AQUAEXCEL

Aquaculture Infrastructures for Excellence in European Fish Research

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Biofiltration model including validation

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Dissemination Level	
PU Public	**X**
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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Glossary

MBBR:	Moving Bed Biofilm Reactor
TAN:	Total Ammonium Nitrogen
COD:	Chemical Oxygen Demand
CFD:	Computational Fluid Dynamics
SSA:	Specific Surface Area
RAS:	Recirculation Aquaculture Systems
BOD:	Biological Oxygen Demand
HRT:	Hydraulic Retention Time
CFST:	Continuous-Flow Stirred-Tank
OUR:	Oxygen Uptake Rate

Nomenclature

A	Biofilm surface area (m^2)
r_{TAN}	TAN removal rate ($g.m^{-2}.d^{-1}$)
k_LA	Volumetric oxygen transfer coefficient (s^{-1})
V_s	Superficial air velocity ($m.h^{-1}$)

Summary

Objectives:

- Establish a matrix of factors affected by scaling of bioreactors
- To validate the matrix by testing effects on these factors due to bioreactor scale
- To transfer and extrapolate the results on the kinetics using uncertainty analysis of the obtained matrix, and to develop correction factors
- To further validate the model and correction factors for biofiltration performance.

Rationale: In aquaculture Moving Bed Biofilm Reactors are used increasingly in closed systems for farming of fish. Scaling is an important issue in general bio-reactor design since mixing behaviour will differ between small and large scale. Research is mostly performed on small scale and the question is to what extent this can be upscaled to a commercial level.

Teams involved: AQUAEXCEL partners involved in this task are:

- SINTEF/ACE
- Nofima
- NTNU
- Wageningen University
- DLO-Imares

Geographical areas covered: The geographical areas covered by this deliverable are Norway and The Netherlands.

Results:

MBBR scale has a significant effect on TAN removal rate. In general, the larger the scale the better the performance.

TAN removal at small scale (0.8 L) is app. 80% compared to that at medium scale (200 L). The difference between small scale and large scale (>20 m^3) is even higher.

These findings warrant further studies on whether a plateau is reached in rTAN at a certain scale; a study which will have considerable impact for RAS suppliers.

Superficial air speed is not a good scaling factor for MBBRs. Upscaling while maintaining geometry implies increasing air injection depth and therefore increased energy input at comparable air speed.

Air speed and media filling percentage have a strong effect on mixing time at small scale. An airspeed below a threshold of 5 $m.h^{-1}$ decreases TAN removal at both small and medium scale.

Intense mixing at small scale increases TAN removal at low TAN concentration. At a high TAN concentration, the small scale MBBR always performs at app. 80% of the capacity of the medium scale system irrespective of the mixing conditions.

Capacity of full scale systems will be under-estimated when based solely on small scale experiments.

1. Introduction

1.1 Background and objective of research on upscaling of Biofilters

The general objective of WP8 on Upscaling and validity of research results is to determine the effects of physical scale of experimental units on fish and biofilter performance. In research, preferably small scales are used to increase the number of experimental units and concominant statistical power. In commercial situations, large units are employed which would be uneconomical for research. The question arises to what extent results from small experimental units can be extrapolated (scaled up) to commercial scale. Reversely, taking the commercial situation as a starting point, the question is whether large units can be scaled down to answer specific research questions in a representative way. Differences in performance caused by scale could possibly be related to the hydro-dynamics (mixing), and microbial biology.

This report deals solely with Task 8.3 of WP8, which covers effects of scale on performance of a specific type of biofilter, the Moving Bed Biofilm Reactor (MBBR). Biofilters are used as a water treatment unit in Recirculating Aquaculture Systems (RAS) for removal of ammonia (nitrification) and soluble organic matter produced by fish. This type of biological water treatment is widely used in treatment of sewage, and fish farming can be viewed as a minor application of the technology. In general, use of micro-organisms like bacteria, fungi or algae in bioreactors for production of useful substances is another huge field of research. This area includes for example fermentation systems for food production (beer, vitamins) or bio-medical application for production of vaccines and cell cultures.

The specific objectives as originally identified in the AquaExcel WP8 Description of Work (DoW) are:
- To establish a matrix of factors affected by scaling of bioreactors
- To validate the matrix by testing effects on these factors due to bioreactor scale
- To transfer and extrapolate the results on the kinetics using uncertainty analysis of the obtained matrix, and to develop correction factors
- To further validate the model and correction factors for biofiltration performance

1.2 The role of biofilters in practice and research

Biofilters are used in RAS in order to remove harmful substances from the fish tank effluent and allow re-used water to be piped back to the fish tanks. Fish excrete ammonia as an end product of their amino acid and nucelotide metabolism. Ammonia, especially its unionized form NH_3, is toxic for fish at low levels but can be removed by nitrification in biofilters. This is economically interesting when water is scarce and/or control of water temperature and quality is needed.

Biofilters both nitrify ammonium and oxidise soluble COD. The latter function is never taken into account in design since data on soluble COD production are generally lacking and no water quality limits are available for this parameter. Moreover, nitrifiers have a much lower growth rate compared to heterotrophs needed for removal of COD and nitrification is considered the rate limiting step. In this project nitrification is the only process studied in up-scaling of MBBR’s and is used as a read-out parameter.

Application of biofilters in aquaculture started in the 1960’s and has gradually developed since that time. The farming of eel (*Anguilla anguilla*) and African catfish (*Clarias gariepinus*), both species which need a relatively high water temperature, has been a strong impetus for application of RAS and biofilters in Western Europe. The status and

development of RAS have recently been reviewed by (Martins, Eding et al. 2010) and (Dalsgaard, Lund et al. 2013). RAS is common in hatcheries these days since it gives additional control of bio-security.
Although RAS for Atlantic salmon smolt production have been studied for a long time (Risa and Skjervold 1975), it is mainly during the last decade that RAS has shown a strong development in farming of salmon smolts and this application is getting most attention these days (Bergheim, Drengstig et al. 2009; Terjesen, Summerfelt et al. 2013). On-growing of Atlantic salmon (i.e. post-smolts) in RAS is also starting to develop on a commercial scale in a few places (Terjesen, Ytrestøyl et al. 2013).

In research, biofilters are used basically in two ways. Firstly as tool to support research which needs a controlled environment in situations where water and heating or cooling costs are prohibitive. In this case the filters are often oversized to assure a good water quality and design is not critical. Secondly, as part of research where water quality and performance of RAS are studied. This can involve effects of waste production or treatment efficiency on system performance. In this type of research, design and scaling of biofilters plays a critical role. The latter application is the main focus of this project.

There is an enormous variety in type of biofilters and media used in aquaculture. In all cases a biofilm on a fixed medium is used in order to retain enough biomass of slow growing nitrifiers. Media range from sand to a wide variety of plastic media. Biofilters can be operated in a down-flow or up-flow mode or in a submerged versus a trickling mode. Moving Bed Biofilm Reactors (MBBR) are a relatively recent development (Odegaard 1994) and are the dominant type of biofilter applied in new RAS. Therefore this research has been limited to this type of biofilters.

2. Literature review

2.1 Up-scaling of bioreactors

Three main scales can be distinguished in the scale-up of bioprocesses (Ju, Chase, 1992):
i) laboratory; for elementary studies on kinetics
ii) pilot scale; for process optimisation
iii) production scale; for commercial application

For general design of bioreactors information on five different aspects is needed: stoichiometry, thermodynamics, microbial kinetics, mass transport and economics. Stoichiometry, thermodynamics and kinetics are scale independent. Both mass transport and economics are highly scale dependant. Economics are not considered in this study.

Scale-up of bioreactors can be approached in four different ways (Garcia-Ochoa and Gomez 2009):
i) Fundamental methods
ii) Semi-fundamental methods
iii) Dimensional analysis
iv) Rules of thumb

With fundamental methods like computational fluid dynamics (CFD), mathematical models are created of operating conditions within the reactor. These methods are complicated and many simplifications are often needed.
In semi-fundamental methods, the equations used are simplified to obtain practical solutions to predict performance. Dimensional analysis is based on keeping certain dimensionsless parameters constant during scale-up. A problem here is that a selection has to be made with regard to the most important parameter since these can never all be kept constant during scale-up. The rules of thumb method is most often applied.

Parameters used most in up-scaling in the fermentation industry are: constant volumetric oxygen transfer coefficient (k_La), constant specific power input (P/V) and constant impeller tip speed (shear).

Ju and Chase (1992) give an overview of process characteristics which have been proposed as factors to keep constant during scale-up:

1. Reactor geometry
2. Volumetric oxygen transfer coefficient, k_La
3. Maximum shear
4. Power input per unit volume of liquid, P/V
5. Volumetric gas flow rate per unit volume of liquid, Q/V
6. Superficial gas velocity, v_s
7. Mixing time
8. Impeller Reynolds number, $Re_i=qND_i^2/\mu$
9. Momentum factor

In the following these factors and their relation to MBBR and the current study, are discussed briefly:

1) Reactor geometry is kept constant in most research on up-scaling because effects of deviations in geometry are difficult to predict.
2) Oxygen is often a rate limiting substance in aerobic bioreactors and oxygen transfer coefficients have proven to be a good parameter for scale-up (Garcia-Ochoa and Gomez 2009). These authors give a detailed review of methods to determine k_La and its use in upscaling of bioreactors. In MBBR's in aquaculture oxygen is not a rate limiting factor for nitrification since these systems are mostly operated around oxygen saturation in situation where TAN is rate limiting.
3) Maximum shear is relevant when sensitive organisms are cultured. This is not directly relevant for MBBR's since the biofilm is largely protected within the media.
4) Power input has been used succesfully as a scaling factor in operations with shear-sensitive organisms. Most biorectors use mechanical stirring for mixing combined with gas dispersion. In MBBR's used for denitrification mechanical mixing is used. In commonly applied aerobic MBBR's only aeration is used for mixing.
5) Volumetric gas flow rate has been largely accepted as a good criterion for scale-up in systems without mechanical agitation. Since MBBR's in aquaculture are mixed with air, this seems a logical factor to consider in up-scaling.
6) The superficial gas velocity is defined as the gas flow per unit (floor) area. The superficial gas velocity can be a critical factor in designs with mechanical mixers. Above a certain velocity the mixing system is unable to disperse the gas. Superficial gas velocity will increase when volumetric gas flow rate is kept constant during up-scaling. This seems to be the most promising scale-up factor considering current practice.
7) Mixing time is defined as the time a liquid needs to be stirred to obtain a certain degree of homogeneity after adding a pulse signal from a tracer. This is one of the criteria most widely used to characterise mixing intensity. The effect of scale-up on mixing time has been studied by Bonvillani et al. (2006) to predict scaling from a 2.5 l bioreactor to systems with a diameter of 4 m. The mixing system is based on mechanical stirring combined with aeration. In this situation mixing time can be predicted from fluid viscosity, tank diameter, power input fom stirrer and diameter of the stirrer. Gill et al. (2008) studied scale-up from a miniature bioreactor (100 ml) to a fermentation system of 2 l. Although mixing time is a good proxy for mixing intensity, it's relevance for reaction rates of biological processes is limited since these are relatively slow processes.

8) and 9) are only relevant for mechanical mixing with shear sensitive organisms. This is not relevant for aerated MBBR's in aquaculture.

In summary it can be concluded that a combination of geometry and superficial gas velocity are the most promising factors for design of scale-up in MBBR's. These criteria can be applied relatively easy when scaling-up from laboratory-scale to pilot-scale.

2.2 MBBR's in aquaculture

Conversion of the toxic ammonia produced by fish to the relative harmless nitrate by nitrification is a key-process in Recirculation Aquaculture Systems (RAS). Therefore, this process is the read-out parameter in up-scaling of biofilters for aquaculture. In this chapter the factors affecting nitrification kinetics will be reviewed.
A large body of literature has become available on different nitrification systems and the kinetics of the process. In general, fixed biofilm reactors are applied because these can retain the needed biomass given the high flows of low strength waste water to be treated. A large variety of filter types and configurations are used in aquaculture for nitrification utilising media ranging from fine sand, micro-beads, beads and plastic packed media. Most reactor types are operated in a plug flow mode e.g trickling filters, sand filters and Rotating Biological Contactors (RBC's). Each type of filters has its own set of advantages and disadvantages.
A relative new development has been the introduction of the Moving Bed Biofilm Reactor (MBBR)(Odegaard 1994). This patented system (KrugerKaldnes) was developed in Norway in the late 80's and is based on a carrier which floats freely in the reactor. The carrier is kept in movement by air or a propeller and is retained by screens in the tank (Figure 1).

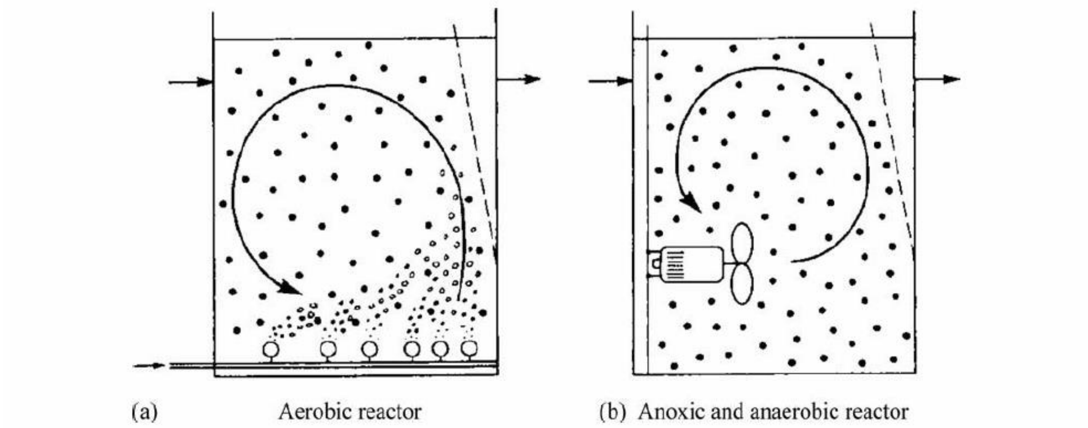


Figure 1. Two types of Moving bed Bioflim reactor, from (Rusten, Eikebrokk et al. 2006)

The advantages of this system are mainly the low pumping costs and the avoidance of clogging of the filter, by constant shearing forces keeping the biofilm thickness relatively constant. The system has been applied world-wide for large-scale treatment of wastewater utilising tanks over 1000 m^3. In commercial fish farms and ornamental fish farms many MBBR's are operational with sizes up to 600 m^3 (Rusten, Eikebrokk et al. 2006). In urban waste water treatment plants, hydraulic retention times in the order of 0,5 to 2 hours are employed. In fish farms retention times can be as low as 5 minutes which causes a high hydraulic load to the screens. This problem can be averted by screening the complete outer perimeter of the tank and applying a central feed with airlifts to move the material. This is a system utilised by InterAqua Advance and Kruger Kaldnes, and induces a toroid flow pattern in the reactor.

The aerobic reactor used for BOD-removal and nitrification is driven by a special designed aeration grid at the bottom of the tank. Diffused aeration systems uniformly distribute the plastic biofilm carriers and meet process oxygen requirement. An air injection in the order of 1 m^3 air per m^3 reactor (under standard conditions) is used with an energy consumption of roughly 30 $W.m^{-3}$ of water treated. For denitrification a propeller or a mechanical mixer is used for mixing using ca. 15 $W.m^{-3}$ of reactor.

Different plastic biofilm carriers exists from different manufacturers and differ in size, shape and bulk specific surface areas (full list in McQuarrie and Boltz, 2011). Figure 2 shows an example of the biofilm carrier used. The material comes in many shapes and is mostly polyethylene (PE) with a the specific density of 0.95 g/cm^3. For marine application a higher density is necessary. Plastic biofilm carriers have channels along the media interior that forms a protected surface. Biofilm primarily develop on the protected surface inside of the plastic biofilm carrier (Bjornberg 2009) where it is protected against abrasion. The 'active' specific surface area (SSA) varies in the case of the Kaldnes carrier from 900 (Biofilm Chip P) to 500 $m^2.m^{-3}$ (K1) and 350 $m^2.m^{-3}$ (K2) although the percentage area of the total which is actually active is under debate. The active surface area used by nitrifying bacteria is reduced under certain circumstances and estimation of nitrification rates based on theoretical surface area can be misleading (Guerdat et al., 2010). In theory, the greater the SSA, the more bacteria are supported and the more TAN removed. In practice, the bacteria create a stratified biofilm with the faster growing heterotrophic bacteria layering over the slow growing autotrophic nitrifying bacteria (Nogueira, Melo et al. 2002). This reduces mass flux of substrate through the biofilm, creates an oxygen diffusion gradient and anoxic processes in deepest zones of the biofilm. The environment below a thick biofilm layer may be totally anaerobic and no nitrification will occur (Schramm, Larsen et al. 1996). Therefore the maintenance of a thin biofilm layer optimises the nitrification efficiency. The amount of biofilm on the carrier will be influenced by the age and history of the biofilm, the influent load, the chemical composition of the substrate, the hydraulic environment of the carrier and the percentage of filling of the reactor. Several of these relationships have not been studied for MBBR's applied in fish farming.

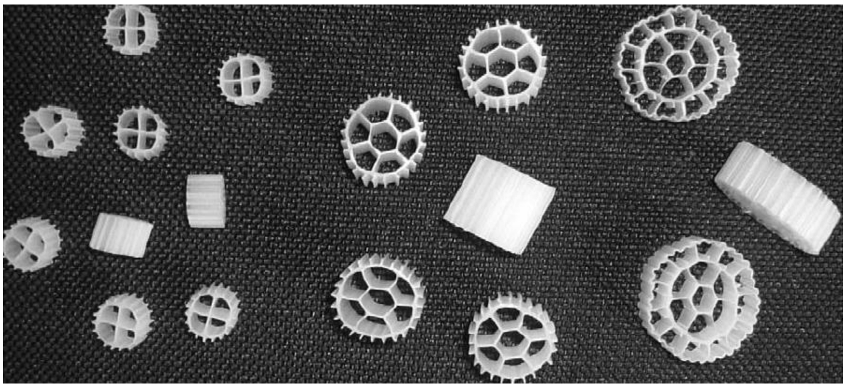


Figure 2. Three types of Kaldnes media from (Rusten, Eikebrokk et al. 2006).

(Pfeiffer 2011) compared 3 types of MBBR media and found a difference of 30% in ammonia removal rate on a volumetric basis at a relative high feed load.

2.3 Factors affecting nitrification kinetics of biofilters

Effects of TAN and Dissolved oxygen.

Nitrification in a biofilm can be adequately describe by a ½-order/0-order kinetic model. The removal rate in the biofilm is determined by diffusion of NH_4-N (TAN) and oxygen into the biofilm. The concentration in the bulk liquid determines the reaction rate (fig. 3). At low

TAN concentration, which is the case in fish farms (<1mg NH_4-N/L), TAN is the rate limiting substrate while O_2 is rate limiting substrate at high TAN (Rusten et al., 2006). The shift from the ammonium to the oxygen concentration being rate limiting occurs for an oxygen to ammonium concentration ratio of about 3 g O_2 (g NH4-N)$^{-1}$. (Hem et al., 1994)

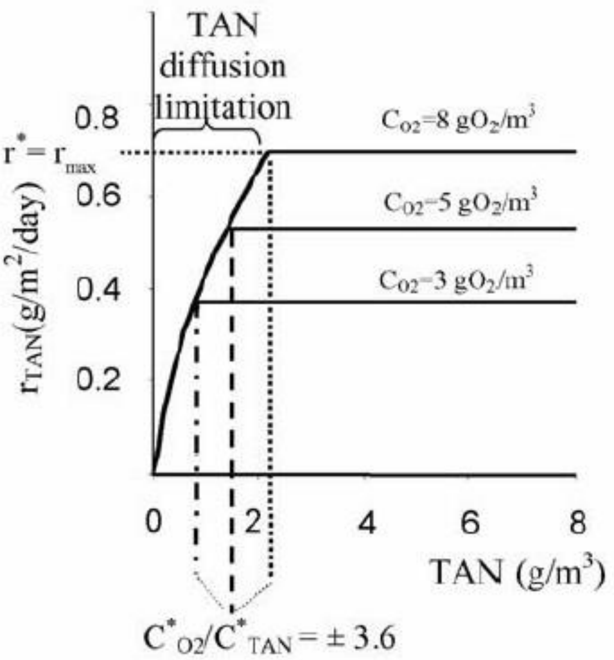


Figure 3. TAN removal rate in relation to the concentration of TAN for a hypothetical biofilm. From (Eding, Kamstra et al. 2006)

Most nitrification systems like trickling filters or MBBR's in fish farming operate at oxygen levels approaching saturation. The MBBRs require a relatively high bulk-liquid dissolved oxygen concentration for nitrification (i.e. 4 to 6 g/m^3), but a 2- to -3 g/m^3 dissolved oxygen concentration has been proven sufficient for carbon oxidation MBBRs treating municipal wastewaters, as a result of the significant particulate and colloidal COD fraction (of the total COD) (Odegaard 2006). For $C_{TAN}<C^*_{TAN}$ the removal kinetics can be described as:
$r_{TAN} = a\ [NH_4\text{-}N]^{0.5}$ in which a is a constant which depends on the characteristics of the wastewater, temperature and other parameters that influence the growth of nitrifying organisms.

Hem et al. (1994) developed a model to predict nitrification rates in MBBR's. Interestingly, the kinetics of nitrification in this model differ significantly from the ½-order, 0 order model described for other nitrifying biofilm reactors (Harremoës 1978; Bovendeur 1989). The relation between nitrification rate and oxygen concentration in a MBBR could be best described by a first order function when oxygen was rate limiting ($C_{O2}/C_{TAN}<2$). The relationship between nitrification and TAN concentration was fitted best by an order of 0.7 when ammonium was rate limiting. The difference in kinetics between an MBBR and other biofilm processes is explained by differences in liquid film diffusional resistance across the biofilm (Hem at al.,1994). Why the diffusional resistance is different in an MBBR is not explained. In general, the performance of MBBR's is more sensitive for fluctuations in oxygen concentration than other type of reactors. It is not clear whether this effect is significant under fish farming conditions. MBBR's in aquaculture will typically be operated at 60 to 110% oxygen saturation, since it usually receives the tank effluent at a quite early stage and this reflects the minimal allowed O_2 concentration in the fish tank

Organic load.

The organic load in waste water treatment is often expressed as Biological Oxygen Demand (BOD).The BOD load to a filter has an effect on nitrification because the heterotrophic activity increase in the presence of biodegradable organic matter and heterotrophic bacteria outcompete the relatively slow growing nitrifiers. Nitrification rates are reduced by increased organic loads and become insignificant with organic load exceeding 5 g total BOD_7 m^2 d^{-1}. These effects have been well-described for high BOD loads common in sewage treatment plants. In fish farming the BOD load is generally low and dependent on feed utilisation and the efficiency of removal of suspended BOD. The effect of BOD load through feed utilisation on the nitrification capacity of MBBR's is currently subject of research in another EU-project (Feed and Treat, project number 286143). The chemical composition of the organic substrate affects also the heterotrophic microbial community composition and the relative concentration of autotrophs. A high C:N ratio increases the number of heterotrophic bacteria and decreases the percentage of ammonia and nitrite oxidising bacteria, leading to reduced nitrification rates. The C:N ratio of the water flow entering the biofilter should be kept as low as possible to ensure sufficient ammonia removal (Ohashi, deSilva et al. 1995; Zhu and Chen 2001). Rusten et al. (2006) studied a few applications of MBBR's at fish farms and concluded that the kinetics of ammonium removal could be adequately described by a 0.7-order model. Two large marine farms were studied of which one had just started-up and showed very low nitrification rates. The performance of the full scale systems could not be completely predicted from bench-scale tests, indicating the need for focus on upscaling processes. Moreover, the results covered only low TAN concentrations and rates of nitrification.

Other environmental factors

Temperature affects greatly the nitrification rate and can be modelled as $k_{T2}=k_{T1}\theta^{(T2-T1)}$, where T_1 and T_2 are temperatures in ^oC, k_{T1} reaction rate constant at T_1, k_{T2} reaction rate constant at T_2 and θ is the temperature coefficient. For MBBR, θ=1.09 (Rusten, Hem et al. 1995)

Alkalinity. Most fish farms control alkalinity by adding alkaline chemicals to keep the pH at a level where nitrification is optimal. In marine fish farms, nitrification rate at pH 6.7 is 50% of the nitrification rate at pH 7.3 (Rusten et al., 2006). However, anecdotal evidence from Dutch RAS farms shows that farms with MBBR's have better nitrification at low pH than farms using trickling filters. High nitrification rates can be achieved at low pH and CO_2 can be a limiting factor for nitrification (Green, Ruskol et al. 2002; Tarre and Green 2004). The efficient CO_2-removal in a trickling filter is probably the reason for the pH-sensitivity of this system. For fish farming applications, no information is available on the relationship between CO2 and nitrification in MBBR's. Autotrophic nitrifying bacteria of the genus *Nitrosomonas* have the capacity to cope with low pH (Tarre and Green (2004).

Salinity. Nitrification rates in marine systems (salinity 21-24 $g.l^{-1}$) are nearly 60% lower than for comparable freshwater system

Composition of the biofilm

Age of biofilters. The relatively slow growth rate of nitrifyers causes a long start-up period for biofilters. Young biofilters have a lower TAN removal capacity than old filters. In bench-scale tests using biofilm carriers from turbot farms, a 2-year old biofilter had more than 10 times higher TAN removal capacity than a 4-month old biofilter (Rusten et al., 2006), with TAN removal rates of 0.07 and 0.95 g $TAN/m^2/day$, respectively. In disinfected marine RAS, for turbot culture, nitrification effectively started after 1 month, indicating the achievement of microbial maturity capable of supporting further nitrification (Rurangwa 2012).

Filling rate (FR) and hydraulic environment. (Calderón, Martín-Pascual et al. 2012) studied the effect of filling rate on biofilter performance. FR had an effect on the microbial community structure, while hydraulic retention time (HRT) and carrier type had no significant influence In

this reseach the carrier filling ratio was the major operational parameter affecting bacterial biofilm formation and diversity in a lab-scale MBBR. An increase in the carrier filling ratio of a MBBR to treat urban waste water increases the surface available for bacteria to attach, thus the bacterial biomass. More mature biofilm and better colonized carrier surface are found with 50% FR, compared to lower FR of 35 and 20%. At 50% FR, the microbial community had also a higher bacterial diversity compared to lower FR. While a HRT between 5 and 15h and biofilm carrier type had no effect on biofilm bacterial community, the shear stresses on biofilm becomes greater and detachment of the biofilm enhanced when the FR is more than the 50% (Gjaltema, Tijhuis et al. 1995). In a MBBR, the ideal biofilm is relatively thin and evenly distributed over the carrier surface to ensure an effective oxygen and nutrient transfer. Since this depth of full substrate penetration is normally less than 100 μm, the ideal biofilm in the moving bed process is thin and evenly distributed over the surface of the carrier (Rusten et al., 2006). COD removal rates had also the upper limit at 50% FR regardless of the carrier type tested (Wang, Wen et al. 2005) Martín-Pascual et al. (2011). Although higher filling ratios up to 67% have been reported (McQuarrie and Boltz, 2011) in MBBRs for wastewater treatment, higher FR restricts the movement of the carrier in the bioreactor and increase collisions and abrasion forces among carrier particles, leading to the selection of bacteria capable of growing on the carrier under these conditions (Wang et al., 2005;(Martin-Pascual, Lopez-Lopez et al. 2012).

The hydraulic configuration of the reactor is an important parameter in the design and performance of nitrification systems (Watten and Sibrell 2006). Systems with a fixed bed like trickling filters or sand filters are mostly designed as plug flow reactors which is an efficient way to utilise the available space. These systems are relatively easy to model for fish farms (Kamstra, van der Heul et al. 1998). An MBBR can be considered a mixed system which will on full-scale show deviations from the ideal Continuous-Flow Stirred-Tank (CFST). By placing a number of CFST's in series, plug-flow performance can be approached. Figure 1 shows that that short-circuiting can easily be induced in MBBR's used in fish farms. There is no information available on the hydraulic performance of MBBR's on fish farms. This information can be obtained through tracer studies.

Bacterial community diversity. Higher diversity of nitrifying communities correspond to higher rates of nitrogen removal (Bernet, Sanchez et al. 2004). In marine MBBR recirculating aquaculture systems, bacterial community of the media has the capacity of carrying out different nitrogen transformation processes—nitrification, denitrification and anammox (Tal et al., 2003). Both ammonia and nitrite oxidizers, *Nitrosomonas cryotolerans* and *Nitrospira marina*, respectively, were found associated with the marine MBBR system as well as a number of heterotrophic bacteria, including *Pseudomonas* sp. and *Sphingomonas* sp (Tal et al., 2003) ref.). In addition, two *Planctomycetes* sp. were detected in the system suggesting the capability for anaerobic ammonia oxidation (anammox).
Biokinetics of heterotrophic and autotrophic biomass in a MBBR have been studied using respirometric tests carried out on both detached and attached biofilm (Ferrai, Guglielmi et al. 2010). Results of the Oxygen Uptake Rate (OUR) profiles indicated the storage mechanism to be prevalent for heterotrophic biomass growth, with a 80% fraction of soluble substrate converted into storage products. The active heterotrophic biomass in detached biofilm corresponded to a 39% fraction of particulate COD. Quantification of autotrophic ammonia and nitrite removal showed an effective specialisation of the nitrifying biomass.

2.4 Conclusions from literature

Based on the review of literature it can be concluded that:
- Constant geometry and volumetric gas flow rate and/or superficial air flow rate are the most promising parameters to that are affected during up-scaling of MBBR's.
- Nitrification rate is the most relevant read-out parameter for up-scaling.

- Kinetics of nitrification are affected by many factors and meaningful comparison of scales can only be performed in a common waste water using an identical bacterial biomass.
- Respirometry is a well-known tool to study kinetics in water treatment processes and can be considered a bench-scale model of a large system. Development of such a tool for use in aquaculture research could be interesting.

2.5 Research objectives

The literature research already indicated factors which should be taken into consideration in upscaling of biofilters. The experimental research focusses on these factors and has the following objectives:
- To establish if there is a scale effect when comparing different scale MBBRs with full scale systems while geometry and superficial air speed are kept constant.
- To estabish the effect of superficial air speed and TAN concentration on scale effects.
- To establish the effect of media filling ratio on mixing and nitrification rate
- To establish the effect of alternative mixing systems on nitrification rate.

3. Experimental research

3.1 Introduction and scope of the reseach

The experimental research on upscaling of MBBRs was focussed on three different scales:

- A small scale, in this case 0.8 L
- A medium scale, in this case 200 L
- A large scale, in this case > 20 m^3.

The small scale is often used in kinetic experiments to test biofilm performance e.g (Bovendeur, Zwaga et al. 1990; Nijhof and Bovendeur 1990). The medium scale we choose was easy to maintain in the laboratory and served as a homogenous pool for biofilter media which were used for the comparison between small and medium scale. Performance of the medium scale system was monitored regularly and this system was maintained over a long period.

The effects of upscaling to a commercial level were studied by bringing the small scale system to a number of farms and test performance in parallel with measurements of the large MBBRs at the site. In both the comparison between small and medium, and small and large MBBR's, biofilter media from a medium/large system was transferred to the small system. Influent of the reactor was taken from a common source while flows of water and air were scaled according to reactor volume. This experimental set-up ensured a true evaluation of scale-effects irrespective of biofilm history or loading.

When comparing the small and medium scale MBBRs, the geometry of the MBBR and the aeration applied was strictly standardised. At farm level this standardisation was not possible since different dimensions and aeration systems were applied.

Below some pictures of the different scales.



At small scale a number of variables were tested for effects on upscaling like TAN concentration and superficial air speed. TAN has a strong effect on nitrification (Figure 3) and is in many cases the limiting substrate. It is expected that under conditions of TAN limitation, mixing becomes more important. Under a high TAN concentration, transport to the biofilm and distribution within the reactor is expected to be of less importance.

3.2 Development and performance of a medium scale MBBR

3.2.1 Introduction

A medium scale reactor of 200 L was developed and maintained at lab scale. This system was continuously fed with a synthetic mix of nutrients. The biofilter media from this system were used in experiments to compare the medium scale system with the small systems and thus eliminate any effects of biofilm composition.

3.2.2 Material and methods

An overview of the experimental system is shown in Figure 4 and Table 1. Twice a week a stock solution was prepared in a glass aquarium of 200 L and added to 160 L of tap water. The mineral mix contained 1200 g NH_4Cl, 3600 g $NHCO_3$ and 1.7 g KP_2O_4 per 186 L. The water surface of the stock solution was covered with a floating sheet to prevent evaporation of ammonia. Sodiumbicarbonate was added to the mixture to ensure suficient alkalinityand pH for nitrification (Table 2). The stock solution was dosed to the outflow of the MBBR using a solenoid dosing pump from FWT (FX C/A with a flow of 15ml/ min). The MBBR was constructed from glass with (wet) dimensions of 59x59x59 cm. Water inflow was distributed evenly over the whole length of one sides by an overflow box. The outlet was situated as an overflow on the whole length of the opposite side and screened. The MBBR was aerated on the bottom below the outlet side through a perforated pipe that was placed on the bottom. The pipe contained holes of 1.5 mm with a spacing of 5 cm. This aeration resulted in a circular water movement as shown in Figure 1. The biofilter media used was Kaldnes K-1 with a specific surface of 500 m^2/m^3 (manufacturer's statement) Most of the time the MBBR was operated at 35% filling but at a later stage a treatment of 50% filling was applied. The total wet volume in the MBBR at 50% filling was 207 L.

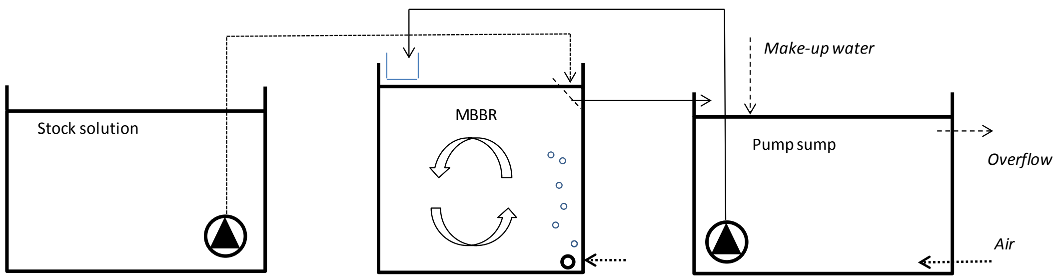


Figure 4. A schematic overview of the experimental system with the medium scale MBBR.

Table 1. Dimensions and operational characteristics of the medium scale MBBR.

Parameters	Value	Unit
Water renewal	562	$L.d^{-1}$
MBBR	207	L
Hydraulic load	20.3	$m^3.m^{-3}.h^{-1}$
Retention time	3.0	min.
Aeration	4062	$L.h^{-1}$
Superficial air speed	12.1	$m.h^{-1}$

The MBBR was aerated using a Secoh EL-S 100 airpump of 92 W with a nominal capacity of 140 l/ min. The outflow of the MBBR was connected to a sump with volume of 125 L. A submerged pump (top-3 (LA) Pedrollo) moved the water back to the inlet of the MBBR. In the sump, make-up water was added continuously to keep the level of nitrate-N around 100 $mg.L^{-1}$. The sump also contained an overflow to the sewer.

The MBBR was in operation over a period of 22 months. Once a week all the flows were measured and adjusted when necessary. Once a week the concentration of NH_4-N was measured in the stock solution. $NH_4/NO_2/NO_3$ were measured weekly in the overflow of the sump. Water temperature and pH were recorded twice a week. The nitrate level in the renewal

water was measured sporadicaly. $NH_4/NO_2/NO_3$ concentrations were measured with testkits of Hach Lange in a Hach Lange fotometer DR2800.

3.2.3 Results and discussion

Table 2. An overview of the operational conditions during maintenance of the medium scale MBBR.

Parameter	*Unit*	*Mean (± SD)*
Temperature	°C	24.9 (0.5)
pH		7.76 (0.23)
Oxygen	$g.m^{-3}$	6.9 (0.9)
[TAN]in	$g.m^{-3}$	77.1 (16.2)
[TAN]out	$g.m^{-3}$	4.6 (8.0)
[NO2-N]out	$g.m^{-3}$	5.44 (8.3)
[NO3-N]out	$g.m^{-3}$	72.4 (35.1)
Water renewal	$L.d^{-1}$	561 (14)
Load	$g\ N.m^{-2}.d^{-1}$	0.85 (0.18)
Removal	$g\ N.m^{-2}.d^{-1}$	0.80 (0.19)

Mean conditions during the period of 22 months in which the MBBR was operated are shown in Table 2 and Figure 1.

Figure 5. The concentration of the different nitrogen compounds in the influent and effluent of the medium scale MBBR, during maintenance.

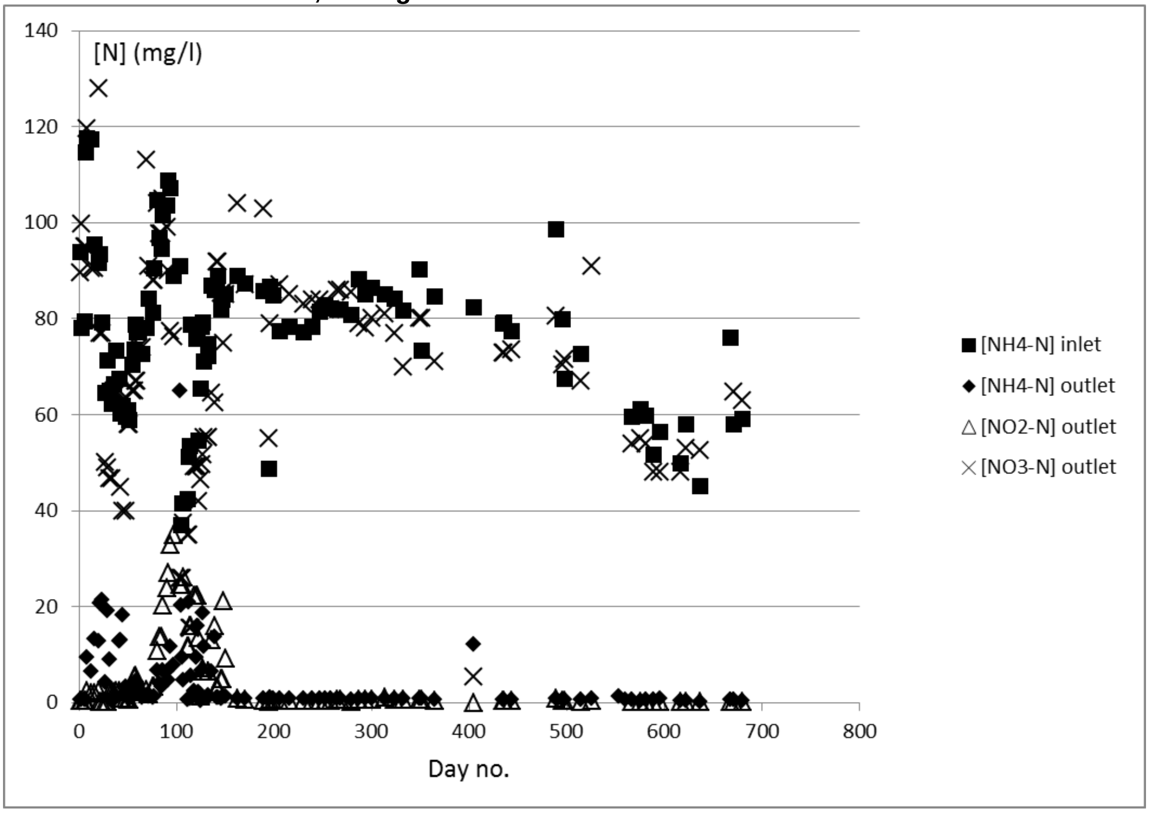
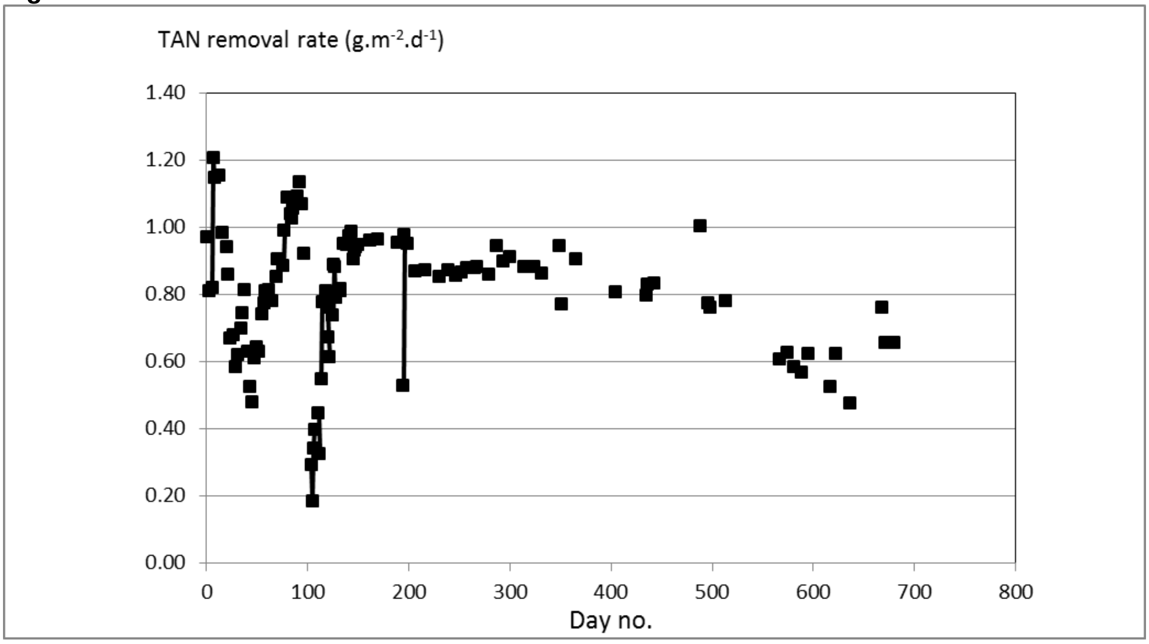


Figure 6. TAN removal rate in the medium scale MBBR over time.



High removal rates before day 100 were accompagnied by relatively high nitrite levels in the outflow. The TAN load was maintained at a level where the level of nitrite in the outflow was below 1 $g.m^{-3}$. Equipment for dosing was prone to blocking by the high solid content of the stock and this was a source of variation. At day 680 the filling rate of the MBBR was increased to 50%.

Figure 7. TAN load versus TAN removal over the experimental period.

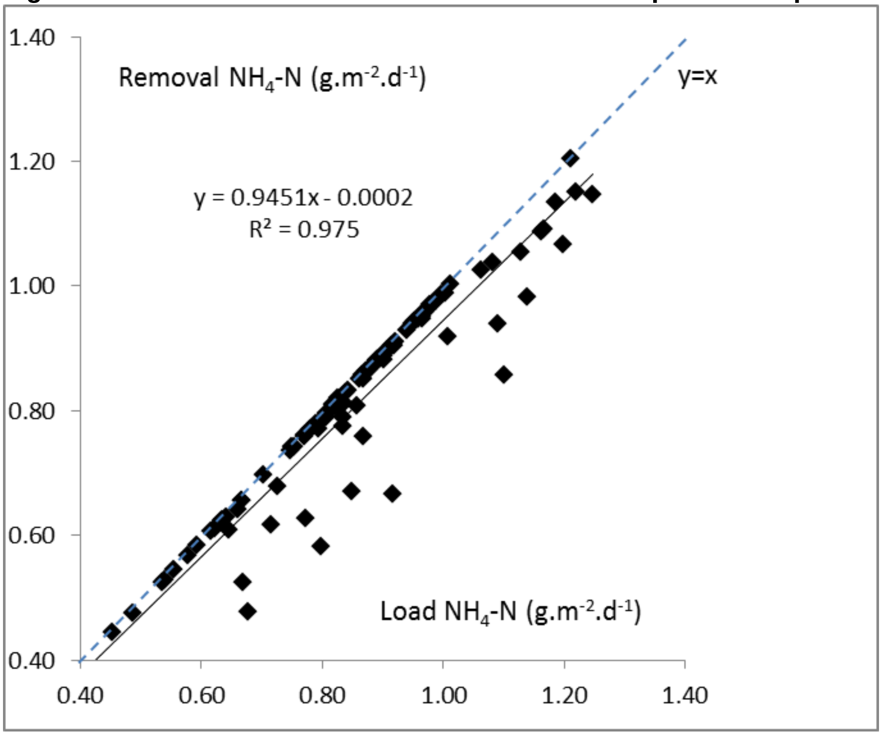


Figure 7 shows that up to a load of 1 $g.m^{-2}.d^{-1}$ TAN all TAN is being removed and above that level roughly 5% is removed in the effluent.

3.2.4 Conclusions

- A medium scale MBBR with a stable performance could be maintained over a period of 22 months.
- Full nitrification could be achieved at a TAN load up to 1 $g.m^{-2}.d^{-1}$.

3.3 Comparison of the performance of medium and small scale MBBRs

3.3.1 Introduction

Small scale units in the order of 1 L are often used to perform experiments on the kinetics in bioreactors. A relevant question is whether the results from these small scale experiments can be extrapolated to larger reactors. MBBRs offer an unique opportunity to answer this question because the biomass is perfectly mixed in the reactor and can be sampled randomly and moved to another reactor. In the comparison between medium scale and small scale reactors we used the medium scale reactor as a baseline (paragraph 3.2) from which media was taken for short-term experiments to small scale reactors. The small scale reactors were then operated in parallel to the medium scale system. This experimental set-up excludes any effects from biofilter history or load. All biofilter media for the small scale had to be handled before the experiments. Therefore, an initial experiment was conducted to assess the effect of handling on nitrification rate. Subsequently a number of experiments was performed in which TAN removal in the medium scale reactor was compared to that at small scale under different environmental conditions. In a separate experiment the effect of air speed and media filling percentage on mixing was established in the small reactors.
The objective of these experiments was to quantify the effect of MBBR scale and elucidate the factors affecting scaling between medium and small scale.

3.3.2 Material and methods

Experimental system
Three small scale reactors of 0.8 L were constructed which had basically the same geometry as the medium scale reactor (3.3.2). The wet (aerated) dimensions (LxBxH) of the reactor 9.4*9.4*9.4 cm which resulted in a wet volume of 0.815 L and a horizontal surface of 0.0088 m^2. The inlet of the reactor was constructed as an overflow over the whole width of the reactor (see pictures page 16). The outlet was opposite the inlet and consisted of a horizontal series of holes of 6 mm. The reactor was aerated through a horizontal PVC pipe placed over the whole width of the reactor underneath the outlet. The aeration pipe contained two holes of 1.5 mm with 4 cm of space in between. The aeration was connected to an airflow meter (Shorate 13-130 LN/h) which allowed exact control of the airflow. During testing, the small reators were operated parallel to the medium scale system and fed from the inlet of the medium scale system (Figure 8).

Experimental set-up handling experiment
An initial experiment was executed to assess the effect of handling of biofilter media on TAN removal rate in the small reactors. Three variants of media handling were tested:
- 1 minutes dewatering in a net and weighing
- 3 minutes dewatering in a net and weighing
- volumetric measurement in water and weighing after the experiment

The latter method is considered to give no handling stress to the media. Each of the small reactors was filled with media following one of the methods above to a filling rate of 50%. The reactors were connected to the inflow of the medium scale MBBR. Flow of water and air to the reactor were comparable to the basic situation in the medium scale reactor. The reactors were filled twice with new media and after each filling a duplicate measurement of TAN_{in} and TAN_{out} was made.

Experimental set-up scale comparison
In order to compare small and medium scale reactors, the small scale reactors were filled with media from the medium scale systems and operated in parallel to the medium scale reactor (Figure 8). The influent to the small reactors was taken from the inlet of the medium scale reactor using a peristaltic pump (Watson Marlow 520S). The water flow over individual

reactors was measured every 15 minutes during experiments using the 'stopwatch bucket' method.The water flow over the medium scale reactor was measured twice a day during experiments in the same way.

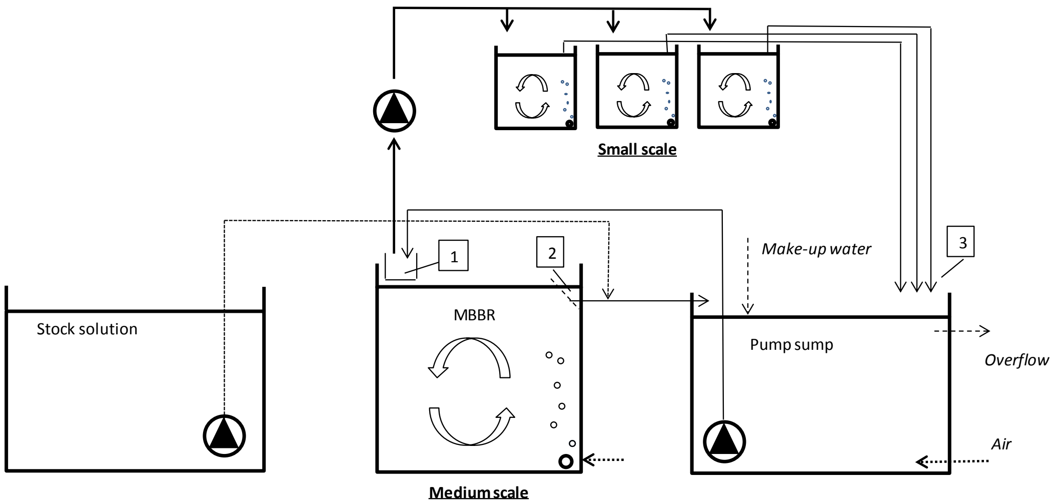


Figure 8. An overview of the experimental system for comparing the small and medium scale MBBRs. 1: sampling TAN_{in} small+medium scale; 2: sampling TAN_{out} medium scale; 3: sampling TAN_{out} small scale.

Air flow to each individual small reactor was measured and controlled by a flow meter (Shorate 13-130 LN/h). The air flow to the medium size reactor was measured with the 'stopwatch bucket' method. A series of experiments was executed according to the experimental design in Table 3. During one experimental day one specific airflow was studied at different TAN concentrations, resulting in six measurement days. The experimental series with 35% filling were performed first and after adding media to a level of 50% filling, the system was allowed to adapt for 4 weeks.

Table 3. The experimental design of the comparison between small and medium scale MBBRs. For Fill ratio, Air flow and TAN the experimental conditions are shown. In the rows after medium and small scale, the number of time replicates is presented.

Fill ratio (%)	35								
Air flow (m/h)	12			8			4		
TAN (mg/l)	0.4	0.8	>2	0.4	0.8	>2	0.4	0.8	>2
Medium scale (n=1)	4	4	4	4	4	4	4	4	4
Small scale (n=3)	4	4	4	4	4	4	4	4	4
Fill ratio (%)	50								
Air flow (m/h)	12			8			4		
TAN (mg/l)	0.4	0.8	>2	0.4	0.8	>2	0.4	0.8	>2
Medium scale (n=1)	4	4	4	4	4	4	4	4	4
Small scale (n=3)	4	4	4	4	4	4	4	4	4

The measurements were started using the medium TAN concentration. Every 15 minutes a sample was taken from the influent and effluent of the medium and small scale reactors. After 4 samplings, the TAN load to the system was reduced by adjusting the pump dosing from the

stock solution. After finishing the series at a low TAN level, the dosage was increased until a level > 2 $g.m^{-3}$ TAN was reached. Since at that concentration the biofilter is not able to handle an increase in TAN, the TAN concentration starts to rise and is difficult to control.The analyses of TAN were performed immediately after sampling using the method described in paragraph 3.2.2.

Calculations and statistics
TAN removal rates rTAN were calculated for each sampling using the formula:

$$rTAN = (TAN_{in} - TAN_{out}) * flow/media\ surface \quad (g\ N.m^{-2}.d^{-1})$$

The effects of media filling rate, aeration, TAN_{out} and scale were studied using a linear mixed model for the mean rTAN. The fixed effects in the model are the main variables mentioned above and all interactions between two variables with one exeption for the interaction between filling rate and aeration. This interaction was omitted to allow testing of the main effects filling rate and aeration against their interaction because these factors are coupled to measurement days. Additional random effects incorporated in the model are 'measurement day' and 'periods (TAN levels) within days' in order to describe dependencies between data. The mixed model used is:

$rTAN_{, mean}$ = c+filling rate+aeration+$TAN_{in.}$+scale+TAN_{in}.filling rate+filling rate.scale+TAN_{in}.aeration+aeration.scale+TAN_{in}.scale+measurement.day+measurement day.period+residuals

The model assumes that effects of measurement day, measurement day.period and residuals are independent and normally distributed with expectancy 0 and variances $\sigma^2_{measurement\ day}$, $\sigma^2_{period.measurement\ day}$ and σ^2_{residu}. respectively. Estimates for fixed effects, the components of variance and approximate F-tests for fixed terms in the model were obtained through the REML procedure in Genstat (International 2013).

3.3.3 Results and discussion

Table 4 presents an overview of the data on the comparison between small and medium scale MBBRs.

Table 4. Mean treatment values for rTAN ($g.m^{-2}.d^{-1}$) for all combinations between filling%, air speed, TAN level and scale.

			TAN_in		
Filling %	Air speed	Scale	Low	Medium	High
35	Low	Small	0.30	0.69	1.13
		Medium	0.38	0.95	1.35
	Medium	Small	0.50	0.74	2.05
		Medium	0.53	0.81	2.63
	High	Small	0.33	0.49	1.66
		Medium	0.34	0.55	2.04
50	Low	Small	0.26	0.55	1.12
		Medium	0.24	0.69	1.33
	Medium	Small	0.33	0.58	0.98
		Medium	0.35	0.72	1.24
	High	Small	0.35	0.62	1.12
		Medium	0.36	0.70	1.39

The estimates for the components of variance are shown in Table 5.

Table 5. Estimates for the components of variance (between brackets the standard error).

	$\sigma^2_{measurement\ day}$	$\sigma^2_{period.measurement\ day}$	σ^2_{residu}

rTAN	0.004(0.030)	0.063(0.046)	0.0041(0.0017)

Table 6 presents the statistics of the mixed model.

Table 6. Calculated F and P values (Fprob) of the F-tests for the fixed terms in the mixed model.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	Fprob[1)]
Filling%	3.80	1	3.80	2.0	0.191
Air speed	1.74	2	0.87	2.0	0.535
TAN_in	64.87	2	32.44	4.0	0.003
Scale	51.73	1	51.73	12.0	<0.001
tan_in.filling%	4.55	2	2.27	4.0	0.219
Filling%.scale	2.42	1	2.42	12.0	0.146
tan_in.air speed	2.43	4	0.61	4.0	0.679
Air speed.scale	0.83	2	0.42	12.0	0.669
tan_in.scale	33.23	2	16.62	12.0	<0.001

Fprob[1)] refers to a F-distribution without considering effects of subsequent rows.

Effects of filling% and air speed on rTAN and all their interactions are not significant ($P>0.05$). Both TAN and scale have a significant effect as as well as their interaction ($P<0.05$).

Based on the test results, predictions are calculated for the rTAN for filling%, air speed and the combinations of TAN and scale (Table 7).

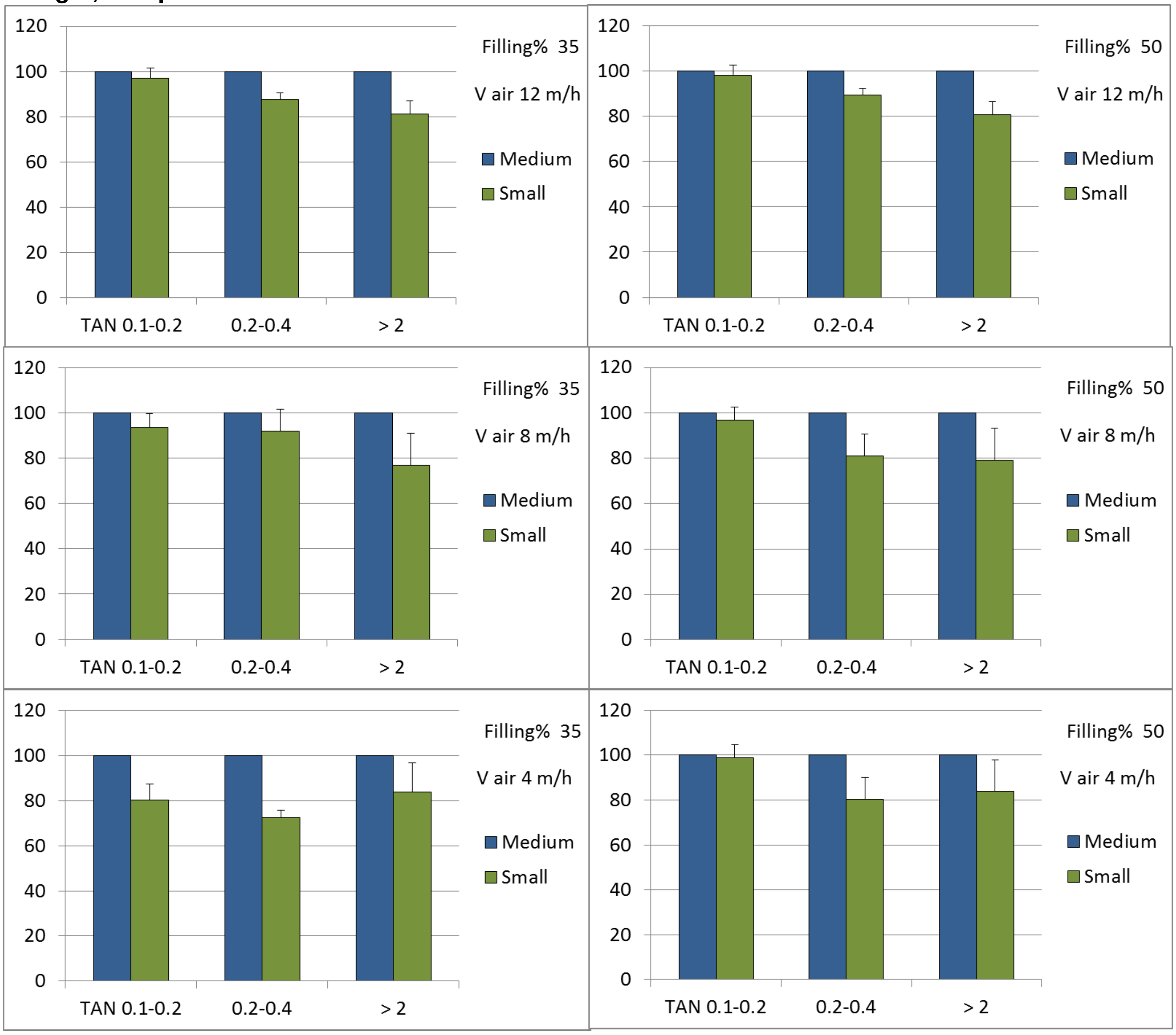
Table 7. Predictions of average rTAN based on filling%, air speed and the interaction between TAN and scale.

Filling%	35	50		SED
rTAN	0.97	0.72		0.13
Air speed	Low	Medium	High	
rTAN	0.75	0.96	0.83	0.159
Interaction TAN and scale				
TAN/Scale	Small	Medium		
Low	0.35	0.37		0.037
Medium	0.61	0.74		
High	1.34	1.66		

Based on the SED's and approaching t-tests there is a significant effect of TAN on rTAN as would be expected. Moreover, the average rTAN is significantly lower on the small scale compared to the medium scale at medium and high TAN level.

The differences between small and medium scale are graphicaly depicted in Figure 9.

Figure 9. The relative difference in rTAN between the medium and small scale system at different filling%, air speed and TAN concentration.



3.4 Comparison of the performance of large and small scale MBBRs

3.4.1 Introduction

Use of MBBRs for biofiltration has become popular in RAS and large systems have been in operation for over a decade. Therefore, upscaling itself is not a specific problem for the application this type of biofilters. However, the relevance of lab-scale research for the design of this type of biofilters still needs to be established and optimisations might still be possible.
In order to study scale effects at full scale, measurements were performed *in situ* at commercial fish farms by running small scale systems parallel to the large bioreactors. The small scale reactors were filled with media from the large system, flows of air and water scaled to the large system and identical influent water used. Like in the comparison between small and medium scale, this approach eliminates effects of biofilm history and capacity and effects of nutrient load.

3.4.2 Material and methods

Performance of large and small scall MBBRs were compared at three different farms. Flows of water and air over the large biofilters were estimated based on the equipment installed and the original design (Table 8).

Table 8. An overview of the characteristics of the large scale biofilters sampled.

	Farm 1	**Farm 2**	**Farm 3**
Species	Hybrid African catfish	Hybrid African catfish	Pikeperch
Production capacity (T/Year)	30-50	750-1000	20-25
Volume reactor (m3)	20	280	20
lxbxh	Ø2,2 x 2,5	Ø6,9 x 3,6	Ø3,5 x 2
Volume media (m3)	10	120	10
Filling bioreactor (%)	50	43	50
Type media	Kaldnes K1	Curler advance X-1	Kaldnes K1
SSA media (m2/m3)	500	800	500
Air flow (m3/h)	80	1000	70
V air (m3/h/m2; m/h)	10.5	13.4	8.3
Water flow (m3/h)	500	2500	450
Retention time water (min)	2	7	3
Feeding level (kg/day)	80	1100	80

The small filters were operated in parallel (Figure 10) to the large biofilter with a hydraulic retention time identical to that of the large filter. Aeration was also adjusted to create identical air speed in the small and large systems. In the small reactors a filling rate identical to the large systems was used. Before transfer of media from large to small scale, the media was left for one minute to dewater in a small net. .

A series of four samples of influent and effluent flows from all reactors were collected over a period of two hours and analysed immediately for TAN.

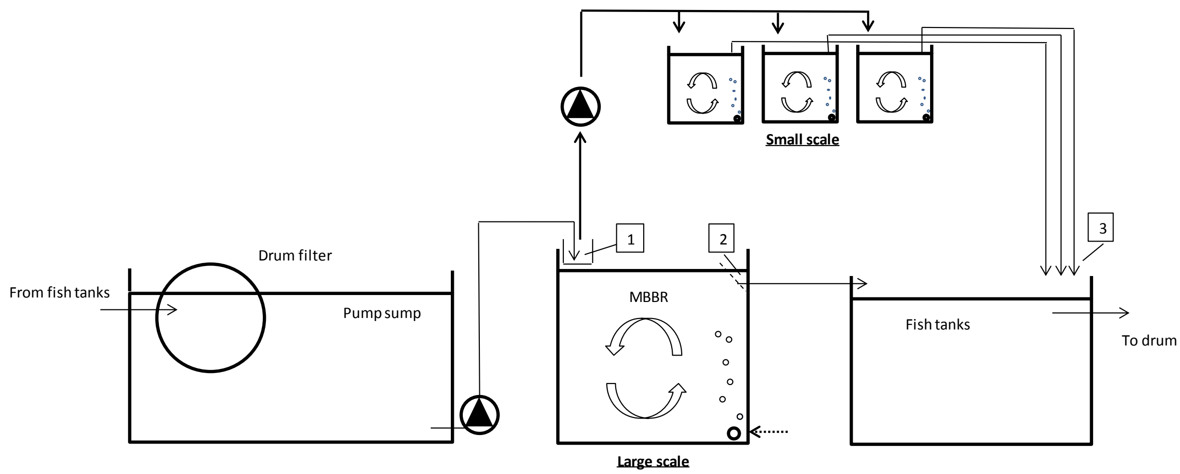


Figure 10. An overview of the experimental set-up for the comparison of small vs large scale. 1: sampling TAN_{in} small+large scale; 2: sampling TAN_{out} large scale; 3: sampling TAN_{out} small scale.

3.4.3 Results and discussion

Conditions during sampling were stable at the farms. TAN concentrations in the outlet of the MBBRs were in the range of 0.1 to 0.3 $g.m^{-3}$ which is in the category low/intermediate as used in the research on comparing small and medium scale. The results show that in all cases mean rTAN in the small systems is 70 to 80% of that in the large ones (Table 9). However, based on the 95% confidence intervals only the difference measured at farm 1 can be considered significant.

Table 9. Mean rTAN, SED* and 95% confidence interval for the comparison between large and small scale MBBRs at three farms.

Farm no.	Mean rTAN ($g.m^{-2}.d^{-1}$)		Difference large-small	SED	95% confidence interval		Ratio S:L	TANout large ($g.m^{-3}$)	TANout small ($g.m^{-3}$)
	Large	Small			Lower limit	Upper limit			
1	0.45	0.31	0.14**	0.005	0.12	0.16	0.69	0.16	0.27
2	0.17	0.14	0.03	0.011	-0.004	0.06	0.82	0.11	0.17
3	0.29	0.22	0.07	0.031	-0.31	0.46	0.76	0.21	0.27

* SED: Standard Error of the Difference
** significant difference ($P<0.05$)

The relatively higher rTAN in the large MBBRs results in lower TAN concentration in the outlet of the MBBRS compared to that of the small reactors. If we consider the MBBRs completely mixed, the outlet concentration will be the concentration in the bulk fluid in the reactor. When rTAN is limited by the TAN concentration, this implies that under the same TAN concentration the difference between the reactors scales would even be larger.

3.4.4 Conclusions

- The TAN removal rate in small MBBRs is in the order of 70 to 80% of that of large MBBRs at a low to intermediate TAN level.

3.5 Mixing in small scale reactors

3.5.1 Introduction

Mixing behaviour of fluids in a reactor was *a priori* considered to be an important aspect of upscaling (see paragraph 2.1). In order to quantify the effects of experimental variables used in our experiments like media filling% and air speed on mixing behaviour, a set of separate experiments was performed with the small scale reactor.

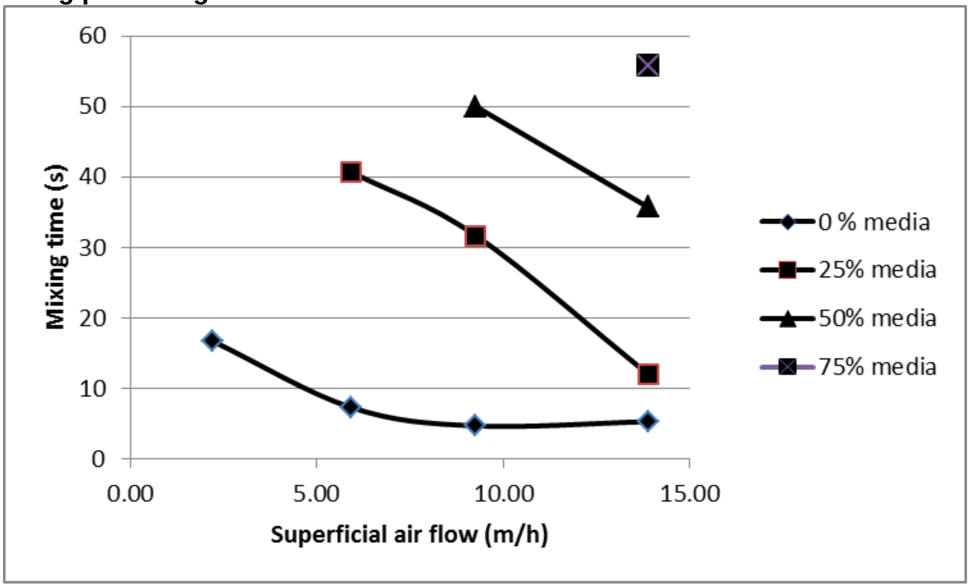
3.5.2 Material and methods

In this experiment the mixing time in a small scale MBBR was determined accoding to the pH-response technique (van 't Riet, van der Lans et al. 2011). The mixing time is defined as the time it takes the pH to reach a value of ±5% of the final value after spiking the MBBR with a concentrated acid. After spiking the value oscillates towards a final value which is recorded over time. A small scale reactor as described in 3.3.2 was used and filled with tap water and media (Kaldnes K-1). A range of air speeds (2.2, 5.9, 9.3, 13.9 m/h) was tested at four different media filling percentages (0, 25, 50 and 75%). Air speed was set at a predetermined level before each experiment. Each combination was tested in triplicate. At T=0, 0.2 ml of 1 M HCl was added to the inflow of the reactor. At the same time the change in pH over time was recorded with a Hach pH meter (HQ 40d; probe IntelliCal PHC 101) positioned in the centre of the reactor until the pH stabilized at a final level. From the recordings, the time to reach a pH value of 5% from the final level was calculated.

3.5.3 Results and discussion

Both air speed and media filling% have an effect on the mixing time (Figure 11). With no media in the reactor, the mixing time is only increased at the lowest air speed. Adding media has a strong effect on mixing time. At 25% media filling, the mixing time without media can be approached only at high air speed. At 75% media filling, the media are not moving at all and mixing time approaches one minute.

Figure 11. The relationship between superficial air flow and mixing time at four different media filling percentages.



Under operational conditions, the flow of water into the reactor also improves mixing. The hydraulic retention time was ca. 3 minutes under all experimental conditions used in the comparison between the small and medium scale. This means that mixing can be considered complete in the small reactor.

3.5.4 Conclusions

- Air speed has a strong effect on mixing time in the small reactor.
- Increased media filling percentage increases the mixing time in the small reactor.
- The mixing time is well below the hydraulic retention time used, which is an indication that complete mixing occurred under normal experimental conditions.

3.6 Effects of TAN, filling rate and superficial air speed on TAN removal in small and medium scale reactors

3.6.1 Introduction

In chapter 3.3 the effects of scale on nitrification rate were studied under a few selected conditions with regard to TAN concentration, air speed and media filling percentage. The different scales were compared simultaneously.
In this chapter the effects of individual factors are elaborated in more detail. In a first trial the effect of air speed on TAN removal was tested in the small reactors at three different media filling% at a constant TAN influent concentration. In a second trial the effect of air speed on TAN removal at the medium scale was tested. In a third trial, the effect of the TAN concentration was measured at three different air speeds in the small scale reactor and at one air speed in the medium scale reactor. In a fourth trial, the effect of additional mixing was tested at different TAN levels.

3.6.2 Trial 1: effects of filling rate and air speed at small scale

Material and method
In Trial 1, three small scale reactors were each filled with 25, 35 and 50% media from the medium scale reactor. Aeration was started at a level of 14 $m.h^{-1}$ and controlled with flowmeters. The reactors were operated in a side loop from the medium scale system as described in paragraph 3.3.2. Throughout the experiment a constant TAN concentration of 0.76 ± 0.01 $g.m^{-3}$ (mean± SD) was maintained in the influent. TAN concentration and flow of water and air was measured simultaneously. After each measurement the air speed was reduced and the system allowed to adjust for 15 minutes.

Results
At first sight there is a marked effect of % media filling on TAN removal Figure 12. However, since all reactors are operated on the same influent concentration, the TAN concentration in the reactor will differ according to the %filling. In Figure 13 the TAN concentration in the outlet, which can be considered the concentration in the bulk fluid in a well mixed system, is plotted against TAN removal. It is apparent that the differences in TAN removal rate are partly caused by small differences in TAN concentration. However, if we consider the dose-response curve from Figure 15 which has also been plotted in Figure 13, we can conclude that an increase in %filling has a negative effect on TAN removal. Compared to the “standard curve” shown in Figure 15, the TAN removal is 91%, 80% and 71% at 25, 35 and 50% media filling respectively.

Figure 12 also shows that below an air speed of 5 $m.h^{-1}$ TAN removal is reduced. Above this level of aeration, TAN removal is constant. This effect seems to independent from the % filling.

Figure 12. The effect of superficial air speed on TAN removal in the smal reactors at three levels of media filling.

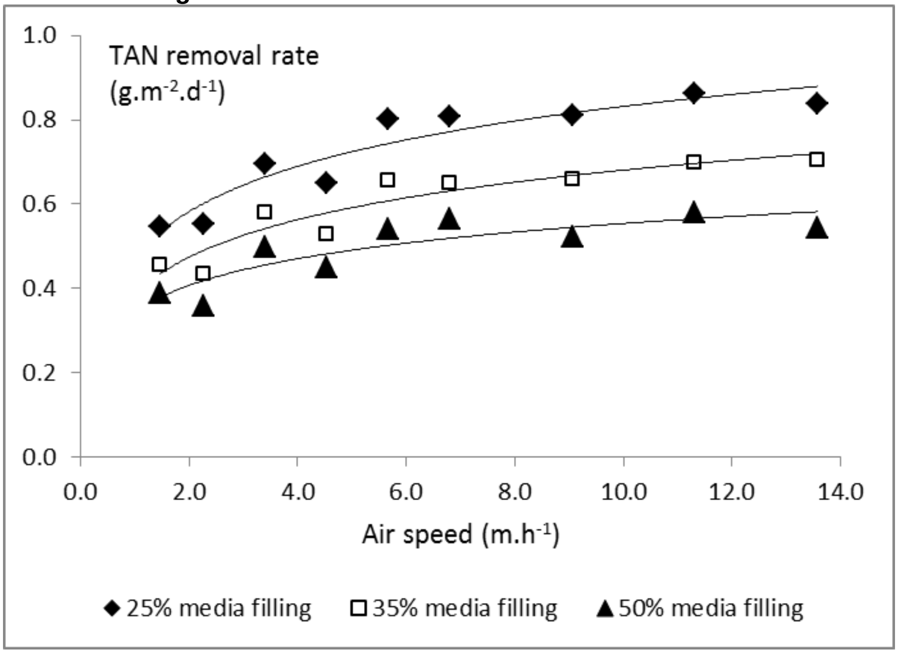
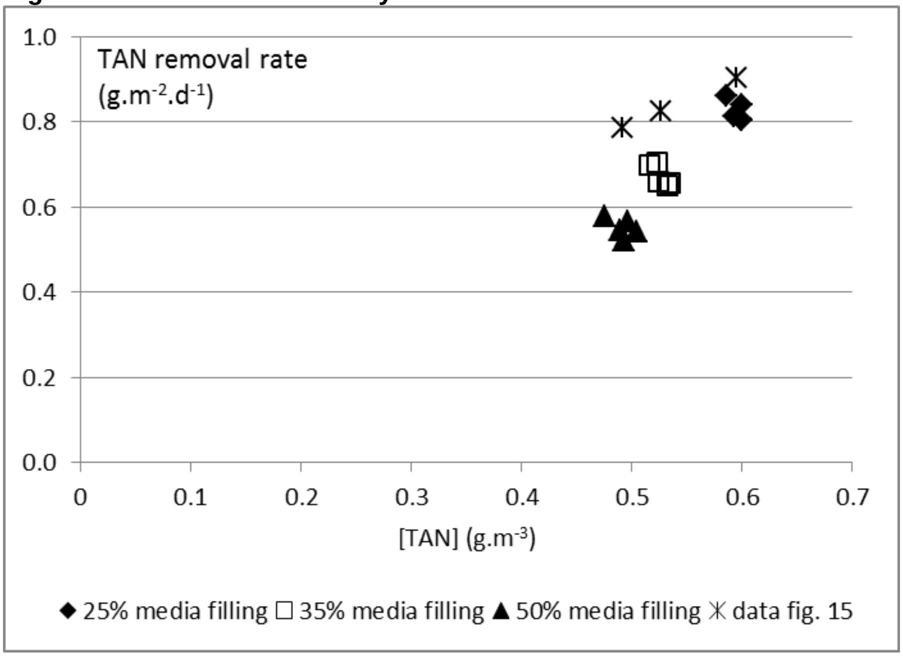


Figure 13. The effect of TAN concentration on TAN removal for three small scale reactors operated at different % media filling. The TAN removal rates for the small scale reactors from Figure 15 are also indicated by X.



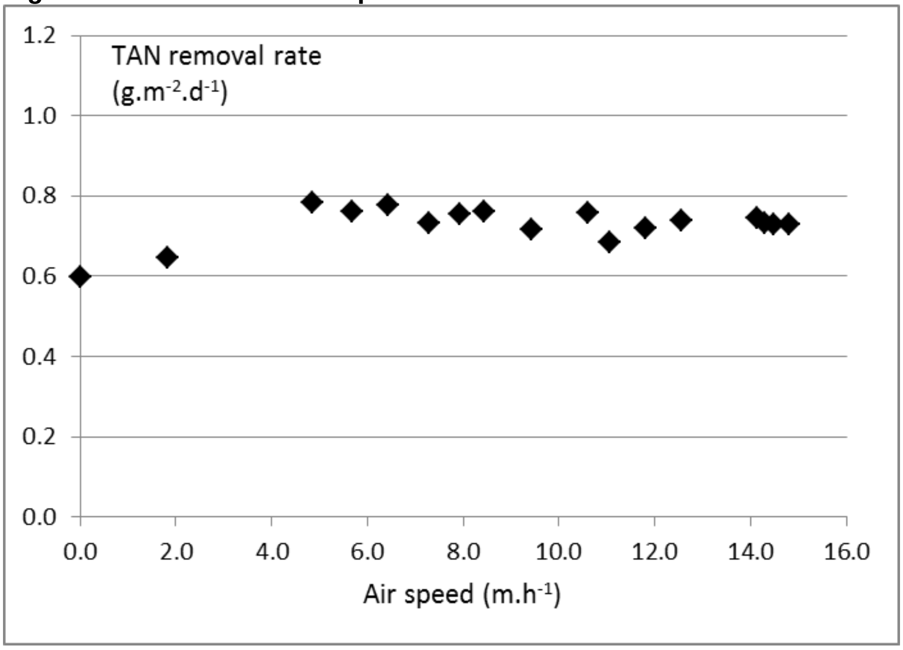
3.6.3 Trial 2: the effect of air speed at medium scale

Material and methods

In Trial 2, the medium scale reactor was operated at default condition (50% media filling) at an influent TAN concentration ranging from 0.73 at the start to 0.84 g.m^{-3} at the end of the experiment. The trial was started at maximum air speed attainable in the system (14.8 m.h^{-1}). After each measurement of TAN in influent and effluent of the reactor, the air flow was reduced in 17 steps to zero. The system was allowed to adjust for 15 minutes after each reduction of air speed.

Results
In the medium scale reactor there is also a distinct effect of air speed on TAN removal (Figure 14). Increased air speed results in increased TAN removal up to a speed of app. 5 $m.h^{-1}$ as in the small scale reactors. However, even without any aeration the TAN removal rate is still 75% of the maximum value.

Figure 14. The effect of air speed on TAN removal in the medium scale reactor.

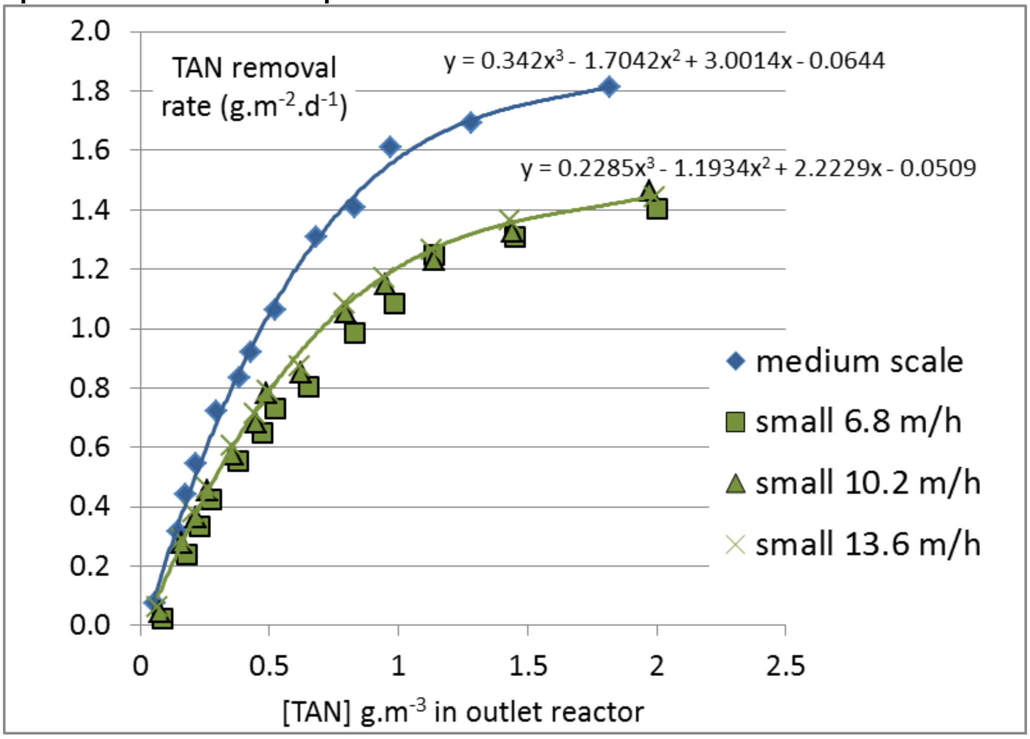


3.6.4 Trial 3: the effect of TAN on rTAN at small and medium scale (dose–response)

Material and methods
In Trial 3 the small reactors were operated at 50% media filling and an air speed of 6.8, 10.2 and 13.6 $m.h^{-1}$ respectively. The small reactors were operated in parallel to the medium scale system. Before the start of the experiment the dosing of TAN to the system was stopped to attain a low TAN level at the start. After measuring TAN in influent and effluent, the dose was slightly increased and the system was allowed to adjust for 15 minutes. This was repeated 13 times until a TAN concentration of 2 $g.m^{-3}$ was attained in the outflow.

Results
In the third trial, the small scale systems were compared at different air speeds and with the medium scale system, all above the threshold air speed of 5 $m.h^{-1}$ (Figure 15). The curves in Figure 15 show a typical increase in removal rate with increasing TAN concentration which reaches a plateau at about 2 $g.m^{-3}$ TAN. The results shown in Figure 15 confirm that air speed has no effect on TAN removal above the threshold. Figure 15 shows a marked difference in TAN removal rate between small and medium scale at all TAN concentrations. The removal rate at small scale is roughly 75% of that at medium scale (at 50% media filling).

Figure 15. The effect of TAN concentration (outlet) on TAN removal rate in small scale reactors operated at different air speed and on medium scale.



3.6.5 Trial 4: effects of strong mixing at small scale

Material and methods

In Trial 4, the three small scale systems were operated in parallel to the medium scale system as indicated in Figure 8. The medium scale system was operated at the basic aeration level of 12 $m.h^{-1}$. The three small scale systems were each aerated differently: a basic version of 12 $m.h^{-1}$, a version with double aeration and a version wich was strirred by a magnetic vortex mixer in addition to the aeration. The TAN concentration at the inlet was kept at 0.5-0.6 $g.m^{-3}$ for the first 5 samplings and in four steps increased to 1.3 $g.m^{-3}$. The interval between samplings was approximately 15 minutes. At the first two samplings, the additional aeration was not used. Analytical procedures were identical as mentioned before.

Results

In the fourth trial, TAN removal rate did not differ much between the small scale systems during the first 2 samplings but was app. 85% of the removal rate in de medium scale system (Figure 16 and Figure 17).

Figure 16. The TAN removal rate in a medium scale reactor and three different small scale reactors over time. At sampling 1 and 2 small scale reactors were operated identically. Starting at sampling 3, additional aeration was applied in two reactors. The TAN concentration in the inlet was gradually increased from 0.5 g.m^{-3} to 1.3 g.m^{-3} starting after sampling 5.

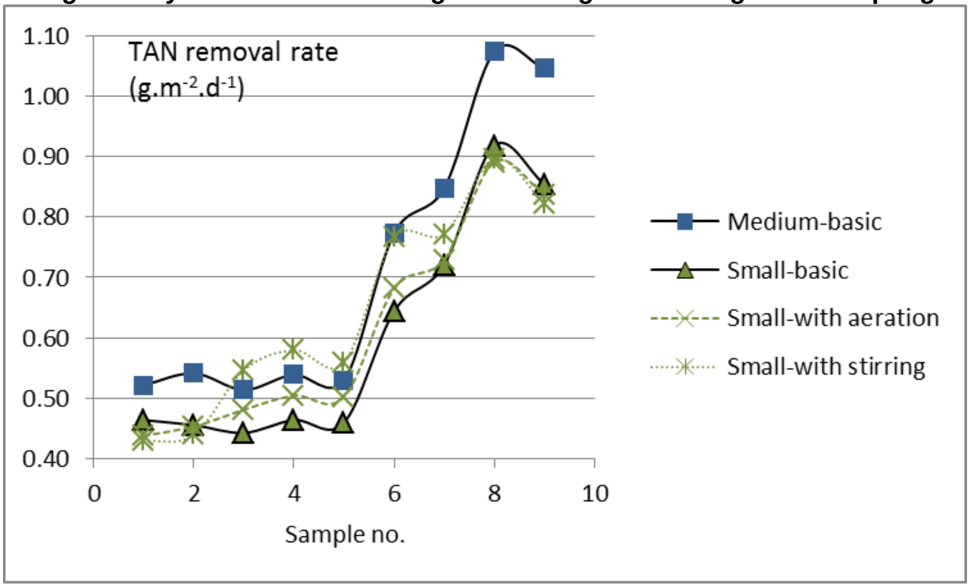
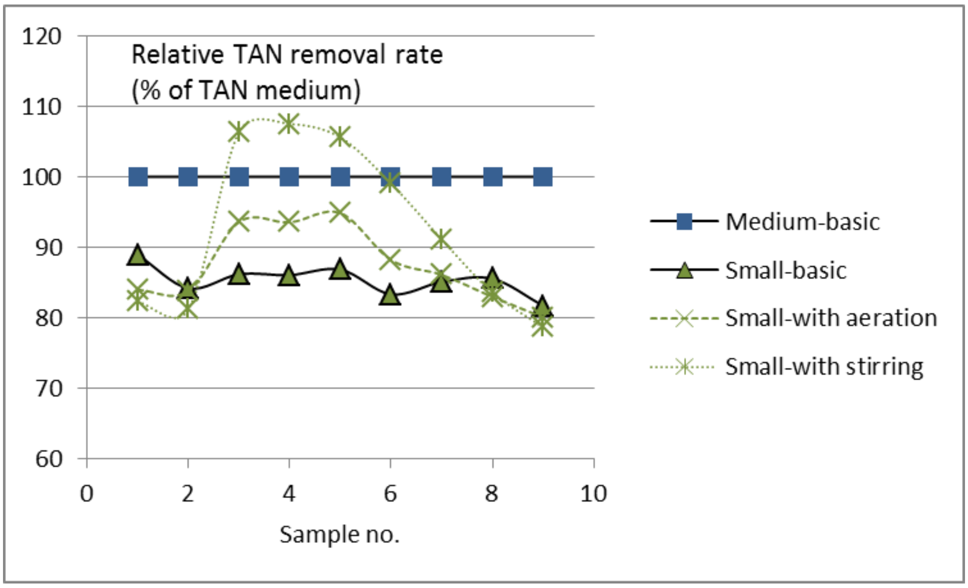


Figure 17. The relative TAN removal rate from figure 16 expressed as a percentage of the rates at medium scale.



Increased stirring during sampling 3 to 5 results in a strong increase in nitrification rate. In the system with double aeration the TAN removal rate increases from 0.46 (± 0.01) to 0.50 (± 0.01) g.m^{-2}.d^{-1}. In the system with additional vortex mixing the rate increases to 0.56 (± 0.02) g.m^{-2}.d^{-1}. The small system with vortex mixing shows in this case an even higher TAN removal rate than the medium scale system (0.53 ± 0.01).

An increase in the TAN concentration after sampling 5 results in a strong increase in TAN removal rates. However, the relative removal rate in the systems with additional mixing decreases at increasing TAN levels. At a TAN level of 1.17 g.m^{-3} in the inlet (0.6 outlet and reactor internally) the TAN removal is again identical in all small systems.

3.6.6 Conclusions

- An increase in media filling percentage has a negative effect on TAN removal rate. Relative (compared to dose-reponse curve) TAN removal rate decreases from 91% at 25% filling to 71% at 50% filling in small scale reactors.
- Below a threshold of 5 $m.h^{-1}$ superficial air speed, TAN removal is decreased. Above the threshold the level of TAN removal in both small and medium scale systems is constant in relation to air speed.
- There is a significant effect of scale on the dose response curve of TAN (at 50% media filling). At all TAN levels the removal rate of TAN is only 75% at small scale compared to medium scale.
- At a low TAN levels (0.3 $g.m^{-3}$), intense mixing increases TAN removal in the small scale reactors, so to equal or in fact surpass the medium scale system
- At TAN levels above 0.6 $g.m^{-3}$ however, intense mixing has no effect on TAN removal rate.

4. Synthesis of the effect of scaling on biofilter performance in aquaculture

In chapter three, different aspects of TAN removal were studied at different scales and in varying combinations. In this chapter we will discuss the individual variables and link the different levels. Finally, some conclusions on scaling will be drawn and discussed in relation to literature.

Media filling percentage
Effects of media filling were studied in the experiment on mixing time (section 3.5), in the multi-factorial comparison between small and medium scale (section 3.3) and in the single factor experiments on small scale (section 3.6). Figure 11 shows that media filling% has a strong effect on mixing time in a small reactor. In Figure 13, effects of media filling% are compared to a standard dose-reponse curve. Figure 13 shows the effect of filling% on rTAN and indicates a reduction to 91% on rTAN at 25% filling which reduces further to 71% at 50% filling. The multi-factorial comparison between small and medium scale did show a decrease in rTAN from 35% filling to 74% at 50% filling (Table 7). However, this experiment lacks the statistical power to test individual variables and therefore the difference is not significant. Effects of filling rate of MBBRs on nitrification have been studied by several authors (Calderon et al.,2012; Gjaltema et al., 1995). Significant effects have been demonstated on biofilm community structure and biofilm turn-over rate. However, in our research identical biofilms were used and results can only be explained by hydraulic effects on biofilm level. Probably higher media filling% results in decreasing turbulence at the boundary level of the biofilm which increases diffusional resistance for the TAN substrate. Zhu and Chen (2001) demonstrated the effect of flow rate expressed as Reynolds number on nitrification rate and presented a theoretical framework. A problem working with moving media is that the actual water speed at the boundary level is unknown and impossible to measure with current technology. Theoretically, if water and media in an MBBR would move at exactly the same speed in the same direction, diffusional resistance would be very high and nitrification rate low.

Superficial air speed
In all experiments superficial air speed was taken into account since this is an important determinant for upscaling of bioreactors (Ju and Chase 1992). The mixing time is strongly affected by air speed as shown in Figure 11. Experiments (Figure 12 and Figure 14) on both small and medium scale demonstrate that there seems to be a threshold of approximately 5 $m.h^{-1}$ at which rTAN reaches a plateau. Above this threshold, mixing time will still be reduced with an increase in air speed but this has no clear effect on rTAN. However, if we increase mixing intensity far above 12 $m.h^{-1}$ as shown in Figure 16, rTAN increases again, provided that TAN is at low concentrations. Probably this effect can again be attributed to the effect of turbulence on the thickness of the biofim boundary water layer. In the comparison between small and medium scale, air speed and its interactions have no significant effects on rTAN (Table 6).

Since there are significant effects of scale on rTAN at identical superficial air speed, superficial air speed can not be considered a good scaling factor for TAN removal in MBBRs. Superficial air speed does not taken into account the depth at which the air is injected. In upscaling, depth of air injection and pressure will increase. At identical superficial air speed, the mixing power and energy input will increase linearly with depth. In the small scale system and the medium system the depth of air injection is 9 and 50 cm respectively. This would mean that energy input in the medium scale system is 5 fold higher compared to the small scale system.

A better scaling factor would be $V' = F.P/A$ in which

V' normalised air speed	$m^3.h^{-1}.N.m^{-2}.m^{-2}$ or reduced as $N.\ h^{-1}.m^{-1}$
F: air flow	$m^3.h^{-1}$
P: air pressure	$N.m^{-2}$

A: top area (footprint) m^2

In general, the role of aeration of MBBRs in aquaculture is different from that in processing high strength waste water. In the latter case, the oxygen demand is high and intensive and continuous aeration is necessary to supply the respiring biofilm. In aquaculture, the oxygen demand can be covered by the oxygen arriving in the flow coming from the culture tanks, which are generally nearly saturated with oxygen. Maintenance of a thin biofilm and prevention of clogging is seen as a great benefit of MBBRs in aquaculture. However, this effect could also be generated with short term intermittent aeration. The management could be identical to that of a bead filter. Potentially, this could result in large energy savings in operating MBBRs in aquaculture.

TAN concentration

The removal rate of TAN is up to a certain transition concentration limited by the concentration of TAN in the bulk fluid as explained in 2.2 and illustrated in Figure 3. Since TAN removal rate was chosen as the read-out parameter for nitrification in this research project, effects of TAN concentration on scaling have to be taken into account. As would be expected, the effect of TAN is highly significant in the multi-factorial comparison between small and medium scale.
In this research all comparisons between scales and within scales were made using an identical influent TAN load. However, differences in TAN removal rate will result in differences in the TAN concentration in the bulk fluid. Since all the reactors used in this research can be considered completely mixed, the TAN concentration in the outlet can be considered the actual concentration in the bulk fluid which the biofilm is experiencing. This implies that a higher TAN removal rate is counter-acted by the effect of the lower TAN concentration. An experimental set-up with uniform TAN levels in the outflow would result in larger differences in TAN removal rate. However, such an experimental set-up would require upfront knowledge of TAN removal rates which is not possible.

Table 6 and Figure 9 show that there is a significant interaction between TANin and scale. At low TANin (mean: 0.35) the rTAN ratio between TAN small/medium is 0.95, at medium TAN (0.44) this is 0.82 and at high TAN (4.21) 0.81. This result is some what counter-intuitive since at a high TAN concentration mixing is expected to be less important since the reactor is flooded with substrate contrary to the situation at low TAN. A reasonable explanation is difficult to produce. The dose-response curve (Figure 15) between TANout and rTAN still shows a considerable gap between scales at low TAN. However, since the curves for the different scales have a different slope in the low TAN range there could be difference in the response to TAN based on TANin or TANout.

In

Figure 18 the data on dose-response from Figure 15 were plotted against TANin and new regression equations were calculated. In Figure 19 the ratio's of rTAN between small and medium scale were plotted against the concentration of TAN.

Figure 18. The relationship between TANin and TAN removal rate for the small and medium scale reactors.

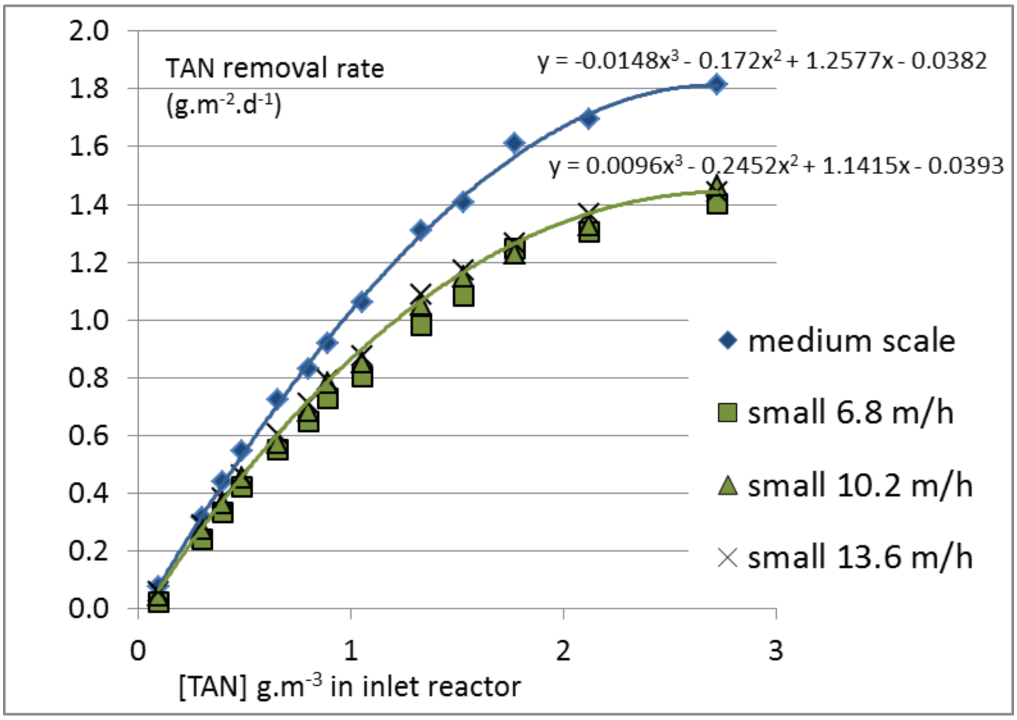


Figure 19. The relationship between TAN concentration (in and out) and the ratio between rTAN in the small and medium scale reactor. OUT/IN is the ratio between Small/Medium_out (S/M out) and Small/Medium_in (S/M in). OUT and IN represent the mean concentrations at which the multi-factorial experiments were performed.

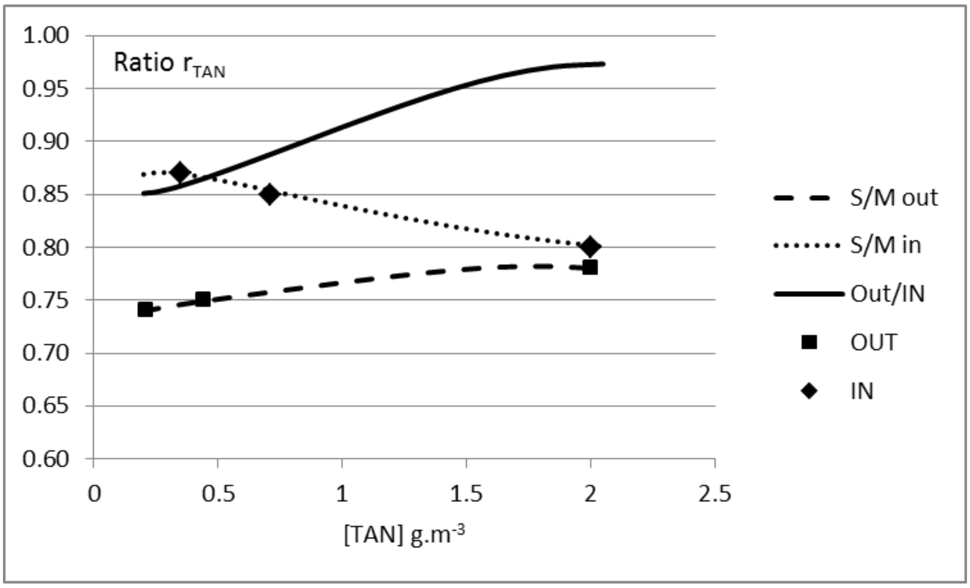


Figure 19 shows that the ratio between rTAN small and medium based on TANout increases from 0.74 to 0.78 while that based on TANin decreases from 0.87 to 0.8. At a high concentration the ratios come close together. Therefore, the true scale effect depends on whether TANin or out is used to calculated the dose response curve. Since we have used TANin the mixed model, the scale effect is somewhat under-estimated at low TAN.

The comparison of small scale and large scale systems was done at TAN levels in the outflow varying from 0.11 to 0.27 $g.m^{-3}$. Even at these levels we find a very strong scale effect with ratio's small/large varying from 0.69 to 0.82. Here again the true scale effect is underestimated and masked by the differences in TAN out.

Effects of scale on TAN removal
The comparison between small and medium scale reactors shows that scale is a significant factor (Table 6, Figure 15). Small reactors show a TAN removal which is at high TAN concentration in the order of 80% of a medium scale system. The comparison between small scale and large scale systems shows an even more pronounced difference as mentioned above even at low TAN (Table 9). It is difficult to predict the differences between scales at higher TAN concentrations since TAN cannot be manipulated easily under farm conditions.
Superficial air speed has been used as a scaling factor. It is obvious that this factor is insufficient to describe scaling of TAN removal over a range of reactor sizes. However, as mentioned in the discussion on the effect of superficial air speed, the mixing effect strongly increases with scale at a constant superficial airspeed. Therefore, full scale systems are much more turbulent than smal scale systems at the same superficial air-speed.
Apart from the differences in energy input for mixing at different scales, the friction experienced by the fluid increases at decreasing scale and mixing will be more difficult at small scale.

Small scale MBBRs are an interesting research tool to study COD production and removal in RAS. This can be done by measuring nitrification rate and oxygen consumption simultaneously in a closed vessel which is not mixed by air but in an alternative way. As we have seen in this research (Figure 17) mixing intensity has a strong effect on TAN removal. Defining mixing conditions which are representative of full scale aerated MBBRs would be a prerequisite for this type of research.

5. Conclusions

- MBBR scale has a significant effect on TAN removal rate. **In general, the larger the scale the better the performance**.

- TAN removal at small scale (0.8 L) is app. 80% compared to that at medium scale (200 L). The difference between small scale and large scale (>20 m^3) is even higher.
  These findings warrant further studies on whether a plateau is reached in rTAN at a certain scale; a study which will have considerable impact for RAS suppliers.

- Superficial air speed is not a good scaling factor for MBBRs. Upscaling while maintaining geometry implies increasing air injection depth and therefore increased energy input at comparable air speed.

- Air speed and media filling percentage have a strong effect on mixing time at small scale. An airspeed below a threshold of 5 $m.h^{-1}$ decreases TAN removal at both small and medium scale.

- Intense mixing at small scale increases TAN removal at low TAN concentration. At a high TAN concentration, the small scale MBBR always performs at app. 80% of the capacity of the medium scale system irrespective of the mixing conditions.

- Capacity of full scale systems will be under-estimated when based solely on small scale experiments.

6. References

Bergheim, A., A. Drengstig, et al. (2009). "Production of Atlantic salmon smolts in Europe--Current characteristics and future trends." Aquacultural Engineering **41**(2): 46-52.

Bernet, N., O. Sanchez, et al. (2004). "Effect of solid hold-up on nitrite accumulation in a biofilm reactor - molecular characterization of nitrifying communities." Water Science and Technology **49**(11-12): 123-130.

Bjornberg, C., Lin, W. and Zimmerman, R. (2009). "Effect of temperature on Biofilm growth dynamics and nitrification kinetics in a full-scale MBBR System." Proceedings of the 82nd Annual Water Environment Federation Technical exposition and Conference, Orlando, Florida **Water Environment Federation: Alexandria, Virginia.**

Bonvillani, P., M. P. Ferrari, et al. (2006). "Theoretical and experimental study of the effects of scale-up on mixing time for a stirred-tank bioreactor." Brazilian Journal of Chemical Engineering **23**(1): 1-7.

Bovendeur, J. (1989). Fixed biofilm reactors applied to waste water treatment and aquacultural water recirculation systems: Engineering Design and management.

Bovendeur, J., A. B. Zwaga, et al. (1990). "Fixed-biofilm reactors in aquacultural water recycle systems: effect of organic matter elimination on nitrification kinetics." Water Research **24**(2): 207-213.

Calderón, K., J. Martín-Pascual, et al. (2012). "Comparative analysis of the bacterial diversity in a lab-scale moving bed biofilm reactor (MBBR) applied to treat urban wastewater under different operational conditions." Bioresource Technology **121**(0): 119-126.

Dalsgaard, J., I. Lund, et al. (2013). "Farming different species in RAS in Nordic countries: Current status and future perspectives." Aquacultural Engineering **53**(0): 2-13.

Eding, E. H., A. Kamstra, et al. (2006). "Design and operation of nitrifying trickling filters in recirculating aquaculture: A review." Aquacultural Engineering **34**(3): 234-260.

Ferrai, M., G. Guglielmi, et al. (2010). "Modelling respirometric tests for the assessment of kinetic and stoichiometric parameters on MBBR biofilm for municipal wastewater treatment." Environmental Modelling & Software **25**(5): 626-632.

Garcia-Ochoa, F. and E. Gomez (2009). "Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview." Biotechnology Advances **27**(2): 153-176.

Gill, N. K., M. Appleton, et al. (2008). "Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up." Biotechnology and Bioengineering **100**(6): 1144-1155.

Gjaltema, A., L. Tijhuis, et al. (1995). "DETACHMENT OF BIOMASS FROM SUSPENDED NONGROWING SPHERICAL BIOFILMS IN AIRLIFT REACTORS." Biotechnology and Bioengineering **46**(3): 258-269.

Green, M., Y. Ruskol, et al. (2002). "The effect of CO2 concentration on a nitrifying chalk reactor." Water Research **36**(8): 2147-2151.

Harremoës (1978). Biofilm kinetics. New York, Wiley.

Hem, L. J., B. Rusten, et al. (1994). "Nitrification in a moving bed biofilm reactor." Water Research **28**(6): 1425-1433.

International, V. (2013). GenStat for Windows 16th edition.

Ju, L. K. and G. G. Chase (1992). "IMPROVED SCALE-UP STRATEGIES OF BIOREACTORS." Bioprocess Engineering **8**(1-2): 49-53.

Kamstra, A., J. W. van der Heul, et al. (1998). "Performance and optimisation of trickling filters on eel farms." Aquacultural Engineering **17**(3): 175-192.

Martin-Pascual, J., C. Lopez-Lopez, et al. (2012). "Comparative Kinetic Study of Carrier Type in a Moving Bed System Applied to Organic Matter Removal in Urban Wastewater Treatment." Water Air and Soil Pollution **223**(4): 1699-1712.

Martins, C. I. M., E. H. Eding, et al. (2010). "New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability." Aquacultural Engineering **43**(3): 83-93.

Nijhof, M. and J. Bovendeur (1990). "Fixed film nitrification characteristics in sea-water recirculation fish culture systems." Aquaculture **87**(2): 133-143.

Nogueira, R., L. F. Melo, et al. (2002). "Nitrifying and heterotrophic population dynamics in biofilm reactors: effects of hydraulic retention time and the presence of organic carbon." Water Research **36**(2): 469-481.

Odegaard, H. (2006). "Innovations in wastewater treatment: the moving bed biofilm process." Water Science and Technology **53**(9): 17-33.

Odegaard, H. R. B. a. T. W. (1994). "A new moving bed biofilm reactor - applications and results." Wat. Sci. Tech. **29**(10-11): 157-165.

Ohashi, A., D. G. V. deSilva, et al. (1995). "Influence of substrate C/N ratio on the structure of multi-species biofilms consisting of nitrifiers and heterotrophs." Water Science and Technology **32**(8): 75-84.

Pfeiffer, T. P. W. (2011). "Evaluation of three types of structured floating plastic media in moving bed biofilters for total ammonia removal in a low salinity hatchery recirculating aquaculture system." Aquacult. Eng. Accepted

Risa, S. and H. Skjervold (1975). "Water re-use system for smolt production." Aquaculture **6**: 191-195.

Rurangwa, E. a. S., E. (2012). The relationship between fish health, water quality and microbial community in RAS. Imares Report C186/11**:** 116pp.

Rusten, B., B. Eikebrokk, et al. (2006). "Design and operations of the Kaldnes moving bed biofilm reactors." Aquacultural Engineering **34**(3): 322-331.

Rusten, B., L. J. Hem, et al. (1995). "NITRIFICATION OF MUNICIPAL WASTE-WATER IN MOVING-BED BIOFILM REACTORS." Water Environment Research **67**(1): 75-86.

Schramm, A., L. H. Larsen, et al. (1996). "Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes." Applied and Environmental Microbiology **62**(12): 4641-4647.

Tarre, S. and M. Green (2004). "High-Rate Nitrification at Low pH in Suspended- and Attached-Biomass Reactors." Appl. Environ. Microbiol. **70**(11): 6481-6487.

Terjesen, B., T. Ytrestøyl, et al. (2013). Effects of salinity and exercise on Atlantic salmon postsmolts reared in land-based recirculating aquaculture systems (RAS). 2nd Workshop on Recirculating Aquaculture Systems. Nordic RAS Net., Aalborg, Denmark, DTU.

Terjesen, B. F., S. T. Summerfelt, et al. (2013). "Design, dimensioning, and performance of a research facility for studies on the requirements of fish in RAS environments." Aquacultural Engineering **54**: 49-63.

van 't Riet, K., R. G. J. M. van der Lans, et al. (2011). 2.07 - Mixing in Bioreactor Vessels. Comprehensive Biotechnology (Second Edition). Burlington, Academic Press**:** 63-80.

Wang, R. C., X. H. Wen, et al. (2005). "Influence of carrier concentration on the performance and microbial characteristics of a suspended carrier biofilm reactor." Process Biochemistry **40**(9): 2992-3001.

Watten, B. J. and P. L. Sibrell (2006). "Comparative performance of fixed-film biological filters: Application of reactor theory." Aquacultural Engineering **34**(3): 198-213.

Zhu, S. and S. Chen (2001). "Effects of organic carbon on nitrification rate in fixed film biofilters." Aquacultural Engineering **25**(1): 1-11.

Zhu, S. and S. Chen (2001). "Impacts of Reynolds number on nitrification biofilm kinetics." Aquacultural Engineering **24**(3): 213-229.

Bergheim, A., A. Drengstig, et al. (2009). "Production of Atlantic salmon smolts in Europe--Current characteristics and future trends." Aquacultural Engineering **41**(2): 46-52.

Bernet, N., O. Sanchez, et al. (2004). "Effect of solid hold-up on nitrite accumulation in a biofilm reactor - molecular characterization of nitrifying communities." Water Science and Technology **49**(11-12): 123-130.

Bjornberg, C., Lin, W. and Zimmerman, R. (2009). "Effect of temperature on Biofilm growth dynamics and nitrification kinetics in a full-scale MBBR System." Proceedings of the

82nd Annual Water Environment Federation Technical exposition and Conference, Orlando, Florida **Water Environment Federation: Alexandria, Virginia.**

Bonvillani, P., M. P. Ferrari, et al. (2006). "Theoretical and experimental study of the effects of scale-up on mixing time for a stirred-tank bioreactor." Brazilian Journal of Chemical Engineering **23**(1): 1-7.

Bovendeur, J. (1989). Fixed biofilm reactors applied to waste water treatment and aquacultural water recirculation systems: Engineering Design and management.

Bovendeur, J., A. B. Zwaga, et al. (1990). "Fixed-biofilm reactors in aquacultural water recycle systems: effect of organic matter elimination on nitrification kinetics." Water Research **24**(2): 207-213.

Calderón, K., J. Martín-Pascual, et al. (2012). "Comparative analysis of the bacterial diversity in a lab-scale moving bed biofilm reactor (MBBR) applied to treat urban wastewater under different operational conditions." Bioresource Technology **121**(0): 119-126.

Dalsgaard, J., I. Lund, et al. (2013). "Farming different species in RAS in Nordic countries: Current status and future perspectives." Aquacultural Engineering **53**(0): 2-13.

Eding, E. H., A. Kamstra, et al. (2006). "Design and operation of nitrifying trickling filters in recirculating aquaculture: A review." Aquacultural Engineering **34**(3): 234-260.

Ferrai, M., G. Guglielmi, et al. (2010). "Modelling respirometric tests for the assessment of kinetic and stoichiometric parameters on MBBR biofilm for municipal wastewater treatment." Environmental Modelling & Software **25**(5): 626-632.

Garcia-Ochoa, F. and E. Gomez (2009). "Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview." Biotechnology Advances **27**(2): 153-176.

Gill, N. K., M. Appleton, et al. (2008). "Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up." Biotechnology and Bioengineering **100**(6): 1144-1155.

Gjaltema, A., L. Tijhuis, et al. (1995). "DETACHMENT OF BIOMASS FROM SUSPENDED NONGROWING SPHERICAL BIOFILMS IN AIRLIFT REACTORS." Biotechnology and Bioengineering **46**(3): 258-269.

Green, M., Y. Ruskol, et al. (2002). "The effect of CO2 concentration on a nitrifying chalk reactor." Water Research **36**(8): 2147-2151.

Harremoës (1978). Biofilm kinetics. New York, Wiley.

Hem, L. J., B. Rusten, et al. (1994). "Nitrification in a moving bed biofilm reactor." Water Research **28**(6): 1425-1433.

International, V. (2013). GenStat for Windows 16th edition.

Ju, L. K. and G. G. Chase (1992). "IMPROVED SCALE-UP STRATEGIES OF BIOREACTORS." Bioprocess Engineering **8**(1-2): 49-53.

Kamstra, A., J. W. van der Heul, et al. (1998). "Performance and optimisation of trickling filters on eel farms." Aquacultural Engineering **17**(3): 175-192.

Martin-Pascual, J., C. Lopez-Lopez, et al. (2012). "Comparative Kinetic Study of Carrier Type in a Moving Bed System Applied to Organic Matter Removal in Urban Wastewater Treatment." Water Air and Soil Pollution **223**(4): 1699-1712.

Martins, C. I. M., E. H. Eding, et al. (2010). "New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability." Aquacultural Engineering **43**(3): 83-93.

Nijhof, M. and J. Bovendeur (1990). "Fixed film nitrification characteristics in sea-water recirculation fish culture systems." Aquaculture **87**(2): 133-143.

Nogueira, R., L. F. Melo, et al. (2002). "Nitrifying and heterotrophic population dynamics in biofilm reactors: effects of hydraulic retention time and the presence of organic carbon." Water Research **36**(2): 469-481.

Odegaard, H. (2006). "Innovations in wastewater treatment: the moving bed biofilm process." Water Science and Technology **53**(9): 17-33.

Odegaard, H. R. B. a. T. W. (1994). "A new moving bed biofilm reactor - applications and results." Wat. Sci. Tech. **29**(10-11): 157-165.

Ohashi, A., D. G. V. deSilva, et al. (1995). "Influence of substrate C/N ratio on the structure of multi-species biofilms consisting of nitrifiers and heterotrophs." Water Science and Technology **32**(8): 75-84.

Pfeiffer, T. P. W. (2011). "Evaluation of three types of structured floating plastic media in moving bed biofilters for total ammonia removal in a low salinity hatchery recirculating aquaculture system." Aquacult. Eng. Accepted

Risa, S. and H. Skjervold (1975). "Water re-use system for smolt production." Aquaculture **6**: 191-195.

Rurangwa, E. a. S., E. (2012). The relationship between fish health, water quality and microbial community in RAS. Imares Report C186/11**:** 116pp.

Rusten, B., B. Eikebrokk, et al. (2006). "Design and operations of the Kaldnes moving bed biofilm reactors." Aquacultural Engineering **34**(3): 322-331.

Rusten, B., L. J. Hem, et al. (1995). "NITRIFICATION OF MUNICIPAL WASTE-WATER IN MOVING-BED BIOFILM REACTORS." Water Environment Research **67**(1): 75-86.

Schramm, A., L. H. Larsen, et al. (1996). "Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes." Applied and Environmental Microbiology **62**(12): 4641-4647.

Tarre, S. and M. Green (2004). "High-Rate Nitrification at Low pH in Suspended- and Attached-Biomass Reactors." Appl. Environ. Microbiol. **70**(11): 6481-6487.

Terjesen, B., T. Ytrestøyl, et al. (2013). Effects of salinity and exercise on Atlantic salmon postsmolts reared in land-based recirculating aquaculture systems (RAS). 2nd Workshop on Recirculating Aquaculture Systems. Nordic RAS Net., Aalborg, Denmark, DTU.

Terjesen, B. F., S. T. Summerfelt, et al. (2013). "Design, dimensioning, and performance of a research facility for studies on the requirements of fish in RAS environments." Aquacultural Engineering **54**: 49-63.

van 't Riet, K., R. G. J. M. van der Lans, et al. (2011). 2.07 - Mixing in Bioreactor Vessels. Comprehensive Biotechnology (Second Edition). Burlington, Academic Press**:** 63-80.

Wang, R. C., X. H. Wen, et al. (2005). "Influence of carrier concentration on the performance and microbial characteristics of a suspended carrier biofilm reactor." Process Biochemistry **40**(9): 2992-3001.

Watten, B. J. and P. L. Sibrell (2006). "Comparative performance of fixed-film biological filters: Application of reactor theory." Aquacultural Engineering **34**(3): 198-213.

Zhu, S. and S. Chen (2001). "Effects of organic carbon on nitrification rate in fixed film biofilters." Aquacultural Engineering **25**(1): 1-11.

Zhu, S. and S. Chen (2001). "Impacts of Reynolds number on nitrification biofilm kinetics." Aquacultural Engineering **24**(3): 213-229.