

# Environmentally friendly use of woodchip bioreactors in aquaculture

Mathis von Ahnen, Kaare Michelsen and Per Bovbjerg Pedersen

DTU Aqua Report no. 383-2021



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By Mathis von Ahnen<sup>1</sup>, Kaare Michelsen<sup>2</sup> and Per Bovbjerg Pedersen<sup>1</sup>

<sup>1</sup>DTU Aqua, Technical University of Denmark

<sup>2</sup>Dansk Akvakultur

DTU Aqua Report no. 383-2021

In collaboration with

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## Colophon

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# Foreword

This is the final report for the project "Environmentally friendly use of woodchip bioreactors in aquaculture" (In Danish: "Miljøvenlig brug af træflisfiltre på dambrug") under the scheme "Fælles indsatser Akvakultur EHFF 2017" and is a collaboration between DTU Aqua and the Danish Aquaculture Association (Dansk Akvakultur).

The first few denitrifying woodchip bioreactors have recently been installed at commercial Danish recirculated trout farms to reduce the discharge of nitrates into the natural environment. Woodchip bioreactors have so far proven to be a reliable method for stable, year-round nitrate removal from aquaculture effluents. However, environmentally friendly bioreactor start-up can be challenging due to dissolved organic matter leaching from fresh woodchips. Moreover, treatment performance regarding several other water pollutants remained unknown so far.

The objective of the project has been to 1) develop a method to reduce the environmental impact of short-term dissolved organic matter leached from newly installed end-of-pipe denitrifying woodchip bioreactors during start-up and to 2) assess the capacity of woodchips bioreactors to remove residual disinfectants (formalin and peracetic acid) from aquaculture effluents in laboratory and field trials.

The project was completed during the period 04-12-2017 to 07-12-2020 by DTU Aqua with participation from the Danish Aquaculture Association.

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Inquiries regarding this report can be made to:

DTU Aqua

Kemitorvet

DK-2800 Ks. Lyngby

Tel: +45 52 58 88 92

Email: [mvah@aqua.dtu.dk](mailto:mvah@aqua.dtu.dk)

[www.aqua.dtu.dk/english/research/aquaculture](http://www.aqua.dtu.dk/english/research/aquaculture)

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Lyngby, March 2021

Project leader

Mathis von Ahnen

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# Executive Summary

The project "Environmentally friendly use of woodchip bioreactors in aquaculture" aimed to 1) reduce the environmental impact of dissolved organic matter leached from woodchips during bioreactors start-up as well as to 2) assess the capacity of woodchip bioreactors to remove commonly used disinfectants, formaldehyde and peracetic acid, from aquaculture effluents.

In work package 1, a laboratory study showed that recirculating water between a woodchip bioreactor and a protein skimmer removed 38% of the dissolved organic matter, measured as dissolved chemical oxygen demand (COD) that leached from woodchips during the first 11 days after bioreactor start-up. Recirculation through protein skimmers can thus be applied as a technically simple method to reduce the environmental impact during bioreactor start-up with removal rates of around 75 g dissolved COD per m<sup>3</sup> woodchips achieved in a laboratory study.

In work package 2, laboratory and field studies have shown that woodchip bioreactors effectively remove formaldehyde (FA), the active substance in Formalin. Removal was due to an instantaneous adsorption (52 g FA/m<sup>3</sup> woodchips) followed by microbial degradation. Concentration independent (0<sup>th</sup> order) removal was 20.5 – 77.1 g FA/m<sup>3</sup> woodchips/d at temperatures of 7-23 C degrees. Microbial removal was dependent on temperature, which could be described with a Q<sub>10</sub> value of 2.27 and an Arrhenius temperature coefficient of 1.086. Removal rates became limited by FA concentration at FA concentrations lower than 11-15 mg FA/l with first order rate constants of 1.8 – 5.2 (1/d) at temperatures of 7-23 C degrees. Removal efficiencies were high, up to 99.8 %, and increased with reduced FA inlet concentration and increased hydraulic retention times in the bioreactor. Removal of formalin lead to an increase in nitrate removal in woodchip bioreactors, indicating that FA was used as a carbon-/energy source for denitrification.

A laboratory study demonstrated that woodchip bioreactors achieved complete removal of peracetic acid (PAA) at inlet concentrations of 50 mg PAA/l, which were much higher than concentration of 1-3 mg PAA/L typically applied in aquaculture. Removal rates of up to 1146 g PAA/m<sup>3</sup> woodchips were achieved at inlet concentrations of 175 mg PAA/l and a hydraulic retention time of 3.4 hours. Woodchip bioreactors continued to remove nitrate even when nitrate inlet concentrations were as high as 350 mg PAA/l.

The high treatment efficiencies for FA and PAA observed in this study were based on the large organic surface areas, long retention times and active microbial communities present in woodchip bioreactors, a combination of factors which may have the potential to promote effective removal of other types of disinfectants as well as antibiotics, too, which deserves further investigations.

# Descriptions and outcomes of work packages

*By Assistant Professor Mathis von Ahnen (DTU Aqua), Senior Scientist Lars-Flemming Pedersen (DTU Aqua), Chief Consultant Kaare Michelsen (Dansk Akvakultur) and Head of Section Per Bovbjerg Pedersen (DTU Aqua)*

In the following, the two main work packages of the project will be described including the background that motivated this work, the experimental approaches used as well as the obtained results and conclusions.

## 1. Developing methods to reduce the environmental impact of end-of-pipe woodchip bioreactors during start-up (work package 1)

*With contributions from Adam Hambly and Colin Stedmon*

### 1.1 Background

Woodchip bioreactors are able to achieve continuous nitrate removal over several years from tile drainage and aquaculture effluents (Schipper et al., 2010; Christianson et al., 2012; Lepine et al., 2016, von Ahnen et al., 2018). It is well documented that, as a negative side effect, leaching of DOM from woodchips occurs during the first year after bioreactor installation with a high initial release of dissolved organic matter (DOM) followed by a rapid decline (Robertson and Cherry, 1995; Schipper et al., 2010; von Ahnen et al., 2016; 2018). Suggested control measures to reduce DOM and nutrient wash-out during start-up include installing post-bioreactor treatment (e.g. sand filter), collecting the initial effluent for disposal elsewhere (e.g. for irrigation in agriculture), and pre-leaching woodchips prior to use (Schipper et al., 2010). However, more practical solutions still need to be found and properly evaluated, as the aquaculture industry is growing rapidly and new woodchip bioreactors are being installed as end-of-pipe treatment for RAS effluents.

Fresh wood contains 1-2 wt% soluble organic constituents (Vogan, 1993), which, when leached into the aquatic environment, can impair water quality. The leachate: lowers pH; contains compounds such as phenols, tannins, lignin, resin acids and terpenes, which can be toxic at high concentrations; and can lead to higher biological oxygen demand in recipient (Taylor et al., 1996; Taylor and Carmichael, 2003; Tao et al., 2005; Hedmark and Scholz, 2008). Lumber yard runoff has also been shown to exhibit acute toxicity to rainbow trout (*Oncorhynchus mykiss*) (Bailey et al., 1999). Furthermore, the leached tannins, lignins and other phenolic substances may cause discoloration of the receiving water bodies, increasing its absorbance and thus decreasing light penetration for photosynthesis in (Kritzberg et al., 2014; Svensson et al., 2014).

DOM leached from woodchip bioreactors typically consists of compounds with low degradability with a BOD<sub>5</sub>/COD ratio of around 0.2 (Tao et al., 2005; von Ahnen et al., 2016; 2018). A number

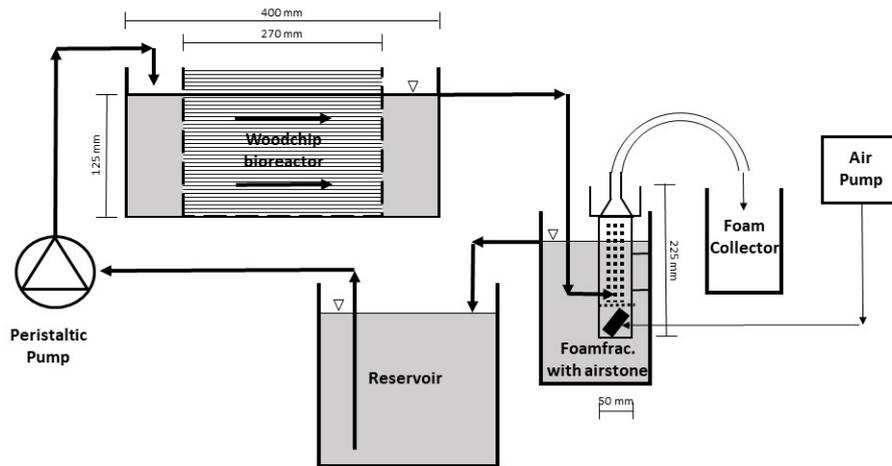
of studies have recently investigated the effect of different bioreactor set-ups and carrier materials on bulk carbon concentrations. For example, both, Feyereisen et al. (2017) and Christianson et al. (2018) ran laboratory set-ups to test the performance of anoxic post-bioreactor chambers filled with inert plastic carrier but found no significant organic matter removal in the post-treatment. These results highlight the low biodegradability of the leached DOM, and as such, removal via physical-chemical treatment appears to be more appropriate than aerobic or anaerobic biological treatment (Forgie, 1988). Abusallout and Hua (2017) were able to show that a recycled steel chip filter removed on average 44.2% of the DOC leached from a laboratory woodchip bioreactor setup, however ozonation has shown to be more effective than biological treatment alone for removing DOM from woodchip filter effluent. For example, in a laboratory study by Svennson et al. (2015), 1.5l of oak wood leachate was exposed to ozone at specific ozone doses between 0.7-7g/l O<sub>3</sub>/g of initial COD removing >90% of polyphenols and up to 73% of COD, 61% of TOC and 97% of colour. A positive correlation between biodegradation and ozone pre-treatment was found. In practice, farm operators would require more technical knowledge and management, as its use entails an inherent risk of overdosing which can cause unwanted, excessive degradation of the woodchips. Though these approaches may be effective, their practical application to fish farms may be limited due to the additional costs and required technical knowledge involved.

A common observation during woodchip bioreactor start-up is the formation of foam in the bioreactor outlet, which may be an indication of the nonpolar/hydrophobic nature of some of the surface-active, DOM compounds (e.g. humic substances, fatty acids, lipids and proteins) leached from the woodchips. Abusallout and Hua (2017) found that the DOC released from woodchips were dominated by hydrophobic and high MW (>10KDa) carbon compounds. The hydrophobic fraction of DOC leached from woodchips may be particularly high during the initial leaching phase as it was shown to decrease from 72.2% to 58% between day 1 and day 240 in a laboratory-scale woodchip bioreactor leachate study (Abusallout and Hua, 2017). This indicates there is strong potential to remove large amounts of the initially leached dissolved organics by utilising foam fractionation. Since hydrophobic natural organic matter is typically rich in aromatic carbon and UV-absorbing compounds (Hua and Reckhow, 2007), we hypothesised that the fate and change in character of leached DOM could be followed using fluorescence excitation-emission matrix (EEM) spectroscopy - a method which has previously been applied to characterise dissolved organic matter in recirculating aquaculture system (RAS) water (Hambly et al., 2015). The objectives of work package 1 were to: (1) ascertain whether a fraction of the DOM released from woodchips after bioreactor start-up, could be removed via continuous recirculation of the water between the woodchip bioreactor and a foam fractionator in a closed system; and (2) Investigate the fate, and change in quantity and quality of the DOM within the entire system, using fluorescence EEM spectroscopy.

## **1.2 Materials & Methods**

### **1.2.1 Experimental set-up**

The experimental setup (figure 1.1; n=3,) consisted of laboratory-scale, horizontal-flow woodchip bioreactors (described in von Ahnen et al., 2016) recirculating water through aquarium foam fractionators (plastic, h: 22.5cm, w: 5cm, MAGT, Wuhan, China).



**Figure 1.1. Schematic of the experimental setup (n=3) consisting primarily of a woodchip bioreactor and reservoir recirculated through a foam fractionator.**

The woodchip bioreactors were filled with willow woodchips (*Salix viminalis*, NyVrå, Denmark) (volume of submerged woodchips: 3l) which had a total porosity of  $66 \pm 2$  % measured according to Christianson et al. (2010). The effluent water from the woodchip bioreactor overflowed into the foam fractionator. The simple aquarium foam fractionators consisted of a tube (W: 5cm H: 22.5cm) with a wooden air stone (Sander, Germany) in the bottom, receiving 2l/min of atmospheric air (HiBlow HP40, Techno Takatsuki CO., LTD., Japan). The top of the tube was connected to a hose (inner diameter: 19mm) with a 180 degree bend (w: 30cm, h: 15cm) directing the foam into a 1l plastic collector. The foam fractionator was located in a smaller reservoir with a total water volume of (3.1 l). Water overflowed from the smaller reservoir with foam fractionator into a larger reservoir (volume: 7l) from where the water was pumped continuously into the woodchip bioreactors using peristaltic pumps (BT 100-2 J, pump heads YZ1515X, Longer Pumps, Langer Instruments Corp., New Jersey, USA). Water was recycled at a rate of 0.6l/h, achieving an HRT of 3.3 hrs and an empty bed contact time of 5 hrs in the woodchips bioreactors.

## 1.2.2 Sample collection and analyses

The experiment was carried out over 11 days, during which grab samples were taken from the larger reservoirs after 24 hours and subsequently on every second day at identical times. Additionally, at each sampling time, the volume of the liquefied foam was noted down. Oxygen, pH, and temperature were measured daily within the reservoir (bioreactor inlet water) using Hach Lange HQ40 multimeters (Düsseldorf, Germany).

Organic matter was analyzed using EEM spectroscopy and water samples were analysed for:

- total ammonia nitrogen (TAN; DS224, 1975)
- total dissolved nitrogen  $N_{total}$  (ISO 7890-1, 1986; ISO 11905-1, 1997)
- nitrite-N
- nitrate-N
- orthophosphate ( $PO_4$ -P)
- sulfate ( $SO_4$ -S)
- total and dissolved  $BOD_5$
- total and dissolved COD

## 1.3 Results

### 1.3.1 System performance

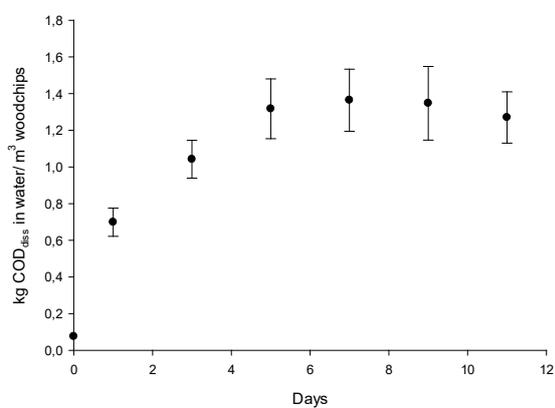
pH decreased in the first two days from  $7.01 \pm 0.02$  to  $6.52 \pm 0.09$ , thereafter rising steadily to reach  $7.98 \pm 0.08$  ( $n=3$ ) by the end of the trial (11 days) (figure 1.2g). Mean DO at the woodchip bioreactor inlet, after the foam fractionation, was  $6.65 \pm 1.08$  mgO<sub>2</sub>/l ( $n=36$ ) throughout the experimental period, whereas DO concentrations at the woodchip bioreactor outlet were much lower,  $0.54 \pm 0.21$  mgO<sub>2</sub>/l ( $n=36$ ) (figure 1.2g). Water temperature in the system was  $18.6 \pm 1.0$  °C ( $n=36$ ). Collection of foam by foam fractionators led to an average total water volume loss of  $27.2 \pm 4.3\%$  ( $n=3$ ) in the replicated experimental systems after 11 days.

### 1.3.2 Chemical Oxygen Demand

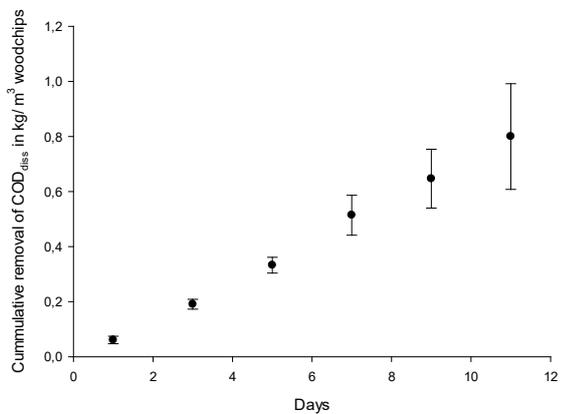
COD<sub>diss</sub> in the water of the experimental setup increased over time reaching concentrations of up to 474 mg/l ( $1.59$  gCOD<sub>diss</sub>/m<sup>3</sup> woodchips) (figure 1.2a). The initial fast increase in COD concentration levelled off over time reaching stable levels by the end of the trial (figure 1.2a). This may be due to leaching of COD<sub>diss</sub> consisting of both the instant, short-term release of pre-existing dissolved organic substances in substrate, as well as dissolved COD originating from degradation of insoluble organic carbon, which then generates further dissolved organic carbon (McLaughlan and Al-Mashaqbeh, 2009. In a pilot-scale woodchip bioreactor operated at a commercial recirculated trout farm and filled with the same type of wood as in the current study, von Ahnen et al. (2016) observed a similar high initial release of COD lasting for about five days. Dissolved COD represented  $98 \pm 4\%$  of the total COD ( $n=18$ ) in the recirculating water as the end of the experiment.

The foam fractionation process removed COD<sub>diss</sub> at a constant rate of  $75 \pm 18$  g COD<sub>diss</sub>/m<sup>3</sup> woodchips/d ( $n=3$ ). The mean COD<sub>diss</sub> concentration in the liquefied foam was  $2.9 \pm 2.2$  ( $n=18$ ) times higher than in recirculating water. This is consistent with Mills et al. (1996), who analysed aquatic foam and stream water samples from two natural streams. Their study observed that the foam had a 10 to 20 fold higher DOC concentration than the stream water, and was particularly enriched in humic substances (90% by weight of DOC, compared to 55-81% in the stream). COD<sub>diss</sub> constituted on average  $90 \pm 3\%$  ( $n=18$ ) of the total COD in the liquefied foam. Foam fractionators removed on average  $11.6 \pm 3.4\%$  ( $n=15$ ) of the mass of COD<sub>diss</sub> present in the water during 48h. The total amount of COD<sub>diss</sub> removed by foam fractionators amounted to  $37.8 \pm 4.7\%$  ( $n=3$ ) of the COD<sub>diss</sub> recovered from the water and foam after 11 days.

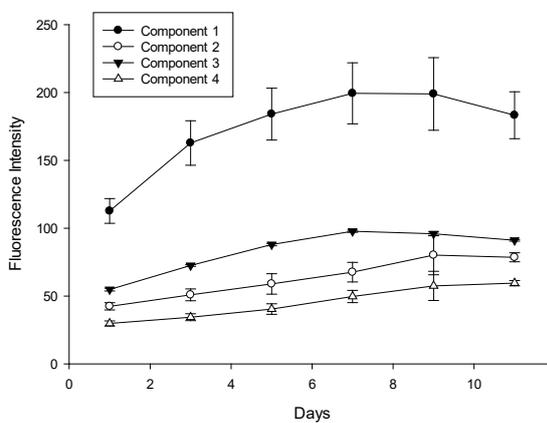
a)



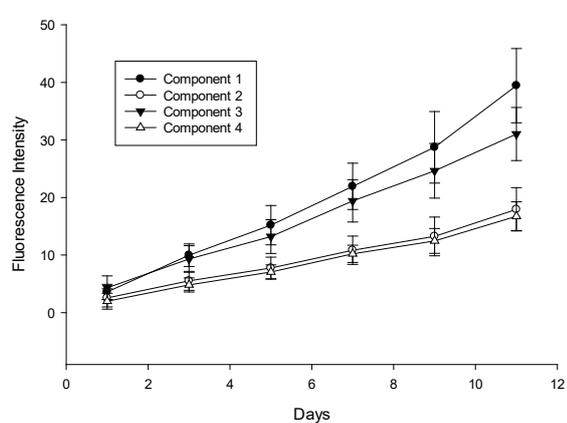
b)



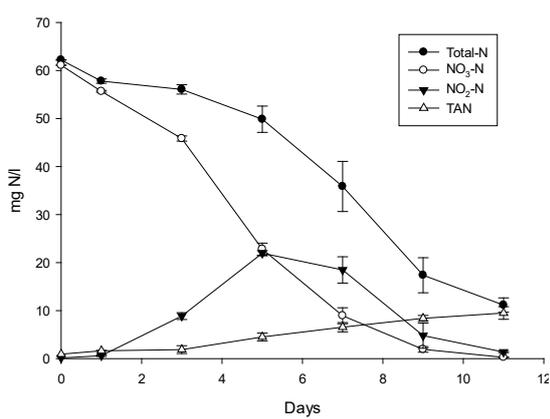
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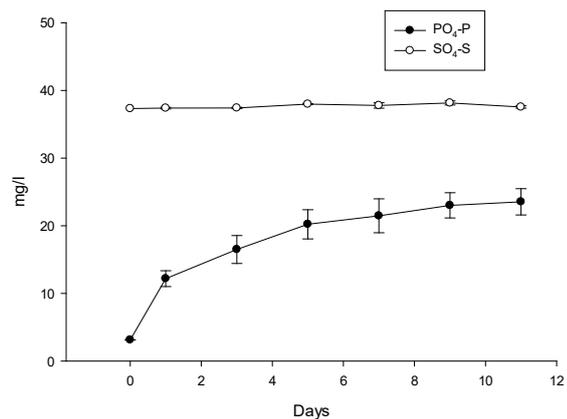
d)



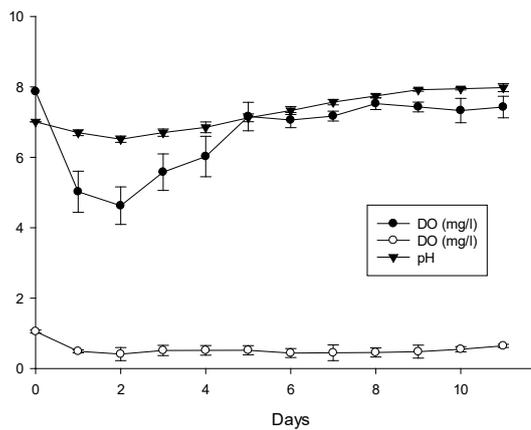
e)



f)



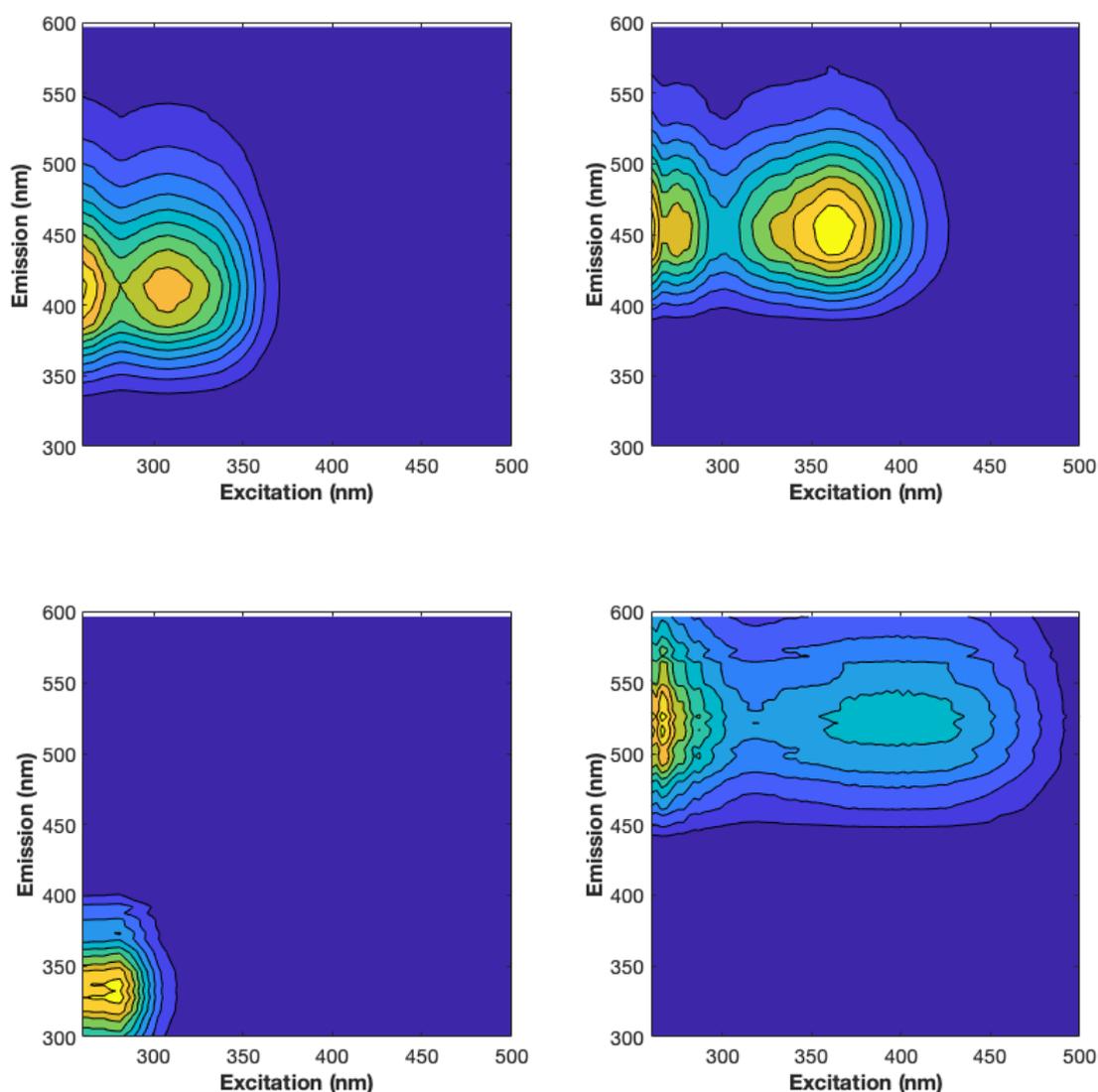
g)



**Figure 1.2. a) Total dissolved COD ( $COD_{diss}$ ) in the water phase; and b) cumulative dissolved COD removed by foam fractionation over time. c) shows the fluorescence intensities of components 1-3 in the water over time and d) shows the cumulative fluorescence intensities of components 1-3 of the collected foam. e) shows concentrations of nitrogenous compounds; total ammonia nitrogen (TAN), nitrite nitrogen ( $NO_2-N$ ), nitrate nitrogen ( $NO_3-N$ ) and dissolved total nitrogen ( $Total-N_{diss}$ ), f) shows concentrations of orthophosphate ( $PO_4-P$ ) and sulfate ( $SO_4-S$ ), and g) shows woodchip bio-reactor inlet and outlet dissolved oxygen (DO) concentrations as well as outlet pH values over time.**

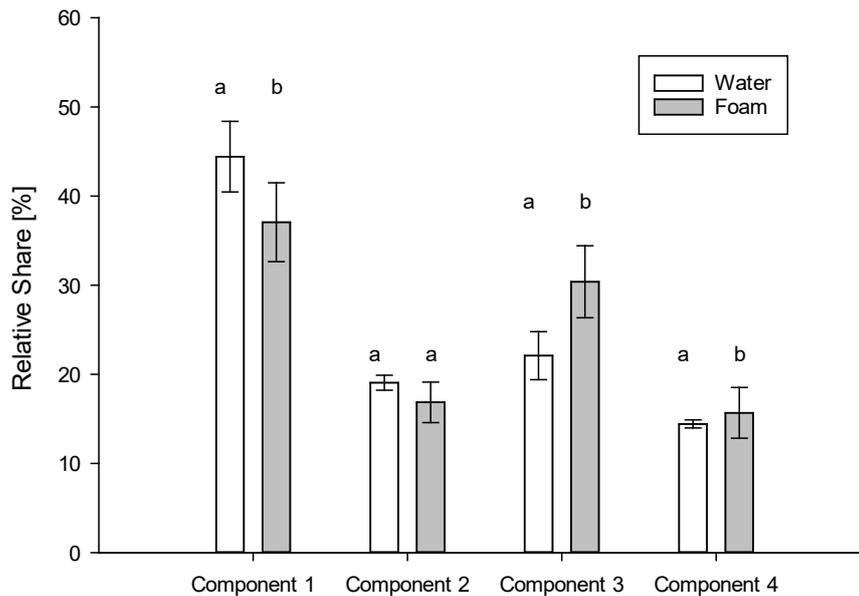
### 1.3.3 Fluorescence EEM Spectroscopy

PARAFAC analysis indicated that the dataset of fluorescence spectra could be mathematically decomposed into four independently varying fractions (figure 1.3), encompassing both the water and the foam. Components 1, 2 and 4 were dominated by visible region emission (>400nm), whereas component 3 was dominated by an emission peak in the UVA region (300-380nm). The fluorescence intensities were generally increasing with COD concentrations.



**Figure 1.3. Fluorescence contour plots with emission (Em., y-axis) and excitation (Ex., x-axis) wavelengths of individual components from the 4-component PARAFAC model (generated from 148 samples for both water and foam samples).**

The ratios of the three components remained similar over time in both the water and the foam, however the ratios between the water and the foam samples differed significantly. In water samples components 1, 2, 3 and 4 were found in ratios of  $44.4 \pm 4.0 : 19.0 \pm 0.8 : 22.1 \pm 2.7 : 14.4 \pm 0.5$  while in foam samples the ratio was  $37.1 \pm 4.4 : 16.9 \pm 2.3 : 30.4 \pm 4.0 : 15.7 \pm 2.8$  ( $n=18$ ) (figure 1.4). Foam samples thus contained a relatively larger share of component 3, and a lower share of component 1, when compared with the water samples. This component 3 is typically characterised as having high bioavailability, and hence is often associated with elevated microbial activity (Hudson et al., 2008). Freshwater RAS have previously been shown to exhibit high levels of this component (Hambly et al 2016).



**Figure 1.4. Mean (+/- s.d.) relative intensities (in %) of fluorescence components 1-3 (figure 1.3) in water samples and foam samples.**

As application of the foam fractionation process on woodchip biofilters has been shown to result in an enriched solution of DOM, this could potentially be used as a resource. Both the foam extract and the remaining water in the system could be applied as fertilizer on fields, as humic substances have been shown to provide valuable nutrients and bio-stimulants for plant growth (Nardi et al., 2007; Canellas et al., 2008). Moreover, at fish farms, both the foam extract and remaining water could be directed into the sludge basins. Here the complex DOM from woodchips could be transformed to simpler organic molecules through anaerobic digestion, which could thereafter be used to fuel heterotrophic denitrification in a subsequent denitrification reactor, constructed wetland or woodchip bioreactor. Field et al. (1988) found that 30-50% of soluble COD from bark water could be acidified into volatile fatty acids (VFAs) and between 7 and 36% could be converted by methanogenesis to methane after 8-9 days of anaerobic digestion. In the case that near complete removal of the remaining DOM was required, post-treatment by combined ozone and biological treatment may be effective at treating woodchip leachate (Zenaitis et al., 2002; Svennson et al., 2015).

#### 1.3.4 Nitrogenous and other compounds

Nitrate concentrations in the water decreased consistently throughout the trial, from 61.11 mg NO<sub>3</sub>-N/l at day 0 to 0.28±0.05 mg NO<sub>3</sub>-N/l at day 11 (n=3). The initial constant nitrate removal rate was observed to increase after 3 days, then levelled off after seven days, where after nitrate concentrations in the water remained below 10mg NO<sub>3</sub>-N/l (figure 1.2e).

Total-N declined in a similar manner as nitrate throughout the trial, decreasing from 62.20 mgN/l (day 0) to 11.16±1.50 mgN/l (day 11, n=3) (figure 1.2e).

Total ammonia nitrogen increased slightly from 0.93 to  $1.87 \pm 0.82$  mgTAN/L during the initial three days, where after concentrations increased more rapidly at a near linear rate, reaching  $9.50 \pm 1.31$  mgTAN/l (n=3) by day 11 (figure 1.2e). ). This may be due to TAN readily leaching from woodchips (von Ahnen et al., 2016).

Nitrite concentrations increased from 0.13 to  $21.95 \pm 0.54$  mg NO<sub>2</sub>-N/l after five days, thereafter decreasing to  $1.38 \pm 0.14$  mg NO<sub>2</sub>-N/l (n=3) at day 11 (figure 1.2e). Von Ahnen et al. (2016) operated a pilot-scale woodchip bioreactor with the same type of willow woodchips, at a commercial trout farm, and a similar nitrite peak was observed on the fifth day after start-up during establishment of the denitrifying bacterial community.

The foam fractionation process did not fractionate nitrogenous compounds. The concentrations of TAN, NO<sub>2</sub>-N, NO<sub>3</sub>-N and total-N in the liquefied foam were equivalent to that in the recirculated water ( $89.0 \pm 14.4$  %,  $106 \pm 28.4$  %,  $108.9 \pm 14.3$  % and  $122.7 \pm 18.3$  %, respectively, (n=18).

## 2. Removal of disinfectants (formaldehyde and peracetic acid) in woodchip bioreactors (work package 2)

### 2.1 Removal of formaldehyde in woodchip bioreactors (work package 2.1)

*With contribution from Lars-Flemming Pedersen*

#### 2.1.1 Background

Formalin is an aqueous solution of formaldehyde (FA,  $\text{CH}_2\text{O}$ ), which is commonly used as a disinfectant in recirculating aquaculture systems (RAS) to treat various bacterial and fungal diseases as well as parasite infections such as *Ichthyophthirius multifiliis* (white spot disease) and *Ichtyobodo necator* (*Costia*) (Masters, 2004; Henriksen and Plessner, 2007). Its treatment effect relies on its ability to react with functional groups of proteins, DNA, RNA, polysaccharides and glycoproteins to inactivate microorganisms (Fox et al., 1985; McDonnell and Russel 1999; Kiernan 2000). Treatments typically include short-term, repetitive applications with formalin at concentrations as high as 100 mg FA/l, depending on the system design, which has shown to be effective against the infective free-living stage of *Ichthyophthirius multifiliis* (Matthews, 2005, Sortkjær et al., 2008a). Efforts have been made to reduce the use of formalin by replacing it with products that have lower environmental impacts and health risks such as for example hydrogen peroxide and peracetic acid (Schreier et al., 1996; Madsen et al., 2000; Masters, 2004). Nonetheless, formaldehyde is still used in significant amounts in Danish aquaculture, with approximately 13 l of formalin (37%) used per ton of trout produced, partly due to insufficient treatment efficiency and risk of biofilter collapse associated with potential substitutes (Madsen et al., 2000; Hohreiter and Rigg, 2001; Henriksen et al., 2008).

Until safer and easier degradable alternatives to formalin are found, it is necessary to investigate the fate of formaldehyde in RAS which may allow to optimize water treatment practices to minimize potential discharge of residual formaldehyde to receiving water bodies. From the moment that formalin is added to the production unit of land-based fish farms, three processes determine the resulting concentration of formaldehyde in the water over time: 1) an instant adsorption onto surfaces, 2) microbial degradation and 3) dilution due to continuous water intake (Masters, 2004; Eiroa et al., 2005; Pedersen et al., 2007; Sortkjær et al., 2008a,b).

Investigations at Danish fish farms revealed that instant adsorption to surfaces caused initial formaldehyde concentrations of around 20 mg FA/l to be reduced by 10% and less in traditional flow-through farms as well as in Danish recirculated Model Trout Farms (MTFs) type 1 (at recirculation in production unit for 4-5 hours) whereas reductions of up to 30% were measured in MTFs type 3 (recirculation of 5-12 hours in production units) (Sortkjær et al., 2008b). The authors discussed that the difference is likely due to the fact that MTF type 3, unlike the MTFs type 1 and flow-through systems, contain biofilters which provide large surface areas for adsorption.

In addition to their good adsorption capacities, aerobic biofilters have also proven to be effective at degrading formaldehyde in aquaculture systems. For example, volumetric removal rates of

220 g F/m<sup>3</sup>/d have been reported for submerged biofilters operated at 20-22 °C (Wienbeck and Koops, 1990), removal rates of 330 g F/m<sup>3</sup>/d in fluidized bed sand filter at 15 to 17.8 °C (Heinen et al., 1996) and rates of 40.8 – 103.2 g F/m<sup>3</sup>/d for trickling filters operated at temperatures ranging from 5.5 to 14.5 °C (Pedersen et al. (2006).

Commonly applied end-of-pipe treatment methods have shown comparatively lower removal rates with sedimentation basins removing 2.88 g F/m<sup>3</sup>/d and constructed wetlands removing 10.8 g F/m<sup>3</sup>/d (instant adsorption + microbial degradation) with dosages of ca. 20 mg FA/l in the production units at temperatures of around 12-14 °C in Danish trout farms (Sortkjær et al., 2008b). The higher removal rates in constructed wetlands compared to sedimentation basins were attributed to plants providing an increased surface area for microbial growth. The authors concluded that the surface area in relation to the mass of water is an important parameter for both adsorption and bacterial formaldehyde degradation and that the removal of formaldehyde at fish farms is mainly due to cleaning devices that involve an increased amount of bacteria, such as the biofilter.

Despite good, combined formaldehyde removal capacities of biofilters, sedimentation basins and constructed wetlands, potentially harmful amounts of formaldehyde may still be discharged, with the extent of discharge being highly dependent on the system design and recirculation intensity. At Danish trout farms, the percentage of the initially dosed amount of formalin exported to receiving water bodies amounted to about 40% for traditional flow-through systems, 27-42% for MTF type 1 and less than 2% for MTF type 3, which are characterized by high degree of recirculation (Sortkjær et al., 2008b). Even though very high removal efficiencies are achieved for the most intensively recirculated MTFs, type 3, the majority of farms may struggle to comply with environmental legislation as water quality requirements for natural water bodies in Denmark demand maximum effluent concentrations as low as 9.2 µg FA/l (Miljø- og Fødevareministeriet, BEK nr. 1022, 2010). Pedersen et al. (2007) discussed that such low effluent concentrations may be difficult to achieve under normal aquaculture management practices. In line with this, investigations on four land-based freshwater aquaculture facilities in Atlantic Canada revealed effluent formaldehyde concentrations of 0.2 -7.1 mg FA/l following treatment with formalin (Lalonde et al., 2015). This implies the need to improve disinfection procedures as well as to develop effective effluent treatment methods that further reduce the discharge concentrations of residual formaldehyde.

Improved disinfection procedures are already in place at many farms. It was found that equal treatment effects, compared to traditional practices, could be achieved with reduced total amounts of formaldehyde when lower dosages are applied at lowered water volumes and increased exposure times in production units (Sortkjær et al., 2008a, Heineke and Buchmann, 2009). In contrast to the advancements made in disinfection procedures, effective, low-cost effluent treatment practices to reduce the amounts of residual formaldehyde discharged into the environment are still lacking (Masters, 2004; Leal et al., 2018).

One potential way to effectively remove formaldehyde from RAS effluents may be through the installation of denitrifying woodchip bioreactors. A few woodchip bioreactors are already implemented as end-of-pipe treatments at Danish recirculated trout farms and have shown to be a reliable method for reducing the discharge of nitrates (von Ahnen et al., 2018). These woodchip bioreactors installed at RAS typically contain several hundreds to thousands of cubic meters of

woodchips, thereby providing large retention times and large surface areas that host an active and diverse microbial community in an anoxic environment (Aalto et al. 2020). There is evidence that formaldehyde degradation also happens during denitrification (Eiroa et al., 2006), but the extent to which formaldehyde is degraded in woodchip bioreactors and how this would affect nitrate removal in woodchip bioreactor remained unknown so far.

It was hypothesized, that the adsorption capacity of woodchip bioreactors was high due to the large surface areas present and that the abundant microbial community would allow for effective formaldehyde degradation. Since the functioning of woodchip bioreactor for removing nitrates is based on microbial activity, operating conditions such as hydraulic retention time (HRT), formaldehyde concentration and temperature were assumed to determine the removal rates and efficiencies of formaldehyde, too. Knowledge on volumetric removal rates and associated degradation kinetics at various operating conditions may allow for better estimates of final formaldehyde outlet concentration under various bioreactor sizes and operating conditions.

This work package consisted of three separate trials, which aimed to investigate 1) the combined effects of formaldehyde inlet concentration and HRT on formaldehyde removal in laboratory woodchip bioreactors, 2) the effect of temperature on formaldehyde degradation kinetics in laboratory woodchip bioreactors and 3) the removal of formaldehyde in replicated full-scale woodchip bioreactors operated at commercial RAS.

## 2.1.2 Materials & Methods

### **Experimental set-up Trial 1: Effects of hydraulic retention time and inlet concentration**

Twelve experimental-scale woodchip bioreactors made from PVC (400 x 89 x 150 mm) with horizontal-flow were packed willow woodchips (*Salix viminalis*) (Ny Vraa Bioenergy I/S, Tylstrup, Denmark). The woodchip packing extended about 4 cm over the water surface and was separated from an inlet and outlet area on both sides via vertically inserted PVC grids (figure 2.1a). The woodchips had been stored dry at room temperature for 1 year and were of irregular shapes ranging from 1 to 5 cm ( $4.1 \pm 0.6$  cm,  $n = 20$ ) in length and from 0.2 to 1.3 cm ( $0.8 \pm 0.5$  cm,  $n = 20$ ) in width. The volume of the submerged woodchip packing was 3 l with dimensions of 279 x 89 x 125 mm. Total woodchip porosity was  $67 \pm 3$  % ( $n=3$ ).

The woodchips were “pre-washed” in RAS effluent for eight weeks at an HRT of 6.7 hours prior to the experiment. The effluent to be treated by the woodchip bioreactors was derived from an 8.5 m<sup>3</sup> experimental, freshwater pilot-scale RAS stocked with rainbow trout (*Oncorhynchus mykiss*) considered to be in steady state. The pilot-scale RAS had been operated under similar conditions for more than six months, receiving a fixed ration of 1 kg feed per day (Efico Enviro 920, 4.5 mm, Biomar, Denmark) and applying a daily amount of make-up water of 1.4 m<sup>3</sup>.

After the eight weeks long pre-washing phase, the experimental bioreactors were run continuously at the operating conditions specified by the central composite design (CCD, as described in 2.1.2) for 10 days prior to conducting the first measurements. Ten days of acclimatization were chosen to mimic the typical repetitive occurring treatments in RAS, which can for example include treatments on every second day for up to two weeks, as well as to calculate removal rates based on measured outlet concentrations that were not affected by dilution. During the 10 days of acclimatization as well as during the measuring period all woodchip bioreactors ( $n=12$ )

received the same RAS effluent water from a continuously fed 700l reservoir. In addition, bioreactors received specific dilutions of formaldehyde in Milli-Q water from smaller 10l reservoirs to achieve the desired formaldehyde inlet concentrations. Dilutions were newly prepared on every third day. The flow rates from the 10l formaldehyde dilutions reservoirs to the woodchip bioreactors were adjusted and regularly monitored, to achieve the desired formaldehyde inlet concentrations and to remain <7% of the RAS effluent flow entering the bioreactors.

The combined effects of HRT and formaldehyde inlet concentration on formaldehyde and nitrate removal rates in experimental woodchip bioreactors was investigated applying a CCD and response surface methodology (RSM) using Design Expert version 12 software (Stat-Ease, Inc., Minneapolis, USA). In accordance with the CCD, each of the two variables were fixed at five coded levels:  $-\alpha$ , -1 (low), 0 (central), +1 (high),  $+\alpha$ , where alpha ( $\alpha$ ) is the relative distance of the axial points from the center point. The total experimental set-up consisted of 12 individual experimental woodchip bioreactors with horizontal flow (Fig. 1a), each run at a specific combination of the two variables according to the CCD (table 2.1).

The investigated HRTs ranged from 3.35 to 15.08 hours and the investigated formaldehyde inlet concentrations ranged from 0-105 mg FA/l (table 2.1). The range of HRTs was chosen to cover relevant HRTs operated at woodchip bioreactors treating the effluents from commercial RAS in Denmark (von Ahnen et al., 2018). Similarly, the range of added formaldehyde inlet concentrations was chosen to include the highest dosages of formaldehyde potentially applied in aquaculture (flow-through) systems (up to 80-100mg/l, Sortkjær et al., 2008a), while still incorporating one treatment without addition of formaldehyde to act as a reference point for estimating the effect of formaldehyde degradation on nitrate removal.

### **Experimental set-up Trial 2: Effects of Temperature**

The experimental set-up consisted of nine experimental woodchip bioreactors which allowed running each of the three investigated temperatures (7, 15 and 23 °C) in triplicates. The bioreactors were filled with willow woodchips (*Salix viminalis*) (Ny Vraa Bioenergy I/S, Tylstrup, Denmark) as specified in 2.1.1. The woodchips have previously been flushed for five weeks on tap water amended with sodium nitrate at nitrate levels of approximately 50 mg NO<sub>3</sub>-N/l and at room temperature of 19-21 °C and an HRT of 10 hours. Ten days before the trial, the woodchip bioreactors were run on effluent water from a freshwater pilot-scale RAS (as describe in 2.1.1) at a water temperature of 23 °C and a HRT of 10 hours.

Before addition of RAS water to the laboratory woodchip bioreactors, the RAS water was amended with sodium nitrate (NaNO<sub>3</sub>, Merck KGaA, Darmstadt, Germany) and formalin (24% formaldehyde, S. Sørensen, Thisted, Denmark). Formalin and sodium nitrate were added to 20l of RAS water and mixed with an aquarium pump (Eheim Universal 300, Eheim GmbH & Co. KG, Deizisau, Germany) for 5min. After five minutes of mixing and just prior to adding the water to the laboratory woodchip bioreactors, a “start/zero” grab sample was taken.

The experimental woodchip bioreactors were recirculated using peristaltic pumps (BT 100-2 J with pump heads YZ1515X, Langer Instruments Corp., New Jersey, USA) at a recirculation flow rate of 2.0 (l/h) corresponding to one complete exchange of the woodchip pore volume per hour.

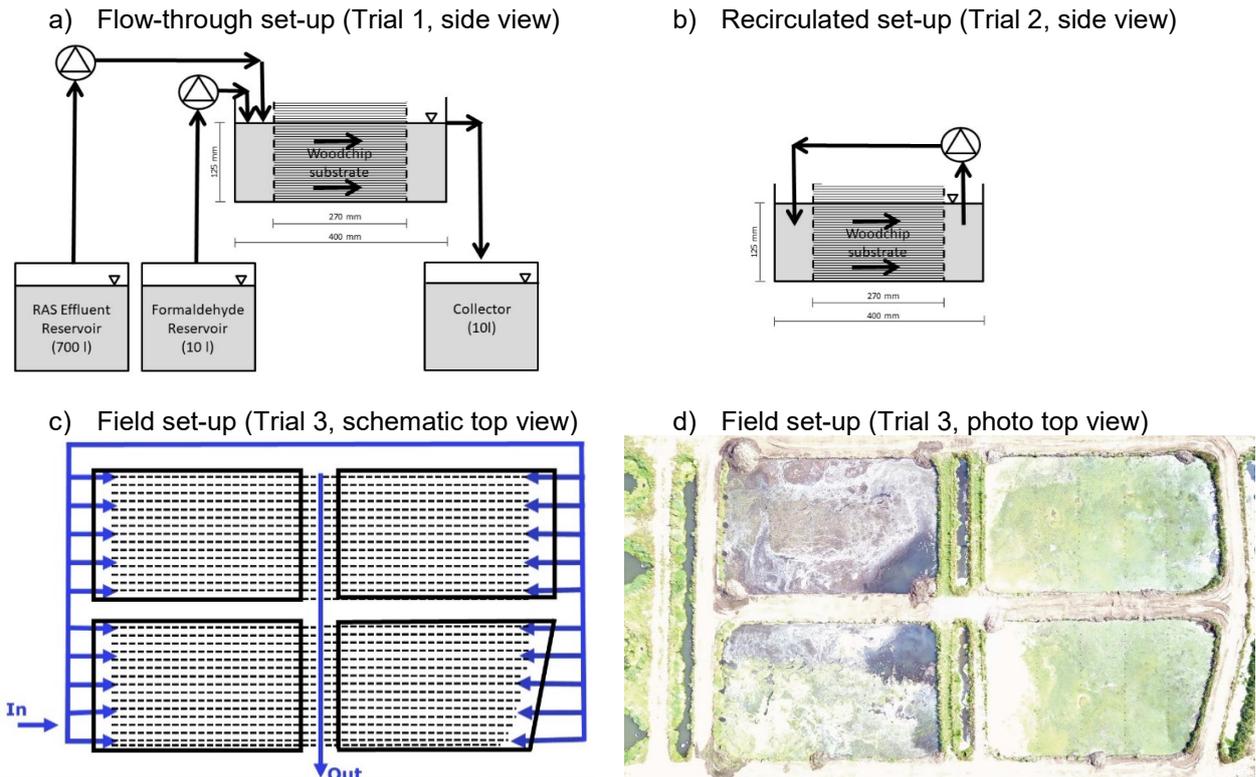
The outlet end of the pump hose was placed below the water surface to avoid input of dissolved oxygen to the system during recirculation (figure 2.1b).

### **Experimental set-up Trial 3: Formaldehyde removal in full-scale woodchip bioreactors**

The field trial was conducted at a commercial Danish MTF type 3 with a production capacity of 1500 tons of rainbow trout per year and a water intake of 100 l/s of groundwater. The farm consisted of 12 independent RAS each containing airlifts, propeller pumps, injections of pure oxygen, sludge cones, drum filters, fixed and moving bed biofilters. The collected sludge was stored in four sludge ponds. The overflow from the sludge ponds and the effluent water from the production units were discharged into a wetland consisting of narrow channels with approximately 1m depth and a total free water surface area of 4000 m<sup>2</sup>.

The discharge from this wetland section entered a distribution channel from where water entered the four parallel woodchip bioreactor through five pipes intersecting an earthen dam (figure 2.1 c, d). Each woodchip bioreactor section contained 1500 m<sup>3</sup> of a hardwood woodchip blend and each section was operated as a vertical down-flow bioreactor.

A commercial formalin solution (S. Sørensen, Thisted, Denmark) containing 24% (w/w) formaldehyde (CH<sub>2</sub>O) dissolved and stabilized in 10% (w/w) methanol (CH<sub>3</sub>O) to prevent formation of highly toxic paraformaldehyde was used. Eight 25 l containers containing formalin were added at once to each bioreactor section by distributing them evenly among the five inlet pipes of each bioreactor section. This corresponded to a total of 51.8 kg of formaldehyde added to each bioreactor section. The added amount corresponded to achieving an initial, average concentration of 34.2 mg FA/l in the water volume of one bioreactor section.



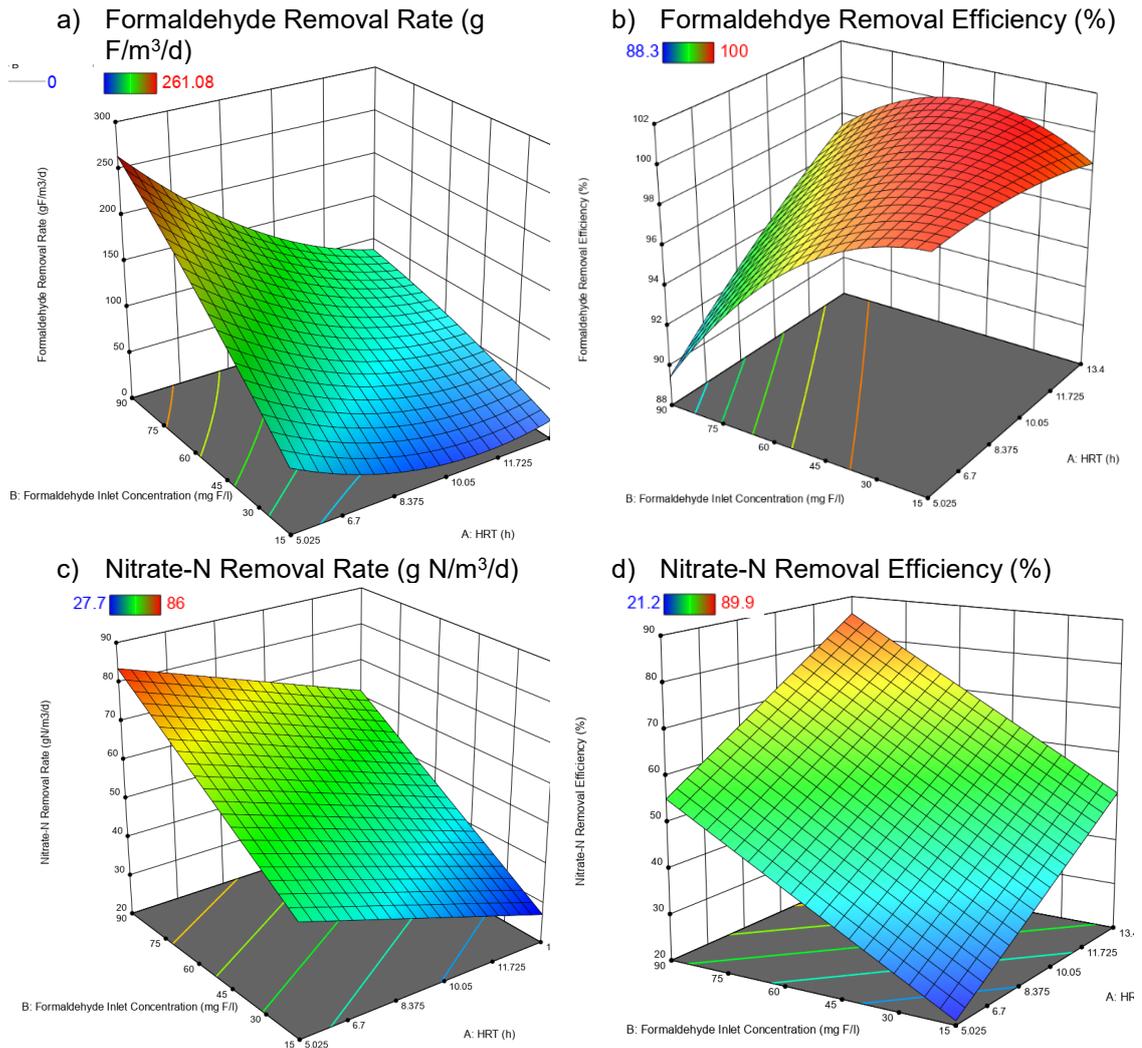
**Figure 2.1. a) Laboratory, flow-through set-up for a central composite design (CCD) to determine the effect of hydraulic retention time and formaldehyde inlet concentration on formaldehyde and nitrate removal in woodchip bioreactors (n=12). b) Laboratory, recirculated set-up to investigate formaldehyde removal kinetics in woodchip bioreactors at different temperatures (n=9). c) Field-scale set-up to investigate formaldehyde removal in four replicated woodchip bioreactor sections with three treatments and one control section without addition of formaldehyde.**

### 2.1.3 Results

#### Effects of hydraulic retention time and inlet concentration

Formaldehyde was removed in all experimental runs receiving formaldehyde. Within the operational conditions tested, formaldehyde removal rates increased with increase in formaldehyde inlet concentration and decrease in HRT, and vice versa. Among those experimental runs receiving formaldehyde, average volumetric formaldehyde removal rates ranged from  $17.6 \pm 0.2$  to  $261.1 \pm 27.2$  g F/m<sup>3</sup>/d (n=3).

In contrast, formaldehyde removal efficiencies decreased with increase in formaldehyde inlet concentration and decreases in HRT. Formaldehyde removal efficiencies were generally high with average efficiencies ranging from  $88.3 \pm 4.6$  to  $99.8 \pm 0.2$  % (n=3).



**Figure 2.2. Response surface plots of a) volumetric formaldehyde (F) removal rates (g F/m<sup>3</sup>/d), b) formaldehyde removal efficiencies (%), c) volumetric nitrate-nitrogen removal rates (g NO<sub>3</sub>-N/m<sup>3</sup>/d) and d) nitrate-nitrogen removal efficiencies at different formaldehyde inlet concentrations (mg FA/l) and hydraulic retention times (HRTs in h) in experimental denitrifying woodchip bioreactors treating the effluent from a pilot-scale recirculating aquaculture system. The displayed models are based on averages of three repeated measurements for each experimental run specified in table 2.1.**

The relationship between the two factors: HRT (h) (factor A) and formaldehyde inlet concentration (mg FA/l) (factor B) and the corresponding volumetric formaldehyde removal rates (figure 2.2a) and formaldehyde removal efficiencies (figure 2.2b), respectively, could be best described by the following quadratic equations:

$$\text{Formaldehyde removal rate (g F/m}^3\text{/d)} = 144.56 - 31.76*A + 4.08*B - 0.20*AB + 1.56*A^2 - 0.004*B^2$$

$$\text{Formaldehyde removal efficiency (\%)} = 99.16 + 0.17*A - 0.03*B + 0.01*AB - 0.03*A^2 - 0.002*B^2$$

Both models were significant (P-values < 0.0001) and R-squared equaled 0.98 and 0.89, respectively.

The nitrate removal rate for the bioreactor not treated with formaldehyde was  $27.7 \pm 1.6$  g N/m<sup>3</sup>/d, while nitrate removal rates for bioreactors treated with formaldehyde were all considerably higher, achieving removal rates of up to  $86.0 \pm 6.1$  g N/m<sup>3</sup>/d at a formaldehyde inlet concentration of 52.5 mg FA/l and a HRT of 3.35 h (table 2.1).

The relationship between HRT (h), formaldehyde inlet concentration (mg FA/l) and the corresponding volumetric nitrate-N removal rates (g NO<sub>3</sub>-N/m<sup>3</sup>/d) and the nitrate-N removal efficiencies (%) were best described by the following linear equations:

$$\text{Volumetric NO}_3\text{-N removal rate (g NO}_3\text{-N/m}^3\text{/d)} = 54.45 - 2.54*A + 0.47*B$$

$$\text{Nitrate-N removal efficiency (\%)} = -4.17 + 3.63*A + 0.46*B$$

Both models were significant (P-values < 0.001) and R-squared equaled 0.85 and 0.88, respectively. Figure 2.2 illustrates the 3-D response surface plots for the investigate factors HRT (h) and formaldehyde inlet concentration (mg FA/l) and the corresponding responses in terms of the volumetric formaldehyde removal rate (figure 2.2 a), formaldehyde removal efficiency (figure 2.2 b), volumetric nitrate-N removal rates (figure 2.2 c) and the nitrate-N removal efficiencies (figure 2.2 d) achieved.

Throughout all sampling events, effluent pH values were slightly lower compared to inlet pH values and outlet dissolved oxygen levels were below 1 mgO<sub>2</sub>/l in all experimental runs (data not shown).

**Table 2.1. Average volumetric removal rates and removal efficiencies for formaldehyde (F) and nitrate-nitrogen (NO<sub>3</sub>-N) obtained at the various combinations of formaldehyde inlet concentrations and HRTs for experimental woodchip bioreactors according to the central composite design (CCD). Nitrate inlet concentrations were on average 56.2 ± 1.4 mg NO<sub>3</sub>-N/l for all experimental runs during the three sampling events.**

Exp. Runs <sup>1)</sup> (mg/l) / (h)	Outlet concentrations (mg/l)		Removal rate <sup>2)</sup> (g/m <sup>3</sup> /d)		Removal Efficiency <sup>2)</sup> (%)	
	Formaldehyde	NO <sub>3</sub> -N	Formaldehyde	NO <sub>3</sub> -N	Formaldehyde	NO <sub>3</sub> -N
F. Inlet conc. / HRT						
52.5 (0) / 3.35 (-α)	2.1 ± 0.8	38.3 ± 0.9	242.0 ± 3.8	86.0 ± 6.1	96.0 ± 1.5	26.0 ± 6.6
15 (-1) / 5.03 (-1)	0.3 ± 0.2	44.3 ± 2.0	47.0 ± 0.8	38.0 ± 3.8	98.0 ± 1.6	21.2 ± 2.3
90 (+1) / 5.03 (-1)	8.4 ± 8.5	33.4 ± 3.0	261.1 ± 27.2	72.8 ± 7.5	90.7 ± 9.5	34.7 ± 12.8
0 (-α) / 9.21 (0)	0.2 ± 0.1	40.3 ± 0.9	-	27.7 ± 1.6	-	28.3 ± 1.2
52.5 (0) / 9.21 (0)	0.5 ± 0.4	22.0 ± 3.0	90.8 ± 0.8	59.7 ± 5.3	99.1 ± 0.9	55.0 ± 13.3
52.5 (0) / 9.21 (0)	0.4 ± 0.3	23.8 ± 3.6	90.9 ± 0.6	56.5 ± 5.8	99.2 ± 0.6	51.8 ± 14.2
52.5 (0) / 9.21 (0)	0.3 ± 0.3	24.3 ± 0.2	91.1 ± 0.5	55.7 ± 2.1	99.4 ± 0.5	45.1 ± 16.4
52.5 (0) / 9.21 (0)	0.7 ± 0.6	24.8 ± 0.6	90.4 ± 1.0	54.8 ± 2.2	98.6 ± 1.1	46.5 ± 13.9
105 (+α) / 9.21 (0)	12.3 ± 4.8	8.2 ± 1.4	161.8 ± 8.5	83.8 ± 3.6	88.3 ± 4.6	86.6 ± 4.1
15 (-1) / 13.4 (+1)	0.3 ± 0.2	27.2 ± 3.4	17.6 ± 0.2	34.7 ± 3.6	98.0 ± 1.2	51.6 ± 5.5
90 (+1) / 13.4 (+1)	0.2 ± 0.2	5.6 ± 1.1	107.8 ± 0.3	60.7 ± 3.1	99.8 ± 0.3	89.9 ± 2.3
52.5 (0) / 15.08 (+α)	0.2 ± 0.3	21.7 ± 3.8	55.8 ± 0.3	36.8 ± 4.7	99.6 ± 0.5	63.7 ± 10.4

<sup>1)</sup> Experimental runs represent combinations of hydraulic retention time (HRT) and formaldehyde inlet concentration (H<sub>2</sub>CO) where values with the original unit (HRT in h; formaldehyde in mg FA/l) are followed by the coded CCD values (section 2.1.2) in parentheses. Removal rates are expressed as g removed per m<sup>3</sup> of woodchip bioreactor volume (i.e. volume of submerged woodchips) per day.

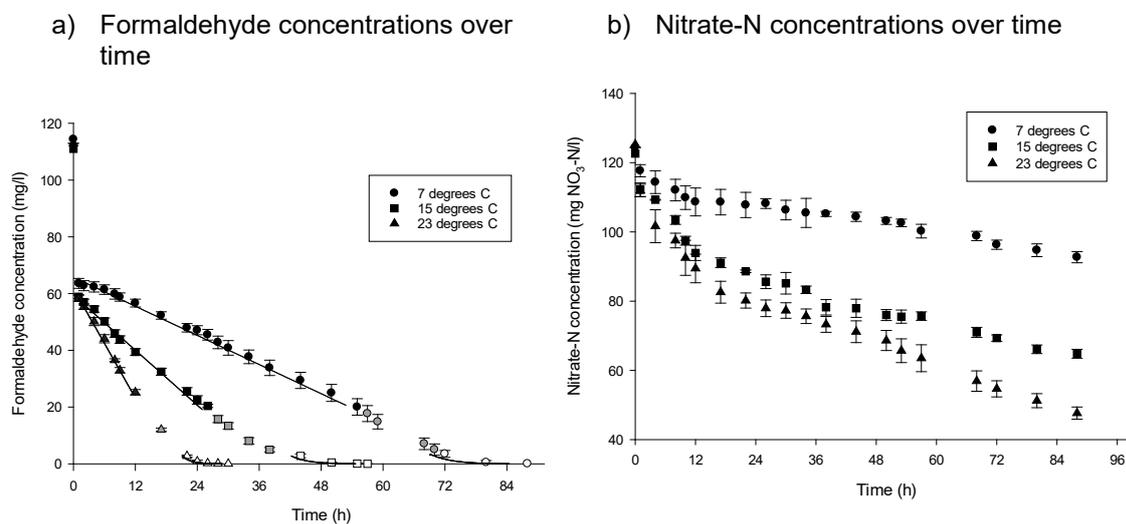
<sup>2)</sup> Removal rates and efficiencies for formaldehyde and nitrate are shown as averages with standard deviation of three repeated measurements on the same woodchip bioreactor. Formaldehyde inlet concentrations are based on concentrations measured in the sumps containing formaldehyde + purified water from a Millipore Milli-Q lab water system and the frequently measured flow rates from both sumps into the experimental woodchip bioreactors (figure 2.1a).

## Effect of temperature

The RAS effluent treated during the temperature trial had BOD<sub>5-TOT</sub> and BOD<sub>5-DISS</sub> values of 2.1 mgO<sub>2</sub>/l and 1.43 (n=1), respectively. The RAS effluent was furthermore characterized by a pH of 7.21, a dissolved oxygen concentration of 7.71 mg O<sub>2</sub>/l and a temperature of 22.5 °C upon collection from the RAS and before adjustment of temperature and amendment with formaldehyde and nitrate.

Dissolved oxygen levels declined at the start of the degradation trials reaching levels below 1 mg O<sub>2</sub>/l after <2 hours at 23 °C and within less than 3 hours at 15 and 7 °C, respectively. Dissolved oxygen levels remained below 1mg O<sub>2</sub>/l for the remainder of the trials (data not shown).

Formaldehyde concentrations in the temperature-acclimatized RAS waters were 114.4, 111.0 and 113.0 mg FA/l at temperatures of 7, 15 and 23 °C, respectively, measured just prior to adding the water to the previously drained woodchip bioreactors. Removal of formaldehyde in the experimental woodchip bioreactors happened immediately after application.



**Figure 2.3. a) Removal of formaldehyde (F) (CH<sub>2</sub>O) in experimental denitrifying woodchip bioreactors at 7 °C (circles), 15 °C (squares) and 23 °C (triangles) over time. Filled symbols represent data points >20mg FA/l used to assess zero order (concentration independent) removal kinetics (excluding data points where time=0), while open symbols represent data points <5 mg FA/l and >0.5 mg FA/l used to assess first order (concentration dependent) removal kinetics. Shaded symbols may in part belong to half-order kinetics (transition zone between zero and first order kinetics) but were not focused on in this study. b) Corresponding nitrate-N concentrations over time during degradation of formaldehyde (as shown in figure 2.3a). Data points represent averages with standard deviation of triplicated runs investigated in three separate experimental woodchip bioreactors.**

The initial adsorption efficiency was similar across the temperatures amounting on average to  $44.7 \pm 2.4$  % (n=9), which corresponded to a total adsorption capacity of  $52.1 \pm 2.8$  g FA/m<sup>3</sup> woodchips. After the initial decline happening during the first hour, formaldehyde concentrations declined linearly for as long as formaldehyde concentrations on the outlet side remained high ~above 20 mg FA/l (figure 2.3a). During the linear decline in concentration, average volumetric zero order removal rate constants  $k_{ov}$  of  $20.45 \pm 1.49$ ,  $38.93 \pm 2.53$  and  $77.13 \pm 4.98$  g F/m<sup>3</sup>/d

(n=3) were found at temperatures of 7, 15 and 23 °C, respectively. In the lower concentration ranges, formaldehyde concentrations declined exponentially eventually being completely removed. Average volumetric first order removal rate constants of  $1.80 \pm 0.15$ ,  $3.25 \pm 0.77$  and  $5.23 \pm 0.31$  (1/d) (n=3) were found at temperatures of 7, 15 and 23 °C, respectively (table 2.2). The ratios of the volumetric zero and first order removal constants ( $k_{0v}/k_{1v}$ ) suggested that the transition zone, at which zero order kinetics switched to first order kinetics, was located around concentrations of  $11.45 \pm 1.32$ ,  $12.76 \pm 3.31$  and  $14.73 \pm 0.14$  mg FA/l (n=3) at temperature of 7, 15 and 23 °C, respectively.

**Table 2.2. Removal Kinetics of formaldehyde (F) (CH<sub>2</sub>O) in experimental denitrifying woodchip bioreactors at different temperatures. Zero order removal kinetics were calculated on formaldehyde concentrations >20 mg FA/l excluding the time 0 sample. First order removal rates were calculated on data points < 5 mg FA/l. The ratio  $k_{0v}/k_{1v}$  represents the estimated formaldehyde concentration at which removal kinetics switch from 0<sup>o</sup> order to 1<sup>o</sup> order. Five-day biological oxygen demand (BOD<sub>5</sub>) was measured before addition of formaldehyde.**

Temperature (°C)	pH (-)	BOD <sub>5-TOT</sub> <sup>1)</sup> (mg O <sub>2</sub> /l)	BOD <sub>5-DISS</sub> <sup>1)</sup> (mg O <sub>2</sub> /l)	0 <sup>o</sup> order rate constant $K_{0v}$ (g F/m <sup>3</sup> /d)	1 <sup>o</sup> order rate constant $K_{1v}$ (1/d)	$k_{0v}/k_{1v}$ (mg FA/l)
7	7.21	2.1	1.43	20.45±1.49	1.80±0.15	11.4±1.3
15	7.18	2.1	1.43	38.93±2.53	3.25±0.77	12.0±3.3
23	7.19	2.1	1.43	77.13±4.98	5.23±0.31	14.8±0.1

<sup>1)</sup> BOD<sub>5-TOT</sub> and BOD<sub>5-DISS</sub> were measured in the RAS effluent one day before the trial, prior to adjusting temperatures.

Temperature correlated positively with both, the volumetric zero- ( $k_{0v}$ ) (Corr. Coeff.: 0.949, P<0.001, n=9) and first order rate constants ( $k_{1v}$ ) (Corr. Coeff.: 0.949, P<0.001, n=9) as shown in figure 2.4. Based on zero-order degradation kinetics, an average Q<sub>10</sub> value for formaldehyde removal in woodchip bioreactors of 2.27 and a corresponding Arrhenius temperature coefficient of 1.086 was found within the investigated temperature range of 7-23 °C.

Nitrate concentrations declined consistently during all treatments (figure 2.3b). Higher nitrate removal rates were observed when formaldehyde was present in the water compared to when formaldehyde concentrations were diminished. At 15 and 23 °C, volumetric nitrate-N removal rates averaged  $20.67 \pm 1.57$  and  $36.81 \pm 0.83$  g N/m<sup>3</sup>/d (n=3), respectively, when formaldehyde concentrations were above 0.5 mg FA/l being significantly higher compared to average volumetric nitrate removal rates of  $8.07 \pm 1.08$  and  $12.86 \pm 0.27$  g N/m<sup>3</sup>/d (n=3), respectively, achieved when formaldehyde concentrations were below 0.5 mg FA/l (figures 2.3 a, b). At 15 and 23 °C, the volumetric nitrate removal rates were thus  $260 \pm 23$  and  $285 \pm 6$  % higher, respectively, when formaldehyde concentrations were above 0.5 mg FA/l compared to when formaldehyde concentrations were below 0.5 mg FA/l

### Formaldehyde removal in full-scale woodchip bioreactors

The inlet water to the four woodchip bioreactor sections had 'start' and 'end' pH values of 7.11 and 7.19, dissolved oxygen concentrations of 6.57 and 6.03 mg O<sub>2</sub>/l and temperatures of 5.7

and 5.5 °C, respectively. Start and end outlet pH, dissolved oxygen concentrations and temperatures in the common outlet of all woodchip bioreactor sections were 7.16 and 7.21, 0.87 and 0.67 mg O<sub>2</sub>/l and 5.6 and 5.6 °C, respectively.

During the entire measuring period, formaldehyde was neither detected in the common inlet water, nor in the outlet of the woodchip bioreactor section that acted as a control.

In the three outlets of the woodchip bioreactor sections treated with formaldehyde, outlet formaldehyde concentrations rose sharply in the beginning and formaldehyde was already detectable in the outlet one hour after addition to the bioreactor inlet. Formaldehyde peak concentrations between 8.17 and 10.91 mg FA/l were detected between three and five hours after addition of formaldehyde. Thereafter, outlet formaldehyde concentrations declined consistently, reaching concentrations around 1 mg FA/l or lower 24 hours after addition of formaldehyde. During these 24 hours on average 7.7 ± 0.3 kg F (n=3) were discharged from each of the three bioreactor sections. This corresponded to an average total formaldehyde removal of 44.1 ± 0.3 kg F/d, an average removal efficiency of 82.5 ± 0.8% and an average volumetric removal rate of 29.4 ± 0.2 g F/m<sup>3</sup>/d.

The occurrence of outflow peak concentrations way ahead of the theoretical hydraulic retention time of 16.8 h for each bioreactor section provided plug-flow, indicated that paths of preferential flow existed in the bioreactors allowing water to short cut on its way from the inlet to outlet. This stresses the importance of optimal woodchip bioreactor design to allow for even water flow through the woodchip packing to improve removal efficiency.

Nitrate inlet concentrations were relatively stable during the measuring period, although slightly increasing during the day, ranging from 8.50 to 10.14 mg NO<sub>3</sub>-N/l and averaging 9.43 ± 0.49 mg NO<sub>3</sub>-N/l (n=25) (figure 2.5b). Outlet nitrate concentrations for the control bioreactor section averaged 6.26 ± 0.40 mg NO<sub>3</sub>-N/l (n=25) and were significantly higher compared to 4.94 ± 0.54, 4.30 ± 0.67 and 3.86 ± 0.62 mg NO<sub>3</sub>-N/l (n=25) measured at the outlets of the three bioreactor sections treated with formaldehyde. Based on average inlet and outlet concentrations and an equal flow of 25 l/s per bioreactor section, the volumetric nitrate removal rates in the control bioreactor section were 4.57 compared to 6.47, 7.40 and 8.02 g N/m<sup>3</sup>/d in the three bioreactor sections treated with formaldehyde. These removal rates corresponded to daily nitrate mass removals of 6.86 kg NO<sub>3</sub>-N/d in the control bioreactor section compared to 9.70, 11.09 and 12.00 kg NO<sub>3</sub>-N/d removed in the three bioreactor sections treated with formaldehyde.

## **2.2 Removal of peracetic acid in woodchip bioreactors (work package 2.2)**

### **2.2.1 Background**

Peracetic acid (PAA), or peroxyacetic acid, is used in aquaculture to improve water quality and to treat fish diseases such as white spot disease caused by the ectoparasite *Ichthyophthirius multifiliis* (Matthews, 2005; Meinelt et al., 2007 a,b). Peracetic acid is a mixture of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), acetic acid (CH<sub>3</sub>COOH) and water, and is a stronger oxidant and disinfectant than chlorine (Cl), chlorine dioxide (ClO<sub>2</sub>), or hydrogen peroxide alone.

Peracetic acid has a high oxidation potential due to its unpaired electrons (free radicals) and formation of hydroxyl radicals characterized by a wide spectrum of antimicrobial activity, which allows it to achieve high treatment efficiencies when applied at relatively low dosages (Baldry, 1983, Wagner et al., 2002; Kitis, 2004). The *in vitro* growth of pathogenic bacteria in aquaculture, such as *Aeromonas salmonicida*, *Flavobacterium columnare* and *Yersinia ruckeri* have shown to be reduced at a relatively low nominal concentration of 1 mg PAA/l (Marchand et al., 2012; Meinelt et al., 2015). Similarly, Rintamaki-Kinnunen et al., (2005a,b) tested PAA against *I. multifiliis* in field trials and found that 1-1.3 mg PAA/l could reduce the parasitic load on the fish. According to Liu et al. (2017) application of PAA in aquaculture systems may thus be via pulse applications at concentrations of 1-2 mg PAA/l or continuous application at concentrations below 0.2 mg PAA/l. Moreover, also in the treatment of secondary and tertiary effluent from municipal wastewater systems, low concentrations of 0.5 to 2 mg PAA/l at short contact times have shown to achieve satisfactory disinfection (Baldry and French, 1989; Stampi et al., 2001; Wagner et al., 2002).

When added to waste water, PAA readily decomposes into acetic acid, hydrogen peroxide, oxygen and water due to chemical oxidation, hydrolysis and transition-metal-catalyzed decomposition (Yuan et al., 1997; Wagner et al., 2002; Kitis, 2004), while both H<sub>2</sub>O<sub>2</sub> and acetate are mainly degraded by bacteria (Rojas-Tirado et al., 2019). When fully degraded, PAA thus leaves no noticeable disinfection by-products, only oxygen and water, and is therefore considered as an environmentally benign disinfection agent (Colgang and Gehr, 2001). Due to these characteristics, PAA represents an environmental friendly alternative disinfectant in aquaculture to the use of formaldehyde (formalin) (Pedersen et al., 2009).

Pedersen et al. (2009) found that PAA effectively degraded in aquaculture biofilters with surface specific removal rates ranging from 4.6 to 13.9 mg PAA/m<sup>2</sup>/h at 17 °C and furthermore concluded that the relation between biofilter surface area and total water volume is the primary influence for the absolute PAA consumption in RAS. The rapid decay of PAA in aquaculture systems typically follows first-order decay functions with half-lives of <5 to 23 min measured at commercial Danish recirculated outdoor trout farms (at COD levels of 19 -24 mg O<sub>2</sub>/l) (Pedersen et al. 2013). Due to the observed rapid decay of PAA in commercial Danish RAS, Pedersen and Henriksen (2011) concluded that most, if not all, PAA will degrade even before entering the biofilter compartment and therefore may not even affect ammonia or nitrite oxidation processes (Pedersen and Henriksen, 2011).

Given its rapid decay in production units, PAA, if applied in correct dosages, may not have any negative environmental impact on the surrounding natural environment at many fish farms. However, due to its rapid decay it can be challenging to apply the right dosage. The active PAA concentration depends on (i) the product formulation (ranging from 2 to 40% PAA with substantial variability between and within products) and the applied dose, (ii) the stability of the product, and (iii) the PAA consumption and decay in the specific aquaculture system. The initial consumption and decay of PAA under aquaculture conditions was shown to be positively correlated to the amount of organic matter in the water as well as existing biofilms on surface (Pedersen et al., 2009; 2013). The abundance of both, suspended organic matter and biofilms may show considerable variations over time, among different farm sites, and even among separate culture units within the same farm (de Jesus Gregersen et al., 2019). Rintamaki-Kinnunen et al. (2005a,b) tested peracetic acid compounds against *I. multifiliis* in field trials and found that PAA

was less effective in earthen ponds compared to concrete tanks. The authors suggested that unaccounted organic matter in earthen ponds could be responsible for the reduced efficacy compared to results from concrete systems. These site-specific differences in organic matter content may lead to difficulties in applying the most appropriate, site-specific, treatment regimen consisting of PAA concentration x contact time (Rach et al., 1997; Rintamäki-Kinnunen et al., 2005a,b; Pedersen and Henriksen, 2011; Pedersen et al. 2013). The challenges and uncertainties in applying the right amounts of PAA may imply the risk of residual PAA left in the effluents when overdosed at low organic matter contents in the water. Pedersen and Lazado (2020) suggested that contact with organic matter and/or biofilm on colonized surfaces (e.g. woodchips) could be a technical solution to further facilitate rapid PAA degradation and thereby reduce the discharge of PAA.

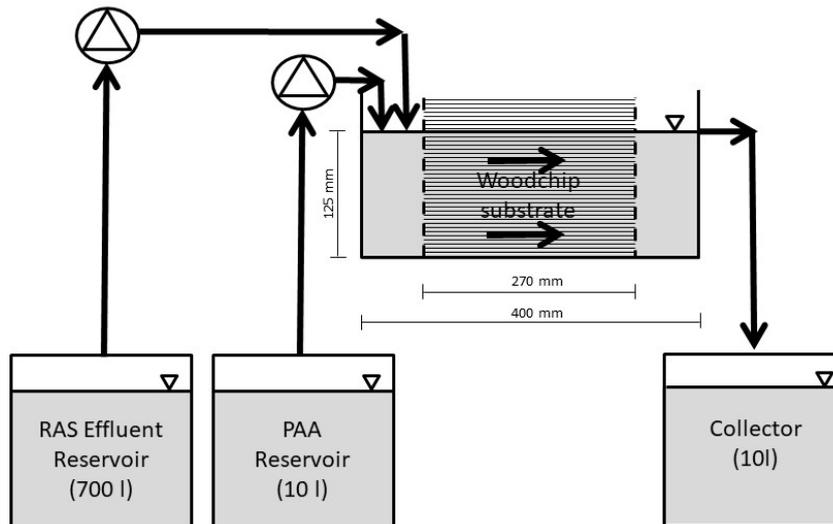
In recent years, some Danish farmers have installed end-of-pipe woodchip bioreactors, which have proven to be a stable and reliable method for removing nitrate from commercial RAS (von Ahnen et al., 2018). More recent investigations have also shown that woodchip bioreactors can achieve high removal efficiencies for formaldehyde due to the long hydraulic retention times, large organic surfaces and active microbial communities provided (von Ahnen et al. unpubl. data). It was hypothesized that due to this combination of factors, woodchip bioreactors would also function as a final polishing barrier for eliminating potential residuals of strong oxidants, such as peracetic acid, used as disinfectants in aquaculture.

This work package investigated the PAA removal capacity of laboratory-scale denitrifying woodchip bioreactors treating the effluent from a pilot-scale freshwater RAS. The experiment was designed according to the central composite design (CCD) to investigate the effects of hydraulic retention times and PAA inlet concentrations on woodchip bioreactor performance.

## 2.2.2 Materials & Methods

### Experimental Set-up

Twelve experimental-scale woodchip bioreactors made from PVC (400 x 89 x 150 mm) with horizontal-flow were packed with willow woodchips (*Salix viminalis*) (Ny Vraa Bioenergy I/S, Tylstrup, Denmark) (figure 3.1). The woodchips had been stored dry at room temperature for one year and were of irregular shapes ranging from 1 to 5 cm ( $4.1 \pm 0.6$  cm,  $n = 20$ ) in length and from 0.2 to 1.3 cm ( $0.8 \pm 0.5$  cm,  $n = 20$ ) in width. The volume of the submerged woodchip packing was 3 l with dimensions of 279 x 89 x 125 mm. Total woodchip porosity was  $67 \pm 3$  % ( $n=3$ ).



**Figure 3.1. Experimental set-up for a central composite design (CCD) to determine the effect of hydraulic retention time and peracetic acid (PAA) inlet concentration on, amongst others, PAA and nitrate removal in woodchip bioreactors (n=12).**

The effluent to be treated by the woodchip bioreactors was derived from an 8.5 m<sup>3</sup> experimental, freshwater pilot-scale RAS stocked with rainbow trout (*Oncorhynchus mykiss*) considered to be in steady state. The pilot-scale RAS had been operated under similar conditions for more than six months, receiving a fixed ration of 1 kg feed per day (Efico Enviro 920, 4.5 mm, Biomar, Denmark) and applying a daily amount of make-up water of 1.4 m<sup>3</sup>. In addition, bioreactors received specific dilutions of PAA (Divosan®) in Milli-Q water from smaller 10l reservoirs to achieve the desired PAA inlet concentrations.

### Experimental Design

The combined effects of HRT and PAA inlet concentration on PAA and nitrate removal rates in experimental woodchip bioreactors was investigated applying a CCD and response surface methodology (RSM) using Design Expert version 12 software (Stat-Ease, Inc., Minneapolis, USA). In accordance with the CCD, each of the two variables were fixed at five coded levels:  $-\alpha$ , -1 (low), 0 (central), +1 (high),  $+\alpha$ , where alpha ( $\alpha$ ) is the relative distance of the axial points from the center point. The total experimental set-up thus consisted of 12 individual experimental woodchip bioreactors with horizontal flow (Fig. 1a), each run at a specific combination of the two variables according to the CCD (table 3.1).

The investigated HRTs ranged from 3.4-15.0 hours and the investigated PAA inlet concentrations ranged from 0-350 mg PAA/l (table 3.1). The range of HRTs was chosen to cover relevant HRTs operated at woodchip bioreactors treating the effluents from commercial RAS in Denmark (von Ahnen et al., 2018). The range of added PAA inlet concentrations was chosen to assess the maximum PAA removal capacity of woodchip bioreactors while still incorporating one treatment without addition of PAA to act as a reference point for estimating the effect of PAA degradation on nitrate removal.

### 2.2.3 Results

#### **PAA removal**

PAA was removed in all treatments. Complete PAA removal was observed in treatments receiving the lowest addition PAA (i.e. 50 mg PAA/l). Removal efficiencies ranged from complete removal (100%) when PAA inlet concentrations were at 50 mg PAA/l down to 50.7% when PAA inlet concentration was at 300 mg PAA/l and HRT at 4.9 h (table 3.1).

**Table 3.1. Average volumetric removal rates, outlet concentrations and removal efficiencies for per acetic acid (PAA), total nitrogen (TN), nitrate-nitrogen (NO<sub>3</sub>-N) and nitrite-nitrogen (NO<sub>2</sub>-N) as well as outlet concentrations for chemical oxygen demand (COD) and dissolved oxygen (O<sub>2</sub>) obtained at the various combinations of PAA inlet concentrations and HRTs for experimental woodchip bioreactors according to the central composite design (CCD).**

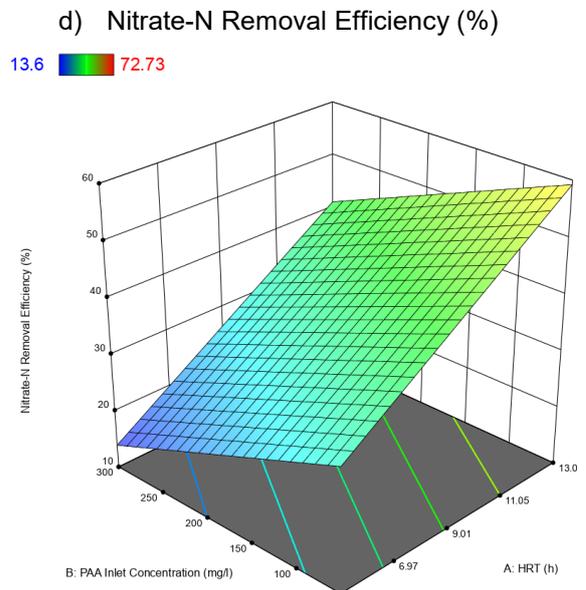
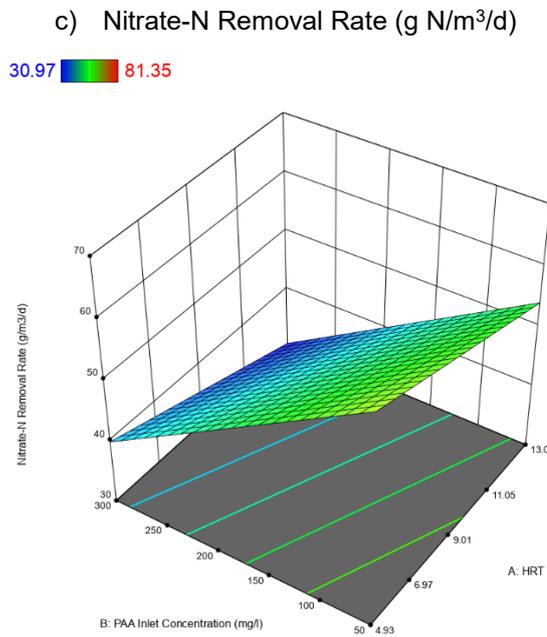
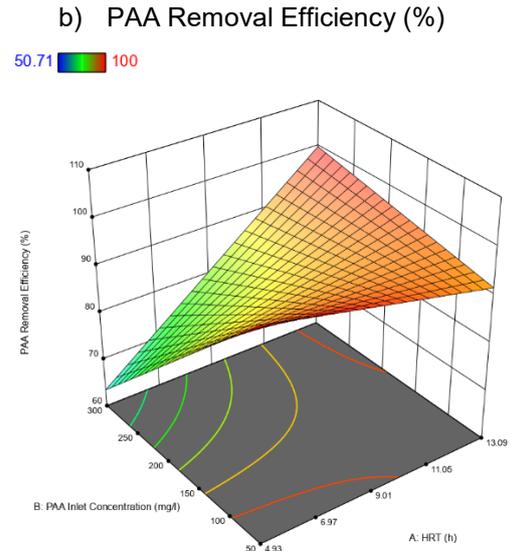
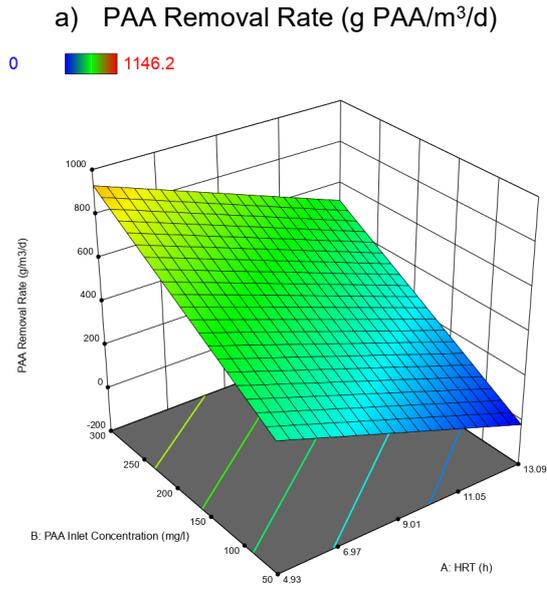
Exp. Runs PAA Inlet conc./HRT (mg PAA/l) / (h)	PAA			Total-N		Nitrate-N		Nitrite-N		COD	Diss. Oxygen	pH
	Outlet Conc. (mg/l)	Removal Rate (g/m <sup>3</sup> /d)	Removal Efficiency (%)	Removal Rate (g/m <sup>3</sup> /d)	Removal Efficiency (%)	Removal Rate (g/m <sup>3</sup> /d)	Removal Efficiency (%)	Outlet Conc. (mg O <sub>2</sub> /l)	Removal Rate (g/m <sup>3</sup> /d)	Outlet Conc. (mg O <sub>2</sub> /l)	Outlet Conc. (mg O <sub>2</sub> /l)	Outlet (-)
175 (0)/ 3.40 (-α)	12.4 ± 1.1	1146.2 ± 7.5	92.9 ± 0.6	35.3 ± 6.4	26.0 ± 2.0	53.1 ± 11.0	16.3 ± 3.4	1.5 ± 1.1	-9.6 ± 8.0	125.0 ± 21.2	3.3 ± 0.8	6.62 ± 0.04
50 (-1)/ 4.93 (-1)	0.0 ± 0.0	243.6 ± 0.0	100 ± 0.0	53.2 ± 7.4	21.2 ± 3.3	81.3 ± 4.2	35.8 ± 1.9	2.7 ± 2.2	-12.5 ± 10.8	39.0 ± 6.8	1.2 ± 0.2	6.67 ± 0.03
300 (+1)/ 4.93 (-1)	147.9 ± 8.3	741.1 ± 40.2	50.7 ± 2.8	33.1 ± 17.1	13.3 ± 6.7	31.0 ± 18.0	13.6 ± 7.9	0.1 ± 0.1	0.2 ± 1.1	731.0 ± 71.2	5.4 ± 0.4	4.28 ± 0.04
0 (-α)/ 8.91 (0)	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	39.8 ± 0.6	28.3 ± 0.2	44.7 ± 6.30	34.9 ± 4.9	0.5 ± 0.4	-1.1 ± 1.4	41.9 ± 1.1	0.4 ± 0.0	6.83 ± 0.02
175 (0)/8.91 (0)	18.4 ± 2.6	421.9 ± 7.0	89.5 ± 1.5	49.1 ± 4.7	55.0 ± 3.8	55.2 ± 2.3	43.1 ± 1.7	0.8 ± 0.5	-2.0 ± 1.5	307.0 ± 47.7	2.4 ± 0.3	6.11 ± 0.10
175 (0)/8.91 (0)	21.0 ± 1.1	415 ± 3.0	88.0 ± 0.6	47.3 ± 4.8	51.8 ± 2.8	49.0 ± 7.2	38.2 ± 5.5	0.2 ± 0.2	-0.3 ± 0.8	402.3 ± 9.0	4.6 ± 0.2	5.90 ± 0.03
175 (0)/8.91 (0)	23.8 ± 4.4	407.2 ± 11.8	86.4 ± 2.5	41.8 ± 10.3	45.1 ± 7.0	42.7 ± 7.9	33.3 ± 6.1	0.5 ± 0.5	-0.9 ± 0.9	422.7 ± 12.2	3.1 ± 0.4	5.91 ± 0.06
175 (0)/8.91 (0)	25.6 ± 6.2	402.4 ± 16.7	85.4 ± 3.5	49.5 ± 5.9	46.5 ± 4.0	48.6 ± 7.2	37.9 ± 5.5	0.0 ± 0.0	0.3 ± 0.4	390.7 ± 68.3	4.9 ± 0.4	5.41 ± 0.06
350 (+α) / 8.91 (0)	29.8 ± 0.6	862.5 ± 1.6	91.5 ± 0.2	31.8 ± 2.1	86.6 ± 2.0	33.5 ± 6.7	26.1 ± 5.3	0.1 ± 0.1	0.1 ± 0.6	850.3 ± 34.9	17.4 ± 0.7	3.59 ± 0.02
50 (-1)/ 13.09 (+1)	0.0 ± 0.0	91.7 ± 0.0	100.0 ± 0.0	57.5 ± 3.8	51.6 ± 5.2	62.0 ± 2.1	72.7 ± 2.8	2.7 ± 1.9	-4.8 ± 3.8	50.5 ± 5.1	2.0 ± 0.1	6.78 ± 0.01
300(+1)/ 13.09 (+1)	29.6 ± 1.3	496.0 ± 2.4	90.1 ± 0.4	25.1 ± 5.0	89.9 ± 5.9	31.5 ± 3.7	37.0 ± 4.5	0.1 ± 0.2	0.0 ± 0.0	525.3 ± 54.2	14.8 ± 0.3	4.40 ± 0.06
175 (0)/ 14.96 (+α)	7.6 ± 1.9	268.6 ± 3.1	95.6 ± 1.1	35.1 ± 4.0	63.7 ± 4.2	38.0 ± 5.8	51.5 ± 7.7	1.3 ± 0.3	-1.9 ± 0.7	214.0 ± 16.8	0.8 ± 0.1	6.29 ± 0.03

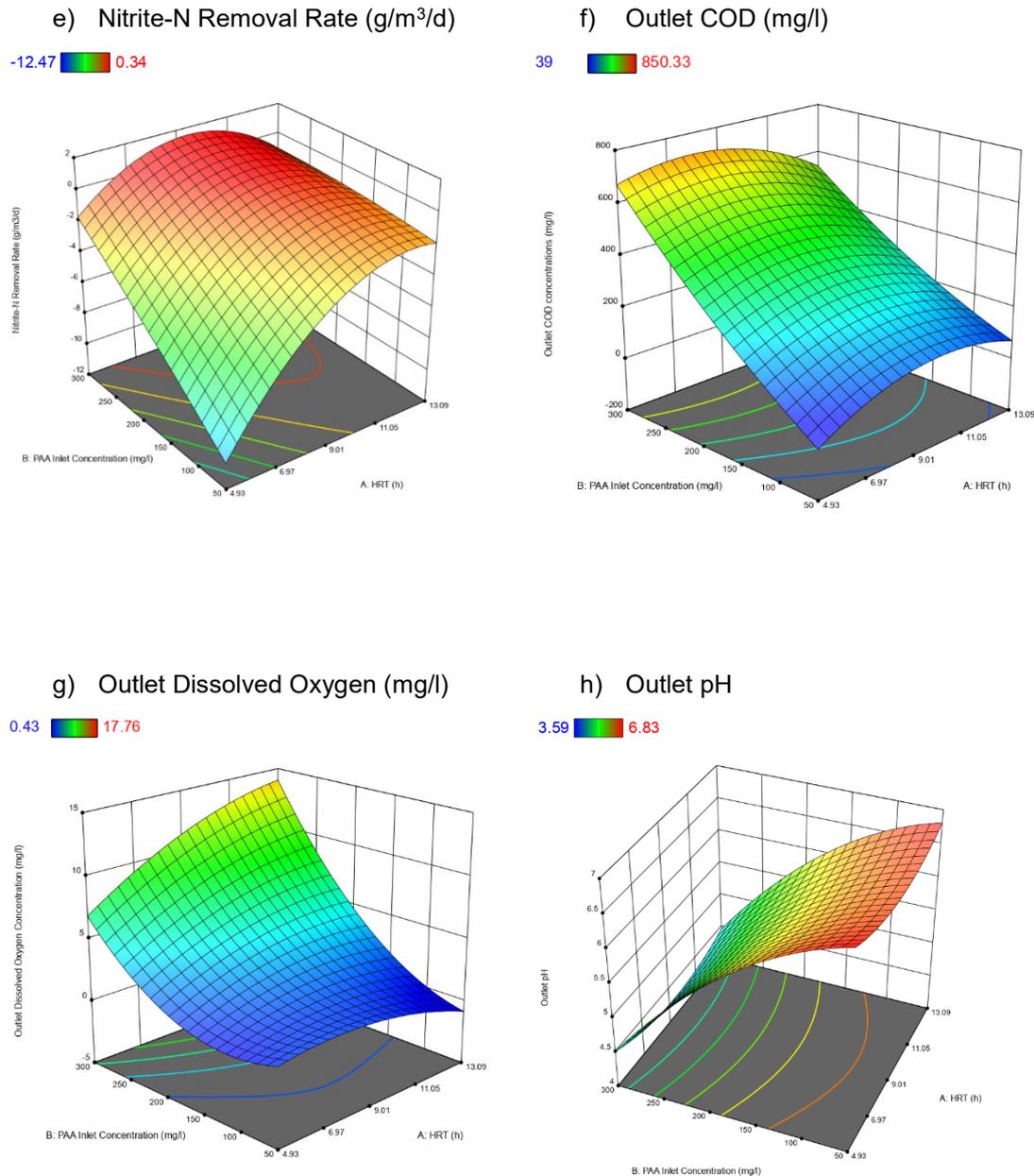
<sup>1</sup> Experimental runs represent combinations of hydraulic retention time (HRT) and PAA inlet concentration where values with the original unit (HRT in h; PAA in mg PAA/l) are followed by the coded CCD values (section 2.2) in parentheses. Removal rates are expressed as g removed per m<sup>3</sup> of woodchip bioreactor volume (i.e. volume of submerged woodchips) per day.

<sup>2</sup> Removal rates and efficiencies are shown as averages with standard deviation of three repeated measurements on the same woodchip bioreactor. Per acetic acid inlet concentrations are based on concentrations measured in the sumps containing PAA + purified water from a Millipore Milli-Q lab water system and the measured flow rates from both sumps into the experimental woodchip bioreactors (figure 3.1).

Removal rates of PAA ranged from 91.7 g PAA/m<sup>3</sup>/d, when PAA was completely removed, to 1146.2 ± 7.5 g PAA/m<sup>3</sup>/d at a PAA inlet concentration of 175 mg PAA/l and short HRT of 3.4 h (table 13.). These removal rates were much higher than PAA removal rates found in the water phase of culture units, distribution channels and sedimentation basins of 0.25 mg PAA/l/h (6 g PAA/m<sup>3</sup>/d), in biofilters of 5 mg PAA/m<sup>2</sup>/h (120 g PAA/m<sup>2</sup>/d, i.e. ~25-100 g PAA/m<sup>3</sup>/d depending on the specific surface area (m<sup>2</sup>/m<sup>3</sup>) of the biofilter media) and constructed wetlands 500 mg PAA/ m<sup>2</sup>/h (i.e. ~10,8 g PAA/m<sup>3</sup>/d at an avg. depth of 0.9 m, Svendsen et al., 2008). The comparatively high removal rates in woodchip bioreactors may be due to the large amounts of organic matter and organic surfaces present. PAA is primarily degraded by chemical oxidation in contrast to the microbial removal of HP by catalase activity (Block, 1991), and the organic surfaces of woodchips may have provided plenty of reaction partners for the free radicals and reactive oxygen species of PAA.

Removal rates of PAA increased with increase in PAA inlet concentration and decreased with increase in HRT (figure 3.2a). In contrast, PAA removal efficiencies decreased with increase in PAA inlet concentrations and increased with increase in HRT (figure 3.2b). For example, at PAA inlet concentrations of 300 mg PAA/L, PAA outlet concentrations were 29.6 ± 1.3 mg PAA/l at long HRT of 13.1 hours, while remaining higher at 147.9 ± 8.3 mg PAA/l when HRT was short at 4.9 hours (table 3.1).





**Figure 3.2. Response surface plots of a) volumetric peracetic acid (PAA) removal rates (g PAA/m<sup>3</sup>/d), b) PAA removal efficiencies (%), c) volumetric nitrate-nitrogen removal rates (g NO<sub>3</sub>-N/m<sup>3</sup>/d), d) nitrate-nitrogen removal efficiencies, e) nitrite removal rates (g NO<sub>2</sub>-N/m<sup>3</sup>/d), f) outlet COD concentrations (mg O<sub>2</sub>/l), g) outlet dissolved oxygen concentrations (mg O<sub>2</sub>/l) and h) outlet pH at different PAA inlet concentrations (mg PAA/l) and hydraulic retention times (h) in experimental denitrifying woodchip bioreactors treating the effluent from a pilot-scale recirculating aquaculture system.**

### Nitrogen removal

Nitrate-N and total-N removal rates in the control treatment (no addition of PAA) were  $44.7 \pm 6.30$  and  $39.8 \pm 0.6$  g/m<sup>3</sup>/d, respectively. Nitrate removal rates were elevated when PAA inlet concentrations were at 50 mg PAA/l (i.e. up to  $81.3 \pm 4.2$  g NO<sub>3</sub>-N/m<sup>3</sup>/d and up to  $57.5 \pm 3.8$  g

TN/m<sup>3</sup>/d). However, when PAA inlet concentrations were elevated further, nitrate-N and total-N removal rates declined with increasing PAA inlet concentrations (table 13., figure 3.2 c). In the modeled range of PAA inlet concentrations of 50-300 mg PAA/l, nitrate removal rates increased with decrease in PAA inlet concentration (figure 3.2 c). Similarly, nitrate-N removal efficiencies increased with decrease in PAA inlet concentration in the modeled range of PAA inlet concentrations (figure 3.2 d). Total-N removal rates and efficiencies were in general similar to the ones for nitrate. However, at two treatments at short HRT (175 mg PAA/l and 3.4 h & 50 mg PAA/l and 4.9 h) total-N and nitrate-N removal showed relatively larger differences as nitrite-N outlet concentrations were elevated at  $1.5 \pm 1.1$  and  $2.7 \pm 2.2$  mg N/l. Nitrite-N was net produced in the majority of treatments, especially when PAA inlet concentrations were relatively low (50-175 mg PAA/l) and when HRT was short (figure 3.2 e).

### **COD, dissolved oxygen and pH**

With increase in PAA inlet concentrations, outlet COD concentrations increased (figure 3.2 f), outlet pH decreased (figure 3.2 h), and outlet dissolved oxygen increased (figure 3.2 g). Outlet COD was  $41.9 \pm 1.1$  mg O<sub>2</sub>/l in the control treatment while it was up to  $850.3 \pm 34.9$  mg O<sub>2</sub>/l at the highest PAA inlet concentration of 350 mg PAA/l.

Dissolved oxygen inlet concentrations measured in the 700l reservoir containing RAS water was  $5.39 \pm 0.27$  (n=3) during the measuring period. Dissolved oxygen concentrations in the outlets of the experimental woodchip bioreactors increased with PAA inlet concentrations expressing a large range of variation over the range of PAA inlet concentrations modeled (figure 3.2 g). Dissolved oxygen outlet concentrations were lowest in the control with  $0.4 \pm 0.0$  mg O<sub>2</sub>/l (4% DO saturation) and with  $17.4 \pm 0.7$  mg O<sub>2</sub>/l (178% DO saturation) highest at the highest PAA inlet concentration of 350 mg PAA/l (figure 3.2 g).

Inlet pH measured in the 700l reservoir containing RAS water was  $7.04 \pm 0.5$  (n=3) during the measuring period. Outlet pH in the experimental woodchip bioreactors decreased with increase in PAA inlet concentration (figure 3.2h). Outlet pH was with pH  $3.59 \pm 0.02$  (n=3) lowest at the highest PAA inlet concentration of 350 mg PAA/L and highest at pH  $6.83 \pm 0.02$  (n=3) in the control bioreactor (figure 3.2 h).

### 3. Conclusions

The environmental impact of newly installed woodchip bioreactors could be reduced if woodchip bioreactors were recirculated through foam fractionators during the start-up period. This would remove large fractions of the harmful DOM initially released, and even the simplest foam fractionators can achieve meaningful reductions of the DOM, which would otherwise be discharged into the environment. These can be built consisting of a tube and an air blower/diffusor, and hence be constructed at relatively low cost on-site. Beyond the start-up period, foam fractionation may also find a useful application in single-pass configurations. This would help to further polish woodchip bioreactor effluents for DOM with little extra cost, as re-aeration of the water is already necessary in most cases before discharge to a receiving body.

Woodchip bioreactors were shown to not only be effective at removing nitrates, but also at retaining and degrading residual amounts of formaldehyde and peracetic acid potentially discharged from the production units. The high treatment efficiencies observed in this study were based on the large organic surface areas, long retention times and active microbial communities present in woodchip bioreactors, a combination which may have the potential to promote effective removal of other types of disinfectants and medicines, too, which deserves further investigations.

The installation of end-of-pipe woodchip bioreactors together with improved formalin application practices, that include lower dosages by treating lowered water volumes at increased exposure times in production units, may enable fish farmers to minimize the amounts of formaldehyde discharged into the natural environment and comply with strict environmental legislations.

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Technical  
University  
of Denmark

DTU Aqua  
Kemitorvet  
DK-2800 Kgs. Lyngby

[www.aqua.dtu.dk](http://www.aqua.dtu.dk)