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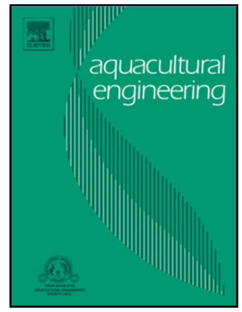


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# Mixing and scale affect moving bed biofilm reactor (MBBR) performance

Andries Kamstra[a*], Ewout Blom[a], Bendik Fyhn Terjesen[b**]

[a]Institute for Marine Resources and Ecosystem Studies (IMARES), Wageningen University and Research Centre, Yerseke, The Netherlands

[*] Present address: Kamstra Consult, Velserbroek, The Netherlands

[b]Nofima, Sjølseng, 6600 Sunndalsøra, Norway

** Corresponding author:

Bendik Fyhn Terjesen

Nofima, Sjølseng, 6600 Sunndalsøra, Norway

Email: bendik.terjesen@nofima.no

## Highlights

- * Superficial air velocity and media filling% have a strong effect on mixing time of a small scale Moving Bed Biofilm Reactor (MBBR);
- * TAN removal rate decreases below an air velocity of 5 $m\cdot^{-1}$ at both small and medium scale MBBR's;
- * Intense mixing increases TAN removal at small scale at low TAN, at high TAN oxygen likely becomes limiting;
- * TAN removal in full scale systems is significantly higher compared to that in small scale systems.


**Abstract**

Moving Bed Biofilm Reactors (MBBR) are used increasingly in closed systems for farming of fish. Scaling, i.e. design of units of increasing size, 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 biofilters and the question is to what extent this can be upscaled to a commercial level. Therefore, the objective of this research was to establish the effect of mixing and scale on MBBR performance. The research was done in two major parts; firstly effects of scale-sensitive factors were studied in small reactors. Secondly, performance of these small reactors was then compared to increasingly large reactor sizes, using the same inlet water quality and biofilm.

Firstly, a 200 L MBBR (medium scale) was operated continuously using a synthetic feed solution. Biofilm carriers from this reactor was used for short-term experiments in 0.8 L reactors (small scale) and compared with the performance of the 200 L medium scale reactor. Reactor geometry and superficial air velocity ($m\cdot h^{-1}$) were identical in these experiments. Subsequently, the small reactors were incubated with biofilm carriers from three commercial farms and performance compared with these large scale reactors. In a number of additional experiments the effect of mixing and Total Ammonia Nitrogen (TAN) was tested at small and medium scale.

The results showed that MBBR scale has a significant effect on TAN removal rate. In general, the larger the scale the better the performance. TAN removal (rTAN) at small scale (0.8 L) is about 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 importance for optimal design and dimensioning of commercial scale RAS. It was further found that superficial air velocity is not a good scaling factor for MBBRs. Upscaling while maintaining geometry implies increasing air injection depth and therefore increased energy input will be required at a comparable superficial air velocity, which is not incorporated in the superficial air velocity term ($m \cdot h^{-1}$). Superficial air velocity and media filling % were found to have a strong effect on mixing time at small scale. An air velocity below a threshold of 5 $m \cdot h^{-1}$ decreased TAN removal at both small and medium scale. Intense mixing at small scale increased TAN removal at low TAN concentration. However, at a high TAN concentration, the small scale MBBR always performed at not more than 80% of the capacity of the medium scale system, irrespective of the mixing conditions. Hence, the capacity of full scale systems will be under-estimated when based solely on small scale experiments.

**Keywords:** Moving Bed Biofilm Reactor; TAN removal; mixing; biofiltration; scale

## 1. Introduction

There is an enormous variety in type of biofilters and media used in aquaculture. In all cases a biofilm on a fixed medium (i.e. carriers) is used in order to retain enough biomass of slow growing nitrifiers. Media range from sand (e.g. Summerfelt, 2006) to a wide variety of plastic media (e.g. Timmons et al., 2006; Greiner & Timmons, 1998; Pfeiffer & Wills, 2011). Biofilters can be operated in a down-flow or up-flow mode or in a submerged versus a trickling mode (Eding et al., 2006; Malone & Pfeiffer, 2006). Moving Bed Biofilm Reactors (MBBR) are a relatively recent development (Odegaard, 1994) and are the dominant type of biofilter applied in new RAS. This patented system 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.

The advantages of this system are mainly the low pumping costs and the avoidance of clogging of the filter, by the 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 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 however, turnover is much faster, with retention times being as low as 5 minutes.

In research, preferably small scales are used to increase the number of experimental treatments, experimental units and concominant statistical power (Colt, 2006). Furthermore, in small-scale units the experimental conditons and treatments are easier to control and manipulate. In commercial situations, in contrast, large units are employed which would be uneconomical for research and are difficult to control and manipulate. The question therefore 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. To our knowledge, these questions have not been studied in a systematic way, except recently for research on salmon growth and physioloy in tanks and cages of differing sizes (Espmark et al., 2016; Føre et al., 2016). In MBBRs differences in performance caused by scale, if any, could possibly be related to the hydro-dynamics (mixing) of the reactors, and the microbial biology.

Since less work has been done on scaling of bioreactors related to aquauclture, other bioreactor research fields may offer information. Ju and Chase (1992) gave an overview of process characteristics which have been proposed as factors to keep constant during scale-up of biorectors. 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.

Nitrification in a biofilm can be adequately described by a ½-order/0-order kinetic model (Harremoës, 1978; Bovendeur et al. 1987). The removal rate in the biofilm is determined by several factors, including diffusion of $NH_3$-N and $NH_4$-N (sum of which is TAN) and oxygen into the biofilm. The concentration in the bulk liquid thereby influences the reaction rate. At low TAN concentrations, which is the case in fish farms (<1 mg $NH_4$-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 in MBBrs occurs for an oxygen to ammonium concentration ratio of about 3 g $O_2$ (g $NH_4$-N)$^{-1}$ (Hem et al., 1994). At the biofilm level, hydraulics determine the thickness of the water boundary level and the diffusion resistance (Zhu & Chen 2001; Prehn et al., 2012). It is expected that under conditions of TAN limitation, mixing becomes more important. At a high TAN concentration, transport to the biofilm and distribution within the reactor is expected to be of less importance; however, these aspects have been little studied for MBBRs used in aqaculture.

The objective of the current study was to determine the effect of mixing and scale on MBBR performance. Since dimensioning of MBBRs in aquaculture is usually based on the area for TAN removal (e.g. Bovendeur et al., 1987; Losordo & Hobbs, 2000; Rusten et al, 2006), being their prime function, this parameter was therefore used as a proxy for MBBR performance in the present study.

## 2. Material and Methods

### *2.1 Experimental design*

The experimental research on upscaling of MBBRs in the present study was focused on three different scales: 1) small scale, in this case 0.8 L, 2) medium scale, in this case 200 L, and 3) large scale (commercial scale), in this case > 20 $m^3$.

The small scale is often used in kinetic experiments to test biofilm performance (e.g. Bovendeur et al., 1990; Nijhof and Bovendeur, 1990). The medium scale used in the present study was easy to maintain in the laboratory and served as a homogenous pool for biofilter media which was 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 size 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. The influent of the reactor was taken from a common source in each experiment, while the magnitude of 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. Many water quality factors have an effect on nitrification (e.g. Chen et al. 2006 ; Eding et al. 2006) and are to a large extent eliminated when using this experimental set-up.

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

At small scale a number of variables were tested for their effects on upscaling like TAN concentration and superficial air velocity.

*2.2 Development and performance of a medium scale MBBR*

A medium scale reactor of 200 L was developed and maintained in the laboratory. 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.

An overview of the experimental system is shown in Figure 2 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 (IJmuiden waterworks, IJmuiden, Netherlands). The mineral mix contained v1200 g $NH_4Cl$, 3600 g $NaHCO_3$ and 1.7 g $KH_2P_2O_4$ per 186 L (after Zhu and Chen, 2001). The water surface of the stock solution was covered with a floating sheet to prevent evaporation of ammonia. The sodium bicarbonate that was added to the mixture was used to ensure sufficient alkalinity and pH for nitrification. 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 15 ml/ min, FWT, Ariccia, Italy). The MBBR was constructed from glass with (wet) dimensions of 59x59x59 cm. Water inflow was distributed evenly over the whole length of one side 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 2. The biofilter media used was Kaldnes K-1 with a specific surface of 500 $m^2 \cdot m^{-3}$ (manufacturer's statement, Kaldnes, Sandefjord, Norway). Most of the time the MBBR was operated at 35 media 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.

The MBBR was aerated using a Secoh EL-S 100 airpump of 92 W with a nominal capacity of 140 $l \cdot min^{-1}$. The outflow of the MBBR was connected to a sump with volume of 125 L. A submerged pump (top-3 (LA) Pedrollo, South Africa) moved the water back to the inlet of the MBBR with a flow rate of 4.1 $m^3 \cdot h^{-1}$. In the sump, make-up water was added continuously to keep the level of nitrate-N around 100 $mg \cdot L^{-1}$, a normal level for a RAS (Colt, 2006). 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. Testkits used were LCK 305/LCK 304 for TAN, LCK 341 for $NO_2$ and LCK 339 for $NO_3$.

The operational conditions (mean ± SD) during maintenance of the medium-scale MBBR were: temperature of 24.9 (0.5) °C, pH of 7.76 (0.23), TAN in the outlet of 4.6 (8.0) g $N \cdot m^{-3}$ ,TAN load of 0.85 (0.18) g $N \cdot m^{-2} \cdot d^{-1}$ , oxygen in the outlet of 6.9 (0.9), $NO_2$-N of 5.5 (8.3), $NO_3$-N of 72.4 (35.1) and a make-up-water addition of 561 (14) $L \cdot d^{-1}$.

*2.3 Small scale system and comparison with medium scale*

Three small scale reactors of 0.8 L were constructed which had the same geometry as the medium scale reactor. The wet (aerated) dimensions (LxBxH) of the reactor were 9.4x9.4x9.4 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. 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 reactors were operated parallel to the medium scale system and fed from the inlet of the medium scale system.

For each measurement on small scale, media was taken carefully from a large or medium scale reactor. An initial experiment was done to assess the effect of this handling of biofilter media on TAN removal rate in the small reactors. No significant effects of handling the biofilter media on nitrification was observed.

In order to compare small and medium scale reactors, the small scale reactors were filled with media from the medium scale system and operated in parallel to the medium scale reactor. Media for the small systems were collected in water on a volumetric basis. After the experiment the exact quantity of media was measured by weight. For each experiment, new media was collected. Measurements were started 15 minutes after stocking the small systems with media. The influent to the small reactors was taken from the inlet of the medium scale reactor using a peristaltic pump (model 520S, Watson Marlow, Falmouth, UK). 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.

The air flow to the medium size reactor was measured with the 'stopwatch bucket' method. With this method, the air was fed into a submerged bucket which was kept upside-down under conditions of identical pressure as measured manometrically.

The experiment for this part of the study included measurements at three superficial air flow rates (4, 8 and 12 m/h), at two different media filling %, and three TAN concentrations. During one experimental day, one specific airflow at one filling % was studied at three TAN concentrations with four replications. Hence, a total of six measurement days was done for this part of the study

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. 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 $g \cdot m^{-3}$ 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.

*2.4 Calculations and statistics used for small scale system and comparison with medium scale*

TAN removal rates (rTAN) were calculated for each sampling using the formula:

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

The effects of media filling%, aeration, $TAN_{in}$ 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% and aeration. This interaction was omitted to allow testing of the main effects filling% and aeration against their interaction because these factors are coupled to measurement days. Additional random effects incorporated in the model were 'measurement day' and 'periods (TAN levels) within days' in order to describe dependencies between data. The mixed model used was:

$$rTAN_{, mean} = constant+filling\%+aeration+TAN_{in}+scale+TAN_{in}.filling\%+filling\%.scale+TAN_{in}.aeration+aeration.scale+TANin.scale+measurement\ day+measurement\ day.period+residuals \quad \text{(Eq. 2)}$$

The model assumes that effects of measurement day, measurement day.period and residuals are independent and normally distributed with expectancy 0 and variancies $\sigma^2_{measurement\ day}$, $\sigma^2_{period.measurement\ day}$ and $\sigma^2_{residu}$. 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).

*2.5 Small scale versus large scale*

Performance of large and small scale MBBRs was compared at three different farms in the Netherlands. Flows of water and air over the large biofilters were estimated based on the specifications of the equipment installed and the original design (Table 2). The small-scale biofilters were operated in parallel to the large biofilter with a hydraulic retention time identical to that of the large filter. Influent for the small scale reactor was taken from the inlet of the large reactor. Aeration was also adjusted to create identical superficial air velocity in the small and large systems. In the small reactors a filling% identical to the large systems was used. A series of four samples of influent and effluent flows from all reactors was collected over a period of two hours and analysed immediately for TAN.

*2.6 Effects of mixing and TAN on small and medium scale*

In a number of additional trials the effects of individual factors were studied in more detail:

*Trial I: The effect of superficial air velocity and filling% on mixing time.*

In this experiment the mixing time in a small scale MBBR was determined according to the pH-response technique (van 't Riet et al., 2011). The mixing time, as defined by these authors (van 't Riet et al., 2011) is the time it takes for the pH to reach a value of 5% (+ or -) 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 before was used and filled with tap water and media (Kaldnes K-1). A range of superficial air velocities (2.2, 5.9, 9.3, 13.9 $m \cdot h^{-1}$) was tested at four different media filling% (0, 25, 50 and 75%). Superficial air velocity 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% above the level was calculated.

*Trial II: The effect of superficial air velocity on TAN removal in the small reactors at three different media filling% at a constant TAN influent concentration.*

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 $m \cdot h^{-1}$. The reactors were operated in a side loop from the medium scale system. Throughout the experiment a constant TAN concentration of

$0.76 \pm 0.01$ 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 superficial air velocity was reduced and the system allowed to adjust for 15 minutes.

*Trial III: The effect of superficial air velocity on TAN removal at the medium scale.*

The medium scale reactor was operated at default condition (50 filling%) at an influent TAN concentration ranging from 0.73 at the start to 0.84 g N·m$^{-3}$ at the end of the experiment. The trial was started at maximum superficial velocity 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 a little, resulting in 17 steps to zero. The system was allowed to adjust for 15 minutes after each reduction of air velocity.

*Trial IV: The effect of the TAN concentration at three different superficial air velocities in the small scale reactor and at one air velocity in the medium scale reactor.*

The small reactors were operated at 50 filling% and an superficial air velocity 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 N·m$^{-3}$ was attained in the outflow.

*Trial V: The effect of additional mixing at different TAN levels at small scale.*

The three small scale systems were operated in parallel to the medium scale system. 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 stirred by a magnetic vortex mixer (IKA® C-MAG HS 7, 1.5 W, Germany) in addition to the aeration. The TAN concentration at the inlet was kept at 0.5-

0.6 g N·$m^{-3}$ for the first 5 samplings and then in four following steps increased to 1.3 g N·$m^{-3}$. The interval between samplings was approximately 15 minutes. At the first two samplings, the additional mixing was not used.

## 3. Results

*3.1 Small versus medium scale*

Table 3 presents an overview of the data on the comparison between small and medium scale MBBRs. It was found that both TAN concentration and reactor scale had a significant effect on performance (rTAN) as well as their interaction ($P<0.05$). The effects of filling% and superficial air velocity on rTAN and all their interactions were not significant ($P>0.05$) (Table 4).

Based on the test results, predictions were calculated for the rTAN for filling%, superficial air velocity and the combinations of TAN and scale (Table 5). Based on the standard errors of the difference between the means (SED) and approaching t-tests, a significant effect of TAN on rTAN was found. Moreover, the average rTAN was significantly lower in the small scale compared to the medium scale, at both medium and high TAN level (Table 5).

*3.2 Small versus large scale*

During sampling, conditions (feed loading, flow, temperature etc) were stable at the farms. TAN concentrations in the outlet of the MBBRs were in the range of 0.1 to 0.3 g $N \cdot m^{-3}$ which was in the category low/intermediate as used in the research on comparing small and medium scale (Table 5). The results show that in all cases the mean rTAN in the small systems was 70 to 80% of that in the large systems (Table 6).  However, based on the 95% confidence intervals only the difference measured at farm 1 can be considered statistically significant.

*3.3 Trial I: effect of superficial air velocity and filling% on mixing time in small reactors*

Both air velocity and media filling% have an effect on the mixing time (Figure 3). With no media in the reactor, the mixing time is only increased at the lowest air velocity. Adding media has a strong effect on mixing time. At 25 filling%, the mixing time without media can be approached only at high air velocity. At 75 filling%, the media are not moving at all and mixing time approaches one minute. Under operational conditions, the flow of water into the reactor also improved mixing. The hydraulic retention time was about 3 minutes under all experimental conditions used in the comparison between the small and medium scale. This means that the small reactors can be considered to be well mixed.

*3.4 Trial II: Effect of superficial air velocity on TAN removal in the small reactors at three different media filling% at a constant TAN influent concentration*

There was a marked effect of filling% in the small scale reactor on TAN removal as shown in Figure 4. At 25 filling% the maximum rate is 0.85 $g \cdot m^{-2} \cdot d^{-1}$ while at 50 filling% the rate is only 0.55 $g \cdot m^{-2} \cdot d^{-1}$. Figure 4 also shows that below a superficial air velocity of 5 $m \cdot h^{-1}$ TAN removal is reduced. Above this level of aeration, TAN removal is nearly constant. This effect appeared to be independent from the filling%.

*3.5 Trial III: Effect of superficial air velocity on TAN removal at the medium scale*

In the medium scale reactor a small effect of superficial air velocity on TAN removal was found (Figure 5). Increased superficial air velocity resulted in increased TAN removal up to a velocity of app. 5 $m \cdot h^{-1}$ as in the small scale reactors. However, even without any aeration the TAN removal rate was still 75% of the maximum value.

*3.6 Trial IV: The effect of the TAN concentration at three different superficial air velocities in small scale reactors and at one superficial air velocity in medium scale reactor*

In the fourth trial, performance of the small scale systems was compared at different superficial air velocities and with the medium scale system, all above the threshold superficial air velocity of 5 $m \cdot h^{-1}$ (Figure 6). The curves in Figure 6 show a typical increase in removal rate with increasing TAN concentration which reaches a plateau at about 2 $g \cdot m^{-3}$ TAN. The results shown in Figure 6 confirm that superficial air velocity has no effect on TAN removal above the threshold. Figure 6 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 filling%).

*3.7 Trial V: The effect of additional mixing at different TAN levels at small scale*

In this trial, TAN removal rate did not differ much between the small scale systems at the first 2 sampling points. However, the removal rate in the small reactors at this time was less than in the medium scale system, at about 85% (Figure 7).

Increased stirring during sampling 3 to 5, however, resulted in a strong increase in the nitrification rate. In the system with additional (double) aeration the TAN removal rate increased from 0.46 (± 0.01) to 0.50 (± 0.01) $g\ N \cdot m^{-2} \cdot d^{-1}$, approaching that of the medium scale system. In the system with additional vortex mixing, the rate increased to 0.56 ((± 0.02) $g \cdot m^{-2} \cdot d^{-1}$. The small system with vortex mixing showed in this case an even higher TAN removal rate than the medium scale system (0.53 ± 0.01). In the rest of the experiment, an increase in the TAN concentration after sampling 5 resulted in a strong increase in TAN removal rates. However, the relative removal rate in the systems with additional mixing, compared to the medium scale system, decreased at increasing TAN levels. At a TAN level of 1.17 $g \cdot m^{-3}$ in the inlet (0.6 outlet and reactor internally) the TAN removal again became identical in all small systems.

## 4. Discussion

*4.1 Experimental set-up*

In the experimental set-up for comparing different scales used in the present study, the biofilter material was exchanged between the reactors, thereby eliminating effects of biofilm history. Furthermore, the influent water from a common source was used, giving identical loading conditions between the scales. This set-up ensured that true effects were measured and other confounding effects were avoided. Use of a synthetic substrate enables accurate manipulation of the load and improves the possibility to provide reproducible results which is not feasible with a natural substrate like fish tank effluent.

The removal rate of TAN is strongly determined by the concentration of TAN in the bulk fluid, as illustrated in Figure 6. However, differences in TAN removal rate will result in differences in the TAN concentration in the bulk fluid. It was not possible to ascertain if the reactors were completely (i.e. ideally) mixed. However, since the acid pulse tracer that was added to the small reactors achieved stable pH tracer readings much sooner than the hydraulic residence time, the reactors can therefore be considered as approaching being well mixed. A tracer study in flow-through mode would yield additional information on short-circuiting and ‘leaching’ of substrate from the reactor. However, in the present study use of tracers was not permitted by the farm managers at large scale so this approach was not used. To some extent therefore, the differences observed in performance between scales could be caused by hydraulic behaviour which cannot be distinguished from processes at biofilm level using the methods employed in the present study.

Since all the reactors used in this study can be considered well mixed, the TAN concentration in the outlet represents 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 therefore result in larger differences in TAN removal rate between scales, than demonstrated in this study. However, such an experimental set-up would require upfront knowledge of TAN removal rates which is not possible. The consequence of this is that differences between treatments are underestimated.

*4.2 Media filling%*

The effects of media filling% were studied in the multi-factorial comparison between small and medium scale, in the experiment on mixing time (trial I), and in the single factor experiment on small scale (trial II). The multi-factorial comparison between small and medium scale did show a decrease in rTAN from 35 filling% to 74% at 50 filling% (Table 5). However, this experiment  lacked the statistical power to test individual variables and therefore the difference was not significant. Figure 3 however, shows that media filling% has a strong effect on mixing time in a small reactor. Trial II demonstrated that an increase in media filling% induces a reduction of rTAN to a certain plateau irrespective of superficial air velocity (Figure 4). In this trial, all reactors were operated on the same influent concentration. Therefore, the TAN concentration in the reactor will differ according to the filling%. In the reactor with 25 filling% the TAN concentration is 0.6 g $N \cdot m^{-3}$ while that at 50 filling% is 0.5 g $N \cdot m^{-3}$. The difference in nitrification rate observed in Figure 4 is overestimated because the TAN concentration in the bulk fluid is lower at the high filling%. Therefore data on TAN removal rate at different filling% (i.e. Figure 4 data) was plotted against the actual TAN concentration in the reactor outlet at each of these filling%, and shown in Figure 8. For comparison, data of rTAN from a dose-response curve at constant filling % (50%, Trial IV, Figure 6) was also added to Figure 8. The figure now indicates that the differences in TAN removal rate of Figure 4 at different filling % were partly caused by small differences in TAN concentration. However, compared to the dose-response curve at constant filling% from Figure 6 which has also been plotted in Figure 8, it is suggested that an increase in filling% has a negative effect on TAN removal. Compared to the standard curve shown in Figure 6, the TAN removal is 91%, 80% and 71% at 25, 35 and 50 filling% respectively.

Effects of filling% of MBBRs on nitrification have been studied by several authors (Gjaltema et al., 1995; Calderón et al., 2012). Significant effects have been demonstrated 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 the biofilm level. Probably, a higher media filling% results in decreasing turbulence at the boundary level of the biofilm which subsequently increases the diffusional resistance for the TAN substrate. Zhu and Chen (2001) demonstrated the effect of flow rates expressed as Reynolds number on nitrification rate and presented a theoretical framework. Prehn et al. (2012) demonstrated that flow velocity in a submerged biofilter decreased the diffusion boundary layer (DBL) of the biofilm and increased TAN removal. A problem when working with moving biofilm media is that the actual water velocity 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 velocity in the same direction, diffusional resistance would be very high and nitrification rate low.

In conclusion, the present study indicates that when increasing the filling% in a small-scale MBBR, although it increases the total capacity of the reactor, it makes the unit process less effective since the higher filling% reduces the TAN areal-specific removal rate.

*4.3 Superficial air velocity and mixing*

In all experiments superficial air velocity was taken into account since this and turbulence is an important determinant for upscaling and efficiency of bioreactors (Ju and Chase, 1992; Zhu and Chen, 2001). The mixing time at small scale was strongly affected by superficial air velocity as shown in Figure 3. This Figure shows only the data points where the media was actually moving. In a flow-through situation mixing would be more intense by the added energy of the water inflow. Visually, movement of media was, however,not much changed by water velocity at superficial air flows above 6 $m \cdot h^{-1}$. Experiments (Figure 4 and 5) on both small and medium scale demonstrate that there seems to be a superficial air velocity threshold of approximately 5 $m \cdot h^{-1}$ at which rTAN reaches a plateau. Above this threshold, mixing time will still be reduced with an increase in superficial air velocity but this has no clear effect on rTAN. However, when we increased mixing intensity far above 12 $m \cdot h^{-1}$, as shown in Figure 7, rTAN increased again, provided that TAN was at low concentrations. This observation can again be attributed to the effect of turbulence on the thickness of the biofim boundary water layer. Zhu & Chen, (2001) and Prehn et al. (2012) have demonstrated the effect of turbulence on TAN diffusion into the biofilm. In the comparison between small and medium scale, superficial air velocity and its interactions had no significant effects on rTAN (Table 4).

Since rTAN is lower in small MBBR if not sufficiently mixed, we propose that superficial air velocity can not be considered a good scaling factor for TAN removal in MBBRs for aquaculture. Superficial air velocity does not taken into account the depth at which the air is injected. In upscaling, when keeping geometrical relationships constant, the depth of air injection and therefore water pressure at this point will increase. At identical superficial air velocities, the required mixing power and energy input will increase linearly with depth. In the small scale system and the medium scale system in the present study, the depth of air injection was 9 and 50 cm, respectively. This would mean that the energy input in the medium scale system was five-fold higher compared to the small scale system.

We propose that a better scaling factor for aquaculture MBBR's would be:

$$V' = F \cdot P / A \qquad \text{(Eq. 3)}$$

in which,

V' normalised aeration — $m^3 \cdot h^{-1} \cdot N \cdot m^{-2} \cdot m^{-2}$ or reduced as $N \cdot h^{-1}.m^{-1}$

F: reactor air flow — $m^3 \cdot h^{-1}$

P: air pressure — $N \cdot m^{-2}$

A: reactor top area (footprint) — $m^2$

*4.4 Effects of TAN concentration*

The removal rate of TAN was highly dependent on the concentration of TAN in the bulk fluid (Figure 6). Since TAN removal rate was chosen as the read-out parameter for nitrification, effects of TAN concentration on scaling have to be taken into account. As would be expected from several studies (e.g. Chen et al., 2006), the effect of TAN is highly significant in the multi-factorial comparison between small and medium scale.

Table 4 and 5 show that there is a significant interaction between $TAN_{in}$ and scale. At low $TAN_{in}$ (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 was somewhat 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. Possibly, oxygen diffusion into the biofilm becomes the limiting factor at high TAN. 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 $NH_4$-N)$^{-1}$(Hem et al., 1994) in research on MBBRs. Assuming this ratio also applies in our research, the data suggest that oxygen becomes limiting at a TAN (out) of about 2 $g \cdot m^{-3}$. At the highest dose in the dose-response experiment (Figure 6) a Δ TAN of 0.7 $g \cdot m^{-3}$ is measured over the reactor implicating an oxygen consumption of 3.2 $g \cdot m^{-3}$. This consumption will only be partly covered by aeration of the reactor.

The dose-response curve (Figure 6) between $TAN_{out}$ 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 $TAN_{in}$ or $TAN_{out}$.

The comparison of small scale and large scale systems was done at TAN levels in the outflow varying from 0.11 to 0.27 $g \cdot m^{-3}$. 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 $TAN_{out}$.

*4.5 Effects of scale on TAN removal*

The comparison between small and medium scale reactors showed that scale is a significant factor (Table  4, 5, Figure 6). 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 6). However, direct measurement of the flow in the large scale systems proved to be difficult by the configuration of the piping. The pumping systems in the farms were well designed and maintained and estimated flows have therefore been used. Figure 7 shows that using a different mixing system at small scale, the gap between scales could probably be closed, but only under some circumstances. It is difficult to predict the differences between scales at higher TAN concentrations since TAN cannot be manipulated easily under farm conditions. In this study we have looked at TAN removal since biofilter sizing is based on this. It is however possible that nitrite removal and nitrate production rates correlate differently with scale compared to TAN removal.

Superficial air velocity has been used as a scaling factor. It is proposed 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 velocity, the mixing effect strongly increases with scale at a constant superficial air velocity. Therefore, full scale systems are much more turbulent than small scale systems at the same superficial air velocity.

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.

*4.6 Future research*

The findings in the present study warrant further experiments on whether a plateau is reached in rTAN at a certain scale. It will have considerable interest for commercial scale RAS dimensioning to know whether TAN removal rate increases with larger bioreactors or if the removal rate levels off with size. Further, the finding that the effect of scale was more pronouced at high TAN concentrations was unexpected, and a satisfactory explanation is lacking. It would therefore be interesting to study the role of oxygen at high TAN, and other potentially limiting factors. Another aspect for future studies was the finding that without aeration TAN removal was still maintained at 75% of the rate in the medium scale reactor (Figure 5). One of the great benefits of MBBRs in aquaculture is the use of carrier mixing to maintain a thin biofilm and prevention of clogging. However, given the relatively high TAN removal without aeration seen in the present study, it can be hypothesized that the benefits of

MBBRs can also be achieved with intermittent aeration, and this hypothesis could be tested in future studies. Management of such a biofilter could be similar to that of a bead filter. Potentially, this could result in energy savings in operating MBBRs in aquaculture. Moreover, the shed biofilm could be captured easily with intermittent aeration while continuous aeration requires addional treatment to remove the fine solids produced. The hydraulic load to a (temporary) fixed bed would be an important determinant for nitrification rate and solids capture and would be worthwhile studying.

## 5. Conclusions

MBBR scale can have 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) was about 80% compared to that in medium scale reactors (200 L). The difference between small scale and large scale (>20 $m^3$) is even higher. Superficial air velocity is not an adequate scaling factor for aquaculture MBBRs. Upscaling while maintaining geometry implies increasing air injection depth and therefore increased energy input at comparable superficial air velocity. Superficial air velocity and media filling% had a strong effect on mixing time at small scale. A superficial air velocity below a threshold of 5 $m \cdot h^{-1}$ decreased TAN removal at both small and medium scale. In one experiment, intense mixing using a vortex mixer at small scale increased TAN removal at low TAN concentration. At a high TAN concentration, the small scale MBBR always performed at app. 80% of the capacity of the medium scale system irrespective of the mixing conditions. Possibly, oxygen diffusion into the biofilm becomes limiting at high TAN. The study suggests that the capacity of full scale systems will be under-estimated when based solely on small scale experiments.

## 6. Acknowledgements

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**Figure legends Kamstra et al. 2017.**

**Figure 1.** Pictures of the small scale (a), the medium scale (b) and a large scale system (c).

**Figure 2.** A schematic overview of the experimental system with the medium scale MBBR. The MBBR was constructed from glass with (wet) dimensions of 59x59x59 cm, and total wet volume at 50 filling% was 207 L. The stock solution was dosed to the outflow of the MBBR using a dosing pump at 15 ml/ min. Water inflow was distributed evenly over the whole length of one side by an overflow box. The outlet was situated as an overflow on the whole length of the opposite side and screened, and further connected to a sump with volume of 125 L. The sump also contained an overflow to the sewer. A submerged pump moved the water back to the inlet of the MBBR with a flow rate of 4.1 $m^3 \cdot h^{-1}$. The MBBR was aerated on the bottom below the outlet side (nominal 140 $l \cdot min^{-1}$).

**Figure 3**. The relationship between superficial air flow in a range of 2 to 15 $m \cdot h^{-1}$ and mixing time at four different media filling% (0, 25, 50, 75) in small scale reactors (0.8 L).

**Figure 4.** The effect of superficial air velocity on TAN removal in the small reactors at three levels of media filling%. The TAN concentration in the influent was 0.76 $g \cdot m^{-3}$. At maximum air velocity the TAN concentration in the effluent was 0.59, 0.52 and 0.48 $g \cdot m^{-3}$ for a filling% of 25, 35 and 50 respectively.

**Figure 5.** The effect of superficial air velocity in a range of 0 to15 $m \cdot h^{-1}$ on TAN removal in the medium scale reactor (200 L).

**Figure 6.** The effect of TAN concentration in a range up to 2 $g \cdot m^{-3}$ (outlet) on TAN removal rate in small scale reactors (0.8 L) operated at different superficial air velocity (6.8, 10.2 and 13.6 $m \cdot h^{-1}$) and in medium scale (200 L) at 12 $m \cdot h^{-1}$.

**Figure 7.** The relative TAN removal rate in a medium scale reactor (200 L) and three different small scale reactors (0.8 L) over time. At sampling 1 and 2 the small scale reactors were operated identically. Starting at sampling 3, additional aeration was applied in two reactors; one reactor using 12 $m \cdot h^{-1}$ aeration (small basic), and one reactor with double aeration (small with aeration). The third reactor was stirred by a magnetic vortex mixer (IKA® C-MAG HS 7, 1.5 W, Germany) in addition to the aeration (small with stirring). The TAN concentration in the inlet was gradually increased from 0.5 g $N \cdot m^{-3}$ to 1.3 g $N \cdot m^{-3}$ starting after sampling 5.

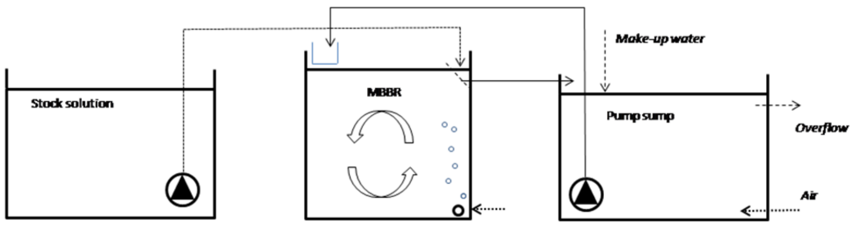
**Figure 8.** The effect of $TAN_{out}$ concentration on TAN removal for three small scale reactors operated at different media filling% (i.e. Fig. 4 data, plotted against bulk TAN concentration). In addition, rTAN data from a dose-response curve in which filling% was constant (50%, Trial IV), is also included. Hence, for the TAN removal rate data at different filling% the reduction in TAN concentration was induced by the filling% itself. In contrast, for the data of rTAN from the dose-response curve at constant filling % (crosses and stippled line), the reduction in TAN concentration was only due to composition changes made to the inlet flow.

Figr-1

Fig. 1 Kamstra et al. 2017

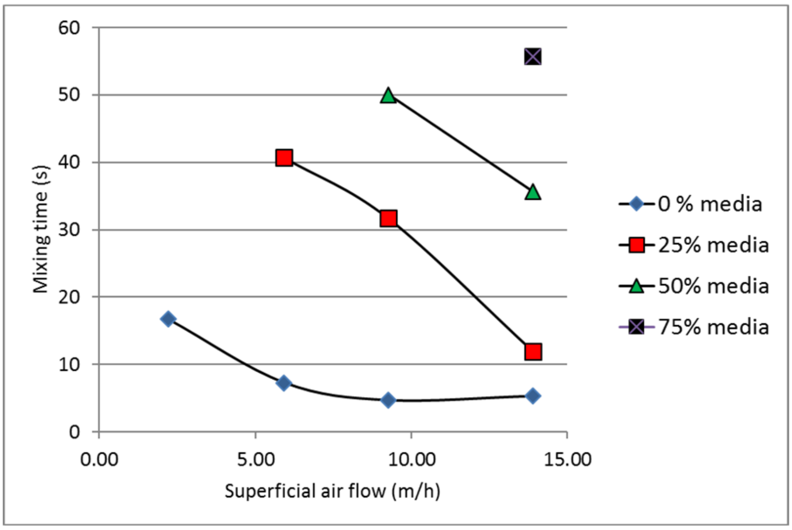
Figr-2

Fig. 2 Kamstra et al. 2017



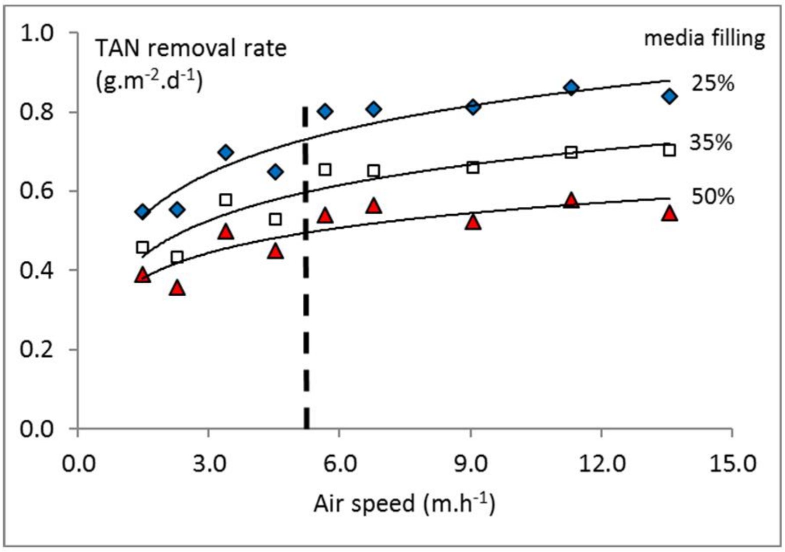
Figr-3

Fig. 3 Kamstra et al. 2017



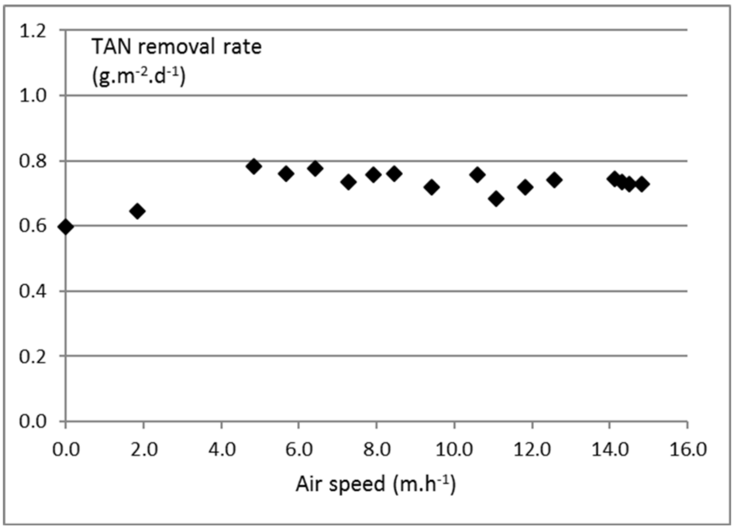
Figr-4

Fig. 4 Kamstra et al. 2017



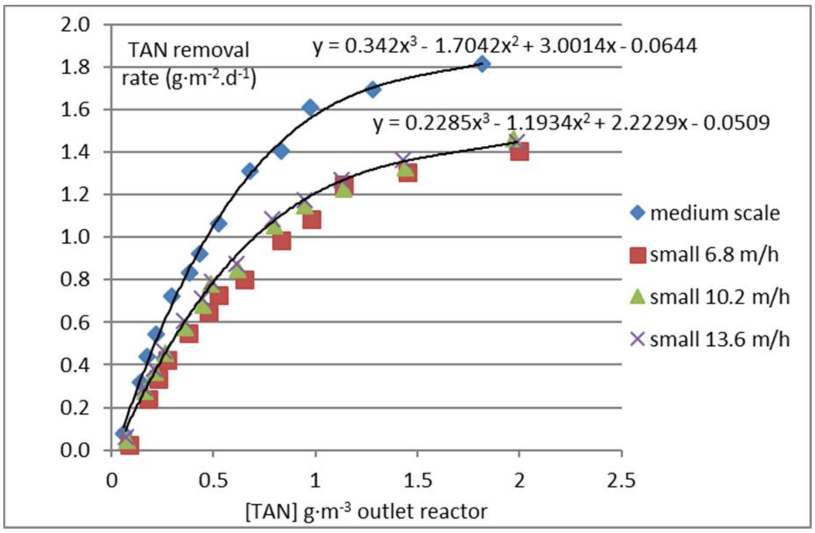
Figr-5

Fig. 5 Kamstra et al. 2017



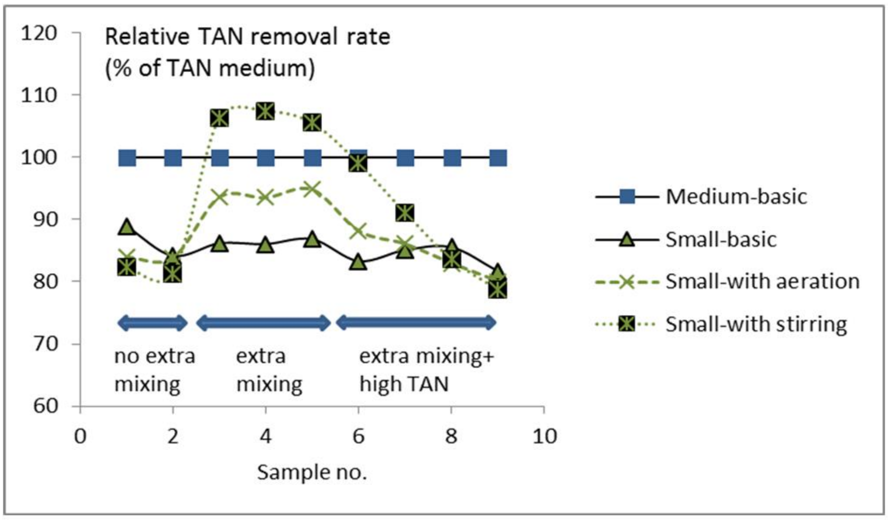
Figr-6

Fig. 6 Kamstra et al. 2017



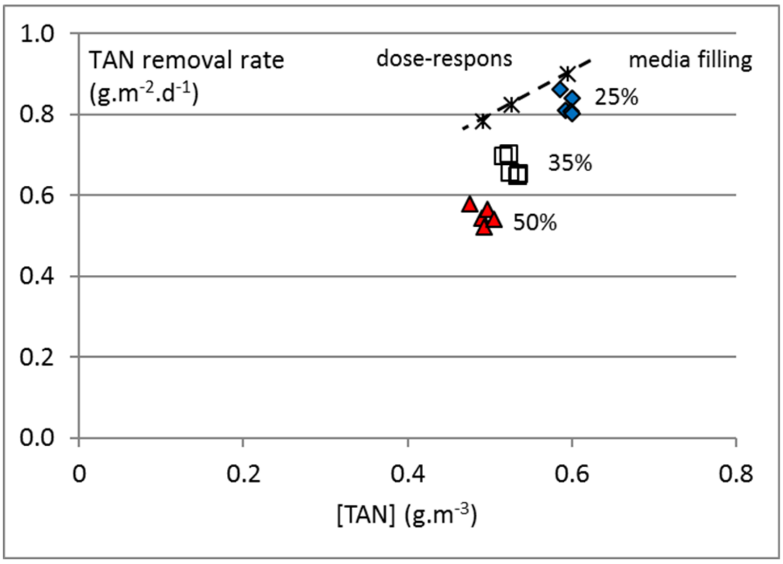
Figr-7

Fig. 7 Kamstra et al. 2017



Figr-8

Fig. 8 Kamstra et al. 2017



**Tables Kamstra et al. 2017**

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

| ***Parameters*** | ***Value*** | ***Unit*** |
|---|---|---|
| Make-up-water | 562 | $L \cdot d^{-1}$ |
| MBBR | 207 | L |
| Hydraulic load | 20.3 | $m^3 \cdot m^{-3} \cdot h^{-1}$ |
| Retention time | 3.0 | min. |
| Aeration | 4062 | $L \cdot h^{-1}$ |
| Superficial air velocity | 12.1 | $m \cdot h^{-1}$ |

**Tables Kamstra et al. 2017**

**Table 2.** 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 ($m^3$) | 20 | 280 | 20 |
| lxbxh | Ø2,2 x 2,5 | Ø6,9 x 3,6 | Ø3,5 x 2 |
| Volume media ($m^3$) | 10 | 120 | 10 |
| Filling% bioreactor | 50 | 43 | 50 |
| Type media | Kaldnes K1 | Curler X-1 | Kaldnes K1 |
| SSA media ($m^2 \cdot m^{-3}$) | 500 | 800 | 500 |
| Air flow* ($m^3 \cdot h^{-1}$) | 80 | 1000 | 70 |
| V air ($m^3 \cdot h^{-1} \cdot m^{-2}$; $m \cdot h^{-1}$) | 10.5 | 13.4 | 8.3 |
| Water flow* ($m^3 \cdot h^{-1}$) | 500 | 2500 | 450 |
| Retention time water (min.) | 2 | 7 | 3 |
| Feeding level ($kg \cdot day^{-1}$) | 80 | 1100 | 80 |

* Based on the specifications of the equipment installed and the original design.

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**Table 3**. Mean treatment values for rTAN ($g \cdot m^{-2} \cdot d^{-1}$) for all combinations between filling%, superficial air velocity, TANin level and scale

| | | | | TAN_in[2] | |
|---|---|---|---|---|---|
| Filling % | Superficial air speed[1] | 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 |

[1]Air velocities used were: 4, 8 and 12 $m \cdot h^{-1}$ (low, medium high).
[2]The TANin levels applied were 0.4, 0.8 and >2 $g \cdot m^{-3}$, for low, medium and high, respectiviely

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**Table 4.** Calculated F and P values ($F_{prob}$) 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 | 0.191 |
| Superficial ir velocity | 1.74 | 2 | 0.87 | 2 | 0.535 |
| TAN_in | 64.87 | 2 | 32.44 | 4 | 0.003 |
| Scale | 51.73 | 1 | 51.73 | 12 | <0.001 |
| tan_in.filling% | 4.55 | 2 | 2.27 | 4 | 0.219 |
| Filling%.scale | 2.42 | 1 | 2.42 | 12 | 0.146 |
| tan_in.air velocity | 2.43 | 4 | 0.61 | 4 | 0.679 |
| Superficial air velocity.scale | 0.83 | 2 | 0.42 | 12 | 0.669 |
| tan_in.scale | 33.23 | 2 | 16.62 | 12 | <0.001 |

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

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**Table 5**. Overall average rTAN (g·m$^{-2}$·d$^{-1}$) calculated from data in Table 3 based on filling%, Superficial air velocity and the interaction between TAN and scale. Superficial air velocities used were: 4, 8 and 12 m·h$^{-1}$ (low, medium high). The TANin levels applied were 0.4, 0.8 and >2 g·m$^{-3}$.

| **Filling%** | **35** | **50** | | **SED** |
|---|---|---|---|---|
| rTAN | 0.97 | 0.72 | | 0.13 |

| **Superficial a ir velocity** | **Low** | **Medium** | **High** | **SED** |
|---|---|---|---|---|
| rTAN | 0.75 | 0.96 | 0.83 | 0.159 |

Interaction $TAN_{in}$ and scale

| **TAN/Scale** | **Small scale** | **Medium scale** | **SED** |
|---|---|---|---|
| Low | 0.35 | 0.37 | 0.037 |
| Medium | 0.61 | 0.74 | |
| High | 1.34 | 1.66 | |

**Tables Kamstra et al. 2017**

**Table 6.** Mean rTAN, SED and 95% confidence interval for the comparison between large and small scale MBBRs at three farms. The small scale was used in triplicate at each farm while four repeated measurements were made with each small and farm scale biofilter.

| Farm no. | Mean rTAN ($g \cdot m^{-2} \cdot d^{-1}$) | | Difference large-small | SED | 95% confidence interval | | Ratio S:L | $TAN_{out}$ large ($g \cdot m^{-3}$) | $TAN_{out}$ small ($g \cdot m^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|
| | 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 |

* significant difference ($P<0.05$)