Towards rational design of redox-stratified biofilms

A novel aproach for developing robust biotechnologies for nutrient removal from wastewaters

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A Novel Approach for Developing Robust Biotechnologies for Nutrient Removal from Wastewaters

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PhD Thesis
May 2009

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Preface

This thesis is based on research for a PhD project undertaken at the Department of Environmental Engineering, Technical University of Denmark, from October 2005 to May 2009. The thesis is composed of a summary and four publications in scientific journals.

(I) **Lackner S.**, Terada A. and Smets B.F. (2008) Heterotrophic activity compromises autotrophic nitrogen removal in membrane aerated biofilms: Results of a modeling study. *Water Research* 42(4-5), 1102-1112

(II) **Lackner S.**, Holmberg M., Terada A., Kingshott P. and Smets B.F. (2009) Enhancing the formation and shear resistance of nitrifying biofilms on membranes by surface modification. *accepted for publication in Water Research*

(III) **Lackner S.**, Terada A., Horn H., Henze M. and Smets B.F. (2009) Operation regimes compromise nitritation efficiency in nitrifying biofilms. *submitted*

(IV) **Lackner S.**, Terada A., Merkey B. and Smets B.F. (2009) The kinetic parameters of ammonium and nitrite oxidizing bacteria may determine nitritation success or failure in Membrane Aerated Biofilm Reactors. *submitted*

The publications are not included in this www-version but can be obtained from the Institute's library, Department of Environmental Engineering, The Technical University of Denmark, Miljøvej, building 113, 2800 Kongens Lyngby (library@env.dtu.dk).

Publications co-authored and closely related to the topic of the thesis, but not explicitly comprised here are listed below and include three publications in scientific journals and several presentations at international conferences.

Terada A., **Lackner S.**, Kristensen K., Wang R. and Smets B.F. (2009) Initial composition of ammonia- and nitrite- oxidizing bacterial populations may compromise nitritation success in counter-diffusion biofilms. *submitted*

Wang R., Terada A., **Lackner S.**, Smets B.F., Henze M., Xia S. and Zhao J. (2009) Nitritation performance and biofilm development of co- and counter-diffusion biofilm reactors: modeling and experimental comparison. *in press, doi:10.1016/j.watres.2009.03.017*

Terada A., **Lackner S.**, Tsuneda S. and Smets B.F. (2007) Redox-stratification controlled biofilm (ReSCoBi) for completely autotrophic nitrogen removal: The effect of co- versus counter-diffusion on reactor performance. *Biotechnology and Bioengineering* 97(1), 40-51

Terada A., **Lackner S.**, Smets B.F. (2008) Do different initial microbial communities converge in identically operated ANAMMOX biofilm reactors? Oral Presentation *Biofilms III, 3rd International Conference, Munich, Germany*

Smets B.F., Terada A., **Lackner S.** (2008) Redox stratification controlled biofilm reactors for completely autotrophic nitrogen removal. Oral Presentation (Invited) *IWA North American Membrane Research Conference, Amherst, MA, USA*

Lackner S., Nàcher C.P., Terada A., Lardon, L., Smets B.F. (2008) Redox Stratification Controlled Biofilm Reactors For Completely Autotrophic Nitrogen Removal. Poster Presentation *5th IWA Leading-Edge Conference, Zurich, Switzerland*

Lackner S., Holmberg M., Terada A., Kingshott P., Smets B.F. (2008) Effect of membrane surface functionalization on formation and shear resistance of nitrifying biofilms. Oral Presentation *IWA Biofilm Technologies Conference, Singapore*

Wang R., Terada A., **Lackner S.**, Lardon L., Smets B.F., Henze M. (2008) Start-up Strategies for Stable Autotrophic Nitrogen Removal in Redox-Stratification Controlled Biofilm Reactor (ReSCoBiR). Oral Presentation *IWA Biofilm Technologies Conference, Singapore*

Terada A., **Lackner S.**, Tsuneda S., Smets B.F. (2006) Redox-stratification controlled biofilm for completely autotrophic nitrogen removal: modeling the effect of substrate co- versus counter-diffusion on performance. Oral Presentation *IWA Biofilm Systems VI Conference, Amsterdam, The Netherlands*

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Lyngby, May 2009 Susanne Lackner

Abstract

Biological nitrogen removal is one of the key processes in advanced wastewater treatment. This thesis investigated the applicability of Membrane Aerated Biofilm Reactors (MABRs) for completely autotrophic nitrogen removal. This rather new nitrogen conversion pathway is based on partial conversion of ammonium to nitrite (nitritation) by aerobic ammonium oxidation coupled with anaerobic ammonium oxidation (Anammox) converting ammonium together with nitrite to di-nitrogen gas and some nitrate. Mathematical modeling and experimental investigations were conducted to study different aspects of this process.

Application of Anammox for ammonium removal requires stable but partial conversion of ammonium to nitrite and no further oxidation of nitrite to nitrate. A simple two-species 1-d biofilm model was constructed in Aquasim to investigate the influence of kinetic parameters on nitritation efficiency in MABRs. This exhaustive simulation study revealed that nitritation efficiencies strongly depend on the chosen kinetic parameters of AOB (ammonium oxidizing bacteria) and NOB (nitrite oxidizing bacteria), the chief microbial groups. The relative maximum specific growth rates ($\mu_r = \mu_{max,AOB} / \mu_{max,NOB}$) of AOB vs. NOB were most predictive of nitritation efficiency. At μ_r >1.5, 100 % nitritation efficiency was obtained independent of all other parameter combinations. At unfavorable values of μ_r (0.75 - 1.25), the absolute and relative values of the oxygen affinity constants were most predictive. High nitritation efficiencies were clearly not solely explained by the oxygen concentration at the membrane or oxygen flux through the membrane.

Experimental investigations with lab-scale MABRs confirmed these results. Nitritation success in these reactors highly depended on the microbial community composition, but not on the membrane oxygen concentration. Batch tests indicated that the oxygen to ammonium flux ratio (J_{O2} / J_{NH4} [g-O_2/g-N]) might be a more suitable control parameter. A decrease in J_{O2} / J_{NH4} also increased nitrite accumulation in the MABRs. Comparing the nitritation performance of those reactors to conventional biofilm systems, a conventional co-diffusion system was clearly superior.

Further simulation work, extending the nitritation model with reactions for Anammox and also aerobic and anoxic growth of heterotrophic bacteria (HB), explored the impact of HB on completely autotrophic nitrogen removal. Simulations suggested that the COD/N ratio had a high impact on Anammox activity, and neglecting growth of HB (even when growing only on autotrophic cell decay products) significantly overestimated nitrogen removal in a counter-diffusion biofilm. Co-diffusion biofilms (and Anammox activity therein) seemed to be much less affected by presence or absence of HB or changes in the influent COD/N ratio. Implementing sloughing events showed that even though reactor performance was strongly affected by a 1 day increase in the detachment rate, recovery was faster than after changes in the COD/N ratio.

As demonstrated in the modeling study, biofilm sloughing events can cause severe impairment of reactor performance. An experimental study was performed to evaluate whether targeted chemical modification of the substratum surface can increase biofilm shear resistance and biofilm thickness control. Plasma polymerization and grafting of poly(ethyleneglycol (PEG) chains with different functional groups (-NH_2 and -CH_3) was explored to modify a standard microfiltration membrane. Laser scanning microscopy and protein measurements revealed that a –PEG-NH_2 modified surface showed more biofilm growth with increased shear resistance (determined from detachment tests), whereas the –PEG-CH_3 modification exhibited a large decrease in biofilm formation with very low shear resistance.

Overall the MABR concept, wherein oxygen and ammonium counter-diffuse into a membrane-supported biofilm from the membrane and bulk liquid side, respectively, is applicable for high rate nitrogen removal. However, advanced process control is essential to control and optimize reactor performance. The microbial community composition, especially of the AOB and NOB, has to be monitored carefully, because it seems determinant for treatment efficiency.

Dansk Resumé

Biologisk kvælstoffjernelse er en central proces indenfor avanceret spildevandsrensning. Denne afhandling beskriver anvendelsen af beluftede membran biofilm reaktorer (eng: MABRs) til fuld autotrof kvælstoffjernelse. Denne ret nye kvælstofomsætningsvej baseres på delvis omdannelse af ammonium til nitrit (nitritation) ved aerob ammonium oxidation koblet med anaerob ammonium oxidation (Anammox) der omdanner ammonium sammen med nitrit til frit kvælstof. Matematisk modellering og eksperimentelle undersøgelser blev brugt til at studere forskellige aspekter af denne proces.

Anvendelse af Anammox til ammonium fjernelse kræver stabil men kun delvis omdannelse af ammonium til nitrit uden videre oxidation af nitrit til nitrat. En simpel to-substrat 1-d biofilm model blev udviklet i Aquasim med henblik på at undersøge betydningen af kinetik parametrene for nitritations effektiviteten i MABRs. Dette omfattende simuleringsstudie afslørede at nitritations effektiviteten afhænger stærkt af de valgte kinetikparametre for ammonium oxiderende bakterier (AOB) og nitrit oxiderende bakterier (NOB) som er de to væsentligste grupper af mikroorganismer i processen. Den relative maximale specifikke væksthastighed ($\mu_r = \mu_{max,AOB} / \mu_{max,NOB}$) for AOB vs. NOB var den mest afgørende factor for forudsigelse af nitritations effektiviteten. For μ_r >1.5, opnås 100 % nitritations effektivitet uafhængigt af alle andre parameter kombinationer. For ufavorable værdier af μ_r (0.75 - 1.25), var de absolutte og relative værdier for ilthalvmætningskonstanter mest afgørende for modellens forudsigelse. Høje nitritations effektiviteter kunne helt klart ikke udelukkende forklares gennem iltkoncentrationen ved membranen eller ved iltfluxen gennem membranen.

Eksperimentelle undersøgelser i laboratorieskala MABRs bekræftede disse resultater. Succes med nitritation i disse reaktorer afhænger stærkt af den mikrobielle sammensætning men ikke af membran iltkoncentrationen. Batchundersøgelser indikerede at ratioen ilt til ammonium flux (J_{O2} / J_{NH4} [g-O_2/g-N]) kunne være en mere anvendelig kontrolparameter. En reduktion af ratioen J_{O2} / J_{NH4} øgede også nitrit akkumuleringen i membranreaktoren. En sammenligning med konventionelle biofilmanlæg med co-diffusion viser at sidstnævnte har en klart bedre funktion.

Yderligere simuleringsarbejde som udvidede nitritation modellen med reaktioner for Anammox samt aerob og anoxisk vækst af heterotrofe bakterier (HB), undersøgte HBs betydning for fuld autotrof kvælstoffjernelse. Simuleringer antydede at COD/N forholdet har en stor indflydelse på Anammox aktiviteten. Hvis man ser bort fra vækst af HB (selv når de udelukkende vokser på autotrofe celle nedbrydningsprodukter) overestimes kvælstoffjernelse signifikant i mod-diffusions biofilmsystemer. Co-diffusions biofilm (og Anammox aktivitet i disse) syntes at være mindre påvirket af tilstedeværelse eller fravær af HB eller ændringer i COD/N forholdet i tilløbet. Biofilm afrivningshændelser viste at selv om reaktorfunktionen blev stærkt påvirket af 1 døgns

øget biofilm afrivning så var genoprettelse af funktionen hurtige end efter ændringer i COD/N forholdet.

Som illustreret ved modelundersøgelserne, kan biofilm afrivning påvirke rektorfunktionen alvorligt. Der blev udført et forsøg for at vurdere om kemisk modificering af biofilmoverfladen kan øge dens modstandsdygtighed overfor afrivning og give forbedret biofilm tykkelseskontrol. En standard mikrofiltrerings membran blev modificeret gennem plasma polymerisation og indbygning af poly(ethyleneglycol (PEG) kæder med forskellige funktionelle grupper ($-NH_2$ og $-CH_3$). Laser scanning mikroskopi og protein målinger viste at en –PEG-NH2 modificeret overflade fik kraftigere biofilm vækst med øget modstandsdygtighed (vurderet ud fra afrivningstests), mens –PEG-CH3 modifikationen viste et stort fald i biofilmdannelse og lav afrivnings modstand.

Alt i alt kan MABR, hvor ilt og ammonium diffunderer fra membransiden henholdsvis fra væskesiden bruges til kvælstoffjernelse med høj hastighed. Der kræves dog avanceret proceskontrol for at kontrollere og optimere reaktorfunktionen. Sammensætningen af biomassen, specielt hvad angår AOB og NOB, skal følges omhyggeligt, fordi det synes at have afgørende betydning for rensningseffektiviteten.

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1. Introduction and Objectives

Across the world, nutrient enrichment of water bodies continues to be a very serious problem resulting in impairment of the ecosystem functions of the affected environments. In addition, the complexity of domestic and industrial wastewaters mandates the removal of an every increasing array of synthetic organic chemicals to meet more stringent threshold concentrations. Hence, innovative approaches are sought to develop cost-effective technologies that permit removal of nitrogen, the most common and deleterious nutrient in many instances.

Nitrogen removal has become one of the main steps in wastewater treatment, and traditionally includes conversion of ammonium (NH_4^+) to nitrite (NO_2^-) by ammonium oxidizing bacteria (AOB) and further to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB) under aerobic conditions: Heterotrophic bacteria then degrade the NO_3^- to di-nitrogen gas under anoxic conditions.

Another recently discovered process exhibits a new route of nitrogen removal under certain conditions: anaerobic ammonium oxidation, the so called Anammox - process (Jetten et al., 1999; Mulder et al., 1995). Within this new process ammonium is converted together with nitrite to di-nitrogen gas and nitrate under anaerobic conditions. This process relies on the conversion of ammonium to nitrite, i.e. partial nitrification or nitritation. Due to its potential economic benefits, this process has gained increasing attention within recent years. Stable production of nitrite for further Anammox is essential for application in wastewater treatment. Many researchers have studied nitritation also as a shortcut step for more efficient denitrification in wastewater treatment system (Bernet et al., 2005; Fux et al., 2002; Fux et al., 2004; Hellinga et al., 1998; Koch et al., 2000; Pambrun et al., 2006; Wyffels et al., 2004; Yamamoto et al., 2008).

Due to the very low growth rate of nitrifiers and especially of Anammox bacteria biofilm processes, which allow very long biomass retention times, are particularly interesting alternatives to conventional activated sludge systems. Biofilm reactors are employed in many different configurations in wastewater treatment such as trickling filters, moving bed reactors, rotating contactors, and many more. However, a very important factor for robust and stable operation of such systems is the control of homogenous biofilm surface coverage and stable biofilm thickness (Elenter et al., 2007; Morgenroth and Wilderer, 2000).

One rather special biofilm reactor type is the membrane aerated biofilm reactor (MABR). This new biofilm reactor concept has been studied for its applicability to wastewater treatment within recent years (Casey et al., 1999b; Syron and Casey, 2008a). In such a system, e.g. oxygen is supplied through a gas permeable membrane which also serves as biofilm support. Applying this Counter-Diffusion concept, oxygen is provided to the base of the biofilm, whereas the substrate, in our case ammonium is supplied from the bulk liquid phase. The merits of such a system lie in the high and efficient oxygen transfer through the membrane (Ahmed et al., 2004) and also in the potential for more amenable control strategies due to the separation of oxygen and nutrient fluxes.

Several studies have already employed such reactors for N removal via nitrification/denitrification (Satoh et al., 2004; Semmens et al., 2003; Terada et al., 2003; Terada et al., 2006b).
However, the feasibility of combining the MABR technology with Anammox for highly efficient completely autotrophic nitrogen removal is still to be discovered.
The aim of this research was therefore to

(i) develop, optimize and validate a 1-dimensional mechanistic biofilm reactor model for completely autotrophic nitrogen removal with special focus on the feasibility of MABR type systems to achieve nitritation (Appendix IV) and Anammox (Appendix I) in comparison to conventional biofilm systems.

(ii) develop, build and operate a lab-scale membrane biofilm reactor that permits detailed microbial and chemical investigation with special focus on membrane properties to improve biofilm formation and stability (Appendix II and III)

(iii) experimentally test the concept of nitritation for nitrogen removal in the lab scale MABR (Appendix III) by defining suitable control parameters with emphasis on the relative fluxes of oxygen and ammonium

2. Theoretical Background

2.1 Biofilms in Wastewater Treatment

Biofilm reactors are amongst the oldest technologies for wastewater treatment and are employed in many different configurations. Trickling filters, rotating biological contactors, or biofilters are some examples for simple reactor configurations with long tradition and wide application. Moving bed biofilm reactors, where the biomass grows on small carriers, are widely used to expand the removal capacity of existing activated sludge plants. More complex systems such as upflow sludge blanket reactors or granule sludge systems, which are more resent developments, are also used more and more for advanced wastewater treatment.
Stable operation of biofilm reactors requires, however, controlled growth of biomass on the respective substratum material, and resistance of the biofilm against detachment and wash out. Therefore, detailed understanding of the relevant processes that occur during biofilm formation and growth is necessary for optimal reactor design and operation. This chapter introduces the main principles of bacterial adhesion, biofilm formation and growth, and its mathematical representation for wastewater treatment applications.

2.1.1 Mechanisms of cellular attachment and biofilm formation

Bacterial adhesion and biofilm formation is a complex process involving several steps (Figure 1). The initial step for adhesion of bacteria onto surfaces is the adsorption of conditioning components, attachment of proteins, and then of single cells to the surface. The second stage involves microbial transport and co-aggregation including reversible adhesion of single organisms and of microbial co-aggregates. The next step is the anchoring and establishment of biofilm on the surface followed by growth of cells on the surface. At this point irreversible adhesion has been established through exopolymeric substance (EPS) production. In a mature biofilm there is also a balance between attachment and detachment of cells (Bos et al., 1999; Bryers, 2000).

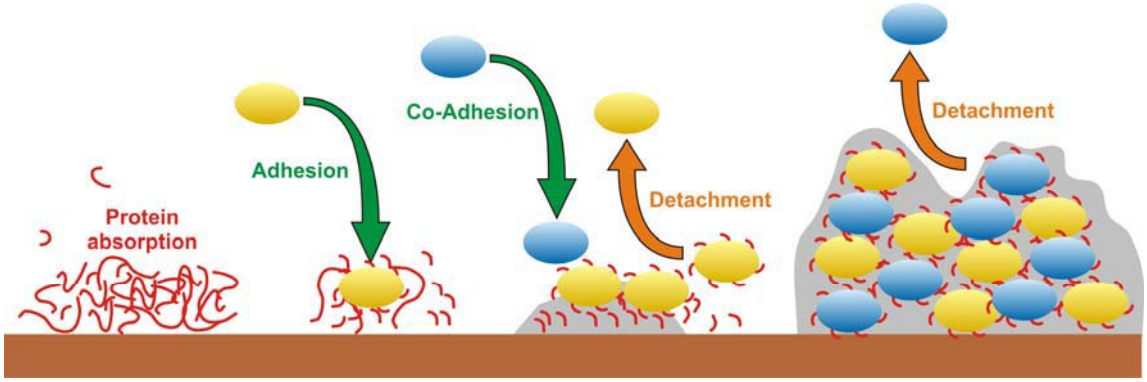

Figure 1: Sequential steps in biofilm formation and maturation

Detachment in biofilms follows different mechanisms. Constant detachment of cells from the outer biofilm layers that outbalances biofilm growth and ensures steady state

biofilm thickness is called erosion. Events, where cell aggregates or larger parts of a biofilm shear off, usually very problematic for reactor performance, are termed sloughing. In systems with floating biofilm carriers or granules the collision of particles also leads to detachment of cells or of parts of a biofilm and is called abrasion (Bryers, 2000; Stewart, 1993).

2.1.2 Molecular interactions between biofilms and attachment surfaces

The interaction between bacteria and surfaces has been studied by many researchers. However, there are still many uncertainties on the relevance and contributions of the various mechanisms involved.
Purely physico-chemical interaction forces, including the Lifshitz-van der Waals forces, electrostatic forces, and acid-base interactions, are probably responsible for initial adhesion of bacteria onto surfaces. These specific interactions are highly directional between molecular groups and consequently operative over very small distances, typically in the nanometer range. The so-called non-specific association in microbial adhesion arises from interaction forces between all molecules of the entire cell and a substratum and are consequently of a more long-range character.
To adequately describe microbe-surface adhesive interactions, both the long-range and non-specific fundamental interaction forces, and the short-range and specific interactions must be taken into consideration.
There are two physico-chemical approaches available to describe microbial adhesive interactions: the first is a kinetic approach (the interacting surfaces are assumed to physically contact each other under conditions of thermodynamic equilibrium, but adhesion is reversible). The second is also called the DLVO (Derjaguin, Landau, Verwey, Overbeek) or extended DLVO theory, an approach which describes the interaction energies between the interacting surfaces, based on Lifshitz-van der Waals and electrostatic interactions (and Lewis Acid/Base forces, for extended DLVO) and their decay with separation distance.
Both approaches have proven useful frameworks to study the contributions of various forces to microbial adhesion, especially in a comparative context, when certain collections of strains and species are compared, but have failed so far to yield a generically valid description and prediction of microbial adhesion (Bos et al., 1999).

2.1.3 Biofilm topology and morphology

Biofilm structure, stability, and cohesiveness is very complex and depends on many factors such as physical (porosity, density, EPS content) and mechanical (viscoelasticity) properties of the biofilm (Körstgens et al., 2001; Möhle et al., 2007; Stoodley et al., 1999; Towler et al., 2003). Biofilm structure also has a significant impact on substrate removal and reactor performance and many environmental factors affect biofilm development and the resulting structural properties. These factors include physical parameters such as, hydrodynamic shear forces, detachment, or mass transfer resistance; chemical factors such as substrate and nutrient composition and concentration, pH; biological factors such as cell physiology, the microbial population

and the produced extracellular polymeric substances (EPS) (Wijeyekoon et al., 2004). The exact and quantitative contribution of these factors (especially different combinations of them) to biofilm structure has, however, not yet been determined. Many studies have tried to shed light on the impact of environmental conditions on biofilm formation and structure. Generally, biofilms grown under flow conditions that generate high shear stress are thinner and more compact, whereas they grow thicker and more fluffy under flow conditions that generate less shear. Exposure to high shear forces is also known to result in stronger and more resistant biofilms (Liu and Tay, 2001; van Loosdrecht et al., 1995). Biofilms behave like viscoelastic materials and are affected by changes in fluid shear. Pereira et al. (2002), for example, showed that flow influences the cellular density inside the biofilm: a higher surficial density (cell per unit area of attachment surface) is observed under laminar flow regimes, but a higher volumetric cell density (amount of cells per unit volume of biofilm) is observed in turbulent regimes.
The effect of substrate concentration on biofilm structure has, in general, been described as follows: at substrate limiting conditions very rough, fluffy, and highly porous biofilms form, whereas at high substrate loadings more smooth and dense biofilms develop (Picioreanu et al., 2000; Wijeyekoon et al., 2004).

2.1.4 Biofilm control

Operation of biofilm reactors relies on effective control of biofilm stratification and thickness. Excessive biofilm loss will negatively affect reactor performance, because detachment and subsequent wash out of large amounts of microorganisms significantly reduce the conversion/degradation capacity of a system (Horn et al., 2003; Telgmann et al., 2004). Especially uncontrolled or spontaneous sloughing events, that sometimes result in substantial biomass loss, can be detrimental to reactor performance (Appendix I; Semmens, 2005).
Biofilm thickness is controlled by the balance between growth and detachment (e.g., minimum flow rate in trickling filters to ensure enough erosion of biomass, certain turbulence in UASBs to ensure enough friction between the granules). Systems like biofilters work with backwashing - removing excess biomass in certain time intervals. However, such biofilm systems may still be subjected to sudden sloughing events that can occur due to hydraulic perturbations.
At steady state, the biofilm thickness in a single substrate biofilm is governed by the interplay of substrate flux J_S and a biofilm specific loss rate ($b + b_D$) (Eqn.1 after Rittmann and McCarty (2001)). Since b is intrinsic to the microorganism and the J_S, an operational parameter priorly chosen for a target pollutant removal rate, the detachment coefficient b_D is the easiest available parameter for biofilm thickness control.

$$L_f = \frac{J_S \, Y}{\left(b + b_D\right) \, X_{B,f}} \qquad \text{Eqn.1}$$

with
L_f = biofilm thickness [m]
J_S = substrate flux [kg m^{-2} d^{-1}]
Y = biomass yield [$kg_{biomass}$ $kg_{substrate}^{-1}$]
$X_{B,f}$ = active biomass density within the biofilm [kg m^{-3}]
b = biomass decay rate [d^{-1}]
b_D = detachment rate [d^{-1}]
(Rittmann and McCarty, 2001)

Active and especially accurate control of biomass detachment is still rather challenging. Some possibilities to mechanically control biofilm thickness include: changes in the hydraulic conditions (via flow or stirring) or controlled gas sparging to cause scouring. Such measures will enable stable reactor operation by keeping the average biofilm thickness at the optimum. However, sloughing phenomena remain possible and can affect reactor performance.

2.1.5 Surface modification to manipulate biofilm formation

Modifying the properties of a surface to influence attachment of proteins and bacterial cells has been studied intensively, especially for medical applications where prevention of protein and bacterial adhesion, e.g. onto implants or catheters, is the main focus. From a wastewater engineering perspective there are two fields where such techniques might be applicable: minimizing clogging of membranes used for solid/liquid separation by adhesion prevention, or applying similar techniques to enhance biofilm formation and detachment resistance in biofilm reactors.
In principal there are two main options to alter bacterial adhesion onto a surface: physical modification of the surface structure (i.e. roughness, porosity…) or chemical surface modification (e.g., modification of the surface charge).
Adhesion prevention in wastewater treatment has mainly focused on finding solutions to prevent or delay membrane clogging (Tan and Obendorf, 2007; Yu et al., 2006), because normally regular cleaning (i.e. backwashing and/or application of chemicals) is required to remove particles from the membrane surface to keep its functionality, and such measures are time consuming and costly. Adhesion prevention has, however, been studied much more extensively in other fields (e.g. medical applications), with special focus on the impact of chemical surface modification on bacterial or protein adhesion. Several surface modification techniques are suggested. Grafting of poly(ethylene oxide) (PEO) brushes (layer thickness approx. 24 nm) revealed much higher biofilm removal from these grafted surfaces compared to unmodified glass in adhesion experiments with *P. aeruginosa* (Roosjen et al., 2006). For effective application of such a grafting technique it is important, though, to optimize PEO chain length and density (Roosjen et al., 2004). Other modification techniques, employing for example deposition of self-assembled monolayers (implementing NH_2^+, CH_3^-, or other functional groups directly onto the target surface), are also reported (Hou et al., 2007; Ploux et al., 2007). Another grafting technique uses poly(ethylene glycol) (PEG) with different functional groups

and PEG structures for protein and bacterial adhesion control/prevention (Kingshott et al., 2002; Kingshott et al., 2003; Park et al., 1998).
Fewer studies explicitly focus on enhancement of bacterial adhesion onto surfaces (Hadjiev et al., 2007; Lee et al., 2005; Wiencek and Fletcher, 1995). Grafting of glycidylmethacrylate (GMA), for example, significantly increased bacterial adhesion (Lee et al., 1997; Terada et al., 2004; Terada et al., 2006c).
Documentation of artificial enhancement of biofilm formation in wastewater engineering applications is even more scarce (Sousa et al., 1997; Terada et al., 2004). Increased biofilm formation on, for example, modified hollow fiber membranes was described by Terada et al. (2004) for nitrifying bacteria. Another approach to enhance nitrifying biofilm formation took advantage of EPS production of another bacterial group (heterotrophic bacteria) (Tsuneda et al., 2001).
However, not only increased biomass adhesion, but also higher detachment resistance is desired in most wastewater treatment applications. Very few studies have focused on the combined effect of fluid flow and surface modification. Roosjen et al. (2005) found, for example, that detachment from PEO brush induced surfaces was much higher compared to unmodified glass surfaces.
A more physical modification approach, in principal similar to applications for bone recovery, e.g. in tissue engineering (Liu and Ma, 2004), is to modify the substratum surface in a way that the biofilm is 'shear-protected' by a scaffold-like structure. The desired thickness can then be maintained by adjusting the thickness of the scaffold layer. An example of such a system is described by Terada et al. (2006a). In their lab-scale reactor a silicone membrane, used as growth surface for the biofilm, was surrounded by a ferro-nickel fibrous slag to immobilize the biomass: rapid immobilization of nitrifying bacteria and high nitrification rates were achieved in this system.
Such approaches have not been studied to a larger extent and have only been applied in lab scale. More research should explore and optimize such techniques of biofilm anchoring and scaffolding.

2.1.6 Biofilm modeling

Mathematical models of complex systems like biofilms, with the various interactions of substrate transport and conversion, biofilm formation and bacterial growth, have been very powerful tools for research and process design. Even though simplifications have to be made to represent such a complex reality, simulating, for example, the impact of environmental conditions on biofilm reactor performance has significant engineering application and modeling approaches provide a rather fast and easy method to assist with often time consuming and expensive experiments.
A number of mathematical models have been developed over the past decades to gain better understanding of biofilm systems and to assist with process design. These models can serve different purposes. Engineers are mostly interested in assessing the performance of biofilm reactors, i.e. effluent concentrations or most critical design parameters (Brockmann et al., 2008; Hao et al., 2002a; Hao et al., 2002b; Koch et al., 2000). Microbiologists tend to be more interested in biofilm structure, interaction and stratification of different microbial species, EPS formation or biofilm composition

(Alpkvist et al., 2006; Chambless and Stewart, 2007; Eberl et al., 2000; Picioreanu et al., 2004a; Picioreanu and van Loosdrecht, 2003; Picioreanu et al., 2004b; van Loosdrecht et al., 2002; Xavier et al., 2005).

Biofilm formation and growth depends on the interaction of many different biological, chemical, and physical processes. Models of very complex systems like biofilms will always be simplifications of reality. Clearly defined objectives are, therefore, essential for every simulation study. Focusing on the most relevant processes and interactions in a specific case is highly recommended and often simple models are sufficient for process design and engineering applications. Certain questions may, however, require a more complex mathematical representation (e.g., simulations of biofilm structure).

The first mathematical expressions of steady state biofilm kinetics were introduced by Rittmann and McCarty (1980) and Harremoës (1978). These approaches were based on many simplifications, describing the biofilm as uniform, single species, steady state film and substrate conversion governed by zero or first order bio-kinetics.

Significant improvement towards multi-substrate and multi-species biofilm models was introduced by Wanner and Gujer (1986). With their model it was now possible to study more complex interactions and kinetics in multi-species and multiple substrate biofilms With the implementation of this 1-d biofilm model in the software package Aquasim (Wanner, 1995; Wanner and Morgenroth, 2004; Wanner and Reichert, 1996) a very powerful tool was provided for biofilm reactor modeling and it has been the base for many studies (Arcangeli and Arvin, 1997; Brockmann et al., 2008; Hao and van Loosdrecht, 2004; Hao et al., 2005; Hao et al., 2002a; Hao et al., 2002b; Horn and Hempel, 1997; Horn et al., 2003; Koch et al., 2000; Lackner et al., 2008; Matsumoto et al., 2007; Morgenroth and Wilderer, 2000; Reichert and Wanner, 1997; Shanahan and Semmens, 2004; Terada et al., 2007; Walter et al., 2005; Wanner and Morgenroth, 2004). Some other 1-d biofilm models were developed by e.g., Casey et al. (1999a), Casey et al. (2000a) and Rauch et al. (1999).

1-d models have limitations when it comes to detailed description of biofilm morphologies and gradients of limiting substrates which occur not only perpendicular to the substratum (as assumed in a 1-d approach). It has also been shown that predicted dynamics especially for slow growing microorganisms and inert biomass in 2-d simulations differ from a 1-d model (Picioreanu et al., 2004a). 1-d models can also not describe the pore structure of a biofilm and they have limitations in representing changes in density and porosity.

Within the last couple of years, 2 and 3-d biofilm modeling has gained increasing attention, also because the computational resources became available to perform more and more complex simulations in a reasonable timeframe. These models have been used to successfully simulate different biofilm structures depending on variations in environmental conditions. A good overview of modeling attempts in 2 and 3-d within the last 10 years can be found in Picioreanu et al. (2004b) and van Loosdrecht et al. (2002).

One of the first 2-d biofilm models was developed by Wimpenny and Colasanti (1997) and it was base on the cellular automation approach (CA). This model could predict different morphological structures of the same biofilm under different substrate

conditions. However, the model had serious limitations because growth only occurred in the top layer of the biofilm but not inside the biofilm matrix which is rather unrealistic.
This limitation was overcome by using a discrete-differential biofilm model also based on CA (Picioreanu et al., 1998). In this model the pressure exerted by biomass growing inside the biofilm matrix would generate displacement of biomass towards the biofilm/liquid interface.
Another biofilm modeling concept has been developed that differs from all previous models in the implementation of biomass growth and spreading (van Loosdrecht et al., 2002). Individual-based modeling (IbM) of biofilms introduced by Kreft et al. (2001) describes biomass as spherical 'single cells' or larger biomass particles (Picioreanu et al., 2004a) with their position in space defined by continuous coordinates. Each of the biomass particles is seen as an individual with its own set of parameters and equations.
Many different variations and combinations (additions) of these biofilm models have been used for a variety of applications (Picioreanu et al. (2004b), and references therein), i.e. simulating detachment events (Picioreanu et al., 2001). In literature the IbM based models are stated as the more promising approach for representing actual biofilm structure and performance due to the greater flexibility and more accurate representation of biomass growth. A review on IbM is given by Hellweger and Bucci (2009).

2.2 Nitrogen removal

2.2.1 Nitrification / denitrification

Nitrogen removal has become one of the essential parts in wastewater treatment over the last few decades. The classical approach to achieve complete biological nitrogen removal from wastewaters applies a two step process, nitrification coupled with denitrification.

The first part, the nitrification, is an aerobic process where aerobic chemolitho-autotrophic bacteria oxidize ammonium (NH_4^+) to nitrate (NO_3^-) in two steps.

Ammonium nitrogen is converted to nitrite (NO_2^-) and further to nitrate by different groups of microorganisms. The conversion of ammonium follows the following stoichiometry and is carried out by ammonium oxidizing bacteria (AOB) (Eqn. 2).

$$2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 4\ H^+ + 2\ H_2O \qquad \text{Eqn. 2}$$

In the second step nitrite oxidizing bacteria (NOB) convert nitrite to nitrate (Eqn. 3).

$$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^- \qquad \text{Eqn. 3}$$

Nitrification requires 4.57 g-O_2 for every gram of NH_4-N oxidized to NO_3-N; nitrite production takes up already 3.43 g-O_2/g-N. Nitrification also requires a substantial amount of alkalinity; 8.64 g of alkalinity (in the form of HCO_3^-) are consumed per g of NH_4-N oxidized. Nitrifiers are autotrophic bacteria and their bicarbonate versus substrate oxidation ratio (as determined by Belser (1984)) is 0.02 for *Nitrobacter* and 0.086 for *Nitrosomonas* and *Nitrosospira sp.*

The dominant AOB and NOB species in wastewater treatment plant environments have often been reported as *Nitrosomonas europaea* and *Nitrobacter*, respectively (Metcalf and Eddy, 2003). However, several studies have shown that especially for AOB several species can be present in WWTPs; for example, *Nitrosococcus mobilis* (Persson et al., 2002; Wagner et al., 2002), *Nitrosospira sp.* (Schramm et al., 1998) and other species from the *Nitrosomonas* linage (e.g. *Nitrosomonas oligotropha*).

Nitrospira is the more frequently observed NOB in wastewater treatment systems because these conditions are more suitable for their survival (Nogueira and Melo, 2006; Schramm et al., 1998; Wagner et al., 2002). However, depending on nitrite concentrations, *Nitrobacter* may also be present (Kim and Kim, 2006), or at certain conditions even be the dominant NOB species (Nogueira and Melo, 2006).

The microbial community structure in a wastewater treatment plant is very complex and highly depends on the environmental conditions, reactor type and reactor operation (Dytczak et al., 2008a; Dytczak et al., 2008b; Lydmark et al., 2007).

The second part of nitrogen removal, the denitrification step, takes place under anoxic conditions and is performed by a large variety of heterotrophic microorganisms that use carbon together with NO_3^- to produce di-nitrogen gas (Eqn. 4).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{Eqn. 4}$$

To accommodate these two processes within a treatment train several technologies have been developed employing different strategies to facilitate the required aerobic and anoxic environments, i.e. configuration of aerobic and anoxic tanks (pre- or post-anoxic), and simultaneous, alternating or intermittent operation (Metcalf and Eddy, 2003).
Nitrification is a sensitive process mainly because of the very low growth rates of the nitrifiers compared to heterotrophic bacteria (degrading carbon under aerobic conditions), and only if the optimal balance between those groups (and between AOB and NOB) is kept, optimal nitrogen removal can be achieved. This requires a high sludge retention time (to avoid wash out of the nitrifiers), but also absence of any inhibitory or growth limitations factors (such as toxic chemical, low pH, high concentration of free ammonia,…).

2.2.2 Anaerobic ammonium oxidation (Anammox)

Within the early 1990s a novel process, anaerobic ammonia oxidation (Anammox), has been discovered offering a radically new way for nitrogen removal. Even though the existence of this process was already suggested by Broda (1977) in the 1970s, experimental evidences of the Anammox reaction was not provided before the 1990s (Mulder et al., 1995).
The Anammox process is based on the oxidation of NH_4^+ to N_2 gas with NO_2^- as the electron acceptor in the absence of molecular oxygen given the following stoichiometry (Schmidt et al., 2002):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow$$
$$0.26\ NO_3^- + 1.02\ N_2 + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad \text{Eqn. 5}$$

The potential benefits of nitrogen removal via this route are enormous: requirements for oxygen are reduced and organic substrate is no longer required, production of biosolids and alkalinity consumption are also reduced significantly.
Molecular studies revealed that the Anammox bacteria (AnAOB) belong to the order of *Planctomycetales*. Two of the well described Anammox strains are *Candidatus Brocadia anammoxidans* (Jetten et al., 1999) and *Candidatus Kuenenia stuttgartiensis* (Egli et al., 2001; Jetten et al., 2002; Jetten et al., 2001; Schmidt et al., 2002).
The preferred pH and temperature range of AnAOB are 6.7-8.3 and 20-43 °C, respectively (Strous et al., 1999). Their growth rate is very low and first estimates by van de Graaf et al. (1996) were 0.001 h^{-1}. Jetten et al. (2001) found a growth rate of 0.003 h^{-1} (three weeks doubling time). In a more recent study Isaka et al. (2005) reported a much higher growth rate of approximately 0.39 h^{-1} which is significantly different from all previous studies, probably also because of its way of determination (in situ from FISH cell counts).
AnAOB are reversible inhibited by nitrite at 0.1 g-N L^{-1} (Strous et al., 1999; Third et al., 2005) and also by oxygen (Jetten et al., 1999; Third et al., 2005). More resent studies showed inhibitory effects of alcohols, methanol being the most potent inhibitor (Guven et al., 2005; Isaka et al., 2008), leading to complete and irreversible loss of activity at

concentrations as low as 0.5 mM. Organic acids such as acetate and propionate were however converted by AnAOB.

Due to the very slow growth rate of AnAOB, reactor types used for Anammox studies are mostly systems with high biomass retention capacities, such as SBRs (Dapena-Mora et al., 2004; Jetten et al., 2001), gas lift reactors (Dapena-Mora et al., 2004), or fluidized bed reactors (van de Graaf et al., 1996; van de Graaf et al., 1997).

Successful implementation of Anammox for ammonium removal in wastewater treatment requires also partial aerobic conversion of ammonium to nitrite. Two stage processes such as the Single High rate Ammonia Removal over Nitrite (SHARON) reactor combined with an Anammox system (Hellinga et al., 1998) are one example of a two reactor system (Jetten et al., 2002). Nitritation in a MBR operated at low oxygen concentrations coupled with an Anammox reactor was suggested by Wyffels et al. (2004).

Nitritation and Anammox combined in one reactor is more challenging. The CANON process (Complete Autotrophic Nitrogen removal Over Nitrite), for example, uses SBR granular technology (Nielsen et al., 2005; Sliekers et al., 2002). Another single reactor concept for complete autotrophic nitrogen removal is proposed by Pynaert et al. (2004) applying a RBC biofilm for oxygen limited autotrophic nitrification denitrification (OLAND) (Egli et al., 2003; Pynaert et al., 2003).

Full scale applications of Anammox are also reported. The first reactor started in Rotterdam (van der Star et al., 2007). Other reports from successful implementation of Anammox in full scale are e.g. from Austria (Innerebner et al., 2007; Wett, 2006; Wett, 2007).

2.3 Membrane aerated biofilm reactors

The Membrane Aerated Biofilm Reactor (MABR) is a rather new technology employed in wastewater and waste gas treatment, and is based on growing biofilms on permeable membranes with the possibility to apply substrate flows from both sides of the membrane to the bacteria inside the biofilm.

2.3.1 Principle

Membrane technologies have seen increasing use in different aspects of water and wastewater treatment over the past few decades. The main functions of membranes within the water treatment context can be divided in solid/liquid separation and solute transport (Figure 2). The application of membranes for separation purposes is the most common one, with an application range from membrane bioreactors (MBRs) in wastewater treatment for solid/liquid separation and biomass retention to reverse osmosis for drinking water purification. In MBR systems, the membrane module - either immersed directly in the activated sludge tank or as an external module - is used as replacement for the secondary clarifier. Such a configuration saves space and also decreases the concentration of suspended solids in the effluent. With proper membrane choice, removal of pathogens can also be achieved.
The other main application of membranes lies within solute transport. Here the distinction must be made between liquid/liquid and gas/liquid applications (Figure 2). The first are, for example, membranes used in industrial wastewater treatment, where they serve to extract pollutants that are not or weakly biodegradable, to avoid toxic effects on the biofilms, and for high quality water purification (for process water) (Stephenson et al., 2000).

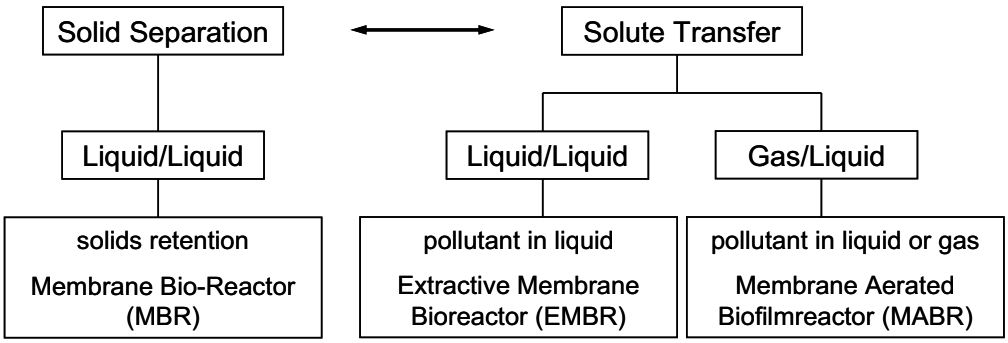

Figure 2: Grouping of the different membrane applications in water treatment

In the latter context membranes are used for gas supply. The first application of membranes in a gas/liquid separation mode is membrane aeration. In that case the membrane module is used for bubble-free aeration, for example, of an activated sludge system. Biofilm growth on these membranes was initially unwanted, because it negatively affects the gas transfer (decrease in oxygen mass transfer due to oxygen uptake inside the biofilm and clogging of pores) into the liquid phase. However, biofilms growing on such membranes can be advantageous when grown intentionally and controlled in so-called membrane aerated biofilm reactors (MABRs) (Casey et al.,

1999b; Stephenson et al., 2000; Syron and Casey, 2008a). In these systems, the membrane is used to support the bacteria (biofilm growth), but also to directly supply the microorganisms in the biofilm with substrate (oxygen or other gaseous compounds). The most widely studied configuration of the MABR uses gaseous oxygen supply and relies on diffusive mass transfer of oxygen through the membrane into the biofilm. In such a system, air or pure oxygen is supplied through a gas permeable membrane to the base of the biofilm whereas the wastewater is supplied from the outer side (bulk liquid). In contrast to membrane aeration where biofilm growth is unwanted on the membrane, the MABR system aims for stable biofilm formation on the membrane surface; the oxygen is consumed by the microorganisms inside the biofilm, leading to very low oxygen concentrations in the bulk liquid.

The principal configuration and gradients of substrates in a biofilm developed in an oxygen based MABR for COD and ammonium removal is shown in Figure 3. The diffusion of substrate (e.g., COD, NH_4^+) and the gas (e.g., oxygen) as shown in Figure 3 (right) is distinctly different from what is observed in a conventional biofilm or activated sludge floc (Figure 3, left). In the MABR, oxygen and the substrate enter the biofilm at opposite ends, leading to counter-current substrate and oxygen gradients in the biofilm.

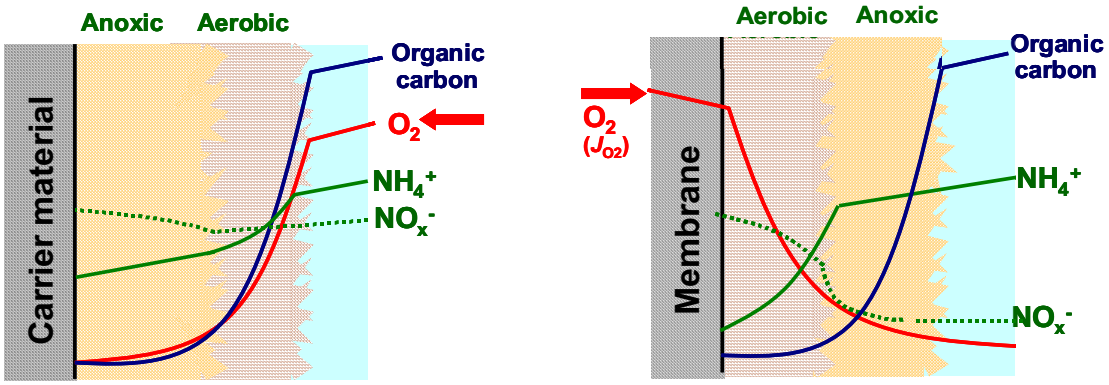

Figure 3: Co- (left) versus counter (right) -diffusion biofilms for wastewater treatment

Characteristic for the MABR configuration is the oxygen depletion towards the bulk liquid, leading to very low oxygen concentrations in the bulk, making this application inverse compared to conventional membrane aeration. In a more sophisticated operation of the MABR different redox zones (e.g., oxygen gradient) inside the biofilm are established and are controlled by the air pressure and gas flow rate. This additional degree of freedom in fine tuning of the oxygen supply allows the establishment of different environments inside the same biofilm to achieve, for example, complete nitrogen removal via denitrification (Semmens et al., 2003; Terada et al., 2003). Furthermore a more divers microbial community establishes in a MABR as the range and gradients of oxygen in the biofilm are different compared to conventional biofilms: oxygen is supplied at the base of a MABR biofilm where it is at saturation level (= 9.1 mg L^{-1} at 20 ºC) compared to approx. 2 mg L^{-1} oxygen in the bulk liquid of a conventional biofilm system.

Another type of MABR is based on supplying hydrogen gas instead of oxygen through the membrane lumen, which then serves as the electron donor to support, for example, denitrification (Lee and Rittmann, 2002). Experiments supplying methane as electron donor together with oxygen for denitrification have also been introduced (Modin et al., 2008).
A third application of MABRs is the treatment of gaseous waste streams and volatile organic compounds. In such a system, the waste gas itself is supplied to the base of a biofilm growing on a semi-permeable membrane. The biofilm is supplied with nutrients from the bulk liquid side, while the contaminant diffuses into the biofilm where it is degraded (Figure 4) (Reij et al., 1998).

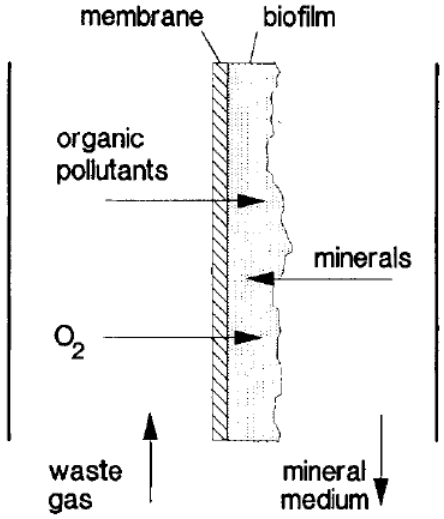

Figure 4: Principle of a membrane biofilm reactor for a gaseous waste treatment (Reij et al., 1995)

In comparison to conventional activated sludge systems, MABR-based systems present several advantages and a few disadvantages.
One of the biggest advantages of an MABR is the very efficient oxygen transfer. Aeration is one of the most costly units in aerobic bioreactors and increasing its efficiency is money saving. A more efficient oxygen supply makes the MABR also suitable for treatment at high substrate loadings.
Applying the MABR to, for example, nitrification/denitrification can be facilitated in one single reactor. Conventional plants, on the other hand, need either two separate reactors or some kind of alternating operation to achieve the necessary oxic and anoxic conditions. In a MABR the zonation inside the biofilm provides oxic/anoxic stratification within one reactor, yielding a smaller footprint system.
Due to the more compact and closed design, stripping of volatile organic compounds by aeration is prevented. MABRs are even explicitly used to treat gases or liquids with high VOC content, which is not possible with conventional activated sludge systems (Stephenson et al., 2000).
Disadvantages of the MABR technology might be the need for biofilm thickness control. A stable biofilm on the membrane, which results from a balance between net growth

and detachment, is important for stable performance of the system. Too thick biofilms as well as sloughing (loss of parts of the biofilm), for example, due to suboptimal flow conditions or rapid changes in flow conditions may jeopardize reactor performance.
The initial costs for the membrane material may also be a disadvantage of the MABR. Very few full-scale (only one H_2 based) or pilot-scale plants (Semmens, 2005) have been installed, which renders final judgment of the controllability and performance difficult.

2.3.2 Application

MABRs have been studied and applied in several - (mostly) lab scale - configurations for removal of many different compounds. An overview of the application range of MABRs is given here and a summary of the most relevant applications and their technical and operational details is provided in Table 1.
The removal of COD and nitrogen from wastewaters by use of MABRs has been documented by several researcher groups (Downing and Nerenberg, 2008a; Matsumoto et al., 2007; Satoh et al., 2004; Semmens et al., 2003; Terada et al., 2007; Walter et al., 2005), using different configurations: some studies supply pure oxygen versus air; differences in reactor type (surface area, membrane material, but also loading rates). Terada et al. (2006c) report simultaneous removal of carbon, nitrogen, and phosphorous in a sequencing batch type MABR. Denitrification in MABRs based on hydrogen gas supply was also proven successful by several research groups (Lee and Rittmann, 2002; Terada et al., 2006a). A rather new approach is the used of methane gas together with oxygen for denitrification in a silicone based MABR (Modin et al., 2008).
Process combinations such as the hybrid process described by Downing and Nerenberg (2008b), combining the membrane bound biofilm with suspended biomass systems, for total nitrogen removal, or the M2BR reactor by Chen et al. (2008), a combination of the classical MABR with membranes for separation purposes proved interesting applications.
Another application field of the MABR technology is the degradation of synthetic and/or volatile organic pollutants. The most relevant examples are summarized in Table 1. MABRs have been used to remove volatile compounds like propene (Reij et al., 1995); toluene or dimetyl-sulphide (van Langenhove et al., 2004) from waste gases.
One group has extensively studied the applicability of MABRs based on hydrogen gas supply for removal of heavy metals or oxyanions, including chromate (Chung et al., 2006b) (reduction of Cr(V) to Cr(III) under denitrification conditions); selenate (Chung et al., 2006c), and arsenate (Chung et al., 2006a).

Table 1: Documented use of MABRs for removal of various contaminants (examples).

Loading/Influent Concentration and Conditions	Removal Efficiencies	Reference
COD removal		
brewery wastewater: influent 1700 - 3000 mg COD L^{-1}; loading rate 4 - 36 kg COD m^{-3} d^{-1} BOD to N to P ratio of 100:5:1 pH around 7.6; temperature 25°C; HRT between 1.8 and 10.7 h	80-88 % (at 27-28 g COD $m^{-3}d^{-1}$)	(Brindle et al., 1999)
COD and N removal (with nitrification and denitrification)		
synthetic medium: organic load: 0.2 - 1.8 gCOD m^{-2} d^{-1} total nitrogen load: 0 - 1.1 gN m^{-2} d^{-1}; pH around 7; HRT 2.22 d (dilution rate of 0.45 d^{-1});	90 % COD 95 % nitrification denitrification achieved	(Satoh et al., 2004)
filtered primary effluent supplemented with nutrient broth influent concentrations: TOC = 70-90 mg L^{-1}, org-N = 17-27 mg L^{-1} NH_4-N = 20-30 mg L^{-1}, NO_3-N < 1 mg L^{-1} pure oxygen	35-50% TOC 55-75% Org-N	(Timberlake et al., 1988)
synthetic medium: COD 218 – 394 mg L^{-1}, NH_4 49 – 87 mg L^{-1} pH maintained between 8-8.3, HRT 6 or 12 h biofilm thickness 1-2 mm	90 % COD (after 40 d); 80 % nitrogen (in first 60 d then > 90 %) Removal rates: N: 2 g m^{-2} d^{-1}; COD 10g m^{-2} d^{-1}	(Semmens et al., 2003)
primary effluent: COD approx. 300 – 800 mg L^{-1}, NH_4 10 – 25 mg L^{-1}	40 – 60 % COD removal, 60 – 90 % NH_4 removal	(Semmens, 2005)
denitrification with H_2 based hollow fiber reactor synthetic medium: NO_3-N 10-15 mg L^{-1}	up to 92% (0.4 mg NO_3-N L^{-1})	(Lee and Rittmann, 2002)

Table 1: continued

Loading/Influent Concentration and Conditions	Removal Efficiencies	Reference
synthetic/volatile organic chemicals		
biodegradation of PCE (perchloroethylene) (influent 70 mg L^{-1}), no accumulation of intermediates; addition of 1.25-2.5 gCOD L^{-1} as glucose; migration of chlorinated compounds and VFAs into gas compartment	with removal rate of 247 mmol PCE h^{-1} m^{-3}, COD removal 85-92 %	(Ohandja and Stuckey, 2007)
perchlorate removal (synthetic influent with 1600 µg L^{-1}) and contaminated groundwater (6-100 µg L^{-1})	reduction nearly 100 %	(Rittmann et al.)
removal of Xylene (influent: 40 gCOD m^{-2} d^{-1}); toluene (influent 170 gCOD m^{-2} d^{-1})	removal rates 95-99 %	(Debus, 1993)
degradation of 1,2-dichloroethance by *Pseudomonas* sp. strain DCA1 (anoxic groundwater as water phase)	removal rate approx. 400 g m^{-3} h^{-1}	(Hage et al., 2004)
degradation of phenol in liquid/liquid inter-phase MBR; wastewater with phenol at the inner side, nutrient solution outer side of membrane (tubing); influent concentration 1000 mg L^{-1}; good solution for industrial wastewater with high salt content/low pH	removal rates up to 98.5% (in all cases 80% conversion to CO2,	(Livingston, 1993)
removal of phenol and sodium salicylate, biofilm and suspended growth - substrate from inside the lumen, (liquid on both sides)	biofilm accounted for up to 78% to removal; also adds to prevent inhibitory effects (tolerance enhancement)	(Juang and Tsai, 2006)

2.3.3 Reactor and membrane configurations

For use in MABR application, membranes need to meet certain criteria. While the exact properties may vary according to the specific applications, certain criteria are universal. When considering MABRs in a gas/liquid mode (e.g., for membrane aeration), the membrane module can be operated in a dead-end or flow-through mode. The advantage of a dead-end configuration is that all gas has to pass through the membrane into the biofilm, which makes it more suitable and efficient when using an expensive gas such as pure oxygen or H_2. The problem with dead-end operation is the potential for condensation inside the lumen, which will result in decreased gas transfer efficiency. The flow-through mode is better to prevent condensate inside the lumen, and also allows for better removal of CO_2 or other produced gasses. At the same time, however, the potential loss of volatile organic compounds may pose a disadvantage. In case of membrane aeration one also needs to decide between the use of air versus pure oxygen, which usually depends on the oxygen mass transfer of the membrane and the required oxygen flux.

For aeration purposes (i.e., membrane aerated biofilms) the membranes must have a hydrophobic layer, to prevent water penetration into the pores while the pore size must be around 0.01-0.1 µm. The membrane material mainly determines the oxygen transfer rate. Microfiltration membranes (with pore sizes of 0.01-0.1 µm) present one option. The limitation of these membranes is the limited pressure that can be applied before bubble formation occurs. Hence, those membranes need to have a high bubble point pressure and can normally not be pressurized above approx 0.5 bar. The other option are composite membranes, membranes composed of a porous and a non-porous layer which makes it possible to pressurize the membrane lumen more and increase the oxygen flux. A third possibility are membranes made of only non-porous material, for example, silicone.

For non-composite membranes the bubble point pressure should be at least >1 bar; the overall membrane thickness should be 200-500 µm. For application within wastewater treatment the reachable oxygen transfer rate must be at minimum 2-5 but better 10-20 g-O_2 m^{-2} d^{-1}.

The membranes can be used in different configurations: tubular, hollow fiber, flat sheet, (frame, plate) (Stephenson et al., 2000). The specific surface area can vary between 20 - 5000 m^2 m^{-3} depending on the configuration (hollow fiber membranes usually have the highest specific surface area). In any case, a large specific membrane surface area will ensure a high level of biofilm attachment area (and biomass in the reactor) and therewith stable and high performance (substrate conversion).

An overview of membranes and configurations that have already been used in different types of membrane biofilm reactors is given in Table 2.

Table 2: Membrane materials and configurations that have been applied in MABRs

Membrane Material	Reactor and Membrane Configuration	Specific Surface Area and Other Conditions	Reference
hollow fiber			
porous composite hollow-fiber membrane (MHF 200TL, Mitsubishi Rayon Company Ltd., New York)	4080 fibers, diameter 280 µm, fiber length 0.84 m, pore size 0.04 – 0.1 µm; reactor working volume 6,75 L, void space 97 %; dead end hollow fiber module (pure oxygen) for treating high strength WWT	4 47 m^2 m^{-3}	(Brindle et al., 1999)
polyurethane hollow fiber membrane	fiber inner diameter 237µm, outer diameter 275µm), arranged as a sheet (250 mm long, 300 mm wide); membrane surface area 0.25 m^2; volume 4.5 L; intra membrane air pressure 0.01 or 0.04 MPa	0.0013 m^2 m^{-3} (calculated) membrane oxygen flux: 0.28 m^3 m^{-2} h^{-2} MPa^{-1}	(Satoh et al., 2004)
Polyethylene hollow fiber membranes (Mitsubishi Rayon Corp., NY)	fibers 280 µm OD; 400 fibers per bundle; reactor volume 7 L	422 m^2 m^{-3} - less then 3.2 % of reactor vol.	(Semmens et al., 2003)
hydrophobic hollow fiber membrane Mitsubishi Rayon model MHF 200TL	32 fibers, 70,4 cm^2 fiber surface area; reactor volume 23.9 ml	-	(Chung et al., 2006b)
non-porous Silastic® (Dow Corning) hollow fiber membrane	115 fibers with a ID 0 155 cm OD 0.318 cm	-	(Rector et al., 2006), (Rector et al., 2004)
polyacrylonitrile hollow-fiber membranes for ultrafiltration (Asahi Kasei Chemicals, Tokyo)	OD 1.0 mm, ID 0.7 mm, (dead end configuration) air pressure 23, 45, 100 kPa;	290, 660, and 1190 m^2 m^{-3}	(Terada et al., 2006b)
polypropylene hollow fiber membrane	membrane for liquid/liquid extraction; ID 1.8 mm, OD 2.7 mm, effective pore size 0.2 µm	8.3 m^2 m^{-3}	(Juang and Tsai, 2006)

Table 3: continued

flat sheet				
	flat sheet microporous polypropylene membrane (3M Corporate Process Technology Center, Minneapolis)	membrane thickness 1.4 mm, bubble point pore diameter of 0.36 µm; dead end air permeability of 6355 g m^{-2} h^{-1} at 8.6 kPa	4.048 m^2 m^{-3}	(Ohandja and Stuckey, 2007)
	waste gas treatment - hydrophobic polypropylene (PP) accurel membrane, Type 1E-PP from Enka AG (Wuppertal, Germany)	porosity 70-75 %, average pore size 0.1 µm, thickness 75-110 µm, membrane area 40 cm^2, volume of compartments 8 cm^2 each	5 m^2 m^{-3} calculated	(Reij et al., 1995)
	hydrophobic membrane (Millipore Durapore, Bedford, Mass)	pore size 0.22 µm, porosity 75%; thickness 125 µm; effective membrane area 40 cm^2; reactor size 2 compartments 8 ml each		(Hage et al., 2004)
other				
	Gore-Tex™	0.025 mm thick expanded polytetra-fluoroethylene layer with 0.2 µm pores, sandwiched between two layers nylon woven fabric		(Timberlake et al., 1988)
	Silicone membrane	3.175 mm OD, wall thickness 0.6 mm		(Casey et al., 2000b)
	silicone rubber	3 mm ID, wall thickness 0.5 mm		(Livingston, 1993)
	waste gas treatment - commercially available composite membrane (GKSS, Germany); hydrophobic dense top layer polydimethyl-siloxane (PDMS), thickness 1 or 2.5 µm; polyvinylidene fluoride (PVDF, 210 µm) as hydrophobic support layer material	air- and MM-side both have a membrane contact area of 40 cm^2	500 m^2 m^{-3}	(van Langenhove et al., 2004)

2.3.4 Economic feasibility and technical hurdles of the MABR technology

New process technologies, such as the MABR, can provide promising alternatives or improvements to existing reactor/process design. However, the step from successful lab scale application to actual large scale implementation can be rather challenging (Semmens, 2005; Syron and Casey, 2008a) and is governed by many factors.

Up to date, there are no full scale applications of MABRs and, therefore, economic evaluations are solely based on theoretical assumptions. However, some general criteria on the economic feasibility of the technology can be given based on theoretical investigations (Syron and Casey, 2008a) and pilot plant studies (Semmens, 2005).

The following illustrates, based on a random example, the comparison of the expected performance of a MABR to a conventional activated sludge (CAS) plant. If not stated otherwise the following specifications are assumed: wastewater flow rate 4 m^3 d^{-1}, influent NH_4-N concentration 500 g-N m^{-3} (yielding a mass loading: 2 kg-N d^{-1}); the required reactor volume in the base case (for a CAS plant) 2 m^3.

Several points distinguish the MABR technology from CAS units. One main issue is the space requirement for each process: CAS plants usually have the highest space and tank volume requirements. For complete N-removal two tanks are needed for nitrification and denitrification, respectively. For standard conditions the required tank volume is about 200 L per population equivalent (1 PE = 0.2 m^3 d^{-1}, 120 g-COD d^{-1}, 11 g-TKN d^{-1}). In case of a high N-load wastewater the volume needed will be higher. The process also requires a sedimentation unit for solids/liquid separation and sludge recycle.

With a MABR for high rate N removal, the tank volume can be reduced by approx. 60%. A separate membrane unit may be used in this case for solids separation. This volume reduction is possible because, both steps for complete N removal take place in the same reactor inside the biofilm. By facilitating the biofilm in the reactor a very high biomass concentration can be sustained, making this large volume reduction possible.

From lab-scale experience it is also expected, that a MABR - like other biofilm-based processes - produces much less excess sludge than a conventional suspended growth plant (although the exact mechanism behind this are not known). This can have a large economical impact on plant operation since sludge processing and disposal contributes a large part (up to 50%) of the total operation costs of the CAS systems.

Probably the highest advantage of the MABR is the much more efficient oxygen transfer to the biomass. To estimate aeration costs for a CAS compared to a MABR the following scenario is considered. Oxygen requirement for a wastewater with a high N load (2 kg-NH_4-N d^{-1}) is 4.57 g-O_2/g-N plus an extra 50% for endogenous respiration. The amount of oxygen needed comes to approx. 14 kg-O_2 d^{-1}. With 1 m_N^3 air containing 300 g O_2, the amount of air needed to provide the required amount of oxygen comes to 46.7 m_N^3 d^{-1}. To determine the required air flow rates, the oxygen delivery efficiency (ODE) must be determined. In the current case ODEs are chosen to be 5% per m submergence for the CAS and 10 or 20 % for the MABR, respectively (see Figure 5 for further details). To estimate the aeration costs for these cases the power requirement has to be calculated. The main factor here is the head loss: in a CAS with diffusers, head losses are generally much higher because the air has to be compressed to overcome the hydrostatic head (normal depth of an activated sludge tank is 3-5 m) whereas the hollow

fibers in a MABR are exposed to atmospheric conditions on both ends, only leading pressure loss due to friction in the pipe system and the fibers. The head losses for the different scenarios were calculated using the Hagen-Poiseuille equation (Semmens, 2005).

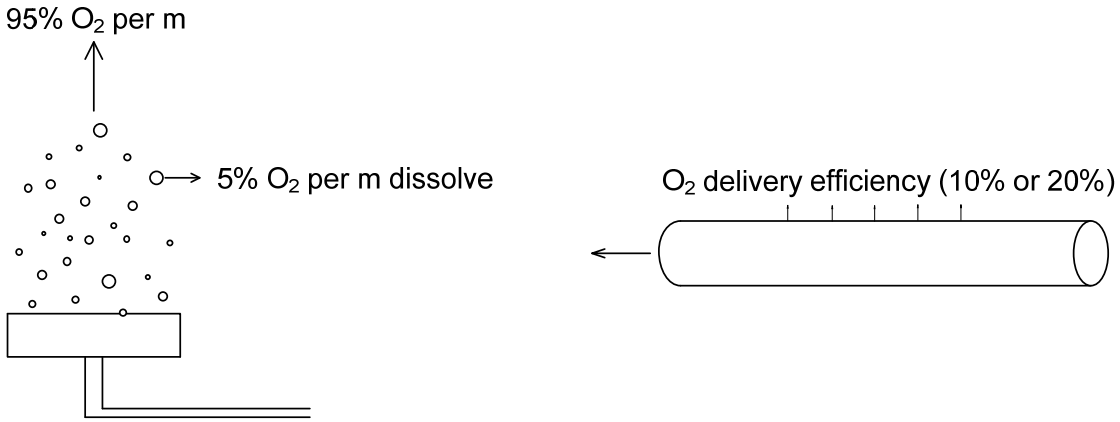

Figure 5: Schematic drawing of the oxygen delivery efficiencies for a diffuser (left: activated sludge and membrane bioreactor systems) and a PMBR fiber (right)

The power requirement of a blower can then be calculated as (Metcalf and Eddy, 2003)

$$P_W = \frac{wRT_1}{29.7ne}\left[\left(\frac{p_2}{p_1}\right)^{0.283} - 1\right] \qquad \text{Eqn. 6}$$

with

P_W = power requirement of the blower [kW]
w = weight of flow of air [kg s^{-1}]
R = gas constant for air [8.314 kJ $kmol^{-1}$ K^{-1}]
T_1 = absolute inlet temperature [K]
p_1 = absolute inlet pressure [atm]
p_2 = absolute outlet pressure [atm] (inlet + head loss)
n = 0.283 (for air)
e = efficiency (usual range 0.70 - 0.90)

The results of these calculations are summarized in Table 4. This simple, rather short example clearly illustrates the economical motivation for implementation of the MABR technology in large scale wastewater treatment, especially under high loading conditions.

However, the technical implementation of the MABR still has to cope with several hurdles. One of the most crucial parts will be the membrane module design. Research on lab scale systems uses either membrane modules that are commercially available for micro/ultrafiltration or employ self-made units customized to their specific research needs with little attention on scale-up. The specific membrane surface area is a crucial factor for efficient high rate removal (Syron and Casey, 2008b) and dense membrane packing might be difficult to operated. Further more Semmens (2005) described wetting

problems with some types of membranes that significantly affected reactor performance. Membrane leakage also has to be prevented because a wet lumen negatively influences the oxygen transfer rates.

Table 4: Comparison of aeration costs for an activated sludge system and a MABR

	Activated Sludge System		MABR	
oxygen delivery efficiency	10 % (diffuser at 2m depth)	20 % (diffuser at 4m depth)	10%	20%
total air required [m^3 d^{-1}]	466.7	233.3	466.7	233.3
flow velocity [m s^{-1}]			0.18	0.09
head loss [m]	3	5	0.17	0.08
Power required [kW]	0.182	0.285	0.012	0.006
Annual cost [€] (assuming 0.1 €/kWh)	160	250	10.5	5.3

Another aspect in stable operation of MABRs is control of the biofilm thickness. To ensure robust operation and high removal efficiency the biofilm thickness must be maintained around the average optimal steady state thickness. The key issue is to minimize large sloughing events that destroy biofilm structure. The biofilm thickness is the result of the ongoing competing processes of biofilm decay and detachment and re-growth. Semmens et al. (2003) experienced that the combination of high specific surface areas, which is desirable, has negative effects. Changes in bulk volume and insufficient shear stress to effectively remove biomass - and produce excess sludge - were the cause. A combination of optimized hydraulic conditions and a biofilm support matrix might be a solution. Clearly, investigations on the influence of reactor configuration, i.e. membrane packing density, specific surface area, membrane support material, or membrane module design are necessary (also stated in Syron and Casey (2008a)).

Lab-scale research on MABRs also did not yet address the influence of wastewater composition, especially suspended solids content, on reactor performance (Semmens et al., 2003). Too high solid contents in the influent can adversely affect reactor performance or might be harmful to the membrane integrity. Within this context, it should also be addressed that essentially no information on biomass (or sludge) production in MABR systems is available. Sludge treatment can be costly and depends on the amount and sludge properties. Even though it is usually assumed that biofilm systems produce much less excess sludge, research is needed to get a better estimation of amounts and characteristics of the MABR biomass production rates.

Another critical issue relates to development of careful process control approaches. Work to date has largely been heuristic, and it has not been identified which control strategies are optimal for stable biological performances of MABRs to guaranty good effluent quality under varying conditions.

3. MABRs for completely autotrophic nitrogen removal

MABRs have been applied in a large variety of setups for the removal of many different compounds (Chapter 2). Nitrogen removal via nitrification/denitfication has, for example, been successfully implemented in MABRs (Terada et al., 2003). The potential application of MABRs for completely autotrophic nitrogen removal has, however, yet to be demonstrated.

The MABR concept, which supports complementary redox zones in a biofilm, theoretically provides an ideal environment for completely autotrophic nitrogen removal within a single reactor system. Growing biomass on a gas permeable membrane and adjusting the oxygen transfer into the system makes it possible to control oxygen penetration depth and aerobic and anoxic/anaerobic zones inside the biofilm.

Shortcut nitrification, i.e. conversion of ammonium only to nitrite, has gained increasing interest over the past years (Schmidt et al., 2003; Sin et al., 2008) also due to the potential economic savings, especially on aeration costs. The produced nitrite can then either be denitrified or, together with residual ammonium, serve as substrate for AnAOB. Implementation of Anammox in MABRs, however, requires stable nitrite production.

Strategies to achieve partial nitritation are mainly based on preventing growth of NOB, i.e. wash out, inhibition, or out-competition of the NOB population. Adjusting the sludge retention time to a minimum, for example, will select only for AOB which usually have higher growth rates than NOB (Hellinga et al., 1998). Inhibition of NOB by free ammonia (FA) is also an option, since inhibitory concentrations for AOB are higher than for NOB (Anthonisen et al., 1976; Yamamoto et al., 2008). However, selection based on FA inhibition alone might not be sustainable since different NOB species are not necessarily affected at the same FA concentration (Blackburne et al., 2007) and NOB might also adapt to higher FA concentration over time (Turk and Mavinic, 1989).

The most practical approach to achieve nitritation, however, seems to be reactor operation under oxygen limited conditions. Because AOB typically are assumed to have higher oxygen affinities than NOB, oxygen limitation has proven successful to achieve nitritation while, simultaneously, maintaining high ammonium conversion (Blackburne et al., 2008a; Blackburne et al., 2008b; Park and Noguera, 2004; Pynaert et al., 2003; Wyffels et al., 2004)

Conceptually, the MABR system, with its independent control of the oxygen and ammonium flux, seems highly suitable for partial nitritation, establishing an optimal growth environment for AOB, but not for NOB, by limiting the oxygen supply. The first part of the presented study, therefore, assessed the applicability of nitritation and the potential combination of nitritation with completely autotrophic nitrogen removal (Anammox) in a MABR using a mathematical modeling approach (1-d biofilm model). In a second stage, this feasibility was explored using lab scale experiments.

3.1 Modeling Approach

3.1.1 Simulating Nitritation in MABRs (Appendix IV)

One of the main hypotheses in controlling nitritation in MABRs is that by controlling the oxygen concentration at the biofilm base (which subsequently controls the oxygen flux), gradients within the biofilm establish and create aerobic and anoxic zones (Downing and Nerenberg, 2008a).

The main outcome of this first modeling exploration was that the ratio of the maximum specific growth rates of AOB and NOB ($\mu_r = \mu_{max,AOB} / \mu_{max,NOB}$) has the highest influence on nitritation efficiency in MABRs, followed by the affinity constants for oxygen. Independent of the membrane oxygen concentrations a ratio of $\mu_r > 1.5$ resulted in complete nitrite production, effectively suppressing nitrate formation. When the μ_r ratio becomes more favorable for NOB survival, the best predictor for successful nitritation is a low (< 1) ratio of the oxygen affinity constants ($K_{O,r} = K_{O,AOB} / K_{O,NOB}$).

The most important conclusion of this study was, however, the discovery that the oxygen concentration at the base of the biofilm could *not* be used as the solely control parameter or indicator for nitritation efficiency: nitritation success or failure is not guaranteed by setting a certain membrane oxygen concentrations or flux (Figure 6)

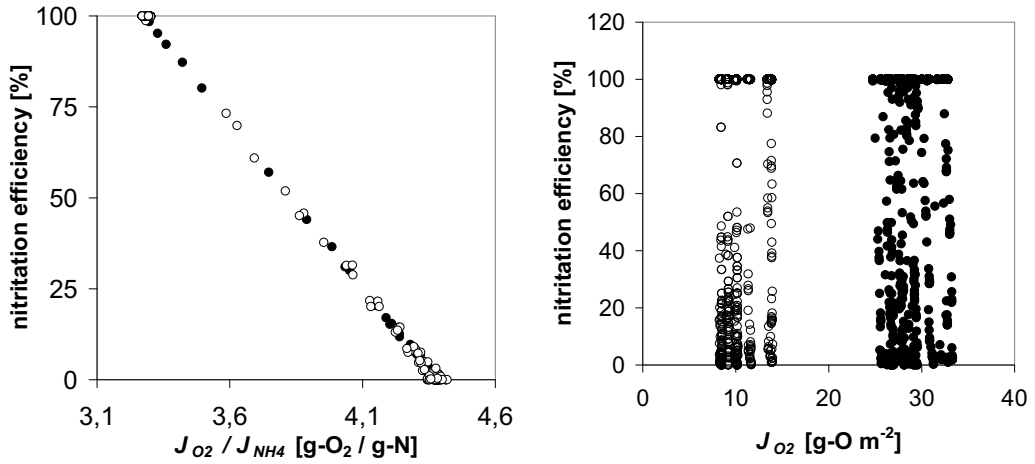

Figure 6: Nitritation efficiency versus the relative fluxes of oxygen and ammonium (left) and versus the oxygen flux (right); white circles $S_{O2,m}$ = 2 g-O_2 m^{-3}, black circles $S_{O2,m}$ = 9 g-O_2 m^{-3} (each circle represents the result of simulation with a certain/different combination of kinetic parameters (μ, K_O, K_N).

The J_{O2} / J_{NH4} ratio (Figure 6, left) seemed more suitable for predicting and potentially controlling nitritation efficiency in MABRs, whereas adjusting solely J_{O2} will lead only to random success. Both fluxes are set by the microbial kinetics (i.e. the substrate conversion) inside the biofilm. Enforcing $J_{O2} / J_{NH4} < 3.6$, favoring high nitritation efficiency (>75%), is more promising to result in high nitrite concentrations, but this result might not be guaranteed. For practical use, ammonium influent concentrations might follow certain dynamics which will also alter J_{NH4}; lowering J_{O2} too much will compromise ammonium removal rates. For optimal reactor operation nitritation can

probably only be controlled by adjusting the oxygen flux based on the ammonium conversion in the biofilm (which sets J_{NH4}).

3.1.2 Impact of COD and sloughing on MABRs for completely autotrophic nitrogen removal (Appendix I)

A modeling study by Terada et al. (2007) had already indicated that MABRs are not only very suitable for completely autotrophic nitrogen removal but may even be superior to conventional (i.e., co-diffusion) biofilm reactors due to a wider optimal application range. However, all models of completely autotrophic nitrogen removal in biofilm reactors (MABR and conventional biofilm systems) (Hao and van Loosdrecht, 2004; Hao et al., 2005; Koch et al., 2000; Terada et al., 2007) have sofar ignored the effect of heterotrophic growth on reactor performance.
The second modeling exercise of this study, therefore, focused among other things on the impact of heterotrophic bacterial (HB) activity on reactor performance (total nitrogen removal) comparing a co-diffusion (conventional biofilm) with a counter-diffusion (MABR) system. Even when only autotrophic decay products supported HB growth, a significant different outcome was prediced in simulations with and without HB. At high ammonium surface loadings a model that neglects HB signficantly overestimates TN removal in the MABR whereas no such error occurred in the conventional biofilm system. (Appendix I).
During completely autotrophic nitrogen removal in a MABR, AnAOB will grow in the outer part of the biofilm, the anoxic zone. This part of the biofilm is, however, subject to detachment, which can jeopardize reactor performance (Horn et al., 2003; Morgenroth and Wilderer, 2000). On the other hand, in a conventional co-diffusion biofilm, the slow growing AnAOB are at the biofilm base and are, therefore, protected from shear. The impact of detachment (sloughing events) on the fate of AnAOB in a MABR is highly important to asses the suitability of these systems, and this was done by simulating short-term changes in the biofilm detachment velocity.

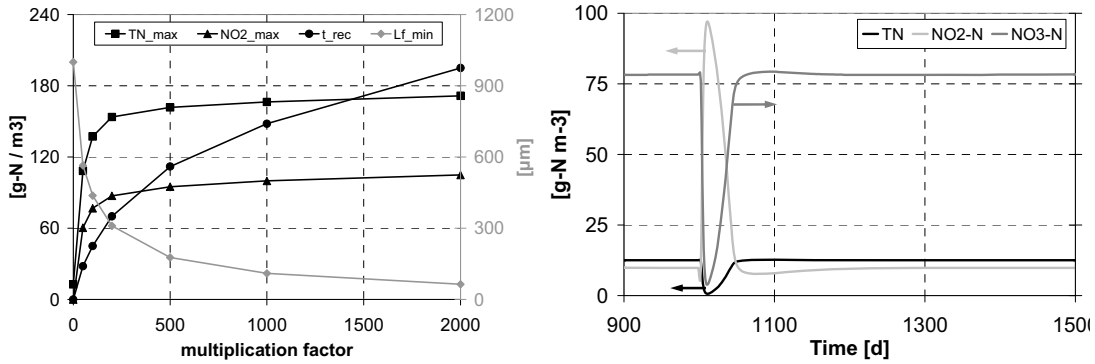

Figure 7: Left: Maximum values of total nitrogen (TN_{max}), nitrite ($NO_{2\,max}$), recovery time (t_{rec}), and minimum biofilm thickness ($L_{f,min}$) for different detachment factors. Right: Effluent concentrations of TN, NO_2-N and NO_3-N for a detachment event with factor 500, duration 1 day.

Sloughing events of different intensities (velocity multiplication factor) and lasting for 1 day were considered and the resulting influence on the nitrogen species, biofilm thickness and recovery time are presented in Figure 7. A dramatic detachment event causes severe deterioration in the system performance, with a nitrite peak of > 100 g-N m^{-3}, and a recovery time (t_{rec}) of more than 3 months. In real engineering applications such a disturbance is not acceptable. In addition, real recovery times might even be longer because of nitrite inhibition to AnAOB (Strous et al., 1999), which was not considered in the model. Also the AnAOB concentration is always finite in the model, but complete loss of AnAOB activity could occur in reality. For stable operation of MABRs with AnAOB, controlled biofilm thickness seems, therefore, essential for successful implementation.

3.2 Experimental Approach

The simulation work revealed that the MABR technology is suitable for completely autotrophic nitrogen removal. However, it also became clear from these modeling efforts that certain conditions (and many parameters) influence nitritation/Anammox success or failure in MABRs. Setting adequate conditions to make proper use of the MABR's advantages is therefore essential. Experimental reactor systems were designed and operated to verify or disprove some of the insight gained from the model-based scenario analyses. Special focus was on the membrane (chemical modifications) and oxygen mass transfer coefficient. The goal was to operate lab-scale reactors to produce an effluent with a composition suitable for subsequent Anammox inoculation.

3.2.1 Surface Modification of Membranes (Appendix II)

When designing biofilm reactors, one important aspect is biofilm growth and control on the substratum (section 2.1.4). Minimizing the effect of detachment events would be valuable especially for biofilms containing slow growing members like AnAOB (Appendix I). Section 2.1.5 already introduced some possibilities of chemical surface modification to alter biofilm formation. Applications of such approaches are scarce within the wastewater treatment field.

A method was sought for more rapid and controlled biofilm formation on membranes ultimately to be used in MABRs. In addition, enhanced shear resistance of those biofilms was desirable. Such a method could provide a significant asset for later engineering applications of MABRs. A plasma-induced grafting technique combined with wet chemistry was applied to enhance biofilm formation on micro-filtration membranes.

The study used nitrifying biofilms because of their high applicability in MABRs. Nitrifiers also seem more subject to sloughing events because they tend not to form strong biofilms (Tsuneda et al., 2001). The surface used for the experiments was a commercial micro-filtration membrane from Alfa Laval (200 µm non woven polypropylene (PP, $-(-CH_2-CHCH_3-)_n-$ supporting layer) and 60 µm polyvinylidenfluoride (PVDF) ($-(-CF_2-CH_2-)_n-$ functional layer)). Figure 8 shows the modification steps and the resulting four functionalizations of the membrane surface. The two PEG grafted surfaces (step 3) showed the highest impact. A clear decrease in biofilm growth was detected for the –PEG-CH3 modification (Figure 9, left) and a significant increase in biofilm formation on the –PEG-NH2 modification was observed (Figure 9, right).

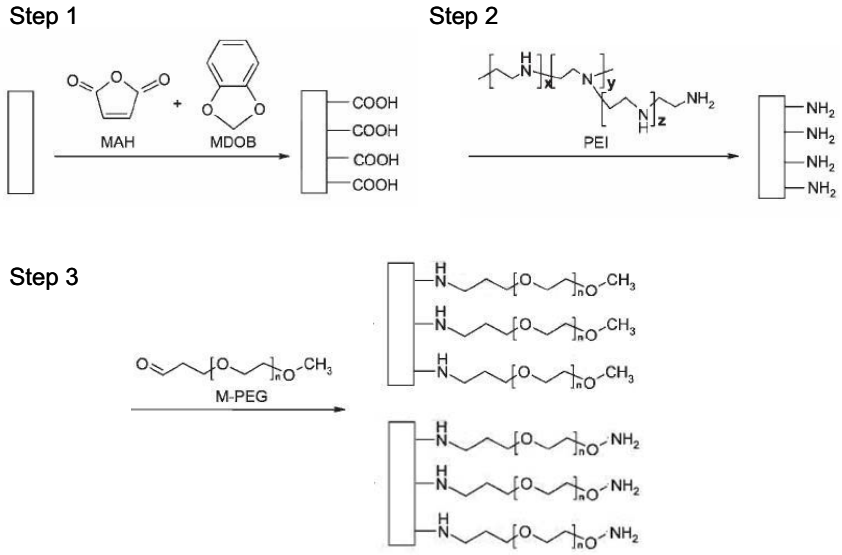

Figure 8: Surface modification steps: (1) plasma polymerisation with MDOB (1,2-(methylenedioxy)benzene) and MAH (maleic anhydride) co-monomer; (2) PEI (polyethylenimine): -(-CH_2-CH_2-NH-)$_n$-NH_2; (3) PEG (poly(ethyleneglycol)): -(-O-CH_2-CH_2-)$_n$-O-CH_3 and PEG-NH_2: -(-O-CH_2-CH_2-)$_n$-O-NH_2 grafting (Appendix II)

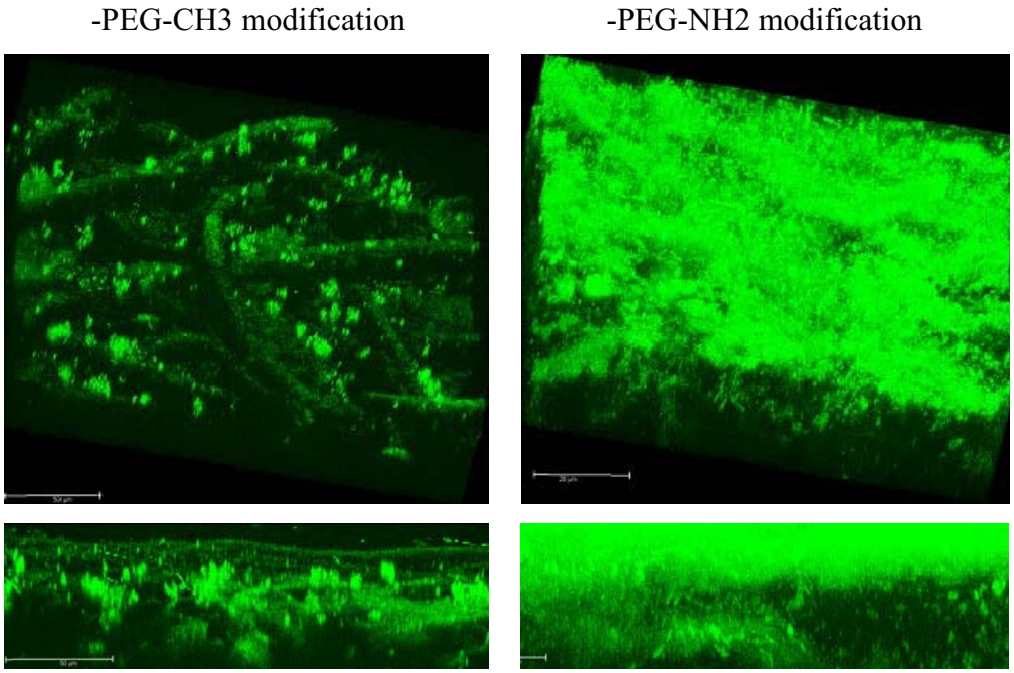

Figure 9: Confocal Laser Scanning Microscopy images from biofilms grown on differently modified membranes after 2 weeks. Bacteria are shown in green (staining with Syto 9)

In addition to rapid formation, the resistance of a biofilm to elevated shear stress is important for stable reactor performance. Detachment tests indicated similar trends as the biofilm growth experiments. Stronger, more shear resistant biofilms formed on the –PEG-NH2 modification compared to unmodified samples (Figure 10, right). Grafting a

PEG with a different functional group (–PEG-CH3) resulted in less biofilm formation and poorer shear resistance (Figure 10, left). In conclusion, biofilm formation and stability was enhanced by a combination of PEG grafting with the NH_2 functional group. Such an approach can be applied to other membranes, suitable for MABRs, and can yield biofilm reactors with better control of biofilm formation and less detachment.

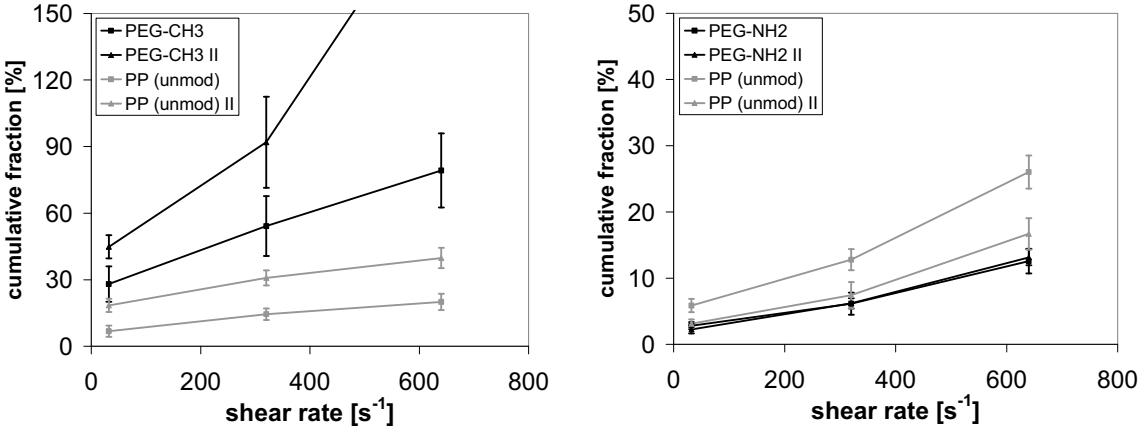

Figure 10: Cumulative fraction of detachment biomass versus the shear rate [s^{-1}]; left: –PEG-CH3 modification and right: –PEG-NH2 modification of the micro-filtration membrane

3.2.2 Operation parameters of MABRs for nitritation (Appendix III)

The model-based scenario analysis, indicated that nitritation efficiencies were strongly governed by the microbial community composition (i.e. by actual kinetic parameter of the AOB and NOB within a range of typically reported values), and only secondarily by the operational parameters, especially the membrane oxygen concentration. MABRs were, therefore, initiated with different AOB-NOB microbial inocula and operated to gain more insight into the importance of kinetic composition versus operational parameters (i.e. intra membrane pressure / membrane oxygen concentrations). The focus was on nitritation and the MABR operation was compared to conventional co-diffusion biofilms, to examine whether certain trends were specific to the counter-diffusion geometry.

An additional system parameter, which has a large impact on overall reactor performance modeling is the membrane oxygen mass transfer coefficient (Terada et al., 2007). Literature suggests that the observed membrane oxygen mass transfer coefficient is usually much higher in the presence versus the absence of biofilm (Casey et al., 2000b; Jacome et al., 2006; Shanahan and Semmens, 2006, Appendix III). The latter is typically measured in clean water tests, where a liquid boundary layer on the bulk side impacts on oxygen transfer. For stable reactor control, an accurate determination of the effective oxygen mass transfer is essential, and a simple approach to do such is introduced here, accounting for the effect of the liquid boundary layer thickness (Appendix III).

The imposed membrane gas pressure influenced the absolute oxygen concentration at the base of the biofilm, but it did not impact on nitritation success. This observation confirms the modeling outcome, that oxygen concentrations are not useful control parameters or predictors for nitritation efficiency.
The microbial community composition, on the other hand, had a significant impact on nitritation efficiency in the lab MABRs. Reactors initiated with different inocula, showed significant deviations in nitritation performance. Furthermore, the co- diffusion biofilms were superior in achieving and maintaining high nitritation efficiencies compared to the counter-diffusion systems (MABRs). Batch experiments in both reactor geometries at different initial ammonium concentration demonstrated these difference (Figure 11): The co-diffusion reactors exclusively yielded nitrite even at the lowest initial ammonium concentration for both inocula, whereas almost all ammonium was converted to nitrate in the counter-diffusion systems. In the latter system, nitrite formation became dominant only with initial ammonium concentrations of 600 g-NH_4-N m^{-3} and above. These experiments revealed that nitritation performance in MABRs is more sensitive to variations in the microbial community and also to the ammonium loading than conventional biofilm process. Hence, the experiments supported the model predictions with respect to the determinant influence of the actual AOB and NOB kinetic parameters on nitritation efficiency.
Microsensor investigations of both biofilm geometries, indicated that the absolute oxygen concentration and also the oxygen gradients were distinctively different (even though oxygen flux was adjusted equally in both geometries) in the two systems, which seemed to be at least one of the very essential parts for explaining the observed differences.

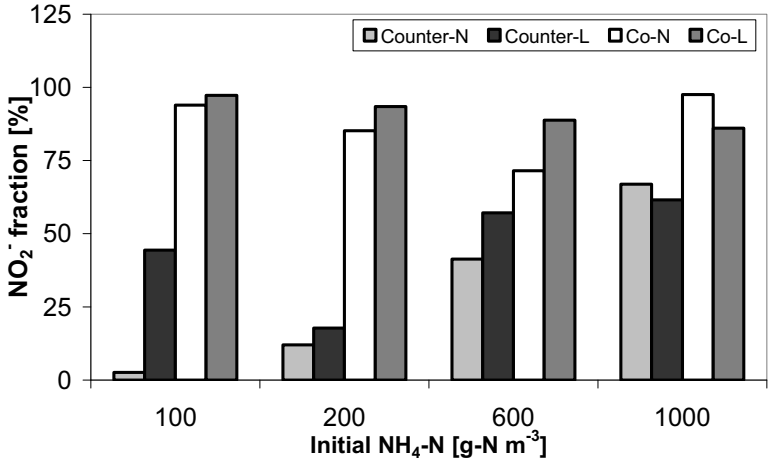

Figure 11: Fraction of NO_2^--N produced / NH_4^+-N removed comparing the N - and L - biomass inocula for Co- and Counter-Diffusion geometries

4. Conclusions and Future Perspectives

Applying nitritation in combination with anaerobic ammonium oxidation (Anammox) in MABRs is a new concept that has yet to be fully investigated. Concrete studies on which factors determine the success or failure of nitritation in MABRs are scarce and comparisons to conventional biofilm systems under certain operational conditions have not yet been presented. This thesis illustrates, through extensive mathematical modeling in combination with experimental observations, which factors are most determinant for nitritation and subsequent Anammox in MABRs. Modeling analysis involved complete autotrophic nitrogen removal. Experimental runs focused only on the nitritation step, and in addition, the use of surface modification to enhance biofilm formation on membranes was investigated.

The main conclusions from the simulation work on nitritation in MABRs are that the bio-kinetics of the system are the most important determining parameters for nitrite production. The nitritation efficiency is, interestingly, independent of the oxygen concentration at the membrane/biofilm interface and the oxygen flux. The complexity of the microbial community composition and its specific bio-kinetics requires more sophisticated control. The relative fluxes of oxygen and ammonium into and out of the biofilm are more suitable to assess nitritation success or failure. These fluxes (especially J_{NH4}) are, however, also dependent on the microbial community in the biofilm.

Experimental observations with different biomass inocula confirmed these modeling results: variations in the microbial community composition also resulted in differences in the nitritation efficiency. The observed impact of the microbial kinetics on nitritation seemed to be specific to the MABR, because different inocula did not influence nitration in conventional biofilm systems, which were also in general superior in their nitritation performance compared to the MABR.

The modeling efforts and the experimental observations both suggest that the oxygen concentration at the membrane/biofilm interface, which has been recommended as a control parameter by several researchers, does not provide enough regulatory influence to assure nitritation in MABRs.

Selection against NOB growth is difficult to achieve and compromises the benefit of high volumetric oxygen input in MABRs. However, the separate control of oxygen and ammonium fluxes in MABRs also offers more flexibility to optimize control strategies.

Correct and accurate experimental determination of the membrane oxygen mass transfer coefficient under operating conditions is essential for controlled reactor performance. The new method introduced in this thesis provides a simple tool for more accurate determination of oxygen mass transfer.

An extended mathematical model revealed that the MABR is a suitable technology for completely autotrophic nitrogen removal. However, neglecting heterotrophic biomass, commonly done for such systems, significantly affects model predictions, especially in MABRs.

To improve growth and detachment resistance of, e.g. nitrifying biofilms, surface modification has proven successful to enhance biofilm formation but also detachment resistance.
Objectives of future research should be mainly in these three areas:

(i) Nitritation in MABRs

The composition of the microbial community has a large impact on nitritation efficiency in MABRs, and it cannot be controlled easily. Up to now, it has been impossible to effectively suppress growth of NOB in MABRs, which can strongly compromise nitritation performance. To further pursue nitritation in MABRs, new startup strategies need to be developed that will minimize growth of NOB right from the beginning. Investigating the impact of the initial bio-kinetic composition of the inoculum on nitritation experimentally and defining criteria an inoculum sludge has to meet, should be the first step. To sustain NOB suppression, the ratio of oxygen and ammonium fluxes should be considered for process control, e.g. adjusting the oxygen flux according to the measured ammonium flux. Mathematical modeling should be used to assist with applying a wider range of ammonium concentrations, since this was limited to one value in the thesis.

(ii) Completely autotrophic nitrogen removal in MABRs

Implementing Anammox in MABRs should test whether simultaneous inoculation is more feasible. AnAOB as competitor might also help in minimizing growth of NOB. The modeling studies clearly suggested the potential of this process in a MABR. No studies have so far explicitly investigated the impact of COD on AnAOB. Experiments dealing with some of the potential problems suggested by the simulation work, e.g. different COD/N ratios or detachment events, should be tested experimentally to confirm or disprove the conclusions from the simulation work. Impact of COD on nitrogen removal in MABRs is also highly valuable for future engineering application.

(iii) Reactor design and scale up

There is still a long way to go from lab-scale observations to large scale application of the MABR technology. The specific membrane surface area is a crucial reactor design parameter, because it will define the removal capacity of the system. An optimal specific membrane surface area in combination with the optimal biofilm thickness for e.g., nitritation/Anammox should be defined. The choice of membrane material and module configuration has to be considered within this context, because it defines the oxygen transfer capacity and shear rate in the system. Membrane surface modification could be explored further to enhance and optimize biofilm formation and stability.

Overall, the MABR technology with its powerful concept of Counter-Diffusion and separation of fluxes seems feasible for high rate nitrogen removal. However, more research is needed to reach large scale applicability of such a reactor.

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Appendix

(I) **Lackner S.**, Terada A. and Smets B.F. (2008) Heterotrophic activity compromises autotrophic nitrogen removal in membrane aerated biofilms: Results of a modeling study. *Water Research* 42(4-5), 1102-1112

(II) **Lackner S.**, Holmberg M., Terada A., Kingshott P. and Smets B.F. (2009) Enhancing the formation and shear resistance of nitrifying biofilms on membranes by surface modification. *accepted for publication in Water Research*

(III) **Lackner S.**, Terada A., Horn H., Henze M. and Smets B.F. (2009) Operation regimes compromise nitritation efficiency in nitrifying biofilms. *submitted*

(IV) **Lackner S.**, Terada A., Merkey B. and Smets B.F. (2009) The kinetic parameters of ammonium and nitrite oxidizing bacteria may determine nitritation success or failure in Membrane Aerated Biofilm Reactors. *submitted*

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