ammonia nitrogen ($\text{NH}_3\text{-N}$)
FISH	Fluorescent in situ hybridization
FNA	Free nitrous acid nitrogen ($\text{HNO}_2\text{-N}$)
HRT	Hydraulic retention time
MBBR	Moving bed biofilm reactor
MEG	Mono-ethylene glycol
NAR	Nitrite accumulation ratio
$\text{NH}_4\text{-N}$	Ammonium nitrogen (see TAN)
$\text{NO}_2\text{-N}$	Nitrite nitrogen
$\text{NO}_3\text{-N}$	Nitrate nitrogen
NOB	Nitrite oxidizing bacteria
$\text{NO}_x\text{-N}$	Sum of $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$
OCT	Optical coherence tomography
OTE	Oxygen transfer efficiency
PCOD	Particulate COD (larger than $1.6\text{ }\mu\text{m}$)
PNA	Partial nitrification and anammox
PSA	Protected surface area
SCOD	Soluble COD (filtered through $1.6\text{ }\mu\text{m}$ pore size)
SND	Simultaneous nitrification and denitrification
SRT	Solids retention time
SS	Suspended solids
TAN	Total ammonium nitrogen
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids
VSS	Volatile suspended solids

Table of contents

Abstract	vii
Populärvetenskaplig sammanfattning	ix
List of papers	xi
My contributions	xii
Abbreviations	xiii
Table of contents	xv
1 Introduction	1
1.1 Aims and objectives	2
1.2 Scope and overview	3
2 The Moving Bed Biofilm Reactor	5
2.1 What is a biofilm?	5
2.2 Biological wastewater treatment	6
2.3 Introducing the MBBR	7
2.4 Biofilm characteristics	8
2.4.1 Diffusion and concentration gradients	9
2.4.2 Biofilm composition and structure	10
2.4.3 Biofilm growth and detachment	12
3 MBBR carriers and development	13
3.1 Established MBBR carriers	13
3.2 Carrier surface area	14
3.3 The Z-carrier	15
4 Function and role of carriers	17
4.1 Mixing and mass transfer	17
4.2 Carrier clogging	18
4.3 Biofilm attachment and growth	18
4.4 Oxygen transfer	19
4.5 Material and production	20
5 Studying the MBBR process	21
5.1 Lab-scale reactors	21
5.2 Comparing parallel MBBRs	22
5.3 Activity trials	23
5.4 The carrier cage	24

5.5	Biofilm evaluation	25
6	The role of suspended biomass	27
6.1	Suspended biomass in the MBBR	27
6.2	Loading rate, HRT and filling degree	28
6.3	Considerations related to detached biomass	31
7	Biofilm thickness – does it matter?	33
7.1	Definitions and measurements	33
7.2	Can we control biofilm thickness?	34
7.3	Biofilm thickness and nitrification	37
7.3.1	Abundance of nitrifiers in different biofilms	41
8	Applications of biofilm control	43
8.1	Case 1: Nitrification at increasing C/N ratio	43
8.1.1	Observations at increasing C/N ratio	44
8.1.2	Suggested explanations for the observations	46
8.2	Case 2: Simultaneous denitrification	47
8.3	Case 3: Scaling in biofilms	51
9	Nitritation with thin biofilms	53
9.1	Partial nitrification and anammox	53
9.2	Mainstream PNA processes	54
9.3	Suppressing NOB under mainstream operation	55
9.4	NOB suppression in thin biofilms using reject water	57
9.4.1	Challenges related to the configuration	59
10	Conclusions and outlook	61
11	Acknowledgements	65
12	References	67
13	Appendix	77
	Appendix A: Nitrification at increasing C/N ratio	77
	Appendix B: Simultaneous nitrification and denitrification	78
Paper I:	Organic Removal Activity in Biofilm and Suspended Biomass Fractions of MBBR Systems	
Paper II:	Evaluating the Effect of Biofilm Thickness on Nitrification in Moving Bed Biofilm Reactors	
Paper III:	Partial Nitrification in MBBRs for Mainstream Deammonification with Thin Biofilms and Alternating Feed Supply	
Paper IV:	Minimizing Clogging and Scaling Issues in the Moving Bed Biofilm Reactor Using Biofilm Control	
Paper V:	The Inhibitory Effects of Reject Water on Nitrifying Populations Grown at Different Biofilm Thickness	
Paper VI:	The Importance of Biofilm Surface Area and Suspended Biomass in the MBBR	

1 Introduction

Wastewater treatment acts as a barrier between pollutant-rich effluents and our sensitive aquatic recipients, averting negative environmental impact such as oxygen depletion and eutrophication of lakes and oceans. An important part of wastewater treatment is the biological treatment process, which can be used for most types of wastewater. In biological wastewater treatment, microorganisms grow spontaneously in the treatment process, degrading and transforming soluble compounds in the wastewater and turning them into harmless metabolites and biomass.

The moving bed biofilm reactor (MBBR) is a biological wastewater treatment process which is used for treating most types of wastewater streams. The basic principle of the MBBR is the use of plastic carriers on which microorganisms can grow in biofilms, where different bacterial groups compete and co-exist in different niches. With microorganisms growing in biofilms rather than in suspended flocs, it is possible to fit more active biomass into the treatment plant, hence creating very compact treatment solutions.

Today, the concept of wastewater treatment is gradually being replaced by “resource recovery”, referring to the high energy and nutrient content in different wastewater streams. In view of planetary boundaries and global warming, the energy potential and nutrient content in wastewater can no longer be neglected, and recovery of these resources has become crucial in the design and development of new wastewater treatment plants. Concurrently, the capacity and effluent quality of treatment plants must improve in order to meet the increased wastewater load, caused by growing populations, and yet protect our aquatic environment from pollution. In addition to this, treatment plants often need to be compact, odour-free and almost invisibly incorporated into the city environment.

As the MBBR has grown in popularity, the demands for accurate designs, high treatment efficiency and low price has increased considerably, and several new companies have emerged on the market, vending their specific MBBR processes and novel carrier designs. Most carrier development has focused on enlarging the carrier surface area in order to optimize process efficiency. However, it is known that the carrier can play several other, crucial, roles in the process; studies have

shown how the carrier can affect both mixing and aeration requirements, and there is a dynamic relationship between carrier design, reactor operation and biofilm characteristics, such as biofilm thickness and biological community, which is yet to be understood in its full complexity.

Increasing knowledge in the field of microbiology, together with a growing environmental concern, has resulted in several new biological process solutions for wastewater treatment. The MBBR process is no exception from this recent development in microbiological specialization, and there is hence an urgent need for an in-depth understanding of the process, in order to ensure accurate designs, stable treatment performance and energy efficiency. The biofilm carriers play a central part in this understanding, as their functionality will both affect, and be affected by, the overall activity in the MBBR.

1.1 Aims and objectives

The purpose of this research project was to evaluate the role of carriers in the MBBR process, with the intention to improve the overall process performance and develop new MBBR applications. The work focused on three core aims, as listed below.

A. Achieve a better understanding of the functions of MBBR carriers.

- i. Evaluate the conventional approach to MBBR design and carrier development.
- ii. Review different carrier functions in the MBBR and how they may affect the overall performance.

B. Evaluate possibilities to control the MBBR process based on biofilm properties.

- i. Examine the relative contribution from suspended biomass in the MBBR process, related to the seeding effect from the biofilm.
- ii. Explore the effect of biofilm thickness on the overall performance as well as on the microbial composition of biofilms.
- iii. Identify MBBR processes for which biofilm control may be essential.

C. Develop and test new MBBR configurations, incorporating the concept of biofilm control.

1.2 Scope and overview

To achieve the aims listed above, several experimental studies have been conducted. The research project started with a lab-scale evaluation of suspended biomass in the MBBR (**Paper I**), in which organic removal activities were compared in suspended biomass and biofilm. This evaluation was later complemented with additional experiments, where the contributions to the overall performance from the different biomass fractions could be quantified and put in relation to operational strategies and design parameters (**Paper VI**).

In order to obtain biofilm control, a new carrier type was developed, with which it was possible to predefine and maintain a maximum biofilm thickness. Several different versions of the carrier were produced to evaluate the effect of biofilm thickness on reactor performance. This effect was studied in nitrifying MBBR systems, for which both the overall performance (**Paper II**) and the biofilm composition (**Paper V**) were evaluated. The findings obtained in **Paper II** resulted in the development of a new MBBR configuration, which was tested in lab-scale (**Paper III**), and further evaluated in **Paper V**.

The potential benefits with biofilm control were studied further in MBBR applications where carrier clogging has previously been an issue, due to scaling (i.e. inorganic precipitation on the carriers) (**Paper IV**). Other potential applications of biofilm control, mainly related to nitrification, were tested in case studies, comparing the new carrier type with conventional carrier types (not published).

This thesis aims to present the full work performed within the scope of this research project. Chapter 2-4 will introduce the reader to the MBBR process, biofilm characteristics and the related role of the MBBR carrier. Issues related to the conventional MBBR design approach will be addressed in relation to various aspects of biofilm and carrier characteristics, to point out crucial areas for further evaluation. Chapter 5 gives an overview of the methodologies used in this project, while Chapter 6-9 present and evaluate the research performed: Chapter 6 focuses on the suspended biomass in the MBBR, Chapter 7 addresses the potential importance of biofilm thickness, Chapter 8 evaluates applications for biofilm control in relation to conventional carriers, and Chapter 9 presents a potential application for the new carrier in a novel MBBR configuration. Finally, Chapter 10 concludes the project and lists areas of interest for future studies.

2 The Moving Bed Biofilm Reactor

The MBBR is a biological wastewater treatment process that was developed by professor Ødegaard and colleagues at the Norwegian University of Science and Technology in the late 80's. There are several different types of biological processes for wastewater treatment, many of which can be used for both municipal and industrial wastewater treatment. The goal of the MBBR was to combine advantages from these various processes into one system, making it a flexible and useful building block for most treatment applications. In the MBBR, active biomass grows in biofilms on the surfaces of plastic carrier elements, which are kept in suspension and movement in the process.

2.1 What is a biofilm?

Biofilms are defined as clusters of microorganisms adhered to solid surfaces, growing in almost any thinkable aqueous or humid environment. The microorganisms are embedded in a coating of extracellular polymeric substances, which are produced by the microorganisms to hold the biofilm together, protect the bacteria from dehydration and toxic substances, and to facilitate the adhesion of the biofilm to the substratum.

The ubiquitous occurrence of biofilms is often considered a nuisance; for example, dental plaque is the formation of biofilms on teeth, which can result in tooth decay and caries, and the formation of biofilms in water and sewer pipes may eventually cause issues with clogging and corrosion. But biofilms can also be beneficial, for instance in the microbiome, where biofilms can protect the body against infection (Robertson & McLean, 2015), and one of the most beneficial usages of biofilms can be found in wastewater treatment.

2.2 Biological wastewater treatment

Although the configuration of wastewater treatment plants can vary greatly, conventional wastewater treatment can usually be divided into primary and secondary treatment. In primary treatment, particulate matter is removed by screening and primary settling, while secondary treatment aims to remove soluble pollutants through biological treatment.

In biological treatment, microorganisms degrade and transform soluble pollutants in the wastewater into harmless metabolites such as carbon dioxide and water, and into biomass which can be separated by for example settling and/or filtration. Microorganisms grow spontaneously in the wastewater treatment plants, but their growth can be controlled by different operational conditions such as aeration, hydraulic retention time (HRT), solids retention time (SRT) and load. Biological treatment usually includes the removal of organic matter (measured as chemical or biochemical oxygen demand – COD or BOD, respectively) through aerobic conversions by heterotrophic bacteria, and the removal of nitrogen through aerobic and anoxic conversions by autotrophic and heterotrophic bacteria (commonly referred to as nitrification and denitrification), respectively. Phosphorous compounds can also be removed in biological wastewater treatment. The level of efficiency of the treatment can be adjusted depending on loading and effluent requirements.

There are several types of technologies for biological wastewater treatment, often categorized into attached (fixed-film) and suspended growth systems based on the mechanism by which the biomass is retained in the process. Attached growth systems, such as trickling filters, are based on the microorganisms growing in biofilms on some kind of support material applied in the process. In suspended growth systems, on the other hand, the microorganisms grow in flocs (i.e. activated sludge) which are retained in the system by being separated from the treated wastewater, normally by settling, and re-circulated to the treatment reactor (Henze et al., 2002). The activated sludge process has existed for over 100 years and has been fundamental for the development of numerous wastewater treatment technologies, design configurations and operation schemes (see Stensel and Makinia (2014) for a thorough overview).

There are benefits and drawbacks with both attached and suspended systems. Activated sludge processes are generally flexible and require little maintenance. These plants are, however, sensitive to high fluxes of wastewater, which can result in loss of biomass, and to toxic disturbances (Jönsson, 2001), as the biomass is directly exposed to the bulk water. Most activated sludge processes also require

relatively large reactor volumes and need a careful process control to maintain acceptable separability of the biomass. In comparison, fixed-film processes are more compact and less sensitive to varying environmental conditions, but may be susceptible to clogging of the support material and may thus be sensitive to suspended solids in the incoming wastewater. In response to this, the MBBR was launched in the 80's, aiming to combine advantages from both attached and suspended growth systems, by growing biofilms on a support material in the form of plastic carrier elements, which are kept in suspension and continuous movement in the process (Ødegaard, 1999).

2.3 Introducing the MBBR

The MBBR (Figure 1) is a biofilm-based biological wastewater treatment process that has increased considerably in popularity over the last decades, with 90 plants distributed in 17 different countries in 1999 (Ødegaard, 1999) expanding to more than 600 plants in 50 countries 10 years later (McQuarrie & Boltz, 2011). The popularity of the MBBR is related to its flexibility, where the technology can be used for most biological applications, including municipal and industrial wastewater treatment, aquaculture and potable water treatment (McQuarrie & Boltz, 2011). The MBBR is especially ideal when upgrading existing wastewater treatment plants (typically activated sludge systems) to meet future, increasing loads in existing volumes (Javid et al., 2013).

In the MBBR, microorganisms grow on carriers which are suspended and kept moving in the reactor by mechanical mixing and/or aeration (Ødegaard et al., 1994). Most carriers are designed to provide a large protected surface area inside voids and cavities (see Chapter 3), where biofilms can grow in a sheltered environment, and the capacity of the MBBR can be adjusted by changing the volumetric filling degree of the carriers, to meet the specific removal requirements at the treatment plant. The continuous mixing in the reactor keeps the carriers in constant movement, in the attempt to prevent carrier clogging and to enhance substrate availability in to the biofilm, hence improving treatment capacity (Ødegaard, 1999).

The carriers are kept in the MBBR by retention sieves over the reactor outlet (McQuarrie & Boltz, 2011), which ensure that biomass is contained in the process independent of the flux through the reactor (Figure 1). Due to this independence, MBBRs can be operated at shorter HRTs, and are therefore considerably more compact, compared to activated sludge systems (Javid et al., 2013; McQuarrie &

Boltz, 2011; Ødegaard et al., 1994). Furthermore, the long sludge age in the biofilm enables the cultivation and effective retention of slow-growing bacteria, such as AnAOB (see Chapter 9) (Christensson et al., 2013), and the removal of slowly-degradable compounds such as micropollutants (Falås et al., 2012).

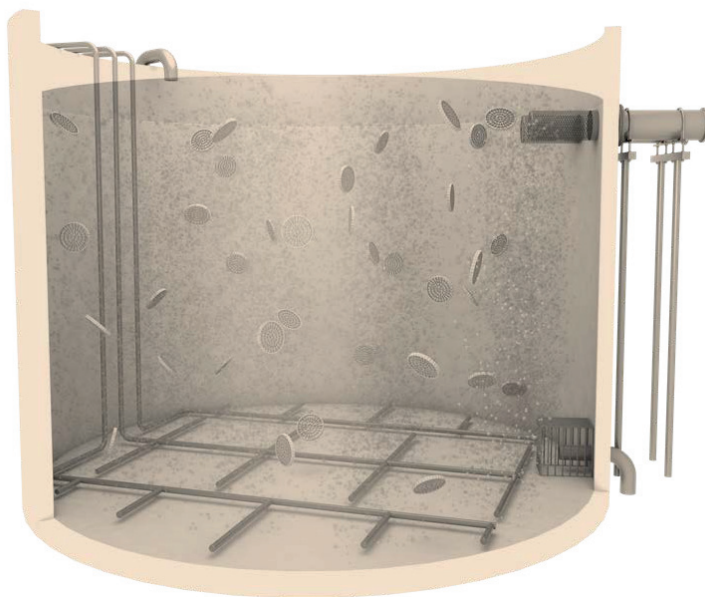


Figure 1

Schematic image of an aerated MBBR (not to scale), showing aeration grids at the bottom of the reactor and the retention sieves over the reactor outlet (top right). In the real process, the carrier filling degree is usually considerably higher. Image by Veolia Water Technologies.

2.4 Biofilm characteristics

Since the MBBR is a biofilm-based process, its capacity and efficiency relies on the growth dynamics of biofilms. Biofilms differ considerably from suspended biomass, both in their microbial composition and governing transport mechanisms, and can vary considerably between different applications. Below follow some general descriptions of crucial biofilm characteristics in the MBBR process.

2.4.1 Diffusion and concentration gradients

Diffusion is generally considered to be the major transport mechanism in MBBR biofilms (Herrling et al., 2015; Rusten et al., 1992). Since diffusion is triggered by concentration differences, the access to substrates and the disposal of metabolites throughout the biofilm will depend on the concentration gradient between the biofilm and the bulk liquid. As bacterial conversion rates rely on the access to substrates, the treatment efficiency in MBBRs will hence be limited by the diffusion rate and the bulk liquid concentration of substrates (Boltz & Daigger, 2010).

Figure 2 exemplifies the concentration gradient of oxygen through a biofilm, where anaerobic zones are created at the innermost layers of the biofilm, as a result of limited oxygen diffusion. The oxygen penetration depth in biofilms will vary depending on the bulk liquid concentration, reactor hydrodynamics and biofilm density, and can range from 50 to 500 μm (Gieseke et al., 2003; Hibiya et al., 2004; Horn & Hempel, 1995; Schramm et al., 1996). Similar gradients can be observed for other substrates, while gradients of metabolites will have a reverse direction (Okabe et al., 2002). As indicated in Figure 2, the biofilm is covered by a boundary layer, i.e. a stagnant layer of liquid surrounding the biofilm. The boundary layer thickness is critical for the access of substrates into the biofilm, and will vary depending on bulk liquid turbulence (Mašić et al., 2010). In addition, the structure and thickness of the biofilm may also affect the flow pattern and the boundary layer surrounding the biofilm, further influencing substrate availability (Herrling et al., 2015).

Many studies have pointed out the strict diffusion limitations in MBBRs, especially related to nitrification, where the availability of dissolved oxygen (DO) and/or ammonium will limit the overall activity of the process (Christensson & Welander, 2004; Gapes & Keller, 2009; Hem et al., 1994). Generally, nitrifying MBBRs are operated at oxygen limiting concentrations, and studies have shown how nitrification rates can be gradually improved up to DO concentrations of 20 mg/L, provided that other substrates are available (Bonomo et al., 2000; Gapes & Keller, 2009). This increase can be explained by an increased specific activity of the bacteria due to higher substrate availability, as well as an increased activation of bacteria in the deeper layers of the biofilm (i.e. a deeper oxygen penetration) (Gieseke et al., 2003).

Due to diffusion limitations, aerobic MBBR processes must generally operate at higher DO concentrations and/or higher mixing intensities than the activated sludge process, in order to ensure sufficient substrate availability (Rosso et al., 2011). This may result in higher energy requirements of the MBBR process, since

both mixing and aeration require considerable energy input. But although diffusion limitation is often considered a drawback of the MBBR process, it can be used as an advantage; the concentration gradient will result in several different microbial niches, enabling co-existence of many different functional groups in the same system (as further discussed below). In addition, microorganisms in the deeper layers of the biofilm will be less exposed to sudden toxic disruptions in the process, potentially making the MBBR more resilient to microbial inhibition than suspended growth processes (Borghei & Hosseini, 2004).

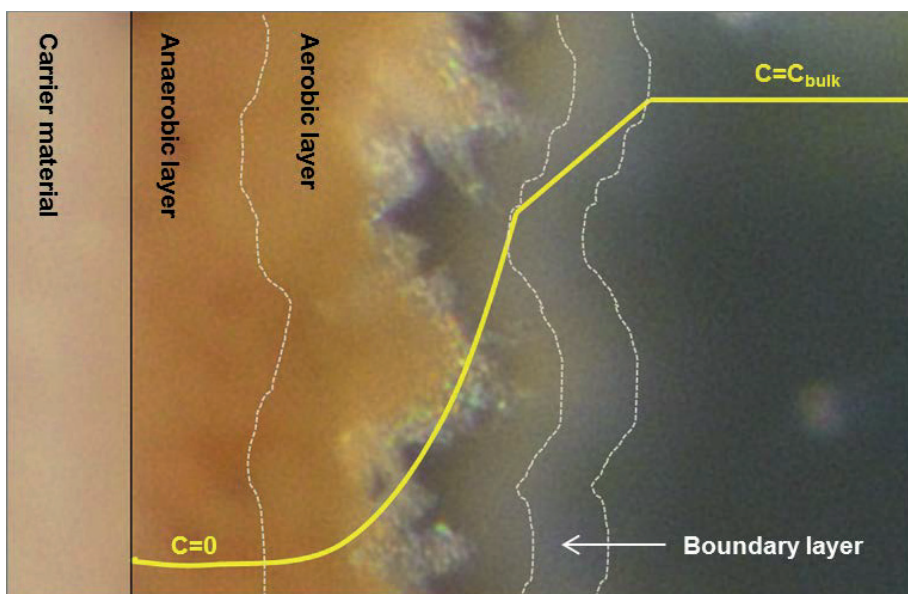


Figure 2

Image of a biofilm where diffusion limitations create a concentration gradient of oxygen (yellow line) through the biofilm, which in turn results in a stratified biofilm composition with aerobic and anaerobic niches (not to scale).

2.4.2 Biofilm composition and structure

In response to diffusion gradients in biofilms, different bacterial groups will compete for the limited substrates and a stratified biofilm structure will develop (Zhang et al., 1994). Naturally, aerobic bacteria will grow in the top layers of the biofilm, while anoxic and anaerobic bacteria can develop in the deeper layers (Figure 2). But, in addition, the combination of different substrate gradients throughout the biofilm will create numerous microbial niches, which also enables the co-existence of bacteria with very similar functions (Gieseke et al., 2003).

The structure of the biofilm can vary considerably depending on reactor conditions such as loading rate, DO and reactor hydrodynamics. Generally, the biofilm structure will depend on the microbial population, where fast growing organisms create more porous biofilms (Van Loosdrecht et al., 1997). A porous structure is further induced by high substrate availability, while the biofilm density may increase if the turbulence is high, as a result of an elevated shear on the biofilm surface (Beyenal & Lewandowski, 2002; Van Loosdrecht et al., 1995).

Depending on biofilm structure, the availability of substrates may vary; the diffusivity may decrease in a denser biofilm (Beyenal & Lewandowski, 2002; Feng et al., 2012; Horn & Morgenroth, 2006), while a rough and flaky biofilm surface increases the biofilm-liquid interface, hence enhancing the substrate availability (Li et al., 2016a). Naturally, a thicker biofilm contains more biomass and therefore has a higher potential, provided that substrate availability is high. At low substrate availability, however, a thin biofilm may be just as effective as a thick biofilm, since only the very top layer is active. The active fraction of the biofilm will vary in different processes, due to microbial stratification, diffusion and varying biofilm structure and density. Usually only a small fraction of the biofilm is aerobically active, and this fraction may decrease as the biofilm thickness increases (Alpkvist et al., 2007; Ødegaard, 1999). However, it has been shown that a thicker biofilm can have a deeper oxygen penetration depth relative to a thinner biofilm, possibly because of an increased biofilm porosity (Hibiya et al., 2004; Zhang et al., 1995).

Although biofilm properties such as thickness and structure may affect the performance of the MBBR, the attempts to compare reactor performance in relation to these properties are limited. Although it has been shown how biofilm properties can differ between different carriers (Forrest et al., 2016; Li et al., 2016b), there is a lack of understanding of how specific carrier designs and/or reactor configurations may affect these properties (McQuarrie & Boltz, 2011). Since there has previously been no means to predetermine and control biofilm thickness and structure, it has been difficult to design experimental studies with the aim of relating MBBR performance to these parameters, and to obtain reliable results. Due to this limited knowledge, MBBRs are normally designed solely based on carrier surface area, independently of the carrier design and biofilm characteristics (Ødegaard et al., 2000).

2.4.3 Biofilm growth and detachment

Biofilm formation on MBBR carriers commonly follows the four stages of attachment, accumulation, re-generation and maturation (Zhu et al., 2015). The growth patterns of biofilms are, however, dynamic, and hence it is practically impossible to reproduce a specific biofilm growth pattern (Lewandowski et al., 2004). In addition, the biofilm thickness and structure may vary in response to operational strategies (see previous chapter) as well as to seasonal variations in temperature, where biofilm mass generally decrease in response to increasing temperatures, due to an increased specific activity (Boltz & Daigger, 2010).

As biofilms grow on the MBBR carriers there is a continuous detachment of biomass caused by i) abrasion, caused by carriers colliding and scraping against each other, ii) erosion, caused by shear forces in the bulk liquid surrounding the biofilm, iii) sloughing, where larger biofilm segments detach from the carrier element, and/or iv) predator grazing (Morgenroth & Wilderer, 2000). While abrasion and erosion result in a continuous detachment of smaller particles from the biofilm surface, sloughing may occur randomly and result in the detachment of whole biofilm segments from the carrier surface (Horn et al., 2003). Simultaneously, predator grazing may affect the overall sludge production of the MBBR process, as predating microanimals consume bacteria in the system (Lee & Welander, 1996).

Assuming that the biofilm system is at steady state, the detachment rate of biomass can be considered equal to biofilm growth. However, for most MBBR systems the shear forces will vary over time, and detachment will vary accordingly (Horn et al., 2003). However, due to detachment, the MBBR always contain some suspended biomass, which is not attached to the carriers but may still contribute to the overall performance (Mašić & Eberl, 2014). The importance of this contribution will vary depending on the specific growth rate and the activity of the microorganisms in the process, as well as in response to loading rate, substrate concentrations and HRT (see Chapter 6).

3 MBBR carriers and development

It is apparent that biofilm carriers are of central importance in the MBBR process, and while the original MBBR carrier design is still in use (see below), several new designs have emerged. Carrier development has mainly focused on increasing the carrier surface area, on the assumption that the available area is a dominating factor for the overall performance of the MBBR process. The effect of biofilm growth on different carrier types has, however, been overlooked. In this research project, a new carrier type was therefore developed, aiming to control and predetermine biofilm thickness and active surface area in the MBBR.

3.1 Established MBBR carriers

The original MBBR carrier was developed in Norway in the 80's (Rusten et al., 1992) and is still used today as the K1 carrier (AnoxKaldnes, see Figure 3). Since then, numerous types of MBBR carriers have been developed and marketed, and most established manufacturers currently supply more than one carrier type. For example, Veolia Water Technologies currently have seven different carrier types in their portfolio (see Figure 3 for examples), differing in surface area, shape and specific design.

Conventional MBBR carriers are generally designed to have a large protected surface area on which biofilm can grow. By increasing the carrier surface area per volume, more biofilm can be fitted into the MBBR process, hence making it more compact and efficient. The available carrier area per packed volume can vary greatly, ranging from 200 to 1,200 m²/m³ for some of the most commonly used carrier types, where the carrier diameter can range from 7 mm to 6.4 cm (Barwal & Chaudhary, 2014; McQuarrie & Boltz, 2011).

Most MBBR carrier designs feature an array of channels or voids (Figure 3) (McQuarrie & Boltz, 2011), but there are also several alternative carrier designs, such as porous media (Bassin et al., 2016; Martín-Pascual et al., 2012), loofa sponges (Zhang et al., 2012), cut pieces of tubing (Orantes & González-Martínez, 2003), gel beads (Levstek & Plazl, 2009) and even cigarette-filter rods (Sabzali et al., 2012).

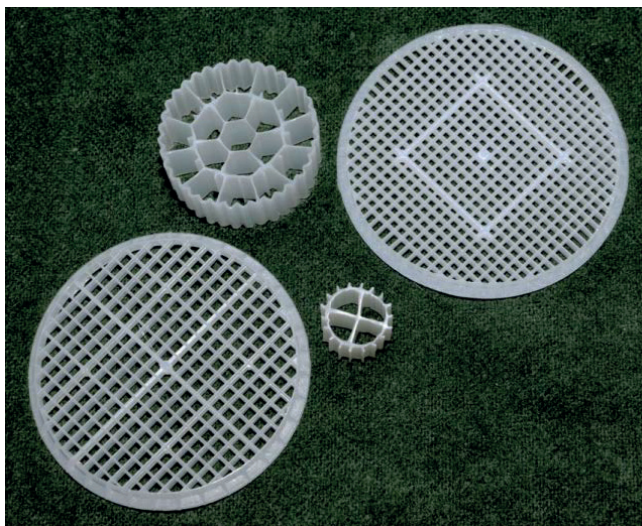


Figure 3
Examples of conventionally used MBBR carriers with inner protected surface area (clockwise from bottom left corner): Chip P, K3, Chip M and K1 (Veolia Water Technologies, AnoxKaldnes).

3.2 Carrier surface area

As mentioned above, the development of MBBR carriers over time has generally focused on increasing the carrier surface area. However, the entire area of the carrier is usually not covered with biofilm. As carriers collide in the MBBR, biofilm growing on the outer, exposed carrier surfaces will be scraped off (Ødegaard, 1999). Due to this phenomenon, the concept of protected surface area (PSA), sometimes also called effective surface area, has been defined as the *carrier surface area which is available for biofilm growth in a protected environment*, and is a commonly used unit to classify MBBR carriers (Ødegaard et al., 1994). In conventional MBBR carriers, which are generally cylinder-shaped with voids (see Figure 3), the PSA mainly constitutes the inner carrier surfaces,

where carrier collisions does not have an effect on biofilm formation (Ødegaard et al., 1994; Ødegaard et al., 2000). Most MBBR designs are based on the PSA, independent of carrier design, and when developing new carriers with higher PSA it is expected that MBBR performance capacity will improve correspondingly (Barwal & Chaudhary, 2014).

The essence of MBBR efficiency, however, is not the carrier area itself, but having a high amount of active biomass performing process specific tasks and degrading targeted substances (Bassin et al., 2016). As discussed in Chapter 2, the active fraction of the biofilm may vary as a result of biofilm thickness and structure, and can differ considerably between different processes. Hence, it is necessary to challenge the use of PSA as a sole measure of carrier efficiency for reliable and predictive MBBR designs. The exposed biofilm area (EBA), defined as the *area of biofilm exposed to the bulk liquid in the process*, may be a more accurate design parameter than PSA. The use of EBA instead of PSA would, however, require a prediction of biofilm growth, which is complex and process dependent (see Chapter 2). Hence, there is a need for tools that enable a better prediction of biofilm development in the MBBR process.

3.3 The Z-carrier

During the course of this thesis work, a new MBBR carrier type – the AnoxKaldnes Z-carrier (Welander & Piculell, 2015) – was developed and tested for several processes. The Z-carrier (Figure 4) was developed only partially focusing on PSA, mainly aiming to target the biofilm thickness (the Z-dimension of the biofilm). Biofilm thickness is controlled on the Z-carriers by utilizing the scouring action from colliding carriers in the MBBR process. Biofilm grows on the outside of the Z-carriers instead of inside voids, and the exposed biofilm is retained on the carrier surface by a thin grid covering the entire carrier. The height of the grid will hence determine the maximum biofilm thickness, as all excess biomass will be scraped off by colliding carriers. Since biofilm grows on the outside of the Z-carrier in small, defined compartments, the EBA can be approximated and will remain similar independent of biofilm thickness.

Five different types of Z-carriers were tested in this study (Table 1). Initially, four saddle-shaped carriers were developed with 200, 300, 400 and 500 μm grid wall height (Z200-Z500, see Figure 4). The saddle shape was selected to enhance mixing and oxygen transfer (see Chapter 4). Later, a fifth Z-carrier prototype was developed with only a 50 μm grid wall height (Z50). This carrier was only

intended for small-scale research purposes, for which mixing and oxygen transfer was not an issue, and was hence shaped as a coin to simplify production.

Table 1 below describes the characteristics of the different Z-carriers, including PSA and EBA. While EBA is defined as the area of the grid compartment *floor*, which is the same for all saddle shaped carriers (Z200 to Z500), PSA is defined as the area inside the grid compartments, *including* the area of the grid walls; hence the PSA will vary with the grid height. The differences in PSA between the different Z-carriers will only be relevant when the biofilm thickness approaches zero, but normally the grid compartments should be filled with biofilm up to the maximum height. As the carriers scrape each other in the process, the biofilm will be thinner in the centre of each compartment compared to along the grid walls. A simple calculation based on grid compartment size and carrier diameter resulted in an estimated scraping depth of less than 90 μm for the saddle shaped Z-carriers (see **Paper II**). On Z50 the compartment size was decreased in order to decrease the scraping depth, and to ensure biofilm growth over the whole compartment.

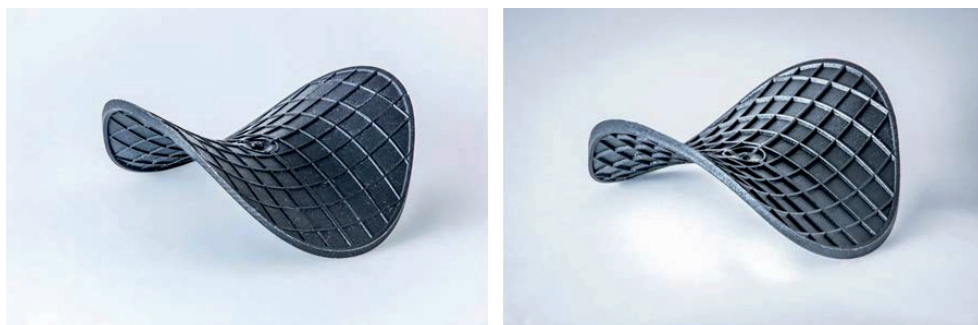


Figure 4

The Z200 with a 200 μm grid wall height (left), and the Z400 with a 400 μm grid wall height (right): two types of Z-carriers developed as part of this research project. Photo by Alan Werker.

Table 1

Z-carriers used in this research project, including parameters defining the carrier shape and dimensions, including exposed biofilm area (EBA) and protected surface area (PSA).

Carrier type	Shape	Projected diameter (mm)	Grid height (μm)	Compartment size (mm)	Carrier area ($\text{mm}^2/\text{carrier}$)	
					EBA	PSA
Z50	coin	32	50	1.5 x 1.5	1120	1270
Z200	saddle	30	200	2.3 x 2.3	1280	1740
Z300	saddle	30	300	2.3 x 2.3	1280	1960
Z400	saddle	30	400	2.3 x 2.3	1280	2190
Z500	saddle	30	500	2.3 x 2.3	1280	2420

4 Function and role of carriers

Although carrier surface area has a considerable impact on the MBBR performance, other aspects of the carrier design should not be neglected. Below follows an overview of different functions related to carrier design, pin-pointing the many aspects to be considered when developing new carrier elements as well as when choosing the right carrier type for a certain process.

4.1 Mixing and mass transfer

In order to keep the carriers in suspension, aeration and/or mechanical mixing is needed in the MBBR. The required mixing intensity, and resulting energy requirement for mixing, depends on reactor dimensions and volumetric load, as well as on carrier design. The density of the carrier material will also affect the mixing requirements; carriers with a density close to that of water are easier to mix since they are less prone to float on the reactor surface (McQuarrie & Boltz, 2011). Furthermore, the filling degree of carriers in the reactor may affect the mixing pattern, where a reactor containing a lower carrier filling degree will enable a better movement of carriers (Barwal & Chaudhary, 2015).

In addition to keeping carriers in suspension, mixing intensity also affects the external mass transfer between bulk liquid and biofilm surface. As turbulence increases in the reactor, the stagnant boundary layer surrounding the biofilm (see Chapter 2) will decrease in thickness, improving mass transfer and consequently improving the treatment performance of the MBBR for both ammonium and COD (Nogueira et al., 2015). The importance of this effect will vary depending on carrier type, where large and open carrier bodies better allow water to flow through and pass the biofilm. In addition to affecting the mass transfer, mixing also has an important role in maintaining thin biofilms on MBBR carriers as the turbulent flow, resulting from the mixing, exerts shear forces on the biofilm, causing continuous detachment.

4.2 Carrier clogging

As mentioned in Chapter 2, only an outer fraction of the biofilm is usually active, as a result of limited substrate diffusion. When the biofilm is only partially penetrated by substrates, any additional increase in thickness will not have any beneficial effects on the process, but rather negative impacts such as i) reduced biofilm area, ii) potentially anaerobic layers causing biofilm sloughing and/or odours and iii) increased weight of the carrier (Boltz & Daigger, 2010). For some processes, the biofilm may grow so thick that it fills the carrier voids entirely and clogs the carrier. Uncontrolled biofilm growth may also be connected to higher levels of inorganic precipitates (i.e. “scaling”) in the biofilm, taking up space in the biofilm and additionally increasing the weight of the carrier material (see Chapter 8).

Different carrier designs are more or less sensitive to clogging; carriers with long and narrow voids are more prone to clogging as turbulence gets very low inside the voids, while large and open carrier bodies facilitates detachment and hence are less prone to clogging (Forrest et al., 2016). The negative impact from clogging on the overall process performance depends on how big the difference is between the EBA in the clogged carrier and the PSA used for the design. For some carrier designs, clogged voids may not affect the EBA considerably, while especially porous carriers with large PSA will have a considerably smaller EBA once clogged with biofilm (Bassin et al., 2016; Martín-Pascual et al., 2012). The risk of clogging increases with loading rate, suggesting that carriers with larger voids are to be preferred in processes operating at high load (Forrest et al., 2016).

4.3 Biofilm attachment and growth

Inevitably, carriers that effectively retain thin biofilms in larger voids, as a result of high shear forces, are less inclined to support initial biofilm colonization. Conversely, a rapid initial biofilm growth can be observed in carriers with deep pores and cavities (Chen et al., 2015; Li et al., 2016b). For some microbial processes, such as anammox, biofilm growth can be very slow, and pre-seeded carriers are sometimes added to the process during start-up, in order to speed up the initial colonization of the system (Christensson et al., 2013). An alternative to using seeded carriers is to pre-treat the carrier surface prior to startup, to increase the surface roughness for better biofilm attachment (Bolton et al., 2007; Chae et al., 2008; Chen et al., 2012). But although some improvement in biofilm

colonization has been observed when pre-treating carriers (Chu et al., 2014), there are only a few biological processes for which the investment would be financially viable, and the importance of having pre-treated carriers will rely on the growth rate of the microorganisms in the targeted process.

4.4 Oxygen transfer

Aerobic MBBRs require continuous aeration for the supply of oxygen, as well as for keeping the carriers in suspension. Aeration is often a major part of the energy consumption at wastewater treatment plants, and the aeration requirement for oxygen supply is directly related to the oxygen mass transfer from air bubbles to the bulk liquid, i.e. the oxygen transfer efficiency (OTE). The OTE depends on bubble retention time, size of the air bubble (i.e. the area of the gas-liquid interface) and the viscosity of the liquid phase.

It has been shown that the addition of carriers in the MBBR can improve the OTE. In studies using conventional, extruded carriers, OTE increases as the filling degree is increased up to 40%, above which OTE levels off or decreases (Barwal & Chaudhary, 2015; Jing et al., 2009). It is suggested that the positive effect of the carriers is due to a combination of i) increased bubble retention time, ii) breakage of air bubbles, hence increasing the gas-liquid interface area and iii) increased turbulence, resulting in high renewal of the gas-liquid interface (Jing et al., 2009). This suggests that the positive effects from carriers on OTE will mainly be observed when carriers can move freely, but will decline when mixing becomes limited by an increased filling degree. Intuitively, carriers will increase the bubble retention time both by prolonging the path of the rising bubbles and by capturing the bubbles inside the carrier voids – two effects that will most likely depend on the carrier design.

The reactor turbulence may be increased by increasing the aeration intensity, hence improving the OTE by enhancing the renewal of the gas-liquid interface (Jing et al., 2009). The aeration intensity is often higher in a compact system such as the MBBR, in comparison to the less compact activated sludge process (Rosso et al., 2011). Hence, carriers may affect the OTE in the MBBR process both directly, by the carrier elements themselves, and indirectly, through the elevated aeration intensities of a more compact system. On the other hand, MBBR processes generally operate at higher target DO concentrations than activated sludge processes, which increases the aeration requirements.

Reported effects of carriers on OTE are not always positive. There are studies showing that the effect can be negligible (Rosso et al., 2011; Viswanathan et al., 2008), or even negative (Pham et al., 2008). However, negative effects on OTE have only been observed when adding carriers to a system with fine-bubble aeration, where carriers are likely to cause coalescence of bubbles, rather than breaking the bubbles into smaller fractions (Pham et al., 2008). In addition, it is possible that different carrier designs have different effects on OTE, due to their shape, density and movement in the reactor. Unfortunately, no detailed information was given on carrier design for the studies above.

Although there are a limited number of studies on carriers and OTE, it has been seen that different carrier designs can have a considerable effect on OTE, and that OTE increases as the carrier design becomes more three-dimensional (Christensson, 2011). As the bubbles rise in the reactor, it has been observed that a flat carrier tends to orient itself in a way parallel to the air flow, hence enabling the fast passing of bubbles, while a more three-dimensional carrier will always change direction as it is hit by the rising bubbles, increasing the reactor turbulence as well as the number of collisions with bubbles in the reactor. Hence, the Z-carrier was designed with a saddle shape (see Chapter 3), which will always spin when hit by air bubbles, to ensure better oxygen transfer. However, more studies are needed to determine the ideal process conditions, and possibly ideal carrier design, to achieve a maximum OTE.

4.5 Material and production

Most carriers types are produced by extrusion or moulding from virgin, or sometimes recycled, high-density polyethylene (McQuarrie & Boltz, 2011). Extrusion is a considerably cheaper option, but depending on carrier complexity and detail, it is not always possible to use extrusion. Generally, production costs will increase with carrier complexity and number of elements necessary per packed volume, but if the carrier design enables a higher maximum filling degree, an improved volumetric performance may reduce the overall cost of the MBBR (Martínez-Huerta et al., 2009). Although small and complex carrier elements may be more expensive to produce, fewer carriers may be needed in the process, as a result of high carrier efficiency. Hence, the final carrier price for a process using small and complex carriers could be the same as for a process using larger, extruded carriers.

5 Studying the MBBR process

There are many ways to assess the MBBR process: from studying the biofilm on one individual carrier to evaluating the overall performance of a full-scale reactor. Comparisons between different systems are, however, not always straightforward, owing to the many different functions of MBBR carriers, as discussed above. Below follow presentations of and comments on the various methodologies used in this research project, and suggestions for future approaches.

5.1 Lab-scale reactors

The main aim of the studies presented in this thesis was to establish trends and principles of the MBBR process, for which a lab-scale setup was sufficient. The majority of studies were performed in small, one-litre lab reactors (Figure 5). There are several advantages with studying the MBBR process in a scale-down lab system: the flexibility is high in terms of loading rates and reactor configuration, and the necessary amount of feed wastewater is relatively low. In addition, no restrictions apply regarding effluent quality. Hence, different feeding schemes can be applied using both real and synthetic wastewaters, and the reactors can be controlled and monitored to a high level of detail, operating at stable loading and reactor conditions, which cannot be achieved when studying a pilot plant operating on real wastewater. On the other hand, due to the small scale of the systems, possibilities for in-depth microbiological evaluations are limited. Before moving into full-scale applications, it is advisable to test the lab-scale findings in an intermediate pilot-scale setup, in order to determine ideal mixing patterns, as well as the system response to varying load and feed composition.



Figure 5
The lab-scale setup used in **Paper III**, with an aerated reactor containing Z200 (left) and an anaerobic, mechanically stirred reactor containing K5 (right).

5.2 Comparing parallel MBBRs

Many studies presented in this thesis compare effects of varying the carrier filling degree or the carrier type in parallel MBBRs. In addition, the reactor operation was varied in some of the studies, by altering loading rate, feed composition, HRT and/or DO concentration over time.

When varying an operational parameter, such as filling degree, between parallel reactors, several factors are affected. Although the reactors may be operated at similar loading rates and mixing intensities, effluent concentrations and reactor hydrodynamics will depend on the activity in the reactors and on the movement of the carriers, which in turn may be affected by carrier design and filling degree. When these conditions differ, substrate availability to the biofilms will vary, resulting in different biofilm performance in the different reactors. Hence, differences in the overall reactor performance will be related both to differences in carrier area (a direct effect of filling degree) in the systems, and to differences in biofilm activity on the carriers (an indirect effect of filling degree). Due to this complex interaction, the interpretation of reactor performance based on a single parameter, such as surface area removal rate, may be ambiguous. Therefore, operational aspects, biofilm characteristics and reactor conditions have all been considered when comparing results from different reactors in the present work.

For MBBR processes, reactor performance is generally evaluated based on the assumption of steady-state conditions. A steady state has been assumed for many of the studies presented in this thesis, even though loading rates and other operational parameters were changed more or less frequently. However, one must be aware that the response time of biofilms to operational changes may vary with the biofilm composition. For instance, growth rates differ considerably between different bacterial groups, where heterotrophic bacteria can have a doubling time of a few hours whereas nitrifiers may require days to duplicate. For heterotrophic systems it may thus be reasonable that the biofilm responds to operational changes in a few days, while for nitrifying systems the change may take much longer. In the presented work, steady-state performance was generally assumed when the observed removal rates were relatively stable over a specified length of time, considering the growth rate of the targeted microbial community as well as the time frame of the overall study.

5.3 Activity trials

When operating reactors in continuous mode, the overall performance can be determined for the actual reactor configuration. However, the real potential is often considerably higher, since the system, in general, is limited by one or more substrates. In addition, the potential of each individual functional group of bacteria may be underestimated due to competition between groups for common substrates, e.g. the competition for oxygen between different nitrifying bacteria (i.e. nitrifiers) (see Chapter 7). Hence, activity trials in batch configuration are often a valid complement to measurements in continuous operation, in order to better understand the underlying reasons for observations made in continuous mode. In addition, a batch trial can be a simple and rapid method for obtaining information about the potential in a full-scale system, without performing an extensive mass balance assessment (see Chapter 8), or for evaluating the activity in separate biomass fractions from the same system (see Chapter 6).

Although batch trials aim to evaluate the maximum capacity of a system under non-limited conditions, usually at high substrate availability, the setup and reactor conditions may still affect the final result. Just as for the continuous setup, mixing can have a considerable effect on the activity in the batch trial, and in addition, oxygen may be limiting (Chapter 2). Hence, it was here considered essential to apply the same mixing configuration, and similar DO concentrations in all batch trials of a study, in order to obtain comparable values.

Results obtained from batch trials should not be regarded as more accurate than results obtained from continuous mode, as they do not include the effects of competition and/or substrate limitations that will always apply in full-scale systems. Rather, the information obtained in batch trials should be used as a complement to the measurements in continuous mode, giving insights into property changes over time or in response to different operational changes.

Continuous, long-term lab trials and short-term batch trials are two conventionally used methods to study MBBRs. A third method, which was frequently used in this project, was to perform studies in short-term continuous trials, in which carriers and/or biomass were removed from their original environment and studied under continuous operation during a limited time span. Alternatively, operational parameters such as DO concentration or feeding scheme were changed during a relatively short time span, in order to evaluate the effect of a temporary change on the overall system (see discussion on steady state above).

5.4 The carrier cage

In order to compare the performance of different carriers under certain operational conditions, a carrier cage, placed directly in a full-scale system, was used in the present work (see Chapter 8). The cage (Figure 6) contained separate, individually aerated compartments, which were filled with different carrier types for comparison. The activity of the carriers in each cage compartment could then be measured and compared in batch trials over time. Compared to operating in a lab- or pilot-reactor setup, the carrier cage was a simple approach to study growing biofilms on different carrier types under full-scale conditions. However, conditions, such as substrate availability and mixing, may differ inside the cage from those in the full-scale reactor, as well as between the different cage compartments. In addition, the cage only enabled observations under prevailing conditions, and no control strategies could be applied to optimize the performance of the carriers. However, for the purpose of assessing the suitability of different carriers for different processes (e.g. comparing a controlled biofilm thickness to unlimited biofilm growth as in Chapter 8), the carrier cage is a useful alternative to bench-scale trials.



Figure 6

The carrier cage used for the study presented in Chapter 8.

5.5 Biofilm evaluation

Since most of the studies presented in this thesis evaluated MBBRs in small lab-scale systems, the amount of carriers available for microbial analyses were limited. Hence destructive microbial analyses of the biofilms, such as fluorescent in situ hybridization (FISH), were often not an option. Stereomicroscopy could, however, easily be performed without destroying the biofilm structure, and it was possible to follow macroscopic changes in the biofilm appearance over time. For the conventional carriers, stereomicroscopy was also used to measure biofilm thickness, by measuring the height of the biofilm perpendicular to the carrier walls. For the Z-carriers, however, the biofilm thickness could not be measured in a similar manner, due to the design of the carrier itself. Instead, an estimate of the biofilm thickness was obtained, based on the height of the grid walls, as explained in Chapter 7. Biomass content on the Z-carriers could also be quantified by measuring the total solids (TS) (see **Paper V** for methodology), although this was a destructive method which required the removal of biofilm from the carriers.

In **Paper V** the biofilm on Z-carriers was evaluated using optical coherence tomography (OCT) (Wagner et al., 2010), giving high-resolution images of the structure of the biofilm that could be used to calculate biofilm thickness as well as volume (see Chapter 7). Since OCT does not give any information on the microbial composition of the biofilm, the OCT analysis was complemented with FISH, performed on both suspended biomass and cryosections of the biofilms (Persson et al., 2014). The FISH analysis gave useful insights into biofilm microbiology by measuring relative abundance of targeted microbial groups

quantitatively, as well as by allowing studies of the microbial stratification of the biofilms with confocal laser scanning microscopy (CLSM). The information gained from OCT and FISH was also combined by using the biofilm volume to quantitate the abundance of different microbial groups (see Chapter 7).

The studies using OCT and FISH gave interesting insights regarding differences between biofilms of different thickness grown in the same environment. Additional studies could be performed in order to evaluate these findings further. Biofilm structures on the Z-carriers should be studied when the carriers are submerged in turbulent water, to ensure more accurate measurements of the actual biofilm characteristics in the process. In addition, DNA sequencing may be useful to obtain a better understanding of the full biofilm community, and not only the targeted groups assessed with FISH. Finally, microelectrodes could be used to determine the concentration gradients in relation to biofilm structure and microbial composition.

6 The role of suspended biomass

There is always some suspended biomass in the MBBR, as a result of biofilm detachment, and although most MBBRs are designed based on the carrier surface area, it has been shown that the suspended biomass can contribute to the overall removal. The activity of the suspended biomass is linked to the activity of the biofilm, but will also depend on the HRT, incoming substrate concentration and loading rate to the MBBR. Hence, it is important to consider the effect of suspended biomass in the MBBR in relation to various operational strategies.

6.1 Suspended biomass in the MBBR

In theory, a continuously stirred tank reactor would not hold any biomass, provided that the HRT is shorter than the critical SRT (solids retention time) for free-growing microorganisms. However, due to the containment of carriers in the MBBR, biofilms grow independently of the HRT, and diffusion limitation and biofilm surface area will determine the activity of the process. But as biofilm detaches from the carriers and enters the bulk phase, it will regenerate and grow in suspension, and hence contribute to the overall performance of the MBBR (Mašić & Eberl, 2014). If the HRT exceeds the critical SRT, biomass will grow exclusively in suspension, with no support from the biofilm.

The importance of the suspended biomass is likely to vary between different MBBR processes. Suspended biomass is less dependent on diffusion, and may hence have a higher access to substrate and – consequently – a higher specific activity than obtained in the biofilm. However, the composition and activity of the suspended biomass will depend on the detachment mechanisms (Chapter 2). If detachment occurs through sloughing (i.e. large biofilm segments detach), the suspended biomass may behave more like a biofilm with active and inactive sections, which are limited by diffusion, while if the detachment is due to abrasion or shear, (i.e. smaller segments detach from the biofilm surface), the suspended biomass may have a high activity, independently of diffusion. Naturally, the activity of the suspended biomass will also vary in response to the microbial

population of the system; in nitrifying MBBRs, where biomass growth is slow, the contribution to the overall removal from suspended biomass may be negligible (Mašić & Eberl, 2014), while it is likely that suspended biomass can play a considerable role in heterotrophic systems fed with easily degradable substrates.

The concentration of suspended biomass in the MBBR, regardless of whether it results from detachment or free growth, should correspond to the removal of incoming substrate. However, the growth yield is related to the sludge age, where a longer sludge age will result in a lower growth yield, due to decay of biomass. Simultaneously, the sludge age will depend on the loading rate to the system, where a high loading rate will result in a shorter sludge age. Hence, the contribution of suspended biomass in the MBBR process may vary considerably with inlet concentrations, but also with loading rate, resulting in a complex and dynamic connection.

Although the suspended fraction of biomass in the MBBR may be important, most MBBR designs assume that all removal activity occurs in the biofilm (McQuarrie & Boltz, 2011; Rusten et al., 2006). This is reasonable considering that the initial MBBR designs were made for municipal applications, with very low substrate concentrations and relatively short HRTs. However, this approach might result in erroneous designs when applied to industrial wastewaters. In addition, studies addressing the concept of detachment generally focus on how detachment mechanisms affect the biofilm composition and structure, rather than the fate of the detached biomass itself (Horn et al., 2003; Morgenroth & Wilderer, 2000). The role of suspended biomass in the MBBR, and its dependence on operational conditions such as HRT, inlet concentration and loading rate, should hence be evaluated further.

6.2 Loading rate, HRT and filling degree

Two separate evaluations of the suspended biomass in MBBRs are presented in **Papers I** and **VI**. For both studies, lab scale MBBRs were operated at relatively high inlet concentrations (1000 mg soluble COD (SCOD) per litre), supplied as acetate to ensure a high growth rate and considerable sludge production in the system. The HRT and loading rate were altered in different phases to evaluate the effect on the suspended fraction.

In the first study (**Paper I**), the role of suspended biomass in a lab-scale MBBR (with the AnoxKaldnes K5 carrier) was evaluated by separating the suspended biomass fraction and the biofilm fraction into two individual systems, for which

the development of COD removal and sludge production was observed over time. In the biofilm system, it was possible to show how the biofilm continuously seeded biomass to the bulk through detachment, while the suspended system gradually lost biomass due to wash out. The results indicated that the suspended biomass fraction could contribute to the overall removal at long HRTs, although the biofilm activity was essential to maintain sufficient removal in the process. The contribution from each biomass fraction was, however, not quantified.

A separate approach to determine the importance of the suspended and biofilm fractions was taken by varying the DO concentration. Since biofilm systems are limited by diffusion, the DO dependency was used as a means to determine the importance of the biofilm activity to the overall removal, where a strong dependence would indicate biofilm dominance, and a negligible dependence would indicate the opposite. This approach showed that the removal was mainly dependent upon the biofilm at short HRTs (linear dependency of DO concentration at an HRT of 1.2 hours, see Figure 7), which agrees with the observations mentioned above.

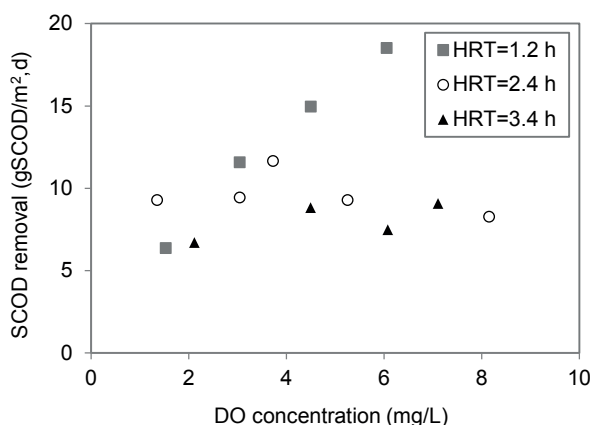


Figure 7

The removal of acetate as a function of DO concentration and HRT in continuously operated MBBRs (from **Paper I**).

In the second study (**Paper VI**), the dynamic relation between biofilm and suspended biomass was evaluated further. The overall removal in the MBBR was measured in two parallel systems, operating at different filling degree (R1 and R2, containing 140 and 70 pieces Z400 carriers respectively), and the specific activity of the suspended biomass was determined in batch trials. By doing so, it was

possible to compare the overall performance of the MBBR in relation to an increased filling degree at different HRTs, as well as to determine the contribution of the suspended biomass in relation to filling degree, HRT and load.

By applying a mathematical expression (**Paper VI**) describing the seeding of suspended biomass from the biofilm, it was possible to show the individual contributions from the biofilm and suspended biomass fraction to the overall removal in the process, as a function of HRT (Figure 8). As seen in the figure, the contribution from suspended biomass was similar in the two reactors, while the biofilm activity differed. Interestingly, the study indicated that the contribution to SCOD removal from the biofilm declines as HRT increases. This was especially noteworthy as the biomass content on the carriers was considerable. This indicates that, although the biofilm is not degrading the SCOD, the biofilm may have a different function in the system at long HRTs, when a high content of suspended biomass is available in the process.

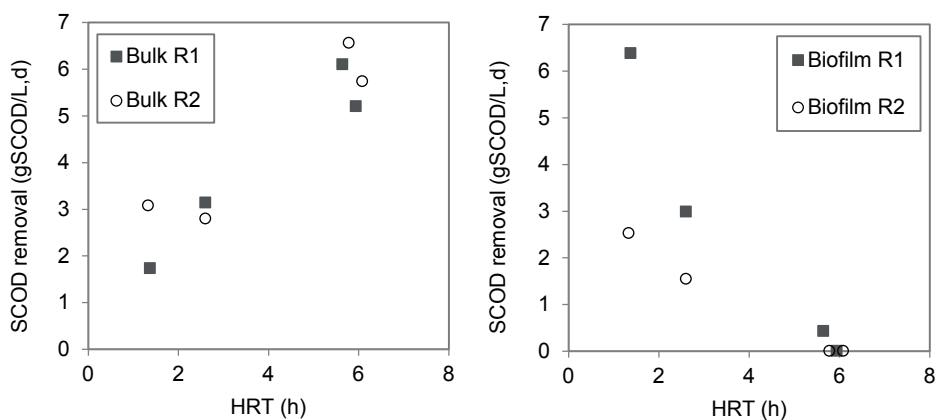


Figure 8

Estimated volumetric removal rate in the suspended bulk biomass (left) and biofilm (right), as a function of HRT in the two reactors: R1 (140 pieces of carriers) and R2 (70 pieces of carriers). After **Paper VI**.

6.3 Considerations related to detached biomass

The studies presented above show that the contribution from suspended biomass may be substantial in the MBBR, although a sufficient amount of carriers are necessary, both to degrade substrate and to ensure the seeding of biomass to the bulk liquid. The significance of the suspended biomass will depend on incoming substrate concentration and HRT, where a longer HRT will increase the growth of biomass and consecutive degradation in the bulk. These observations are especially interesting for industrial wastewaters with high organic content, such as, for example, dairy wastewaters, while the suspended biomass may be negligible in diluted municipal wastewaters. However, the potential activity of suspended biomass in the MBBR may be hard to predict. Hence, the role of suspended biomass should be considered at all times – from the interpretation of experimental data to the design of full-scale processes.

The results in **Paper VI** suggest that there are systems for which the required amount of carriers may be lower than what could be expected based on surface removal rates, since the biofilm activity becomes less considerable at long HRTs (Figure 8). A simple mass balance was set up in an attempt to explain the relationship between HRT and the biofilm area needed for removal (see **Paper VI**). The coupling between biofilm growth, detachment and bulk activity is, however, more complex than described in the paper. For example, potential variations in growth yield in the biofilm and the suspended biomass, in response to bulk liquid concentrations and loading rates, was not considered to any extent in the calculations. More complex models could be used to simulate various scenarios in order to understand this complex behaviour further, and plan for future experiments.

Finally, the effect of substrate concentration, HRT and loading rate on suspended biomass should be evaluated also in relation to sludge properties and settling characteristics, since these are the parameters that govern the total removal efficiency of the process. Indeed, many studies have shown that sludge settleability in MBBRs depend on loading rate and HRT (Karizmeh et al., 2014; Melin et al., 2005; Ødegaard et al., 2000), but not in relation to the available carrier surface area. In combination with this, the composition and activity of the biofilm at low loading and long HRTs should be studied further, in order to determine the role of the biofilm in systems where the majority of the soluble matter is removed by suspended biomass, especially with respect to sludge production and predator grazing (Lee & Welander, 1996).

7 Biofilm thickness – does it matter?

The effect of biofilm thickness on the overall MBBR performance is most likely dependent on the biological conversions involved in the specific process; purely diffusion limited, aerobic processes such as nitrification may mainly depend on active biofilm surface area and diffusion depth, while other biological processes, such as anammox, might be more limited by biomass content and the availability of different microbial niches. With the Z-carrier the biofilm thickness can be pre-defined at a level of precision which has previously not been possible. This enables studies on how the biofilm thickness may affect the overall MBBR performance, as well as the evaluation of biofilm thickness as a possible control parameter for future applications, here exemplified by studies of nitrifying biofilms.

7.1 Definitions and measurements

Depending on reactor conditions, the biofilm structure in MBBR carriers can either be dense and smooth or porous and non-uniform (see Figure 9), and may vary considerably between samples from the same reactor – and even between different carrier voids. Although the estimation of biofilm thickness may be rather straightforward for smooth and uniform biofilms, a more complex biofilm structure can complicate the estimation of biofilm thickness considerably. Hence, the definition of biofilm thickness and the methods used to determine thickness may differ between studies. Most studies measure biofilm thickness visually, as the height of the biofilm from the substratum to the bulk-liquid interface (Karizmeh et al., 2014; Xiao & Ganczarczyk, 2006), but there are examples where biofilm thickness is estimated based on biomass and biofilm density (Horn & Hempel, 1997). The choice in method can vary depending on the purpose of the study, which may be to follow the development of biofilm growth or to quantify biomass in relation to reactor performance. Preferably, biofilm thickness should be estimated in combination with measurements of biomass and biofilm porosity, in order to get the full picture of the biofilm characteristics.

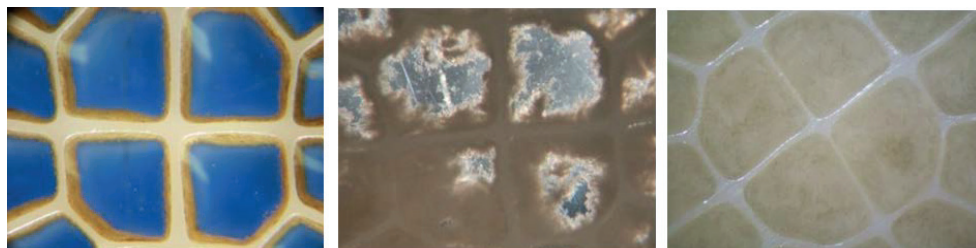


Figure 9

Stereomicrographs of K5 carriers sourced from different MBBR processes, illustrating how the biofilm growth can vary depending on reactor conditions (void size approximately 3×3 mm).

7.2 Can we control biofilm thickness?

The Z-carrier is designed to control the biofilm thickness to a pre-determined maximum height (see Chapter 3) and is produced by moulding in order to achieve high accuracy of the carrier dimensions. Hence, it can be assumed that the height of the grid walls are close to the pre-defined values ($50\text{-}500\text{ }\mu\text{m}$ depending on carrier type) and that biofilm thickness can be estimated on the Z-carriers by using the grid walls as a reference. Images obtained from stereomicroscopy did, however, indicate that the biofilm structure was different in different processes, and that generally the biofilms were thicker along the grid walls, while the centre of each grid compartment contained thinner layers of biofilm (Figure 10). Further evaluations of the biofilm were therefore necessary to ensure the precision of the Z-carrier for biofilm control.

By using OCT analysis it was possible to determine the biofilm thickness of Z-carriers further, by imaging individual grid compartments of the carriers (Figure 11). In **Paper V**, the biofilm thickness was measured with OCT in two different Z-carrier types (Z50 and Z400) from the same pilot reactor, showing that the average biofilm thickness was similar to the pre-defined grid heights of the carriers. In addition to measuring the biofilm thickness, the OCT images also contained information on biofilm volume (i.e. biovolume) and porosity, revealing that, although the carriers were colonized in the same reactor, the biofilms were considerably different. While the Z400 biofilm was porous and non-uniform, the Z50 biofilm was smooth and dense. It is possible that the biofilm grew in a more porous structure in the Z400 carrier to enable better substrate availability (Zhang et al., 1995), while the denser biofilm on the Z50 may be due to a higher shear and lack of protective walls surrounding the biofilm.

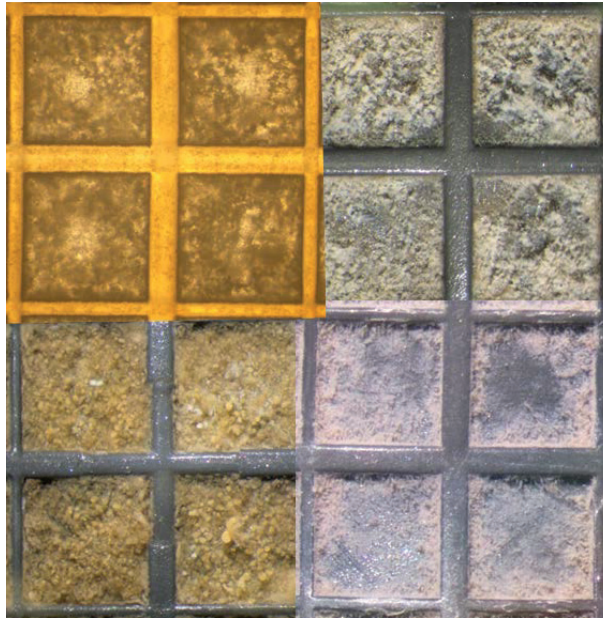


Figure 10

Examples of different biofilm structures on Z400, as observed with stereomicroscopy. The carriers were obtained from the following MBBR processes (clockwise from top left): heterotrophic lab reactor (**Paper VI**), nitrifying full-scale reactor (from cage, see Chapter 8), initial growth on reject water in pilot reactor (not included in this study) and nitrifying pilot reactor (**Paper V**). Compartment size approximately 2.3×2.3 mm.

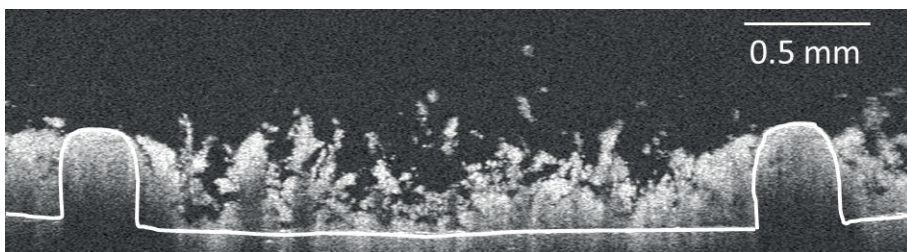


Figure 11

Cross section of a biofilm growing in one grid compartment of the Z400 carrier, as obtained with OCT (see **Paper V**), showing a typical porous biofilm structure. The white line shows the surface of the plastic carrier, above which any signal (white pixels) corresponds to biomass. The protruding shapes in the carrier surface are the grid walls.

In **Paper V**, biofilm thickness was determined in pre-selected grid compartments, studying a sample of five carriers of each type (Z50 and Z400). However, the studied grid compartments were all located at a similar distance from the carrier centre, and did not give any information on spatial variability on the carriers. Hence, an additional OCT study was performed on carriers from the same pilot, where a larger number of grid compartments were imaged on one of each carrier type (Figure 12).

The results indicate that the overall average biofilm thickness on the two carrier types were similar to the pre-defined grid wall heights (346 and 53 μm on Z400 and Z50, respectively), although the spatial variability (both in relative and absolute numbers) was large for Z50 (Figure 12). The variations in Z50 could be related to variations in the carrier wall heights, where some variability over the carrier surface can be expected. If so, there should be a detectable trend between the biofilm thickness and the location of the grid compartment on the carrier. However, although the majority of the measurements exceeding 50 μm were made in compartments closer to the centre of the Z50 carrier (Figure 12), no clear trend could be detected. In addition, the biofilm thickness was distributed evenly throughout the Z400 carrier, suggesting that the carriers were indeed produced with high accuracy. Hence, the measurements exceeding 50 μm in the Z50 were most likely a result of biofilm protruding beyond the grid wall height during imaging. However, it is probable that the biofilm was more compressed during operation in the MBBR process than during imaging, due to the movement of the carriers. Having a more compressed biofilm during operation may explain why the observed protruding biomass had not been scraped off by other carriers in the process.

Natural dynamics of biofilm growth, detachment and attachment may also explain some of the variability within and between carrier samples. It should be noted that the Z-carrier design can only control the *maximum* thickness, and that the biofilm growth below this pre-defined height will be limited by substrate availability, turbulence and shear, just as in any other biofilm carrier. In addition, biofilm density and/or porosity can vary considerably between different Z-carrier types (as shown in **Paper V**), as well as in the same Z-carrier type (see **Paper VI**), although biofilm thickness is maintained below the grid wall height. Nonetheless, although biofilms remain complex and dynamic systems that are not easily restrained, it is clear that biofilm thickness can be controlled to a relatively high degree of accuracy by using the Z-carrier.

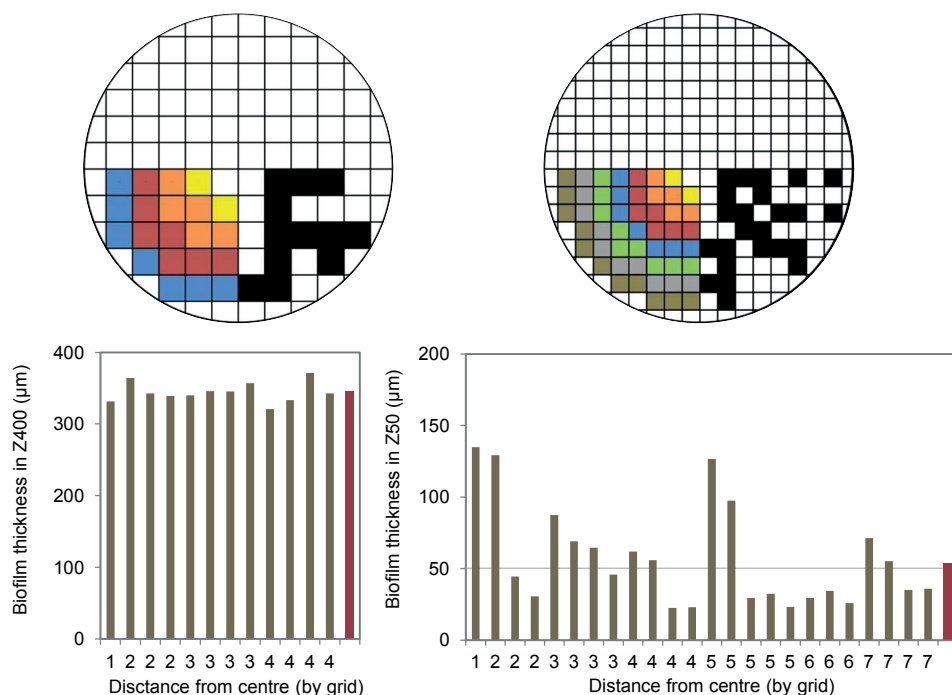


Figure 12

Studied grid compartments in Z400 (left) and Z50 (right) from the same nitrifying pilot reactor (**Paper V**). The top picture shows the location of the imaged grid compartments (black cells) and the estimated distance (by grid) from centre, indicated in different colours. The bottom graphs show the measured average biofilm thickness in each grid compartment, where the red bar indicates the average of all measurements.

7.3 Biofilm thickness and nitrification

Nitrification is the conversion of ammonium to nitrate via nitrite, performed by autotrophic bacteria commonly referred to as ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Biological nitrogen removal in wastewater treatment conventionally relies on nitrification, followed by heterotrophic denitrification where nitrate is reduced to dinitrogen gas. Under normal municipal treatment conditions, NOB grow faster than AOB, and the overall nitrification rate hence relies on the AOB activity, although there are conditions for which AOB can outcompete NOB (see Chapter 9).

Nitrifying biofilms are often stratified, as a result of diffusion gradients and different growth optima for AOB and NOB. AOB are generally located in the top layers of the biofilms, at high oxygen and ammonium concentrations, while NOB are more abundant in the deeper layers (Lydmark et al., 2006; Okabe et al., 1999, 2002; Schramm et al., 1996). However, there are also observations of more heterogeneous distributions of nitrifiers, especially in biofilms which are exposed to varying reactor conditions (Gieseke et al., 2003).

Since nitrifying biofilms are generally limited by ammonium or oxygen rather than biomass, the biofilm thickness should not affect the nitrification efficiency, provided that the thickness exceeds the penetration depth of substrates and oxygen, and does not affect the overall biofilm surface. However, if the oxygen and substrate availability is high and the biofilm is thin, the growth of nitrifiers may be limited by space, resulting in a different competitive situation.

The effect of biofilm thickness on nitrification was evaluated in four parallel lab-scale MBBRs containing Z200, Z300, Z400 and Z500 (Figure 4), in order to limit the maximum biofilm thickness to 200, 300, 400 and 500 μm , respectively (**Paper II**). The results showed that, while ammonium oxidation responded similarly in all systems, nitrite oxidation was seemingly restricted in biofilms thinner than 300 μm , especially at high loading rates. Figure 13 shows how the NOB activity (defined as production of nitrate) varied with the AOB activity (defined as removal of total ammonium nitrogen (TAN)) in two of the reactors, containing Z200 and Z400, for which data clearly suggest different trends. It was hypothesized that the limited NOB activity was a result of space limitation, where AOB could outcompete NOB for space in the thinner biofilms. In the Z200 reactor, NOB activity became limited when AOB activity increased beyond 0.4 gN/L,d (corresponding to approximately 2 $\text{g/m}^2\text{,d}$), suggesting that NOB are outcompeted by AOB at high activity and limited space, while for Z400, the thicker biofilm still contained sufficient biomass to accommodate NOB (Figure 13).

Normally nitrification is limited by AOB activity, and the finding that NOB could be suppressed in thin biofilms was unexpected. However, the finding suggested the potential of using thin biofilms for NOB suppression in the application of mainstream anammox processes, as further discussed in Chapter 9.

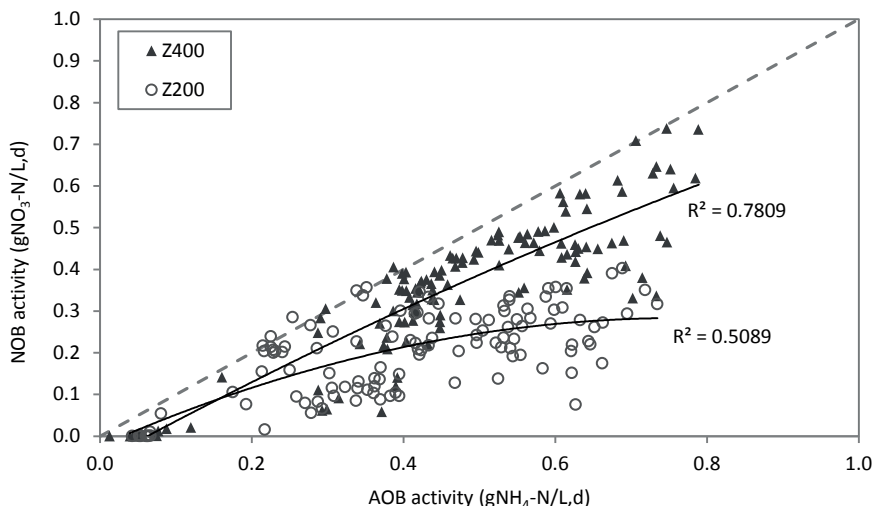


Figure 13

NOB activity in relation to AOB activity as measured in two of the parallel reactors studied in **Paper II**, showing how NOB activity is limited at high AOB activity in the thinner biofilm on Z200 (after **Paper II**). Dotted line represents 100% conversion.

Although the results in **Paper II** indicate that high AOB activity in biofilms thinner than 300 μm may result in NOB being outcompeted, it is important to emphasize that this behaviour is not universal for all nitrifying systems. A separate study on Z50 and Z400 (**Paper V**) showed fully nitrifying biofilms on both carrier types, with no indications of NOB suppression in the thinner biofilms on Z50, although colonization had occurred at high DO and ammonium availability. The two systems did, however, differ considerably in terms of operation; the biofilms studied in **Paper V** were cultivated in a municipal pilot reactor which was subject to varying inlet concentrations, several operational disruptions and some COD in the influent, while the lab reactors in **Paper II** were operated at relatively stable conditions, fed with a synthetic substrate containing easily available ammonium and no COD in the feed.

Potentially, the different reactor conditions between the two studies may have resulted in considerably different biofilm compositions. As mentioned above, stratification of nitrifiers is mainly found in biofilms operating under stable conditions, while stratification is less apparent in biofilms which are exposed to varying reactor conditions. Hence, a stratified structure was probably established in the lab-grown biofilms, for which AOB would dominate the top layers and block the oxygen availability for NOB in the deeper layers, especially at high ammonium load (Brockmann & Morgenroth, 2010). Unfortunately, the biofilm

composition was never determined in the lab study, why this theory cannot be confirmed. For the pilot-grown biofilms, however, a non-homogenous biofilm structure was observed in Z400 (Figure 14) as well as in Z50 by using FISH-CLSM (see **Paper V**). As both AOB and NOB were found throughout the biofilm depth, the oxygen availability would be equal to both groups, independent of loading rate.

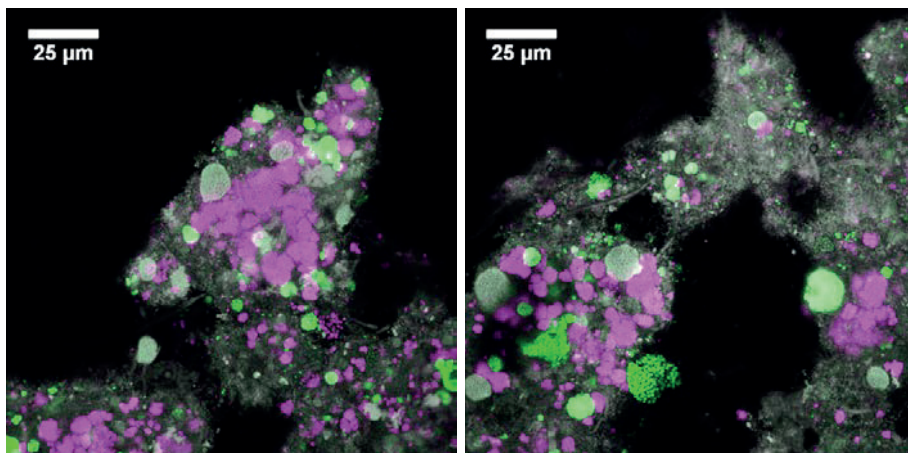


Figure 14

Distribution of AOB (green) and NOB (magenta) in the upper layers of a Z400 biofilm cross-section (see **Paper V**) at two different locations. The water-biofilm interface is oriented to the top of the images. Imaging and cryo-sectioning performed by Carolina Suarez.

Another possible explanation for the different results between the two studies was that the nitrifying activities differed considerably; the lab reactors (**Paper II**) achieved AOB activities above $3 \text{ gN/m}^2\text{,d}$ at maximum loading, while the pilot carriers (**Paper V**) never reached more than half of those rates. As mentioned above, NOB activity in the lab study only became restricted when AOB activities exceeded $2 \text{ gN/m}^2\text{,d}$ (0.4 gN/L,d , see Figure 13), suggesting a possible explanation for the lack of NOB suppression in the pilot-grown biofilms. The lower activity in **Paper V** can be explained by the competition for oxygen with heterotrophs, which should be negligible in the lab-grown biofilms. Microbial evaluations of the pilot biofilm indicated that the very top layers of the biofilms contained neither AOB nor NOB, suggesting that heterotrophs dominated the surface of the biofilm (Figure 14 and **Paper V**).

Provided that the behaviour observed in **Paper II** is indeed related to stratification, a change in DO concentration and/or loading rate could potentially have changed the competition between AOB and NOB, resulting in new equilibria. For example,

it is possible that the NOB could have become more competitive in the deeper layers of the thin biofilm at an elevated DO concentration. The effect of DO on the activity was evaluated, but only during a short time-span, during which no substantial changes in biofilm structure could have taken place. The study does, however, suggest that the competitive advantage of AOB over NOB could be controlled in a biofilm of limited thickness, by ensuring the right substrate availability (see Chapter 9 for further discussion).

7.3.1 Abundance of nitrifiers in different biofilms

In addition to evaluating the configuration of AOB and NOB in the pilot-grown biofilms (**Paper V**), FISH was also used to quantify the relative abundance of AOB and NOB in the two carrier types. The relative abundance obtained with FISH was determined on a volumetric basis, and could hence be related to the measured biovolumes obtained with OCT. However, AOB and NOB were determined in relation to a general bacteria probe mix (i.e. EUB-mix) which only detected bacteria with high ribosomal content. Hence, additional quantification was done by comparing the EUB-mix towards a counterstaining detecting all cells (i.e. SYTO 40). In rough, it can be estimated that the AOB/EUB-mix and NOB/EUB-mix showed the relative abundance of AOB and NOB, respectively, in the “active” bacteria volume, while the EUB-mix/SYTO 40 gave the relative abundance of “active” bacteria over total biomass in the biofilms. By multiplying these fractions with the measured biovolume from OCT-analysis, it was hence possible to quantify the “active” AOB, NOB and other bacteria in the biofilms by volume (Figure 15).

As seen in Figure 15, the total biovolume in Z400 was approximately 6.4 times higher than in Z50, although the total “active” biovolume was only 5.6 times higher. For Z50, the nitrifying population made up almost 60% of the “active” biofilm, while for Z400, only 34% of the “active” biofilm was AOB and NOB. These results suggest that the specific nitrifying activity was higher in the thinner biofilm on Z50, while the thicker biofilm on Z400 enabled a higher abundance of other bacteria, potentially due to more biological niches in the deeper biofilm layers. However, the thicker biofilm contained a considerably higher total volume of nitrifiers than the Z50 biofilm, indicating that the nitrification potential of the thicker biofilm would be higher. However, FISH only reflects the volumetric abundance in the biomass, and does not take biofilm density into account. Intuitively, the number (i.e. cell count) of bacteria per volume should be higher at an increased density. TS measurements suggested that the biofilm density was considerably higher in the Z50 biofilm, suggesting that the cell count per

biovolume would be higher than for Z400 (see **Paper V**). Further evaluations of the two biofilm compositions are underway, where additional information is obtained with DNA sequencing, and the presence of other bacterial groups, besides nitrifiers, are evaluated.

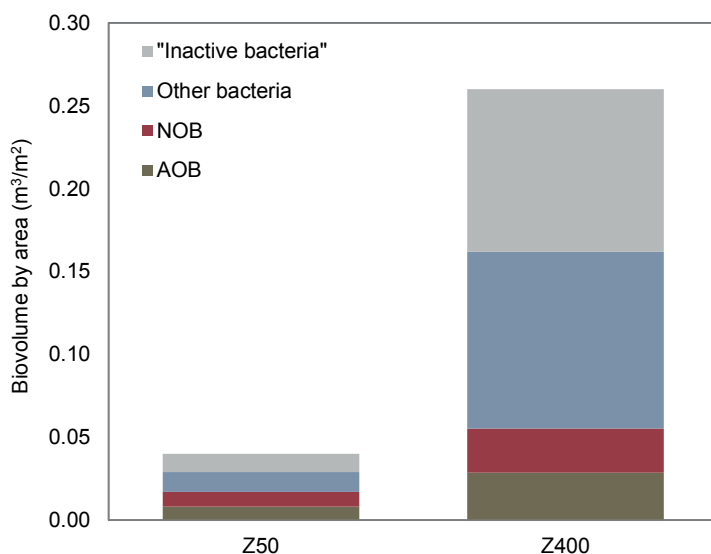


Figure 15

An estimation of microbial distribution of “active” AOB, NOB and other bacteria, as well as the “inactive” fraction, in Z50 and Z400, respectively, calculated by multiplying abundance of AOB and NOB over EUB, respectively, with EUB/SYTO and biovolume as obtained from OCT (after **Paper V**).

8 Applications of biofilm control

For some processes, biofilm thickness may not have a considerable effect on the overall performance. But even when a pre-determined biofilm thickness is not necessary, the EBA (exposed biofilm surface) should still remain close to the PSA (protected surface area) used for designing, in order to ensure the predicted reactor performance (see Chapter 3). For such processes, biofilm control can be essential to avoid a reduced EBA as a result of thick biofilm growth or carrier clogging. As mentioned in Chapter 4, different carrier designs can be more or less prone to clogging, where sufficiently large voids are necessary to facilitate detachment in conventional carriers. On the Z-carriers, however, the design of the carrier ensures that the EBA remain stable, independently of biofilm thickness. In addition, abrasion on the biofilm surface from other Z-carriers may have an effect on biofilm growth, which differs from conventional carriers where the biofilm is only exposed to hydraulic shear. Below follow three examples where the Z-carriers were compared to conventional carriers in environments where biofilm control may be advantageous.

8.1 Case 1: Nitrification at increasing C/N ratio

The nitrifying performance of biofilm systems depends on the availability of oxygen and substrates (as discussed in Chapter 7). When COD is available in the bulk liquid, heterotrophic bacteria will grow in the biofilm and compete with the nitrifiers for oxygen. As heterotrophs grow faster than autotrophs, they will locate themselves at the very top of the biofilm where oxygen is most abundant, thus blocking the oxygen availability for the nitrifiers below (Wanner & Gujer, 1985). This effect will result in reduction of nitrifying activity with increasing COD load, as shown by Hem et al. (1994). In addition, the growth of heterotrophs may clog the carrier material, as the biofilm thickness increases. It is hence reasonable to assume that the observable effect of COD loading on nitrification efficiency may be a result of both the consumption of oxygen by heterotrophic bacteria, and the reduced EBA caused by thick biofilm growth. However, when using the Z-carrier,

the EBA should remain similar to the PSA, independently of the population dynamics in the biofilm, and no clogging can occur. Hence, nitrifying biofilms grown on Z-carriers may show a different response to COD loading than a conventional carrier.

To test this hypothesis, a comparison was made between Z400 and K5 in nitrifying lab-scale MBBR, operated in parallel at similar loading and carrier area ($0.2 \text{ m}^2/\text{L}$) (not published, see Appendix A). The reactors were operated for 257 days on a synthetic feed, containing approximately $90 \text{ mgNH}_4\text{-N/L}$ and no added COD ($\text{C/N} \approx 0$), before acetate was added to the feed at 100 mgCOD/L ($\text{C/N}=1$). The response in nitrifying activity to the changed feed configuration was observed on a daily basis during two weeks, measuring AOB and NOB activity as well as TS (total solids), after which the C/N ratio was further increased by doubling the acetate content to 200 mgCOD/L ($\text{C/N}=2$) (Appendix A), and the reactors were observed for an additional four weeks before the test was terminated. The response to the altered C/N ratios can be seen in Figure 16.

8.1.1 Observations at increasing C/N ratio

Before acetate was added to the feed, both reactors performed similarly at around 80% TAN removal, where TAN removal and the production of nitrite plus nitrate ($\text{NO}_x\text{-N}$) were similar. The biofilm on the K5 carriers, as observed by stereomicroscopy, was dense and even (Figure 17) and was estimated to a thickness of $400\text{-}500 \text{ }\mu\text{m}$. For Z400, no biofilm growth was observed on the exposed grid walls, wherefore it was assumed that the biofilm thickness was less than $400 \text{ }\mu\text{m}$. Biomass content (as TS) was measured the day before changing the feed, and was similar on the two carriers (7.3 and 8.6 gTS/m^2 on Z400 and K5, respectively), confirming the biofilm thickness estimates.

When acetate was added to the feed, the TAN removal decreased gradually in both reactors, and the COD removal rapidly increased (Figure 16). After a bit more than a week of operating at $\text{C/N}=1$, the AOB activity stabilized around $0.5 \text{ gNO}_x\text{-N/L,d}$ in both reactors, while the NOB activity continued to drop to 0.15 and $0.25 \text{ gNO}_3\text{-N/L,d}$, in K5 and Z400 respectively. The considerable repression of NOB activity resulted in nitrite building up in both reactors, especially in the K5-reactor where NOB activity decreased the most.

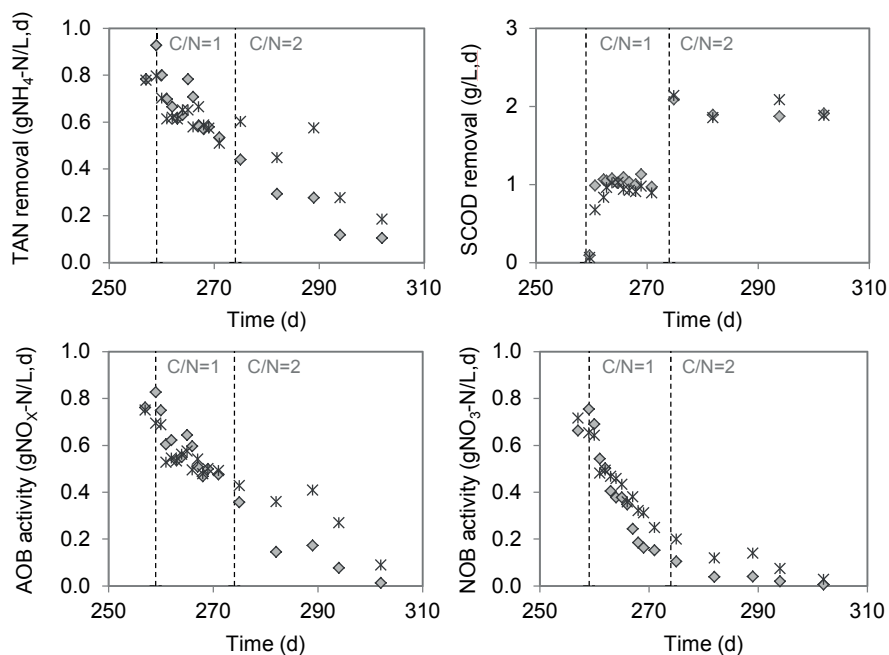


Figure 16
Observed activity in the K5 (diamonds) and Z400 (stars) reactors, in response to an increased C/N ratio over time (first vertical line represents the shift to C/N=1 and the second line represents the shift to C/N=2).

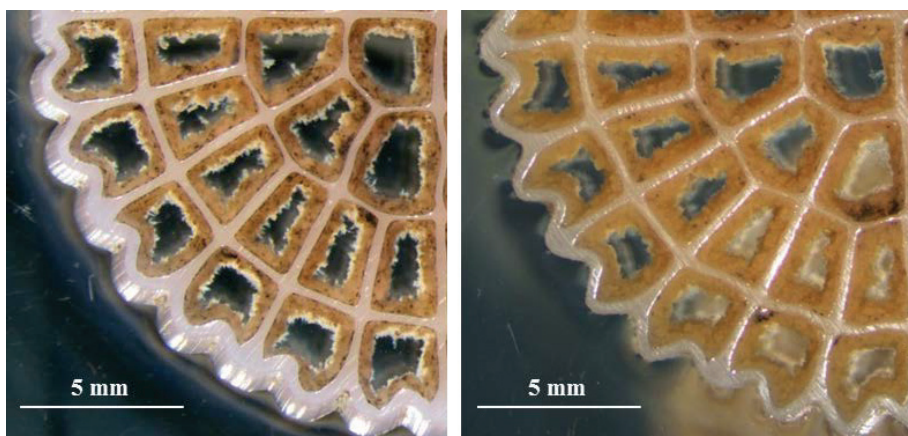


Figure 17
K5 carrier with biofilm as imaged in a stereomicroscope on day 257 prior to changing the C/N ratio in the feed (left), and on day 266 during C/N=1 phase (right).

Biomass increased on both carriers as a result of the increased C/N ratio, with the most noticeable increase in K5 from 8.6 to 12.9 gTS/m² (compared to 7.3 to 9.2 g/m² in Z400). But although TS increased considerably on K5, biofilm thickness only increased slightly (Figure 17), indicating that the increase in biomass was related to an increased biofilm density, rather than an increased thickness in K5. For Z400, no change in biofilm characteristics could be observed by stereomicroscopy.

When the C/N-ratio was doubled, the TAN removal continued to decline, and after 4 weeks of operation the TAN removal was 0.10 and 0.19 gNH₄-N/L,d in the K5 and Z400 reactor, respectively (Figure 16). The decline in TAN removal was steeper for K5 than Z400, resulting in higher TAN removal in the Z400 reactor throughout the phase. COD removal stabilized around 1.9 gSCOD/L,d in both reactors, with an effluent concentration of 15-30 mgSCOD/L. Simultaneously, both AOB and NOB activity continued to decline, with hardly any activity observed at the end of the study, suggesting that the remaining TAN-removal was caused by heterotrophic assimilation. Carrier biomass measurements at the end of the trial indicated that no major increase in TS had occurred in K5 over the C/N=2 phase, compared to the C/N=1 phase (12.2 gTS/m²). For Z400, on the other hand, the biomass content returned to the initial value of 7.3 gTS/m², indicating that the biofilm thickness was relatively stable on Z400 throughout the study.

8.1.2 Suggested explanations for the observations

At high COD availability (e.g. high bulk liquid concentrations), heterotrophs will compete with nitrifiers for oxygen and suppress nitrifying activity (Elenter et al., 2007; Wanner & Gujer, 1985), explaining the elimination of AOB activity towards the end of this study (where C/N=2). In addition, NOB activity was clearly more sensitive than AOB to the COD addition, most likely due to the increased ammonium availability caused by the drop in TAN removal, which in combination with the limited oxygen supply ensured a competitive advantage for AOB over NOB, as also observed by Elenter et al., (2007). Since AOB activity became negligible at the end of the study, any remaining TAN removal would be caused by heterotrophic assimilation. A difference between AOB activity and TAN removal was observed already at C/N=1, but became considerably more obvious when the COD load was doubled (Figure 16).

Although both reactors performed equally at the end of the study, Figure 16 clearly shows that both AOB and NOB activity remained higher in the Z400 reactor compared to the K5 reactor during most of the trial. In addition, the TS

measurements showed that the accumulation of biomass was considerably larger on K5. Combined, these results suggest that the establishment of heterotrophs may have been more rapid in the K5 carriers, where the biofilm was less exposed to shear, compared to the Z400. Although the difference between the two reactors was not major, the Z400 carrier may be a better option in nitrifying processes where the COD load vary temporarily, as the scraping of the biofilm will suppress the establishment of heterotrophic bacteria. To establish this theory, future studies of this phenomenon should include microbial evaluations in response to varying COD load.

8.2 Case 2: Simultaneous denitrification

Total nitrogen removal via simultaneous nitrification and denitrification (SND) may occur in biofilms where the combination of nitrifying activity, anoxic niches and COD availability results in the growth of heterotrophic denitrifiers in the inner biofilm layers. In thick and dense biofilms, the oxygen penetration is limited (as discussed in Chapter 2), and there are oxygen-free sections in the deeper biofilm layers where denitrifying bacteria can thrive. As denitrification only occurs in the absence of oxygen, aerated reactors are not usually intended for total nitrogen removal. However, there are several examples where aerated biofilm systems, originally intended for nitrification and COD removal, achieved considerable total nitrogen removal as a result of SND (Bassin et al., 2016; Feng et al., 2012). In addition, an increased biofilm thickness resulted in an elevated nitrogen removal activity, confirming that the anoxic niche is enhanced with biofilm thickness (Bassin et al., 2016).

Although there are advantages of SND processes, such as compact plants and potential energy savings, SND can cause problems when occurring unwanted. For conventional nitrogen removal, the MBBR process configuration is generally designed with a C-stage for COD removal followed by a nitrifying N-stage, from which nitrate (and/or nitrite)-rich effluent is recycled up to the front of the plant, where it meets the COD-rich influent in a first anoxic reactor, ideal for denitrification (a so-called pre-denitrification configuration, see Figure 18). However, if the nitrification stage does not produce sufficient nitrate and/or nitrite, the pre-denitrification stage will go from anoxic to anaerobic and no denitrification can take place. This, in turn, results in the production of hydrogen-sulphide in the pre-denitrification stage, as well as an increased COD-load on the C-stage. When the C-stage is overloaded, excess COD will enter the nitrification stage, resulting in an increased heterotrophic growth in the system and a succeeding drop in

nitrification. Consequently, the insufficient nitrate and/or nitrite production may result in a negative loop, which gradually suppresses nitrogen removal at the plant. Nitrate and/or nitrite production is of course related to nitrification, but will also be affected by simultaneous denitrification in the biofilms.

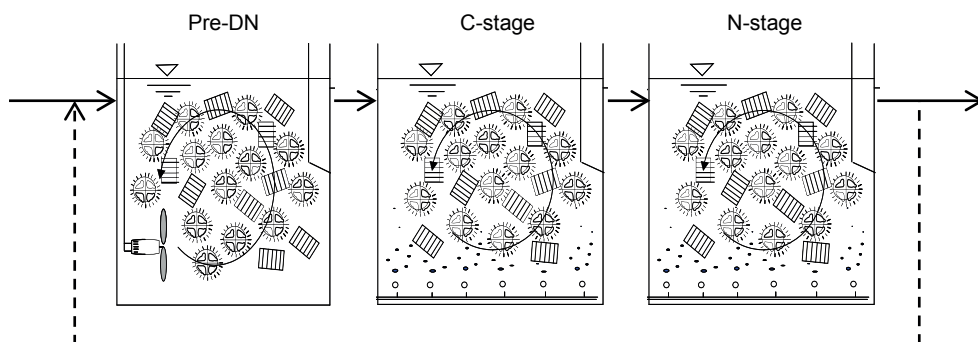


Figure 18

Schematic of a pre-denitrification configuration for which nitrate- and/or nitrite-rich wastewater is recirculated from the nitrifying N-stage to the pre-denitrification (pre-DN) stage, in which heterotrophs convert nitrate and/or nitrite to dinitrogen gas by utilizing incoming COD (not to scale).

Heterotrophic bacteria can be found in high abundance in nitrifying systems, even when COD is negligible in the feed (Bassin et al., 2015; Elenter et al., 2007), and although denitrification requires COD, there have been observations of total nitrogen removal at very low bulk liquid COD concentrations, indicating that SND can also occur by endogenous denitrification (Chen et al., 1992). In such systems, soluble microbial products, produced by decay and hydrolysis of autotrophic biomass, may be utilized as a carbon source for denitrifiers growing in the deeper layers of the biofilms (Rittmann et al., 1994). Hence, simultaneous denitrification may occur in an aerated nitrification stage, in the absence of external COD, provided that the biofilms grow thick enough to accommodate anoxic zones.

A municipal treatment plant in Sweden encountered problems with nitrogen removal, resulting in the negative loop described above. When performing batch trials on the K3 carriers (AnoxKaldnes, see Figure 19) from the nitrification stage, 30% of the TAN removal was not reflected in the $\text{NO}_x\text{-N}$ production, indicating that simultaneous denitrification took place. Since all batch trials were performed on synthetic feed, containing no external COD, it was likely that the denitrifying bacteria utilized an internal carbon source, as discussed above. It was hypothesized that SND was caused by unlimited biofilm growth on the K3 carriers, resulting in thick and dense biofilms (Figure 19). To test this hypothesis, an aerated carrier

cage (see Chapter 5), containing one compartment with Z400 and one with K3, was placed in the nitrifying stage. Over time, the biofilm development (measured as TS) was followed on the two carrier types in the cage, as well as on the carriers in the full-scale reactor, and the nitrifying performance was measured in regular lab-scale batch trials (not published, see Appendix B).



Figure 19

K3 carriers from the municipal treatment plant, as taken from the N-stage on the 11th of April, 2014.

As seen in Figure 20, the ratio between $\text{NO}_x\text{-N}$ production and TAN removal (i.e. the $\text{NO}_x\text{-N/TAN}$ ratio) was initially low (less than 0.5) in the caged K3 carriers, while the ratio was close to 1.0 in the Z400 carriers throughout the study. However, the ratio in the K3 carriers increased gradually, and reached a similar value as Z400 after approximately five months of operation. Meanwhile, the nitrifying performance increased gradually from $0.3 \text{ gNH}_4\text{-N/m}^2\text{,d}$ in April, to $0.9 \text{ gNH}_4\text{-N/m}^2\text{,d}$ at the end of the study (similar for both carrier types), while the full-scale carriers never exceeded $0.6 \text{ gNH}_4\text{-N/m}^2\text{,d}$ in the batch trials.

The biomass on the caged carriers was 20 and 14 gTS/m^2 in K3 and Z400, respectively, after approximately three months of operation. Over time, the biomass content dropped gradually in the caged carriers, as well as in the full-scale reactor, most likely as a result of increasing temperatures in the reactor. The change was, however, most noticeable in the caged K3 carriers, where the biomass dropped from 20 to 11 gTS/m^2 . There was a positive, although not very clear, correlation between TS and estimated biofilm thickness in the caged K3 carriers

(as defined by stereomicroscopy, not shown). Interestingly, TS correlated well with the $\text{NO}_x\text{-N/TAN}$ ratio for the caged carriers, suggesting that SND was indeed enhanced by thick biofilms (Figure 20). However, no such correlation was found for the full-scale carriers, in which the $\text{NO}_x\text{-N/TAN}$ ratio was approximately 0.7 independently of biofilm thickness (not shown). Considering that both the nitrification rate and the $\text{NO}_x\text{-N/TAN}$ ratio differed between the full-scale and the caged K3 carriers, it is likely that the carrier cage did not fully mimic the reactor conditions. However, the difference may also be related to biofilm maturation.

Since the Z400 carriers always maintained a $\text{NO}_x\text{-N/TAN}$ ratio close to 1.0, this study suggests that biofilm thickness control can help preventing unwanted SND in biofilms. Although the caged K3 carriers also achieved a high ratio at the end of the study, the Z400 performance was more stable with less seasonal variability in TS. However, a longer experiment would be necessary to determine whether the performance should remain stable throughout the year, and if the caged K3 carriers would become more similar to the full-scale carriers over time.

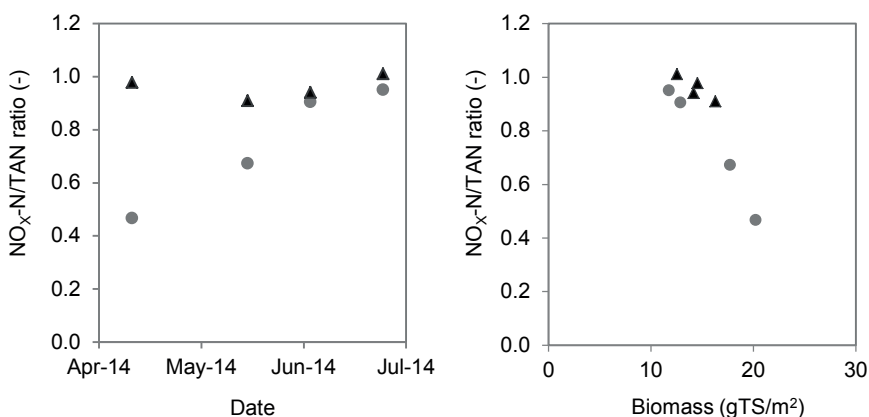


Figure 20

Change $\text{NO}_x\text{-N/TAN}$ in the caged carriers over time (left) and the correlation between $\text{NO}_x\text{-N/TAN}$ ratio from batch trials, as a function of TS (right). Data displayed for K3 (circles) and Z400 (triangles) as cultivated in a cage in the same reactor (start January 2014).

8.3 Case 3: Scaling in biofilms

A third example when uncontrolled biofilm growth may cause issues in the MBBR is when scaling occurs in the biofilms, an issue which was addressed in **Paper IV**. Scaling is the phenomenon where salts (e.g. calcium carbonate or phosphate), precipitate in the biofilm, resulting in heavy carriers which may eventually sink due to the increased biofilm density. The risk of scaling is high in MBBRs used for the treatment of wastewaters containing high calcium content at elevated pH, such as some industrial wastewaters from the mining, pulp and paper and food and beverage sectors. However, it can be hard to foresee the exact conditions where scaling may occur, as the conditions inside the biofilm may differ from the bulk liquid concentrations (Goode & Allen, 2011). In addition, there are indications that scaling may also be a biologically mediated process (Gonzalez-Martinez et al., 2015), which further complicates the understanding of where and when scaling in biofilms occur.

There are indeed two negative effects caused by scaling in biofilms, as i) the biofilm surface may be blocked by the precipitates, thus reducing the EBA, and ii) the density of the biofilm may increase the weight of the carrier so that it eventually becomes hard to maintain in suspension. The blockage of biofilm surface may be addressed by increasing the carrier filling degree in the process, but once carriers become so clogged that they sink, the removal performance will be dramatically reduced. The exact density at which carriers will sink, however, depends on the mixing intensity of the reactor and carrier design. Combined, these scaling-related uncertainties may sometimes result in conservative designs, and although this problem is commonly known by experts, it has not been addressed much in literature.

An experimental study was hence performed to test the effect of scaling on different carriers (**Paper IV**). In an initial lab trial the Z400 carrier was compared to K3 in parallel reactors, operating on a real industrial wastewater with high pH and calcium content, while a second trial compared the response of Z400 and Z200 when combined in the same reactor at extremely high calcium content and high pH. In addition, a simple mathematical balance was set up to evaluate the response of the different carriers to varying fractions of inorganics in biofilms of different thickness (Figure 21).

As seen in Figure 21, the calculations showed that the Z400 carrier could contain a larger fraction of precipitated calcium carbonate than the K3 carrier, while at the same density. This behaviour is mainly determined by the buoyancy of the carrier material, where the plastic content of the Z400 is higher than the K3, in relation to

biofilm content. In addition, the unlimited biofilm growth on the K3 carrier can result in extremely high carrier weight while the biofilm thickness of Z400 is limited to 400 μm (Figure 21). Interestingly, the study also showed that when operated at the same conditions, the Z-carrier developed less scaling than the K3 carrier, potentially related to the constant shear on the biofilm from other carriers. In addition, it was indicated that an even thinner biofilm thickness, as obtained by using Z200, contained even less precipitates (see **Paper IV**). Hence it was suggested that the Z-carriers may be preferable over conventional carriers in MBBR processes at high risk of precipitation, both due to the design of the carrier and due to the thin biofilms obtained.

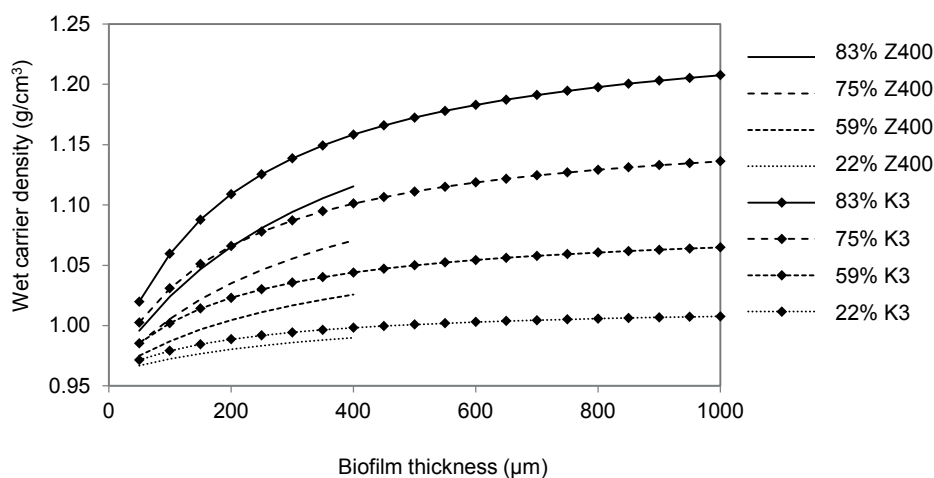


Figure 21

Total density of carriers (Z400 and K3) with wet biofilm, as a function of biofilm thickness at different ratios of scaling in the biofilm (inorganic fraction in dry biofilm), from **Paper IV**.

9 Nitrification with thin biofilms

Incitements to decrease energy consumption and improve resource recovery in the wastewater treatment sector have led to the development of a new, resource efficient process for nitrogen removal, based on partial nitrification and anammox. Full-scale applications of the process exist for treatment of reject water from sludge dewatering, but the real challenge lies in the application on diluted, low-temperature municipal mainstream wastewaters. The major challenge for mainstream application is the suppression of NOB, which thrive under mainstream conditions. The observations presented in Chapter 7 indicated that NOB suppression may be enhanced in thinner biofilms, and a new MBBR configuration was developed to test this potential application.

9.1 Partial nitrification and anammox

Although conventional nitrification-denitrification (see Chapter 7) is a well-established process for nitrogen removal in wastewater treatment, an alternative process has emerged with the discovery of the anaerobic ammonia oxidizing (anammox) bacteria (Strous et al., 1999). In conventional denitrification, heterotrophic bacteria reduce nitrate to dinitrogen gas by consuming organic carbon in the wastewater. Anammox bacteria (AnAOB), on the other hand, remove nitrogen from the wastewater by converting nitrite and ammonium to dinitrogen gas, without the required addition of carbon needed in denitrification. Hence, the anammox process is an attractive alternative for nitrogen removal when the organic compounds in the wastewater are utilized for biogas production.

Since AnAOB use nitrite (rather than nitrate) as electron acceptor, the success of the anammox process relies on achieving partial nitrification, which refers to the first step of nitrification where ammonium is oxidized to nitrite by AOB (i.e. nitrification). As nitrification requires less oxygen than needed for full nitrification, and anammox eliminates the need for an organic carbon source (Hellings et al., 1998; Siegrist et al., 2008), the partial nitrification and anammox (PNA) process is an energy efficient and increasingly popular alternative to traditional nitrogen

removal, for certain applications. The PNA process is especially applicable for the treatment of high strength ammonium (1000 mgTAN/L) wastewaters at low C/N ratios and elevated temperatures (30°C), such as digester centrate (i.e. reject water), and is now successfully established on the market with more than 100 operating full-scale installations worldwide (Lackner et al., 2014). The majority of these PNA installations are one-stage configurations, where AOB and AnAOB co-exist in the same reactor, in the shape of flocs, granules or biofilms.

9.2 Mainstream PNA processes

Today, an increasing number of process solutions are being developed to facilitate bioenergy recovery in wastewater treatment. This is often done by utilizing the majority of incoming organic matter for biogas production, either by mainstream anaerobic treatment or by aerobic treatment followed by anaerobic digestion of the wasted sludge. In addition, energy requirements must be minimized with the aim of reaching energy neutrality. By applying PNA, rather than conventional nitrogen removal, in the mainstream wastewater treatment line, energy requirements for aeration could be considerably reduced and nitrogen removal would not depend on the consumption of organic matter (Daigger, 2014; Gao et al., 2014). But although this concept shows great promise, several challenges arise when applying PNA in mainstream wastewater, which – relative to digester centrate – contains low concentrations of substrate ($< 100 \text{ mgNH}_4\text{-N/L}$) at low temperatures ($< 20^\circ\text{C}$). In addition to being more diluted, mainstream wastewaters generally have a considerably larger daily and seasonal variation in flux and load, compared to reject water, which further complicates the application of mainstream PNA. Several water professionals are studying the application of PNA in mainstream wastewater, using different processes and operation schemes. However, only a few have succeeded in achieving stable PNA at ambient temperatures and low feed concentrations (De Clippeleir et al., 2013; Gilbert et al., 2014; Gustavsson et al., 2014; Lemaire et al., 2014; Lotti et al., 2015), and mainstream PNA still waits for a broader application in the wastewater industry.

The main challenges when applying PNA in the mainstream line are i) the suppression of NOB, which compete with AOB for oxygen and with AnAOB for nitrite, and usually thrive under mainstream conditions, ii) the accumulation and retention of the slow growing AnAOB at low temperatures and diluted influent, and iii) the C:N ratio in the mainstream wastewater enabling the growth of heterotrophic bacteria which compete with AOB for oxygen and with AnAOB for nitrite (Al-Omari et al., 2015; Xu et al., 2015). While ii) is usually addressed by

using biofilms or granular systems, where AnAOB can grow at long SRTs, and iii) can be prevented by applying the necessary upstream treatment (Lemaire et al., 2014), i), the suppression of NOB, is often regarded as the key to achieving successful PNA in the mainstream line.

9.3 Suppressing NOB under mainstream operation

A successful suppression of NOB under mainstream conditions relies on creating a competitive advantage of AOB and/or AnAOB over NOB, and hence many approaches for NOB suppression are related to ensuring ideal reactor conditions for AOB and/or AnAOB growth. Although these ideal conditions are still to be agreed upon, the most common approaches involves some type of DO limitation, as it is generally considered that AnAOB are inhibited at high DO concentrations (Strous et al., 1999) while AOB will grow faster than NOB at low DO concentrations (< 2 mg/L) (Blackburne et al., 2008). This strategy, however, depends strongly on the hypothesis that AOB have a considerably higher affinity for oxygen than NOB, as has been shown in several simulations (Brockmann & Morgenroth, 2010; Lackner & Smets, 2012). However, the affinity constants for AOB and NOB are yet to be determined, and there are observations showing that the roles can be reversed, i.e. a higher affinity for NOB, potentially due to having different types of nitrifiers in the system (Malovanyy et al., 2015; Regmi et al., 2014). In addition, operation at low DO concentrations will considerably limit the nitrification rate, demanding lower loading rates and, consequently, larger reactor volumes.

For suspended growth systems, the suppression of NOB can be facilitated by SRT control, which is not a possibility in biofilm systems, where the SRT will depend on biofilm growth patterns, detachment and attachment (Lackner et al., 2014). Hence, if NOB establish in biofilms they may be very hard to wash out (Kouba et al., 2014; Trojanowicz et al., 2016), and sometimes several months of operation under non-favourable conditions are required to remove them entirely (Isanta et al., 2015). In addition, biofilms often contain several different biological niches due to mass transfer resistance (Chapter 2), which further increases the challenge of NOB suppression compared to suspended biomass. For example, the ideal bulk DO concentration to suppress NOB in biofilms will depend on the oxygen penetration into the biofilm, which in its turn relies on the biofilm thickness, structure and stratification, as well as the thickness of the boundary layer, and can hence vary considerably between different biofilm processes (Brockmann & Morgenroth, 2010). However, biofilm systems do also have advantages over

suspended systems, such as the robustness against hydraulic shifts and the efficient biomass retention (see Chapter 2).

In one-stage PNA systems, where AnAOB and AOB co-exist in biofilms or granules, it has been shown that AnAOB can favour the out-competition of NOB by more efficiently consuming nitrite, especially in larger aggregates where the aerobic fraction is limited (Pérez et al., 2014; Volcke et al., 2010). However, depending on DO and bulk liquid ammonium concentrations, the AnAOB may also compete with AOB for ammonium, hence having a two-sided effect on NOB suppression (Pérez et al., 2014). In addition, nitrite production is usually the bottle-neck for efficient AnAOB activity, and as the aerobic fraction decreases in an enlarged biomass aggregate, the relative AOB to AnAOB activity may decline, hence lowering the overall nitrogen removal capacity (Wang et al., 2014). For discussions on biofilm thickness and NOB suppression in an aerated process, see Chapter 7 and below.

It has been shown that a high availability of ammonium throughout the biofilm will ensure an active AOB population, which can better compete with NOB for oxygen (Brockmann & Morgenroth, 2010; Isanta et al., 2015; Pérez et al., 2014). For such a process, a thin biofilm would be preferable, ensuring high substrate availability throughout the biofilm layers. This also points at an advantage of operating PNA systems in a two-stage configuration, where nitrification at high ammonium availability is achieved in the first stage, followed by a second, anoxic stage for anammox (Pérez et al., 2015).

As the application of DO limitation alone may not be sufficient to ensure NOB suppression, it has been suggested that future research should address the potential short- and long-term inhibition of NOB by using free ammonia (FA) or free nitrous acid (FNA), as an alternative or complementary approach to achieve mainstream PNA (Al-Omari et al., 2015). Both AOB and NOB can be suppressed by elevated concentrations of FA and/or FNA, with NOB generally being more sensitive (Anthonisen et al., 1976). By exposing the biomass to a certain range of FA and/or FNA it may thus be possible to inhibit NOB without affecting AOB. However, the inhibitory ranges for AOB and NOB inhibition varies in different investigations, depending on the biomass composition studied (i.e. pure-cultures, suspended biomass or biofilms), the type of nitrifiers tested and the specific test setup (Blackburne et al., 2007; Chung et al., 2006; Hawkins et al., 2010; Kouba et al., 2014; Simm et al., 2006; Vadivelu et al., 2006a; Vadivelu et al., 2006b; Q. Wang et al., 2014). In addition, no studies could be found evaluating the long-term effect on NOB suppression from FA and/or FNA.

Elevated concentrations of FA and FNA can be achieved at high bulk concentrations of TAN and nitrite, respectively, in combination with high pH for FA or low pH for FNA, according to Anthonisen et al. (1976). Hence, inhibitory concentrations of FA and/or FNA can be challenging to achieve in diluted mainstream wastewaters. However, there are examples where reject water has been used to achieve inhibition in mainstream PNA systems, by temporarily exposing the biomass to reject water conditions either by moving the biomass between different reactors or by switching the feed (Gustavsson et al., 2014; Lemaire et al., 2014; Q. Wang et al., 2014).

9.4 NOB suppression in thin biofilms using reject water

The potential of combining reject exposure and biofilm control for mainstream PNA was evaluated in a two-stage, lab-scale MBBR configuration (**Paper III**). Since previous findings (**Paper II**) suggested that NOB activity was suppressed in thinner biofilms (see Chapter 7), the Z200 carrier was used in a nitrification stage, for which the feed was regularly switched from mainstream to reject water in order to inhibit NOB and/or boost AOB activity further. During mainstream operation, the nitrification effluent was fed to an anoxic anammox stage, to test the function of the full configuration for nitrogen removal.

The configuration maintained stable nitrification, with more than 75% nitrite accumulation during mainstream operation for 250 days (Figure 22), while the nitrite-rich effluent was successfully utilized by the anammox stage. However, AOB activity was occasionally also affected negatively by the reject exposure, resulting in a drop in nitrite production at the start of the mainstream phase. Although no clear correlations could be made, it was hypothesized that the success of the scheme was related to FA and/or FNA concentrations during reject exposure, which ranged between 7.1 to 495 mgNH₃-N/L and 0.2 to 7 µgHNO₂-N/L, respectively.

The ideal reject exposure scheme for NOB suppression was evaluated further, by exposing fully nitrifying biofilms from a municipal pilot reactor to reject water in controlled lab trials, at varying loading rates (**Paper V**). In connection with this, the importance of biofilm thickness to achieve suppression was also evaluated, by using two different carrier types (Z50 and Z400) which contained biofilms of considerably different thickness (see Chapter 7).

The study in **Paper V** showed that both AOB and NOB activity could be suppressed by reject exposure, and that the suppression was most evident for the thinner biofilms on Z50. The higher resilience to exposure in the thicker biofilms was probably related to diffusion gradients and a higher content of nitrifiers (see Chapter 7 and Figure 14). The results indicated that reject water could be used as a means to increase nitrite production in thin biofilms, although none of the tested exposure schemes resulted in the full inhibition of NOB without considerably affecting AOB activity. However, nitrite production increased gradually during three days after the exposure, indicating that AOB activity recovered faster than NOB activity. The suppression seemed to be related to FA, although the concentration ranges required for suppression (FA exceeding 50 and 100 mgNH₃-N/L for Z50 and Z400, respectively) were considerably higher than those previously suggested in literature, where NOB suppression has been observed at FA below 1 mgNH₃-N/L (Anthonisen et al., 1976; Blackburne et al., 2007). Possibly, a sharp concentration gradient resulted in considerably lower FA concentrations inside the biofilm, compared to the measured bulk concentrations.

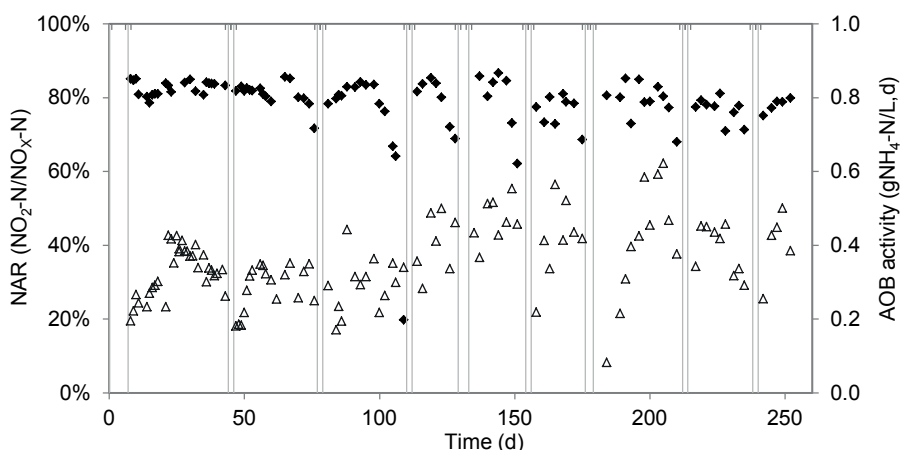


Figure 22

Nitrite accumulation ratio (NAR, diamonds) and AOB activity (triangles) over time in the lab scale reactor from **Paper III**. Vertical lines represent the switch between reject (short periods) and mainstream (long periods) operation.

The results from **Paper II**, **III** and **V** indicate that NOB suppression under mainstream conditions can be facilitated by using thin biofilms, but that a stringent operation scheme may be necessary to ensure stable operation. Probably, considerably different schemes are necessary to maintain NOB suppression in continuous operation than to suppress NOB from a fully nitrifying biofilm. The

repeated disruption of the biofilms by switching the feed between reject and mainstream water has shown promising results, probably due to the high FA concentrations obtained. Further studies are, however, necessary to determine the ideal approach to maintain stable nitrification in the system.

9.4.1 Challenges related to the configuration

The amount of reject water available for the scheme presented above is limited. Depending on the anaerobic digestion of sludge, reject water usually constitute 20-30% of the total nitrogen load to municipal treatment plants (Christensson et al., 2013). Hence, the potential duration of reject and mainstream operation phases will depend on the applied loading rate for each phase. Both **Paper III** and **V** indicated that nitrification could be obtained by exposing the system to 5-6 gTAN/m²,d for approximately 2 days. In **Paper III**, the minimum possible duration at mainstream operation to maintain NOB suppression was ten days, at a loading rate of 3.7 gTAN/m²,d (Figure 22).

Assuming a scheme where reject water is applied for two days at a loading of 5 gTAN/m²,d, followed by ten days of mainstream operating at 3.7 gTAN/m²,d, the reject water stands for approximately 20% of the total nitrogen load, which is within the feasible range. The loading rate of reject could be increased if the duration of mainstream operation was prolonged, while, if necessary, the mainstream phase could also be decreased to 7 days and still remain within a feasible range of reject usage. This indicates that reject available at municipal treatment plants could be sufficient to maintain the proposed scheme for mainstream PNA. However, if NOB colonize the biofilms, an alternative operational scheme may be necessary to get back to stable nitrification.

The results in **Paper V** suggest that a higher frequency of reject exposure may be necessary to suppress NOB from fully nitrifying biofilms (in comparison to what is required for maintaining NOB suppression in **Paper III**). Due to the faster recovery of AOB over NOB after exposure, it was suggested that a regular switch to reject water would be required after only three days of mainstream operation, for which the reject water would constitute approximately 50% of the total nitrogen loading (assuming two days of reject and the same loading rates as suggested above), for which it is not feasible to treat the whole mainstream load. However, this high frequency exposure may only be required for a shorter period, when/if NOB colonize the biofilm, after which the scheme suggested above may be sufficient for normal operation. Potentially, reject water could be used even more efficiently by applying a semi-continuous exposure, i.e. feeding the reactor

with reject water for a limited time and then stopping the feed without starting the mainstream feed, hence exposing the biofilm to high-strength water for a longer time without using any more reject water.

Clearly, the cause of NOB suppression in thin biofilms during reject exposure needs to be evaluated further to optimize the reject exposure loading rate and duration. Apart from the feasibility of using reject as a means to suppress NOB, a few other challenges remain to be evaluated for the suggested PNA configuration; it has been shown that operation at high nitrite concentrations can result in high nitrous oxide emissions (Castro-Barros et al., 2016), which needs to be measured and potentially mitigated in order to prevent substantial greenhouse gas emissions from the process. In addition, the process needs to be tested in pilot/full-scale to evaluate the sensitivity to daily and seasonal changes in load during municipal operation.

10 Conclusions and outlook

In this research project, the MBBR carrier was evaluated in the context of overall process performance and potential future MBBR applications. Conventionally, MBBR design and carrier development are mostly based on having a large carrier surface area, neglecting the potential importance of other, carrier-related aspects, such as biofilm characteristics and the role of detached biomass. This thesis aimed to present an alternative approach, where MBBR performance was evaluated considering several additional aspects, involving both carrier design and operational strategies.

A new carrier type, called the Z-carrier, was developed as part of this research project. With the Z-carrier it was possible to control the biofilm thickness in the MBBR to a pre-defined level. This, in turn, enabled a better control of the exposed biofilm area, as well as of the concentration gradients and microbial niches inside the biofilm. Hence, the new carrier was a useful tool for evaluating possibilities with biofilm control in the MBBR process, and to compare the effect of different biofilm thickness on the overall performance.

The effect of biofilm thickness was compared in nitrifying MBBRs, showing that NOB activity was suppressed in biofilms thinner than 300 μm . It was hypothesized that this behaviour was related to high AOB activity in a stratified biofilm structure, which resulted in limited oxygen availability and space for NOB in the thinner biofilms. Biomass limited conditions in thin biofilms, and their effects on the competition between different microbial groups, should be evaluated further, and the potential usage of controlled biofilm thickness for various biological processes, other than nitrification, should be tested.

A new MBBR configuration for nitrification, to be used for mainstream anammox applications, was developed as a result of the observed NOB suppression in thinner biofilms. By using biofilms of 200 μm and switching the feed between mainstream and reject water, it was possible to maintain high nitrite production under mainstream conditions and high oxygen concentrations, for 250 days. When a similar scheme was applied to fully nitrifying biofilms from a pilot reactor, the suppression of NOB was, however, always linked to a reduced AOB activity. This indicates that the operation strategy to suppress established NOB from a biofilm

may differ considerably from what is needed to maintain NOB suppression in a continuous process with a low NOB population. However, it was also shown that nitrifiers in thin biofilms (50 μm) were considerably more sensitive to inhibition than those in thicker biofilms (400 μm), suggesting that the combination of thin biofilms and a frequent exposure to reject water may enable NOB suppression without sacrificing AOB activity.

Process advantages of biofilm thickness control were also evaluated in relation to conventional carriers, in which biofilm growth could not be controlled. Studies of nitrification at increasing C/N-ratio indicated that the Z-carrier withstood the establishment of heterotrophs better than conventional carriers, although nitrification dropped substantially on both carrier types. In addition, it was shown that the stable biomass content on the Z-carrier may prevent the development of unwanted simultaneous denitrification in biofilms. These findings suggest that a controlled biofilm thickness may be preferable for nitrifying processes, ensuring stable production of nitrite and/or nitrate for denitrification, where temporary changes in C/N ratio do not result in drastic changes of the biofilm composition.

Another advantage of biofilm thickness control was found when comparing the effect of scaling on Z-carriers and conventional carriers. Both experimental studies and calculations indicated that the Z-carriers were less sensitive to scaling, as the biofilms contained lower amounts of inorganic precipitates and the carriers were less prone to sink, in comparison with conventional carriers. Interestingly, the biofilm thickness also seemed to have an effect on this behaviour, where a thinner biofilm contained less precipitates than a thicker biofilm, suggesting that carriers with a controlled, thin biofilm are to be preferred in processes at high risk of scaling. The cause of this effect, however, remains to be evaluated.

Suspended biomass is always present in the MBBR, due to biofilm detachment. In addition to evaluating the role of the carrier and the biofilm characteristics in the MBBR, this research project showed that suspended biomass may contribute considerably to the overall performance of the process. This contribution does, however, depend on the substrate concentration and HRT, and should mainly be considered when designing MBBRs at high inlet concentrations and easily degradable substrates. Nonetheless, the removal capacity in some MBBRs cannot be directly related to the filling degree of carriers, and the biofilm area needed to meet a given removal can depend on the HRT. However, the biofilm may also play an important role for the settling properties and total COD removal of the process, which should be studied further.

This thesis shows that MBBRs are intricate systems, in which biofilm characteristics, carrier type, HRT, filling degree, loading rate and bulk liquid concentrations all play important roles for the overall performance. As seen above, several interesting areas have been appointed, for which biofilm thickness control may be advantageous to MBBR performance, particularly in relation to microbial control and process stability. The potential in using biofilm thickness as a control parameter for the MBBR should be investigated further, especially for complex microbial processes such as nitrification-anammox or micropollutants removal. Experimental studies should be complemented with microbial analysis such as FISH and/or DNA sequencing, in order to better understand the microbial interactions within the biofilms, and mathematical models should be used to simulate and explain the complexity of the process, both overall and in detail. In summation, this work has been an initial evaluation of the many aspects of carrier development, biofilm thickness control, and the contribution from suspended biomass in the MBBR process, all of which should be considered for further studies, in order to improve the performance and develop new configurations of the MBBR process.

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13 Appendix

Appendix A: Nitrification at increasing C/N ratio

Data for the reactors used in the C/N-study (Chapter 8.1) are displayed in Table A1 and A2 below. The two reactors operated in parallel on synthetic substrate (same composition as presented in **Paper II**) for 250 days, leading up to the C/N study. The initial loading rate of 0.5 gTAN/L,d was gradually increased to 1.0 gTAN/L,d leading up to day 250. On day 260, acetate was added to the substrate at a concentration of 100 mgCOD/L, which was increased to 200 mgCOD/L on day 274. The HRT was 2 hours throughout the trial.

NOB activity was defined as the production of nitrate. Since assimilation was expected due to heterotrophic growth, AOB activity could not be defined as TAN removal. Rather, AOB activity was defined as the production of nitrite plus nitrate (see **Paper V**). TAN removal was, however, also measured. Effluent COD concentrations were adjusted for high nitrite content, by subtracting the measured nitrite concentration from the measured COD concentration, according to equation A1. Biomass, as TS, was measured according to **Paper V**.

$$COD_{out} = [COD_{out,meas}] - \frac{[NO_2-N]_{meas} * 16}{14} \quad (A1)$$

Table A1

Reactor dimensions and carrier properties in the study presented in Chapter 8.2.

	Reactor volume (ml)	No of carriers (-)	Carrier surface (mm ² /carrier)	Reactor surface (m ² /reactor)
K5	1150	80	2420	0.19
Z400	1150	150	1280	0.19

Table A2

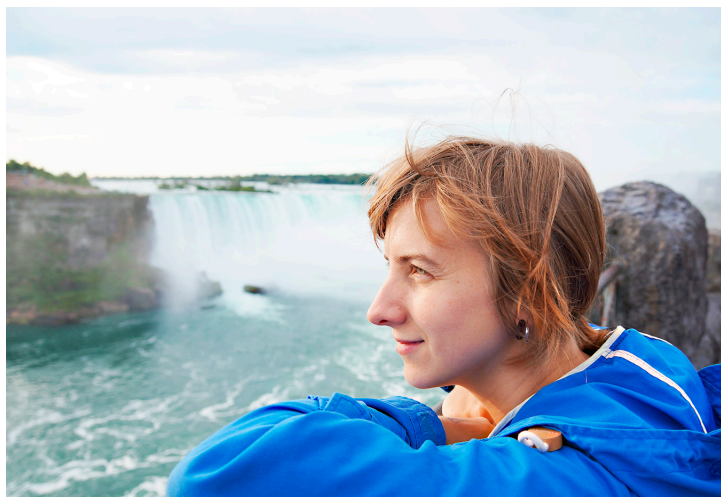
Average values for the two reactors as measured throughout the study, including standard deviations.

	Influent concentration (mgNH ₄ -N/L)	pH (-)	DO (mg/L)	Temperature (°C)
K5	87±7	7.7±0.3	5.5±0.7	21.0±0.3
Z400	87±7	7.7±0.3	5.8±0.7	20.8±0.1

Appendix B: Simultaneous nitrification and denitrification

All results presented in Chapter 8.2 were obtained in batch trials. A carrier cage (see Figure 6), containing K3 and Z400 in separate compartments, was added to the nitrification-stage of the municipal treatment plant in January 2014, and batch trials were performed between April and June. The cage was placed near the edge of the reactor, and floated on the surface of the water. Air was supplied through a separate tube, connected to the full-scale aeration system, and distributed over the bottom of the cage through perforated plastic pipes. Trials were mainly performed on the caged carriers, although the full-scale carriers were also tested on most occasions. For two of the trials, the batch trials were performed in duplicates, for which the average rates are used in Figure 20.

For each batch trial, carriers were removed from the cage and/or the full-scale reactor and carefully rinsed in tap water. A sample of 100 K3 carriers or 300-400 Z400 carriers were placed in a 3 L lab reactor (approximately 0.4-0.5 m²/reactor), which was filled with 1.5 L feed solution, containing 40 mgNH₄-N/L (as NH₄CL), and 0.8 g/L NaHCO₃. The TAN-removal as well as the production of nitrite and nitrate were followed by sampling every 15 minutes over a 1.5 h time-span. The trials were performed at 10°C, and pH was adjusted manually to 7.5-7.8 with H₂SO₄ and NaOH. Air was supplied through the bottom of the reactor and the gas flow was adjusted to maintain a DO of 6.3-7.0 mg/L. The access to N₂-gas was limited, and hence it was not possible to ensure a stable gas flow during the trials, resulting in a varying aeration intensity in the different trials (0.9-2.4 L/min for K3 and 1.9-4.0 L/min for Z400). After the trials were completed, the carriers were returned to the cage. Biomass as TS was measured according to **Paper V**.



In the spring of 2010 I had just returned from studying water resource management in Melbourne, Australia. There, I had experienced the extreme water scarcity that exhausted the region, and I was full of inspiration to finish my degree in Environmental Engineering. But back in rainy Sweden, while waiting to start my master thesis, I was in need of a job for the summer. As it happened, I was offered a job at AnoxKaldnes, and that was how I ended up in the world of wastewater. It was a world full of surprises, and much to learn – so much, indeed, that I returned to AnoxKaldnes after finishing my degree, and eventually I was given the opportunity to start my PhD studies. Since then, I have learnt a lot about the Moving Bed Biofilm Reactor, carriers and biofilms, but also about the requirements of good research. Today, I am still full of curiosity and commitment towards the field of wastewater, and I hope that the findings presented in this thesis can contribute to the joint efforts by water professionals all around the world, in creating a better water environment for future generations.