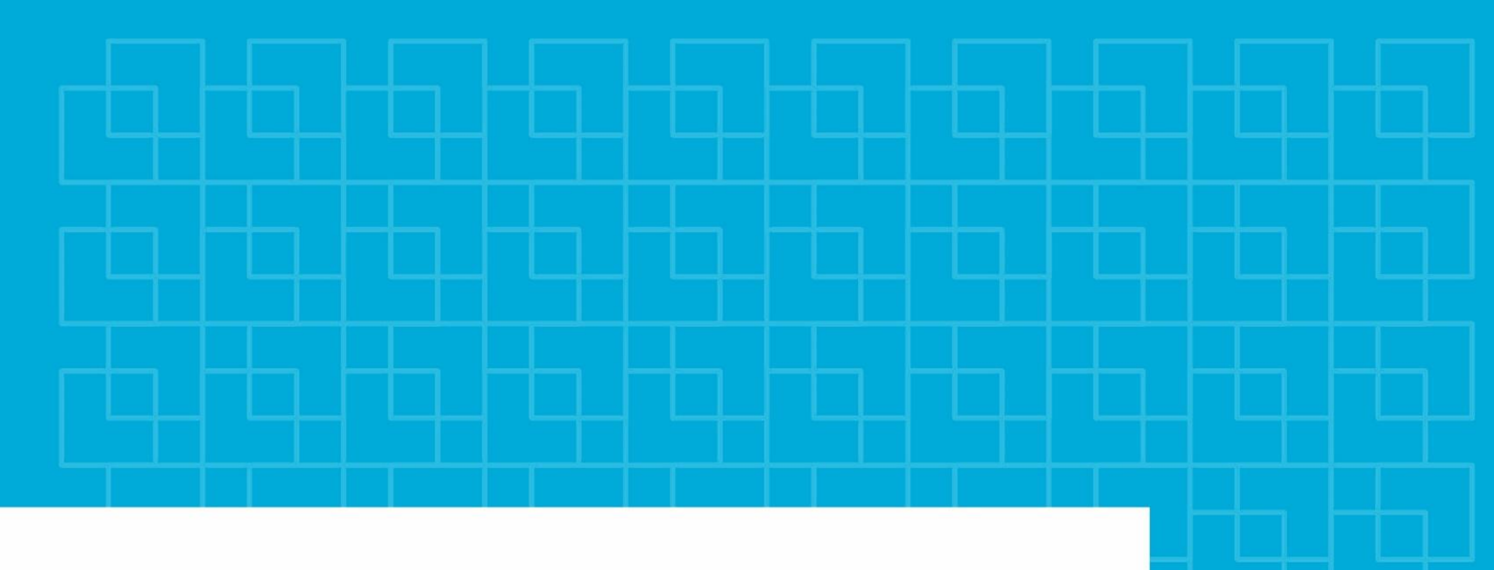

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Assessing the replacement of plastic filtration media with woodchips in biofilters located in recirculating aquaculture systems

Vurdering av treflis som erstatning for filtreringsmedier av plast i biologisk filtrering i resirkulerende akvakultursystemer.

Kristian Haraldsen
Mechanics and Process Technology

Assessing the replacement of plastic filtration media with woodchips in biofilters located in recirculating aquaculture systems

Kristian Haraldsen, 2019

NMBU

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15.05.19

Abstract

There is a growing interest in low-cost filtration media and reducing the environmental effects of aquaculture, by reducing the impact of effluents and reducing the use of plastic. This study evaluated woodchips as an alternative to plastic filtration media used for biological filtration processes. Four 2,24 L laboratory scale reactors were used to test two sizes of woodchip media, and mixes of RK BioElements Light and Mutag BioChips plastic filtration media. One of the reactors had new plastic media, while the other reactor was used for plastic media that already had biofilm established, taken from an operating moving bed bioreactor. The filtration reactors were used to filtrate water from smolt production at the Centre for Fish Research, at Norwegian University of Life Sciences. The average influent concentration of ammonium-nitrogen was 0,279 ± 0,115 mg/L, the average influent concentration of nitrite-nitrogen was 0,033 ± 0,015 mg/L and the average influent concentration of nitrate-nitrogen was 6,372 ± 1,220 mg/L.

The results from the experiment show that nitrification was occurring and that woodchips showed comparable results to plastic filtration media for nitrification. Both oxidations of ammonium and nitrite was occurring. The reactors were compared to a full-scale RAS, and comparable results were achieved for the oxidation of ammonium. Between the reactors, the nitrification rate was found to be higher for the woodchips than the plastic filtration media. No significant change was registered in total nitrogen or nitrate-nitrogen concentration, indicating that denitrification was not occurring at significant levels. This is likely to be due to the presence of dissolved oxygen in the water, known to inhibit denitrification. Visual inspections of the woodchips showed decomposing, indicating that woodchips likely can be used as a carbon source for bacteria. The smallest woodchips particles tested in the experiment were found to increase the levels of total oxygen demand (TOD) in the water, indicating the leaching of organic material.

The filtration media showed comparable clogging and reduction in flowrate. Thus, woodchips cannot be stated to give a higher potential of clogging than plastic media for a bioreactor with static media. The reason for the rapid clogging is believed to be a combination of the reactor design, where access biofilm is not flushed away as in a moving bed filter, and because of high load due to the small volume and the high flowrate. This shows that the reactor design is crucial for the flow and clogging potential.

Keywords: Recirculating aquaculture systems, microbiological filtration, nitrification, woodchips, clogging potential

Sammendrag

Det er en økende interesse for kostnadsreduserende filtreringsmedier, men også for å redusere miljøeffektene av akvakultur, som rensing av avfallsvann og redusere bruk av plastikk. Dette prosjektet tar for seg treflis som et alternativ til filtreringsmedier av plast i biologisk rensing. Fire laboratorieskala filtreringsenheter med volum på 2,24L ble anvendt for å teste to størrelser av treflispartikler, og to mikser av plastmaterialer. Plastmiksen bestod av halvt om halvt med RK BioElements Light og Mutag BioChips filtreringsmedier. Den ene plastmiksen var ubrukt, mens den andre miksen ble tatt fra et opererende filtreringskammer. Filtreringsenhetene ble anvendt til å rense vann fra smoltproduksjon ved Senter for fiskeforsøk lokalisert ved Norges Miljø og Biovitenskapelige Universitet. Inntaksvannet hadde snittkonsentrasjoner på 0,279 ± 0,115 mg/L ammonium-nitrogen, 0,033 ± 0,015 mg/L nitritt-nitrogen og 6,372 ± 1,220 mg/L nitrat-nitrogen.

Resultatene fra forsøket viste at nitrifisering oppstod, og at treflis viste sammenlignbare nitrifiseringsresultater med filtreringsmediene av plast. Det forekom både oksidering av ammonium og nitritt. Filtreringsenhetene ble sammenlignet mot et av anleggene ved forskningssenteret, noe som viste sammenlignbare resultater for oksidering av ammonium. Sammenligning av filtreringsmediene mot nitrifikasjonsrate, viste at treflis hadde høyest nitrifiseringsrate. Ingen signifikante konsentrasjonsendringer ble registrert for gjennomsnittsmålingene av totalt nitrogen eller for nitrat-nitrogen. Noe som indikerer at det ikke har skjedd denitrifikasjon på merkbare nivåer. Årsaken til dette er trolig tilstedeværelsen av oppløst oksygen i vannet, noe som er kjent at forhindrer denitrifikasjon. Inspeksjon av treflisen etter forsøket viste klare tegn til nedbrytning, noe som indikerer at bakterier kan bruke treflis som karbonkilde. De minste treflispartiklene som ble testet viste en økning i TOD (Total Oxygen Demand), som indikerer at filteret tilfører organisk material til vannet.

Filtreringsmediene viste lignende resultater når det kommer til fortetting og reduksjon i volumstrøm. Derfor er det ingen grunnlag til å si at treflis har høyere potensial for å tette seg enn for plastmedier, når en bruker et anlegg med statisk filtreringsmedium. Årsaken til at anleggene tettet seg så raskt, er trolig en kombinasjon av reaktorenes design, med tanke på at overflødig biofilm ikke blir vasket vekk, og høy belastning på anlegget på grunn av stor volumstrøm i forhold til volum. Noe som viser hvor viktig reaktorens design har for stabil volumstrøm og fortetting.

Nøkkelord: Resirkulerende akvakultursystem, mikrobiologisk filtrering, nitrifikasjon, treflis, tettingspotensial

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Equations

#	Description	Equation
[1]	pH is measured as the negative logarithm of H+-ion concentration	$pH = -\log[H^+]$
[2]	pH equilibrium in water	$2H_2O\ (l) \rightleftharpoons H_3O^+(aq) + OH^-(aq)$
[3]	pH equilibrium in water (ii)	$H_2O\ (l) \rightleftharpoons H^+(aq) + OH^-(aq)$
[4]	Dissolved carbon dioxide in water	$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
[5]	Breakdown of organic matter by aerobic bacteria	$CHONS\ (organic\ matter) + O_2 + bacteria \rightarrow CO_2 + H_2O + NH_3$
[6]	Breakdown of organic matter to cell tissue	$CHONS + O_2 + bacteria + energy \rightarrow C_5H_7NO_2(new\ cell\ tissue)$
[7]	Endogenous respiration; Cells consume their own tissue to create energy for cell maintenance	$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O$
[8]	Ammonia and ammonium relationship in water.	$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$
[9]	Simplified stoichiometry for oxidation reaction of ammonium by bacteria of Nitroso-group	$2NH_4^+ + 3O_2 \xrightarrow{(Nitroso-bacteria)} 2NO_2^- + 4H^+ + 2H_2O$
[10]	Simplified stoichiometry for oxidation of nitrite by bacteria of Nitro-group	$2NO_2^- + O_2 \xrightarrow{(Nitro-bacteria)} 2NO_3^-$
[11]	Simplified total stoichiometry for nitrification	$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$
[12]	Stoichiometry for oxidation of ammonium by Nitrosomonas.	$55NH_4^+ + 5CO_2 + 76O_2 \rightarrow C_5H_7O_2N + 54NO_2^- + 109H^+ + 52H_2O$
[13]	Stoichiometry for oxidation of nitrite by Nitrobacter.	$400NO_2^- + 5CO_2 + NH_4^+ + 195O_2 + 2H_2O \rightarrow C_5H_7O_2N + 400NO_3^- + H^+$

#	Description	Equation
[14]	Denitrification steps	$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$
[15]	Denitrification with wastewater as carbon source	$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow$ $5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^-$
[16]	Denitrification with methanol as carbon source	$5CH_3OH + 6NO_3^- \rightarrow$ $3N_2 + 5CO_2 + 7H_2O + 6OH^-$
[17]	Denitrification with Acetate as carbon source	$5CH_3COOH + 8NO_3^- \rightarrow$ $4N_2 + 10CO_2 + 6H_2O + 8OH^-$
[18]	Conversion from concentration of ammonium-nitrogen to ammonium	Ammonium = Ammonium Nitrogen x 1,29
[19]	Conversion from concentration of nitrite-nitrogen to nitrite	Nitrite = Nitrite Nitrogen x 3,28
[20]	Conversion from concentration of nitrate-nitrogen to nitrate	Nitrate = Nitrate Nitrogen x 4,43
[21]	Porosity of material in water. Void volume divided by total volume of the medium.	$\emptyset = \frac{V_V}{V_T}$
[22]	Formula for determining the specific area of woodchips.	$S_s = \frac{2 \cdot m_{ch}}{\rho_w \cdot g}$
[23]	Mass of a volume of woodchips without void volume.	$M_{ch} = V_w \cdot \rho_w$
[24]	Volume of a block of woodchips described with surface area and woodchip thickness	$V_w = g \cdot S_s/2$
[25]	Formula for determining specific surface area based on the formula of specific area.	$S_a = \frac{2 \cdot m_{ch}}{\rho_w \cdot g} \cdot \rho_{bulk}$
[26]	Null hypothesis, all means in a series of *k* groups are equal.	$H_0: \mu_1 = \mu_2 = \mu_3 = \cdots = \mu_k$
[27]	Tukey`s range test, formula for determining statistically significantly difference between two independent groups.	$q_s = \frac{Y_A - Y_B}{SE}$
[28]	Null hypothesis	$H_0: \mu = 0$
[29]	Alternative hypothesis	$H_A: \mu \neq 0$

#	Description	Equation
[30]	One-sample t-test for testing group sample mean against a specified mean.	$t = \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}}$
[31]	Formula for standard deviation S.D. for a series of samples	$SD = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n - 1}}$
[32]	Formula for determining the total standard deviation when comparing two groups.	$Total\ Standard\ Deviation = \sqrt{SD^2 + SD^2}$

1. Introduction

1.1 Background

Plastics have been used worldwide since the 1930s and can be found in almost everything we surround ourselves with, from food packaging to clothes. The overall production in the world is around 300 million tons of plastic a year. Of this around half is used in disposable products, and globally only 5 % of plastic is recycled after use (Avset, 2017). The low recycling rate and bad handling of trash make plastic a global environmental problem. Increasing the problem is the fact that plastic is slowly broken down in nature (Nerland et al., 2014). Plastic finds its way into nature because of many reasons. Poorly secured garbage dumps close to the oceans drives plastic into the ocean by the wind. While storms, flooded rivers, and natural disasters can as well drive unsecured items into the ocean. Every year at least 8 million tons of plastic waste ends up in the ocean, lakes, and rivers (Tyree & Morrison, 2017).

Plastic has a direct effect on marine life, as species can eat plastic or get entangled in litter. This fact has been known for decades, and is seen in stomachs of seabird, who mistake pieces of plastic for food. Later studies show that this is the case for many other marine species, such as seahorses, fish and larger marine animals (Nerland et al., 2014).

The last decade another issue regarding plastics have brought great concern. Plastic in particles less than 5 mm, called microplastic, pollutes much of the marine environment (Nerland et al., 2014). Microplastics can be found everywhere, even in our drinking water. These plastics come from the release of manufactured microplastics and from the breakdown of larger plastic litter (Tyree & Morrison, 2017). An example of this is microplastics from artificial soccer turfs. These fields contain tons of microplastic and are frequently flushed into the drain by rain or spread into nature as the plastic pieces stick to clothes or shoes (Gulden, 2018).

It has been proven that marine organisms ingest microplastics, and laboratory studies show that microplastics can have sub-lethal effects as reduced feeding and increased uptake of certain contaminants. Studies have shown that for fish there has been seen changes in gene regulation. The research field on the long term effects of microplastics is still quite new within marine research. Therefore it still remains a lot of research before the long-term effects are known (Nerland et al., 2014).

Aquaculture is using a lot of plastic in the production of fish, from boxes of Styrofoam to filtration media. A study of aquaculture facilities in Norway estimates that 325-ton microplastics are being released into the sea from plastic pipes used for pumping feed pellets. These are being torn down due to high shear. This is probably only one of many uses

of plastic that is causing the release of microplastics into the ocean from aquaculture (Christensen, 2017).

In recirculating aquaculture systems plastic is also a frequently used material. Pipes bringing water around, fish chambers, filtration chambers, and filtration media are only some of the uses. All of these are potential sources to microplastics. Especially plastic filtration media in moving bed chambers are exposed to high shear forces and friction.

As a way of reducing the use of plastic in filtration-systems, an approach can be to replace the plastic filtration media with a natural filtration media. A range of filtration media have been tested for its effect in biological chambers, woodchips are one of them. Earlier studies have been positive regarding its viability as a replacement. Woodchips are an environmental resource, if harvested sustainable (Svanæs, 2004), and the woodchips may serve other purposes after use in biofiltration, as a fertilizer can be one of them.

1.2 Scope of Thesis

The thesis focuses on the use of woodchips in aquaculture recirculating water systems. A practical study using lab-scale filtration reactors have been carried out at the Centre for fish research at Norwegian University of Life Sciences. The testing included the use of woodchips of two sizes and a mix of two types of plastic filtration media.

The parameres measured in the testing of filtration media were flow rate, pH, NH_4^+-N (ammonium-nitrogen), NO_2-N (nitrite-nitrogen), NO_3-N (nitrate-nitrogen), Tot N (total nitrogen) , TOD (Total oxygen demand), porosity, density, and pressure drop.

1.3 Objective of the thesis

Research if woodchips can replace plastic filtration media in microbiological filtration and/or if there are other potential uses for woodchips in aquaculture water treatment.

The specific aims of the thesis are to:

- Perform an experiment on microbiological filtration where woodchips are compared against plastic filtration media.
- As a part of the experiment, compare flowrate and clogging potential for the filtration media types.
- Design and perform a test on pressure drop for the filtration media
- Determine the physical properties of the filtration media
- Evaluate potential uses of woodchips in aquaculture water filtration based on the studies carried out and an extensive literature review.

1.4 Limitations

Parameters such as COD (chemical oxygen demand), DO (dissolved oxygen), TP (total phosphorus), TAN (total ammonium nitrogen (NH_4^+-N and NH_3-N)), and TSS (Total suspended solids) were excluded due to time and equipment limitations.

The concentration of ammonium was low in the recirculated water at the Centre for Fish Research, this is known to affect nitrification effect. The wastewater from aquaculture is more complex compared to artificial wastewater used in many lab-scale tests of biological filtration. Thus it is more parameters that may affect the growth of bacteria than there would be in a more controlled laboratorium environment. No additional concentrations of ammonium were added to increase the concentration. This was due to the water used for the study was pumped back into the recirculating system after filtration. Thus the concentration of ammonium in the water is a limitation for the thesis, as the systems were only tested under low concentrations.

Another limiting factor is the stability of the system. Where in a controlled laboratory environment with artificially made wastewater for lab-scale use, one will be able to deliver stable concentrations of ammonium, nitrite, and nitrate to the filtration systems, while with use of wastewater from fish chambers the levels of nutrients in the water increases as the fish grow. Thus, it is expected higher effects of nitrification with time.

2. Literature review

In the literature review a more comprehensive presentation of important factors of water quality, how recirculation systems are built up, what processes that are included, but also knowledge about wood and earlier research on the use of woodchips in biological reactors will be presented.

2.1 Fresh-water quality for aquaculture

Water quality is an important factor to achieve optimal growth conditions for fish in aquaculture. Changes in only one or two quality parameters will give an impact on fish well-being and growth (Lekang & Fjæra, 1997). Under the essential parameters for water quality will be presented.

2.1.1 Temperature

The water temperature is important when it comes to activity and comfort for the fish. In wild condition, the salmon uses 2-5 years to reach the fish size called smolt. After this age, the fish have gone through a physiological change that makes it able to live in saltwater. When using heated water, this process is reduced to one year (Gjedrem, 1979).

For salmonids, the lower temperature limit is -0,5^{o}C, and the top limit is 25°C. For rainbow trout, the optimum temperature is 18°C, and for salmon the optimum temperature is 16°C. At temperatures as low as 4-5°C the growth is close to none, while temperatures over 20°C give too little available oxygen for the fish. By maintaining stable optimate temperatures, the operation runs smoother and production time is also shortened significantly (Gjedrem, 1979).

2.1.2 Oxygen and nitrogen gas.

The fish use oxygen in the water in its inhalation, plants use it at night, and it's used in the biological break down of organic material. Because the fish breathes, the oxygen levels in the water are vital for the fish. The oxygen amount decreases with increasing water temperature, which is one of the reasons why high temperatures create problems for the fish. Higher temperatures increase fish activity, thus also the oxygen and water use. It`s known that salmonids need at least 5 mg/l oxygen in the water over a longer period (Gjedrem, 1979).

In freshwater, most of the oxygen comes from the air and photosynthesis of plants, while in salt water most of the oxygen comes from freshwater, from the air or produced by photosynthesis by plankton (Gjedrem, 1979). Ideally, the oxygen saturation should be around 95-100 %, and the nitrogen content should not be oversaturated. 100 % oxygen saturation means that the content of oxygen is at the maximum level of what that the water can hold at atmospheric pressure (Lekang & Fjæra, 1997). In a recirculating aquaculture system, the water is not as exposed to oxygen sources as in nature. Therefore, oxygen must be added by adding air or pure oxygen gas.

Nitrogen (N_2) can be dangerous to the fish when oversaturated. Oversaturation can cause gall bladder disease, which will cause damage or death if levels are over 102-105 % (Lekang & Fjæra, 1997).

2.1.3 Buffer ability and pH

Buffer ability is the ability to maintain the pH value when adding acid or base to the water. The alkalinity and acidity in the water affect this ability. The alkalinity is the ability to neutralize acidic components, while the acidity is the ability to neutralize basic components (Lekang & Fjæra, 1997).

We can define pH in water as the negative logarithm of the H^+-ion concentration

$$pH = -\log[H^+] \qquad [1]$$

The equation for pH is:

$$2H_2O\ (l)\ \rightleftharpoons H_3O^+(aq) + OH^-(aq) \qquad [2]$$

Which can also be written:

$$H_2O\ (l)\ \rightleftharpoons H^+(aq) + OH^-(aq) \qquad [3]$$

The pH-scale is neutral at 7, where pH-values under 7 indicates acidic liquid, and pH-values over 7 indicates alkaline liquid. At 7 the equation is in equilibrium and the amount of OH^- and H^+ ions are equivalent. The logarithmic correlation means that with a pH of 8, then the concentration of OH^- will be ten times the concentration of H^+ ions. For lakes and waterways in Norway the pH lays around (4,5-7) (Lekang & Fjæra, 1997). pH in seawater lays around 8 and is very stable due to the high content of salt which gives the water high buffer ability (Gjedrem, 1979).

For salmonids, the optimum temperature is neutral water (pH = 7) or a little higher (Gjedrem, 1979). Low pH can cause damage to skin, eyes and gill, and give a reduction in growth. It can also increase the solubility of metal ions, that will occur in forms that are toxic. It is recommended that pH should never be lower than 5-6, and in aquaculture facilities the

level is usually set in the range 6,5-9. pH in acid water is adjusted by removing H^+ ions. This can be done by adding a substrate that attracts and binds the free H^+ ions, such as hydroxides (OH^-) or carbonates (Lekang, 2007). The pH can also be raised by adding water with higher alkalinity, such as groundwater, or by using a calk filter.

pH in water is affected by the concentration of dissolved carbon dioxide in water. When CO_2 is dissolved in water, it creates a week acid, carbonic acid, H_2CO_3. This acid can be separated into a hydrogen ion (H^+), and a bicarbonate ion (HCO_3^-) as seen in equation [4] (Patel & Majmundar, 2018).

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad [4]$$

When the concentration of CO_2 is high, there is a shift to the right in the reaction, producing more H^+ ions. Thus, the pH decreases. While at low concentration of CO_2 the reaction shifts to the left and the pH increases (Patel & Majmundar, 2018).

2.1.4 Organic material

Fish feed and excrements from the fish introduce organic material to the water. Organic materials in water give bacteria and fungus in water nutrients to grow on. The microorganisms use oxygen when they break down organic material, and lowered oxygen levels can harm fish and other organisms in the water. Increased organic material gives an increase of particles and nutrients like nitrogen and phosphorus. When it comes to nitrogen components particularly the concentration of ammonium increases (Gjedrem, 1979).

Organic material is decomposed by aerobic bacteria, and there will be decomposing present as long there is material to degrade and enough oxygen. There are three essential activities that occur with organic material. (1) Some of the waste will be oxidized to an end-product; this is to create energy for the maintenance of cells and synthesis of new cell tissue. (2) Some waste will concurrently be converted into new cell tissue using the energy released from oxidation. (3) When the organic material is used up, new cells will start to consume their own cell tissue to create energy for cell maintenance, a process called endogenous respiration. These processes happen by the equations listed under, [5], [6] and [7]. Organic material is described as CHONS (Carbon, Hydrogen, Oxygen, Nitrogen, and Sulphur), and cell tissue as $C_5H_7NO_2$ (Tehobanoglous et al., 2003).

Oxidation:

$$CHONS\ (organic\ matter) + O_2 + bacteria \rightarrow CO_2 + H_2O + NH_3 \quad [5]$$

Synthesis:

$$CHONS + O_2 + bacteria + energy \rightarrow C_5H_7NO_2 (new\ cell\ tissue) \quad [6]$$

Endogenous Respiration:

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O \quad [7]$$

There are several methods used to determine the concentration of organic material in the water. These methods are built on measuring the oxygen demand, which is how much oxygen needed to oxidize all the organic material in a water sample. This gives an estimation of how much oxygen that will be used when the organic material is broken down by microorganisms.

Biological oxygen demand, BOD, is a method where aerobic bacteria break down (oxidize) the organic matter in a sample to CO_2 and H_2O under controlled conditions. Then the amount of oxygen used in the process is measured (Ødegaard, 2014). The BOD method is time-consuming as it takes five days with BOD_5 and seven days with BOD_7. Another disadvantage is that bacteria need to be present in the sample. For wastewater, there is usually enough bacteria present, but for cleaner water bacteria must be added. It can be challenging to know what bacteria that is most dominant in the plant, therefore the bacteria added might not be the same, which will give a less trustworthy estimate (Ødegaard, 2014).

Chemical oxygen demand, COD, is similar to BOD, but instead of bacteria, the oxidizing agent is used. In wastewater analysis, a mixture of sample water, the oxidizing agent potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid are boiled. The amount of potassium dichromate used is measured and converted into oxygen, which gives the oxygen demand. It differs from the BOD analysis where only the oxygen demand of biodegradable material is measured. For COD the total amount of organic material is measured, and not how much of it that is biodegradable (Ødegaard, 2014).

TOD, Total oxygen demand, is a chemical-free way of determining the amount of organic material in water samples. This method is done by evaporating water samples at high temperatures (1200 ^{o}C). The oxidization is catalyst-free, and an oxygen detector determines the amount of oxygen used during the combustion. The analyzer measures the oxygen demand of all oxidizable substances in the water sample. The method is suitable for larger sample series as one sample analysis only takes a few minutes (LAR, 2019).

2.1.5 Phosphorus

In freshwater, phosphorus is often a limiting factor for the production rate. Even a small rise in the concentration can give increased algae and plant growth. Increased algae growth due to increased production can give problems as large day-variations in oxygen levels and pH. The oxygen level decreases at night cause to the algae and plant respiration. It is measured up to a 40 % decrease in oxygen at larger facilities during the night (Gjedrem, 1979).

2.1.6 Nitrogen

Nitrogen can be found in wastewater as organically bound nitrogen and inorganic nitrogen. Organic nitrogen can be found in aquaculture wastewater as urea, which is made by fish when proteins are broken down in the body and is separated with the urine. Inorganic nitrogen is found as ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). The sum of the nitrogen in organic nitrogen and inorganic nitrogen makes the parameter total nitrogen (Tot N), which is often the parameter used for measuring and regulating purification wastewater before it is released in nature (Ødegaard, 2014).

Ammonium and ammonia are produced by fish as it breaks down protein and releases organic nitrogen in the water through excrements, and due to fish feed. Microorganisms as bacteria and fungus convert organic nitrogen to ammonium and ammonia in a process called ammonification. Of these two, ammonia (NH_3) is most toxic. For fish farming using a run-through water system there will be no problem of metabolic ammonia, but for recirculating systems accumulation of ammonia will occur and can be a problem at high pH. Levels of ammonia at 25-300 µg/L is proven to give raised mortality for salmonids, but problems occur at much lower levels. Levels down to 10 µg/L is proven to impact on fish gills. In Norway, a conservative limit for ammonia concentration is set to 3-5 µg NH_3-N/L, dependent on operating temperature (Bjerknes, 2007).

The relationship between ammonia and ammonium is described with the following formula:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad [8]$$

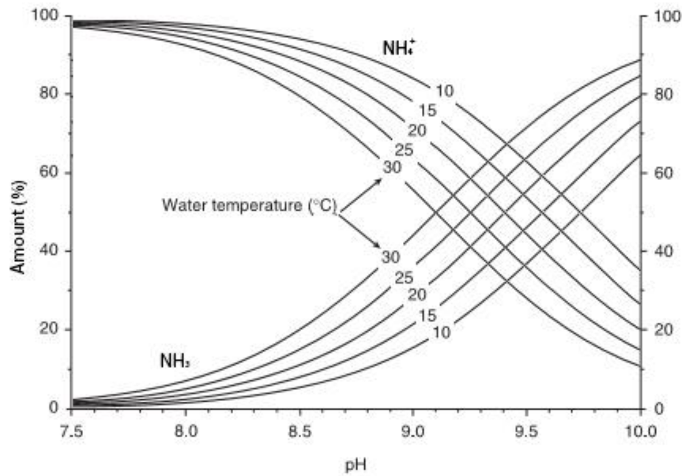

Figure 2.1: The relationship between NH3/NH4+, pH and temperature (Hargreaves & Tucker, 2004)

Ammonia can be described as a week base and ammonium as the conjugate acid. The amount of each component is dependent on pH, salinity and temperature, see figure 2.1. High pH drags the reaction to the right side and gives more ammonium than ammonia. Low pH drags the reaction to the left side and gives more ammonia. The pH in the water at the Centre for fish research is monitored to lay between 7,7 and 8, and the water temperature lays close to 12,8°C, which indicates close to 98,5-99 % NH_4^+ and 1-1,5 % NH_3.

After ammonification, there are two more microbiological processes, called nitrification and denitrification. These are presented under.

Nitrification

Nitrification is the two-step biological process where ammonium is oxidized to nitrite, and nitrite is oxidized to nitrate. This process is useful in water as the toxicity of ammonia, ammonium, and nitrite is higher than for nitrate. Systems designed for nitrification often have longer retention times than systems made for removal of organic material. Most of the organic material needs to be removed before a nitrification process effectively can occur as the heterotrophic bacteria have higher biomass yield and growth and therefore can dominate the surface area on the media in a reactor (Tehobanoglous et al., 2003).

In active sludge and biofilm processes, aerobic autotrophic bacteria are responsible for the nitrification. These bacteria use dissolved oxygen and for their metabolism and growth (Haug & McCarty, 1972). The two steps in the nitrification process are done by two separate groups of bacteria. Both groups are autotrophic, meaning that they can build organic compounds based on simple substances present in the surroundings. The two most known bacteria in nitrification are *Nitrosomonas,* oxidizing ammonium to nitrite, and *Nitrobacter* oxidizing nitrite to nitrate (Tehobanoglous et al., 2003) other bacteria proved to oxidize ammonium to nitrite (prefix *Nitroso*) is *Nitrosococcus, Nitrosospira, Nitrosolobus,* and *Nitrosobrio.* Other bacteria proven to oxidize nitrite to nitrate (prefix *Nitro*) are Nitrocystis, *Nitrococcus, Nitrospira, and Nitrospina* (Painter, 1970; Tehobanoglous et al., 2003).

When establishing a nitrification process, the bacteria use some time to colonize. This is dependent on the amount of ammonium in the water, temperature, and salinity. Colonization in freshwater takes a few to several days, while up to a month in saltwater. The *Nitrosomonas* will become active hours and up to days before the *Nitrobacter*. Therefore it is normal to experience spikes in nitrite concentration in the time before the biofilter becomes fully functional (Stickney, 2000).

Stoichiometry

$$2NH_4^+ + 3O_2 \xrightarrow{(Nitroso-bacteria\ group)} 2NO_2^- + 4H^+ + 2H_2O \qquad [9]$$

$$2NO_2^- + O_2 \xrightarrow{(Nitro-bacteria\ group)} 2NO_3^- \qquad [10]$$

Total oxidation reaction:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad [11]$$

The first step with Nitrosomonas bacteria:

$$55NH_4^+ + 5CO_2 + 76O_2 \rightarrow C_5H_7O_2N\ (cell) + 54NO_2^- + 109H^+ + 52H_2O \qquad [12]$$

Second step with Nitrobacter bacteria:

$$400NO_2^- + 5CO_2 + NH_4^+ + 195O_2 + 2H_2O \rightarrow C_5H_7O_2N\ (cell) + 400NO_3^- + H^+ \quad [13]$$

As seen in equation [11] based on equation [9] and [10] one mole ammonium can give one mole nitrate. The reactions also produce hydrogen ions which will affect the pH. The equations [12] and [13] show that the amount of ammonium and nitrite needed to produce one mole cell is high.

Environmental factors

The nitrifying bacteria are dependent on and can be regulated by several factors, as ammonium availability, pH, temperature, oxygen concentration, bacteria competition, and organic carbon availability. The most important of these factors are pH, temperature and dissolved oxygen concentration (Strauss & Lamberti, 2000).

The higher the concentration of ammonium, the more effective the nitrification process will be (Lekang & Fjæra, 1997). The presence of dissolved oxygen is also a limiting factor, as the two bacteria are aerobic and will only live and perform nitrification when there is oxygen present. If the bacteria are derived from oxygen, even only for a short period, the bacteria will die, and the biofilter will start producing high levels of ammonia and nitrite (Stickney, 2000). For low concentrations of dissolved oxygen (<0,50 mg/L) in systems where nitrification is inhibited, the *Nitrobacter* is shown to be more inhibited than *Nitrosomonas*. This gives an increased concentration of NO_2-N in the effluent. (Tehobanoglous et al., 2003).

Temperature is a limiting factor. The *Nitrosomonas* and *Nitrobacter* have an ideal temperature at about 30 ^{o}C, and temperatures below 10°C give low growth. (Lekang & Fjæra, 1997). pH is also a limiting parameter; the ideal pH-value for the nitrification process is 7,5-8,0, and the rate significantly decline at pH levels below 6,8 (Tehobanoglous et al., 2003). During the nitrification process, the bacteria will produce hydrogen ions H^+, which lowers the pH. In a system with a high load, it will be necessary to add chalk or water to compensate (Lekang & Fjæra, 1997).

A different factor is the presence of organic material, which in a nitrification chamber lead to the growth of heterotrophic bacteria that uses the carbon in organic material to grow. These bacteria can outcompete the nitrification bacteria (Lekang & Fjæra, 1997). A study by (Strauss & Lamberti, 2000) researched the effect of organic material on nitrification rates in stream sediments. Their findings and conclusions are that organic carbon does inhibit nitrification and that the inhibition-effect increases with carbon quality. Their study also showed an increase in microbial respiration of 4-6 times, indicating the growth of other bacteria. They state that organic carbon is an important factor in the regulation of nitrification rates (Strauss & Lamberti, 2000). A different study showed increased

competition of aerobic denitrifying bacteria, and that the degradation rate of ammonium was reduced when the concentration of organic material was increased (Tang et al., 2010).

The nitrification organisms can also be affected by toxicity. The aerobic heterotopic organisms are sensitive to a range of compounds both organic and inorganic. This will in many cases show as inhibition and not a complete elimination of nitrification. Compounds toxic to the nitrifying bacteria are solvent organic chemicals, proteins, amines, tannins, alcohols, phenolic compounds, cyanates, carbamates, ethers, and benzene. (Tehobanoglous et al., 2003)

Metals are also capable of inhibiting nitrification. A study by (Skinner & Walker, 1961) on the effect of metallic ions on the growth of *Nitrosomonas* showed that nitrification could be completely inhibited for levels of 0,25 mg/L nickel, 0,25 mg/L of chromium, and 0,10 mg/L of copper. While metallic ions from iron, cobalt, manganese, and zinc had no effect on nitrification.

Denitrification

In denitrification, nitrate is reduced to nitrite then reduced to nitric oxide (NO) and nitrous oxide (N_2O) and then reduced to dinitrogen (N_2 (g)) (Knowles, 1982). In denitrification nitrate or nitrite are used as electron-acceptors for the oxidization of a range of organic or inorganic electron donors (Tehobanoglous et al., 2003). The nitrification process is enough to make the water safe for the fish; this is because the nitrate is less toxic for the fish than ammonia (Lekang, 2007).

There is a wide range of bacteria that are capable of denitrification, both heterotrophic and autotrophic bacteria. Heterotrophic bacteria that have been believed to reduce nitrogen components are; *Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Azospirillium, Bacillus, Chromobacterium, Corynebacterium, Cytophaga, Flavobacterium, Halobacterium, Hypomicrobium, Methanomonas, Moraxella, Neisseria, Paracoccus, Propionibacterium, Pseudomonas, Rhizobium, Rhodopseudomonas, Spirillum, Thermothrix, Thiobacillus, Vibrio, and Xanthomonas.* The most widely distributed of these species are *Pseudomonas* species (Gayle et al., 1989; Payne, 1981). Most of these bacteria are not strict anaerobes, but facultative anaerobic organisms; they can use oxygen as well as nitrate or nitrite as electron-acceptors. When there are cycles of aerobic and anaerobic conditions, there is a phase with lag, before denitrification occurs. In this phase, nitrate is reduced, but nitrite tends to accumulate *(Gayle et al., 1989).* Some of these can carry out fermentation in the absence of nitrate or oxygen (Tehobanoglous et al., 2003).

Autotrophic bacteria that carry out denitrification use hydrogen and reduced sulfur components as electron donors. Under these conditions, no carbon source is required (Gayle

et al., 1989). Both heterotopic bacteria and autotrophic bacteria can grow heterotrophically if an organic carbon source is present (Tehobanoglous et al., 2003).

Stoichiometry

To make the denitrification process happen there must be a sufficient carbon source available (Brenner & Argaman, 1990). Usually, it is necessary to add organic carbon as nutrients to the bacteria, as there is not enough organic carbon present in wastewater from aquaculture (Lekang & Fjæra, 1997). Almost any compound that degrades with oxygen as the electron acceptor are also able to serve as an electron donor with nitrate. The electron donor in the biological denitrification process is usually one of three sources:
(1) Biodegradable material in the water - equation [15]
(2) Biodegradable material produced by microorganisms - equation [16]
(3) External source as methanol or acetate - equation [17]

Biodegradable organic material in wastewater is often represented as $C_{10}H_{19}O_3N$. The stoichiometric equations for reduction of nitrate with three typical electron donors; (Tehobanoglous et al., 2003)

All steps in denitrification:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad [14]$$

Wastewater:

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \quad [15]$$

Methanol:

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad [16]$$

Acetate:

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \quad [17]$$

Environmental factors

The denitrification process is dependent on several factors to work optimally. This is; removal of oxygen for the bacteria to perform the reactions, presence of organic carbon, presence of nitrogen oxides, pH and temperature in the water. Optimum pH is 7-9 and temperature 20-30 ^oC. (Knowles, 1982) (Lu et al., 2014).

For the nitrogen oxides to be reduced by the bacteria, the oxygen must be removed. This is because the denitrifying bacteria will only use the nitrogen oxides as electron acceptors if

there is an absence of oxygen (Lu et al., 2014). The concentration of dissolved oxygen at 0,2 mg/L or above have been shown to inhibit the denitrification for a *Pseudomonas* culture (Tehobanoglous et al., 2003). The oxygen is efficiently removed by adding methanol (Lekang & Fjæra, 1997).

The denitrification process affects the pH as it produces OH^- ions, which increases the pH. The change of pH in the water has no significant effect on the denitrification rate for pH between 7 and 8, but the denitrification rate decreases at lower values (Tehobanoglous et al., 2003).

2.2 Recirculating Aquaculture Systems

Most of today's salmon smolt production in Europe of around 250 million per year (2009) is done in land-based facilities with flow-through water systems (Bergheim et al., 2009). There has been an increased interest in recirculating water systems, RAS, because of limited water supply during the growing season due to dry periods. This is not the only cause of increased interest. The use of RAS gives increased abilities when it comes to controlling water parameters such as temperature, carbon dioxide, dissolved oxygen, the nitrogen chain, pH, salinity and suspended solids. The water can also be disinfected by using UV irradiation and ozone treatment. RAS makes it possible to maintain optimal rearing conditions for the smolt throughout the entire year, which reduces the overall production time. This also has the potential to reduce problems with sea lice as larger fish are less vulnerable to sea lice (Kristensen et al., 2009), (Dalsgaard et al., 2013).

The downsides with RAS are higher costs considering investment and operation compared to flow-through systems (Dalsgaard et al., 2013). The system has an increased technology demand due to hydraulics, oxygen supply, particle and effluent removal as nitrogen components and CO_2. In a RAS the nitrogen components and CO_2 are limiting for the operation, and this demands a good water treatment system. The complexity also increases as a RAS can be described as a living unit where a change of one parameter in the system will affect other parameters (Terjesen & Rosseland, 2009).

2.2.1 Build up

A recirculating system is typically built such a way that the water leaving the fish chambers goes to a settling chamber or a mechanical filter, such as the rotating drum, to remove solids from the water. The water then flows into a microbiological filter where bacteria detoxify ammonia/ammonium and nitrite. The next step is another settling chamber where loosened flakes of bacteria from the biofilter is removed. After this step, the water is disinfected by UV-light, ozonation, photozone or by heat treatment. Between these steps, there are

aeration chambers to bring the water to the correct saturation of oxygen and nitrogen (Lekang & Fjæra, 1997). In the following chapters, some of these principles are explained.

2.2.2 Particle removal

Removal of particles is used in several instances in a land-based aquaculture facility, in the intake water, in the RAS and removal of particles in the wastewater. The principle of particle removal is to lead water through a particle removal unit and by this get purified water in one drain and particles in the form of sludge in one other. Particles come in different forms, suspended (particles bigger than 10^{-3}), completely dissolved or colloidal (small dispersed particles 10^{-6}-10^{-3}). Only suspended particles are removed in aquaculture due to cost matters (Lekang & Fjæra, 1997). There are different principles for removal of particles in water in aquaculture. Three common ones are mechanical filtration, depth filtration, and settling.

Mechanical filters

Mechanical filters are different forms of sieves that are placed in the water flow and have a mesh that only let through particles under a certain size. The most basic design is a sieve in the form of a plate. A configuration which will get clogged fast. Most mechanical filters are therefore more advanced and have automatic self-cleaning systems. It is common that the sieve rotates to reduce the clogging. The self-cleaning of the sieves is often called backwashing. In an automatic system, the system will backwash with a set interval or by sensor registration. Here water is flushed through the filter in the opposite direction to loosen the particles and fat that have clogged the sieve. There are different forms of rotating sieve systems, among these; axial rotating screen, radial rotating screen (drum filters) as seen in figure 2.2, rotating belt and horizontally rotating disk (Lekang, 2007).

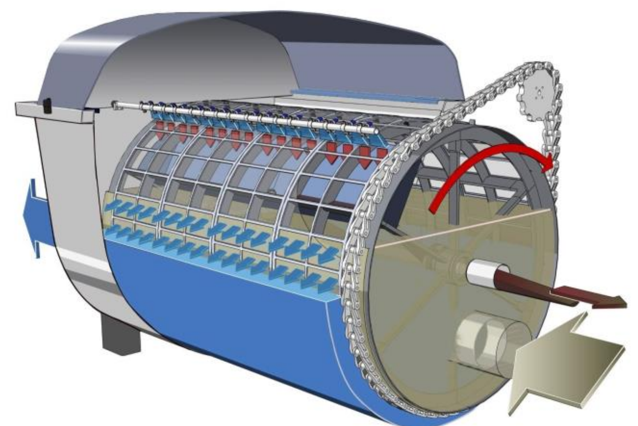

Figure 2.2: Drumfilter with microscreen and self-cleaning (NP Innovation, 2019)

Depth filtration

In depth filtration, larger particles are used to clean the water. Between the particles, there are cavities where the impurities in the water get stuck or are held back (Lekang & Fjæra, 1997). As the filter gets clogged the effect is reduced, and the pressure loss increases. A depth filter must be cleaned when the pressure loss reaches the value for the available pressure head (Bjerknes, 2007). Depth filters are separated in up-stream and down-stream

filters. For the up-stream filters, the water enters under the filter and flows up through the media. For the down-stream, the water enters over the filter and flows down through the media (Lekang & Fjæra, 1997). Depth filters can remove particles far smaller than the pore openings in the filter should indicate. If the pore openings in the filter media are around 35-50 µm the smallest particles that can be removed can have a size down to 1 µm. (Bjerknes, 2007)

The most common filter media is quartz sand with grain size 0,4-0,8 mm (Bjerknes, 2007). The size of the media decides what particles are removed. The smaller media, the more particles are filtrated out, but the faster the media will get clogged (Lekang & Fjæra, 1997).

Settling

Settling uses the density difference between particles and water to separate them. The particles have a density of 1,005-1,2 kg/l, while water has a density of about 998 kg/l, therefore the particles will sink in still water. The bigger the density difference, the faster is the separation. Sedimentation and centrifugal filters both use this phenomenon (Lekang & Fjæra, 1997).

For sedimentation, water flows slowly through a big surface tank; gravity will then separate the particles from the flow if the sinking velocity overcomes the horizontal flow of the water (Lekang & Fjæra, 1997). The system claims little energy but needs much space, and the removal of small particles (<100 µm) is poor (Bjerknes, 2007).

In centrifugal filters, the centrifugal force is also introduced to separate the particles faster. The water enters a cyclone along one side, and the water makes a swirl where the particles are being forced to the edge of the cyclone because of the centrifugal force and exits through a drain at the bottom. Purified water is lighter and seeks the center of the cyclone and exits from an overflow (Lekang & Fjæra, 1997). The effect of removing small particles (<50 µm) is also poor for the centrifugal system (Bjerknes, 2007).

2.2.3 Removing ammonia-ammonium

The nitrogen compounds ammonia and ammonium are toxic for fish, and in recirculating water systems, these compounds will accumulate. It is therefore important to reduce total ammonium (TAN, the sum of NH_4^+ and NH_3). The two most used methods for removing TAN is biological or chemical filtration (Lekang & Fjæra, 1997).

Biological removal

The biological removal of nitrogen happens in several steps from ammonium to nitrogen gas. The nitrification process stands for oxidizing ammonium to nitrite, and nitrite to nitrate, and both steps are aerobic, which means that oxygen must be present. The denitrification process stands for reducing nitrate to nitrogen gas with several intermediate steps. These steps are anaerobic and require abscess of air (Lekang & Fjæra, 1997). Which means that the processes need to happen in separate reactors. Under are some common biological filtration systems described;

Flow-through systems

There are two main types of from flow-through biological systems; trickling filters and submerged systems (Lekang & Fjæra, 1997).

A trickling filter is an over-water system, and water flows (trickles) through a colon with filter media with biofilm, as illustrated in figure 2.3. This system gives good natural aeration and is simply built, but the capacity of nitrification is low compared to other systems (Lekang & Fjæra, 1997). In a trickling filter, the filter material is meant to break up the water flow and allow air to pass into the filter (Bjerknes, 2007).

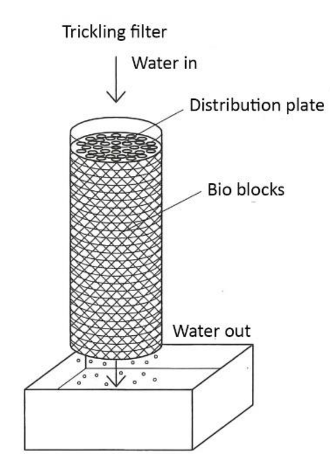

Figure 2.3: Trickling biofilter (Lekang & Fjæra, 1997).

Submerged filters are chambers filled with filter medium, which can be static or moving. In chambers with static material, there will be an accumulation of organic material due to low water flow. This configuration must be cleaned frequently, to maintain good nitrification and prevent the formation of anaerobic zones. To enhance nitrification air is supplied at the bottom in the chamber in a turbulent stream (Bjerknes, 2007).

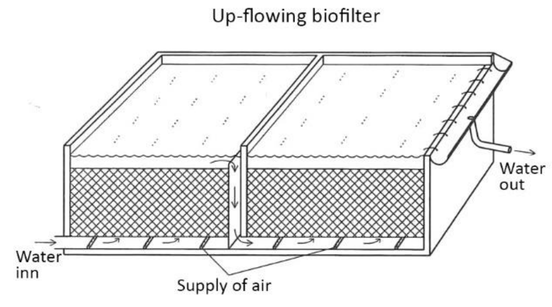

Figure 2.4: Submerged biofilter (Lekang & Fjæra, 1997)

A chamber with moving filter material is called "moving bed" and is commonly filled 2/3 with filter material. In moving bed reactors, the filter material is chosen after density so that they can move even at low flow rates. The media is kept in motion by the aeration and/or water

flow. By continuous flow and aeration, the biofilm is prevented from growing thick, because the excess biofilm is removed (Bjerknes, 2007) (Sterner BioTek AS, 2019).

The system can be configured with either up-flowing water stream or down flowing water stream. Illustration of an up-flowing configuration is shown in figure 2.4. The distribution of water is higher in the up-flowing system, but the contact with the air is better in the down-flowing system due to opposite flow directions for the water and the air. The submerged filter systems have high nitrification effect because of good contact between the biofilm on the filtration media and the water. The negative is that air needs to be added (Lekang & Fjæra, 1997).

Rotating biofilter

Follows the same principle as a submerged filter, but the entire filter rotates at a rate of 2-3 rpm. The filter is partially submerged and partially above the water as the system rotates. The oxygen necessary for the oxidation is provided when the media is above the water and reduction of CO_2 is also achieved. There are two types, one where biofilm grow on plastic biofilter media, and one where biofilm grows on parallel discs. The system has a lower efficiency than submerged filters, but efficiency can be increased further by adding oxygen or air to the tank. (Lekang & Fjæra, 1997).

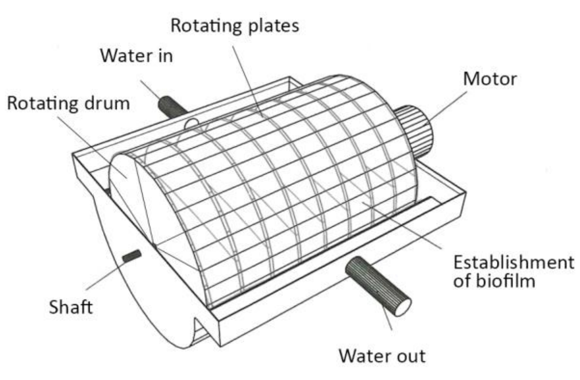

Figure 2.5: Rotating biofilter (Lekang & Fjæra, 1997).

Effect of filtration media, and criteria to fulfill.

Nitrification filters can be measured based on effectiveness, which is often described by the nitrification rate. It is defined as the amount NH_4^+ oxidized per surface area of the filter media and time (mg NH_4^+/ (m^2 min) (Lekang, 2007).

A suitable filtration media for biofiltration can be anything that bacteria will colonize (Stickney, 2000), but the most effective biofilm systems are established on an artificial surface. There are several requirements for the selection of filtration media, to ensure optimal biofilm growth, some of these are; Large specific surface area and good contact between the water and the filtration media surface. The filtration medium must create a low head loss, and not clog too easily. The filter medium must ensure even distribution of water in the filter and the medium must be simple to clean or replace (Lekang & Fjæra, 1997).

The most used media type today is plastic media in different types and forms, optimized to give the highest specific surface, and best flow distribution. The plastic media also have the advantage that does not clog as quickly (Lekang, 2007).

There have been done tests with mechanical filters in place of biofilters. These static filters use sand and gravel with enormous surface areas, but serious water quality problems tend to occur, due to clogging and channeling. When the mechanical filter gets clogged, the organic material in the filter will start to decay, and the microorganisms that have colonized the media will die, as the filter becomes anaerobic. In addition to the stop of biological filtration, there is a risk that the filter will start to release ammonia, hydrogen sulfide, and other toxic substances. The water will also have low oxygen concentrations due to the breakdown of organic material (Stickney, 2000).

Chemical removal

For chemical removal of ammonia in aquaculture, the principle used is ion exchange. The principle of an ion exchanger is to use the fact that the different ions have different electrical charges. An anion exchanger is used to remove negatively charged ions, while cation exchangers are used for removing positively charged ions. Ammonium ions NH_4^+ are positive, and therefore cation exchangers are used for this process. An ion exchanger can be designed as a column filled with ion-exchange substrate, and the water flows through the column (Lekang, 2007). The substrate "clinoptilolite," is a clay material and a natural zeolite, and is used for the absorption of ammonia (Stickney, 2000).

When all the ions have reacted, the exchanger can be regenerated by using a solution with a high concentration of sodium ions. Which will remove all the NH_4^+ bound to the substrate ions (Lekang, 2007).

Denitrification

In some facilities with a very high degree of water re-use and high fish densities, a denitrification filter is used. In a denitrification filter, it is normal to add organic carbon (methanol, ethanol or liquid sugar) as there is not enough carbon for the biofilm to grow. It is also necessary to remove the oxygen from the water to get the denitrification to start. Methanol and ethanol can be used to remove oxygen. When added the free oxygen will be adsorbed (Lekang & Fjæra, 1997).

For denitrification only submerged filter types are used, as oxygen is unwanted. The requirements for the bioreactors and filtration media are otherwise the same for denitrification (Lekang, 2007).

2.2.4 Aeration and oxygenation

In a recirculating water system, oxygen is used by the fish, algae and in the breakdown of organic material by microorganisms. Freshwater can be added to compensate for oxygen use, or we can add oxygen by using aeration or oxygenation. Aeration and oxygenation are also used to reduce the build-up of nitrogen (over-saturation), which can cause gas bubble disease for fish and cause an increase in fish mortality (Lekang, 2007). While some oxygen is necessary for the fish to maintain good health, to high levels are dangerous as some by-products of oxygen metabolism are highly toxic for fish (Stickney, 2000).

In water, the concentration rate between nitrogen and oxygen is 60/40 %, and in air, the content is 79/20 %. Because of the difference, we will get oversaturation of nitrogen if the air is added to the water under pressure. For achieving saturation when the water is over- or undersaturated of gasses, aeration systems are designed to give highest possible contact surface between the air and the water, and with turbulent flow for effective gas exchange. If the retention time is long enough in the aeration system, the equilibrium will be reached (Lekang & Fjæra, 1997).

2.2.5 Heating water

To ensure optimal growth the water in a recirculating system is heated, this is done by either using immersion heaters, oil and gas burners or a heat pump. In larger facilities, it is most common to use heat pumps, while in smaller immersion heaters are more common (Lekang & Fjæra, 1997).

2.2.6 Disinfection

Disease control is difficult in closed water systems. To reduce the number of micro microorganisms such as bacteria's, viruses and fungus, disinfection is used (Stickney, 2000). We want disinfection to inactivate fish pathogenic micro-organisms (which infect the fish and causes diseases) and to reduce the total number of micro-organisms. The wished reduction is the minimum of 99,9 percent of the outgoing concentration (Lekang & Fjæra, 1997).

UV-radiation and ozone are the two most common methods for disinfecting intake water and recirculated water in land-based aquaculture facilities (Bjerknes, 2007). UV-radiation uses electromagnetic radiation of wavelength 1-400 nm to inactivate and kill micro-organisms (Lekang & Fjæra, 1997). Ozone (O_3) is effective for disinfecting bacteria and viruses and is an unstable gas made by sending oxygen through an electrical field (Stickney, 2000). Ozone is toxic for fish and has a half-life of 20 minutes. Aeration is often used to strip ozone from the water which reduces the half-life to 5 minutes (Bjerknes, 2007).

2.3 Use of wood in biofiltration

2.3.1 General about wood

Wood is a renewable raw material from nature. Use of wood from trees have little impact on the environment if harvested sustainably. 90% of the wood harvested in Norway are certified recording to international standards for sustainable forestry, and the forest in Norway is increasing. Which means that the use of wood from Norwegian forests will be a sustainable source for woodchips for filtration purposes (Svanæs, 2004).

As seen in the figure 2.7, a cross-section of a tree trunk, a tree consists of several parts. Some are visible for the naked eye, and some only with a microscope. The tree consists of many types of single cells, and these are attached by a binding component called lignin (Moen et al., 1998).

Figure 2.6: Cross section of a tree stem (Gislerud & Gulliksen, 1998).

The outer bark is the first layer and is protecting against chemical, microbiological and mechanical attacks. While the inner bark, also called the phloem transports water and nutrients. Further in is a layer called the cambium, this is where new wood is produced, by cells dividing. After the cambium are where the parts known as wood are located (Kucera, 1998). The wood is divided into sapwood and heartwood, where sapwood contains sap and water, while hardwood only forms in older threes and only consists of dead cells. The hardwood is mostly preferred for woodwork, as sapwood must be carefully dried before use and is more exposed to fungus and decay (Heartwood Mills, 2019; Moen et al., 1998). Further in is the pith of the three, which are also made of dead cells (Kucera, 1998).

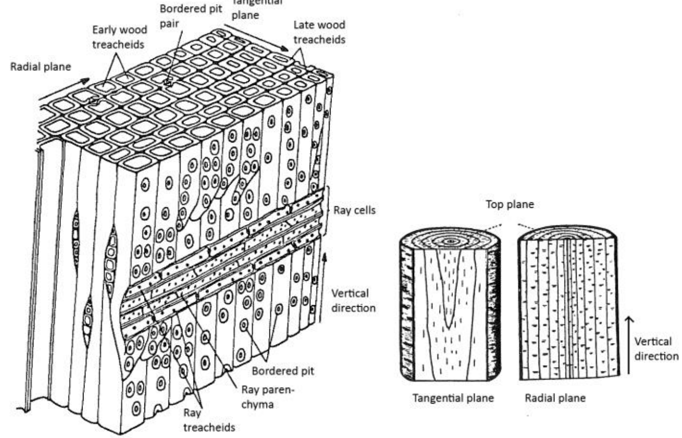

Figure 2.7: Cell structure in wood (Ullevålseter, 1998), (Moen et al., 1998).

Living cells in the wood are called Parenchyma cells. These are long cells that storages and transports nutrients and leads water. These cells are found in the bark, but also as ray cells in wood, as seen in Figure 2.8 these cells cross the wood cells in the radial plane (Moen et al., 1998).

Dead cells are called prosenchyma cells and are defined as the wood. A seen in Figure 2.8 these cells are orientated vertically and shaped as pipes. The prosenchyma cells have the task to lead the water in the sapwood part of the stem. These cells can be further divided into three groups; tracheids, wood cells, and wood channels. These can be seen in Figure 2.9.

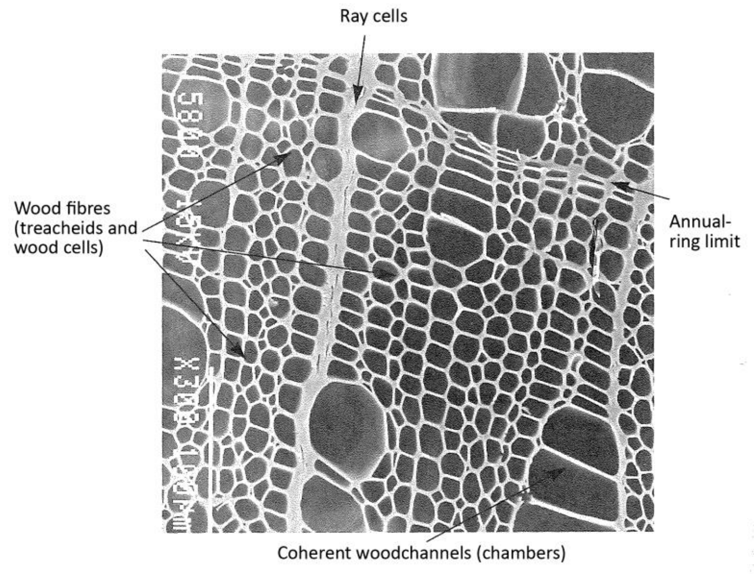

Figure 2.8: Microscopical image of a wood cross section (Kucera, 1998).

Chemical composition

Water makes up about 50 % of the wood raw weight and can be removed by heat treatment. The dry weight is the weight after the heat treatment. This weight is made by about; 50 % Carbon (C), 43 % oxygen (O), 6 % hydrogen (H), 0,1 % nitrogen (N) and 0,4 % Ash (Moen et al., 1998).

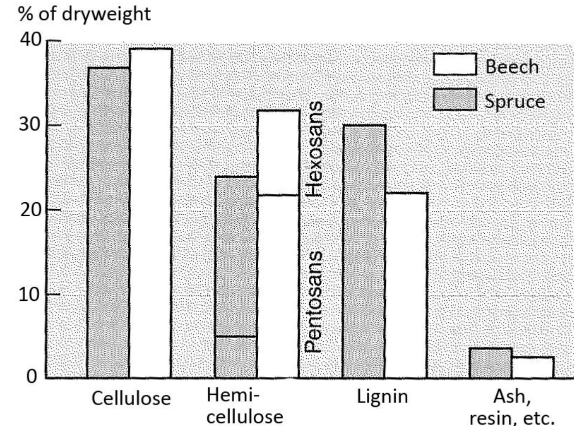

Figure 2.9: Diagram over contents in Beech and Spruce (Moen et al., 1998).

The elements carbon, oxygen and hydrogen make different organic compounds, such as cellulose, hemicellulose, and lignin. The other compounds in the wood are ash, resin and proteins. The amount of each component varies with each tree sort. For beech and spruce, these contents are shown in figure 2.10 (Moen et al., 1998).

Physical properties of wood

The physical and mechanical properties of wood are affected by the water content. Water in the wood can either be found in free form in cell gaps or as a bound form in the cell walls. (Moen et al., 1998).

The point where the wood starts to shrink when dried is called the fiber saturation point, as illustrated in Figure 2.11. The water content of wood decreases without shrinking until the saturation point. First, the cell gaps are emptied, then the bound water in the cell walls starts to evaporate. Because of this, the cell walls shrink. The average fiber saturation point for wood-types is 28 % water content, while beech has fiber saturation points from 32-35 % and higher. The swelling of the wood is proportional to the water uptake until the fiber saturation point; after this, it is not affected by the water content (Moen et al., 1998).

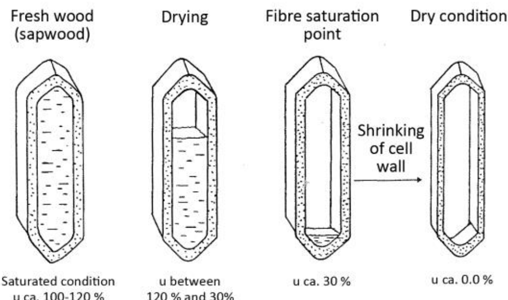

Figure 2.10: Illustration drying wood cells (Ullevålseter, 1998).

Table 2.1: Raw density for some wood types (Moen et al., 1998).

Tree type	Sapwood kg/m^3	Heartwood kg/m^3
Normal spruce	960	520
Pine	980	550
Beech	1060	970
Oak	1000	1000
Birch	950	950

The basis density (weight in the absolute dry state per volume in raw condition) of most wood types lies around 4-600 kg/m^3, and the density of the cell walls is about 1500 kg/m^3 (in a dry state) (Moen et al., 1998).

2.3.2 Beech - *Fagus sylvatica*

Beech grows naturally in the south, west and middle Europe and south in Scandinavia. In Norway, there is some wild growing beech in Vestfold and some occurrences in Telemark, and along the coast in Kragerø, Arendal, and Grimstad. The yearly increase is around 14 000 cubic meters. (Moen et al., 1998)

Beech is a medium-sized tree and can grow to be 30-40 meters high. In figure 2.12 is an illustration of how the tree looks like. The three have a zone without twigs, which can be up to 15 meters. The three is of type diffuse-porous hardwood. Nonimpregnated wood has short durability if kept in contact with soil, but under stable and dry conditions the wood lasts long. It also lasts long under water (Moen et al., 1998).

Figure 2.11: Illustration of an European Beech (Speedtree, 2019) (modified).

Table 2.2: Physical properties for beech, spruce, and birch (Moen et al., 1998).

Properties	Beech	Spruce	Birch
Basis density kg/m^3	570	380	500
Shrinkage (volume) %	17,9	11,7	17,4
Compressive strength MPa	55	43	50
Bending strength MPa	123	78	105
Modulus of elasticity in bending MPa	13,7	11	14,9
Tensile strength MPa	135	90	173
Shear strength MPa	8	6,7	11,7
Impact work kJ/m^2	83	40	94
Hardness (radial) N (Janka)	6500	2100	4400

Beechwood is a relatively heavy wood type with good strength properties. The wood is homogeneous and durable. The hardness of the wood is high compared to the density (Moen et al., 1998).

2.3.3 Earlier studies

Woodchip as a material for use in biofiltration has been researched the last years. In pilot-scale with artificial wastewater, and in full-scale reactors filtrating water from land-based aquaculture. In these studies, woodchip has been applied in denitrification processes as a carbon source for denitrifying bacteria.

Full-scale denitrification

In a study by (Ahnen et al., 2018) three full-scale woodchip bioreactors were monitored from week 28 to 52 after the start-up, to see the initial denitrifying performance of the reactors. The reactors were made as natural ponds outdoor, filled with woodchips, of size 350, 650 and 125 m^3. The bioreactors were used to treat effluent water at three commercial recirculated farms of rainbow trout.

The bioreactors removed nitrogen from the start, and at stable rates during the research period. The system was leaching dissolved organic matter for a half year. Clogging was experienced, and the study state that the accumulation of organic material and bacterial growth can give head loss and affect the long-term performance of the bioreactors. Thus their conclusion was that woodchip bioreactors could be used for full-scale denitrification, but there must be shown caution in the design of the bioreactors, to minimize head loss and reduce clogging (Ahnen et al., 2018).

Table 2.3: Average values for nitrogen components, organic material, phosphorus, oxygen, pH and temperature for three denitrifying reactors of different sizes (Ahnen et al., 2018).

Parameter	Study site 1 (n = 19)			Study site 2 (n = 11)			Study site 3 (n = 17)		
	In	Out	RR	In	Out	RR	In	Out	RR
TN	6.9 ± 0.2	1.9 ± 0.7	5.7 ± 2.5	13.1 ± 2.6	9.8 ± 2.2	5.3 ± 3.0	13.1 ± 3.2	8.2 ± 2.9	8.5 ± 2.6
TN dissolved	6.8 ± 0.2	1.7 ± 0.6	6.0 ± 3.0	13.0 ± 2.7	9.8 ± 2.1	5.1 ± 3.4	12.7 ± 3.1	7.8 ± 2.8	8.3 ± 2.4
NO_3-N	5.3 ± 0.2	1.0 ± 0.5	4.8 ± 2.3	10.5 ± 3.5	7.5 ± 2.6	4.5 ± 1.9	9.5 ± 2.8	4.9 ± 2.5	7.8 ± 2.1
NO_2-N	0.8 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	−0.3 ± 0.7	0.7 ± 0.3	0.5 ± 0.3	0.4 ± 0.7
TAN	1.0 ± 0.3	0.4 ± 0.2	0.7 ± 0.6	1.5 ± 0.8	1.5 ± 0.8	0.0 ± 0.4	2.2 ± 0.6	2.1 ± 0.8	0.2 ± 0.6
$BOD_{5\text{-total}}$ *,**	3.2 ± 0.8	13.8 ± 15.6	−5.2 ± 3.9	3.0 ± 1.2	4.2 ± 3.2	2.0 ± 0.3	4.8 ± 1.9	7.4 ± 6.9	0.2 ± 1.5
$BOD_{5\text{-diss}}$ *,**	2.4 ± 0.8	10.5 ± 9.6	−4.7 ± 3.6	2.5 ± 1.6	3.2 ± 2.3	1.4 ± 0.6	2.3 ± 0.8	4.1 ± 4.5	0.3 ± 3.9
COD_{total} *	8.9 ± 3.4	40.2 ± 50.1	−8.9 ± 7.9	16.8 ± 12.5	17.5 ± 7.3	2.2 ± 0.3	19.4 ± 5.6	21.0 ± 7.8	0.4 ± 5.2
COD_{diss} *	6.8 ± 1.3	37.4 ± 50.7	−7.5 ± 6.8	15.7 ± 10.4	15.9 ± 5.5	2.2 ± 2.2	14.0 ± 2.6	17.0 ± 6.6	4.3 ± 7.2
TP	0.3 ± 0.1	0.3 ± 0.4	−0.2 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.1 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	0.1 ± 0.2
PO_4-P	0.2 ± 0.0	0.2 ± 0.2	−0.1 ± 0.5	0.4 ± 0.2	0.4 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.1
Dissolved O_2	9.0 ± 0.5	0.4 ± 0.2	–	3.2 ± 0.5	0.8 ± 0.3	–	1.9 ± 1.6	0.4 ± 0.2	–
pH	7.2 ± 0.2	7.0 ± 0.3	–	7.4 ± 0.2	7.3 ± 0.2	–	7.5 ± 0.1	7.2 ± 0.2	–
Temperature	10.0 ± 3.2	10.0 ± 3.3	–	9.9 ± 3.2	9.8 ± 3.4	–	10.5 ± 3.3	10.5 ± 3.3	–

For the performance of the reactors in table 2.3, we can see a clear reduction of total nitrogen at the three plants, indicating denitrification. The chemical oxygen demand, COD, and biological oxygen demand, BOD, are increasing for the reactors, which means that organic material is leached to the water from the bioreactors. For dissolved oxygen, we can see that there is a significant decrease from outlet to inlet.

Pilot-scale woodchip denitrification and clogging potential

In a study by (Christianson et al., 2016) woodchips was evaluated as an alternative biological wastewater treatment option. Woodchips were applied as a solid carbon source for denitrification. Their aim was to investigate the relationship between hydraulic retention time and COD/TSS removal and to research the potential for clogging during operation.

Their study was performed with four pilot-scale woodchip denitrification bioreactors over 267 days. They experienced the removal of total suspended solids of over 90 %. The COD removal was found to be most efficient at lower hydraulic retention times. During the study, the flow was decreasing progressively, as a result of woodchip settling, clogging due to wastewater solids and/or accumulated bacterial growth. They state that the use of woodchip was viable and showed excellent removal of nitrate-nitrogen, TSS and notable COD removal. To reduce clogging, they recommend a filtration process before the woodchip denitrification process (Christianson et al., 2016).

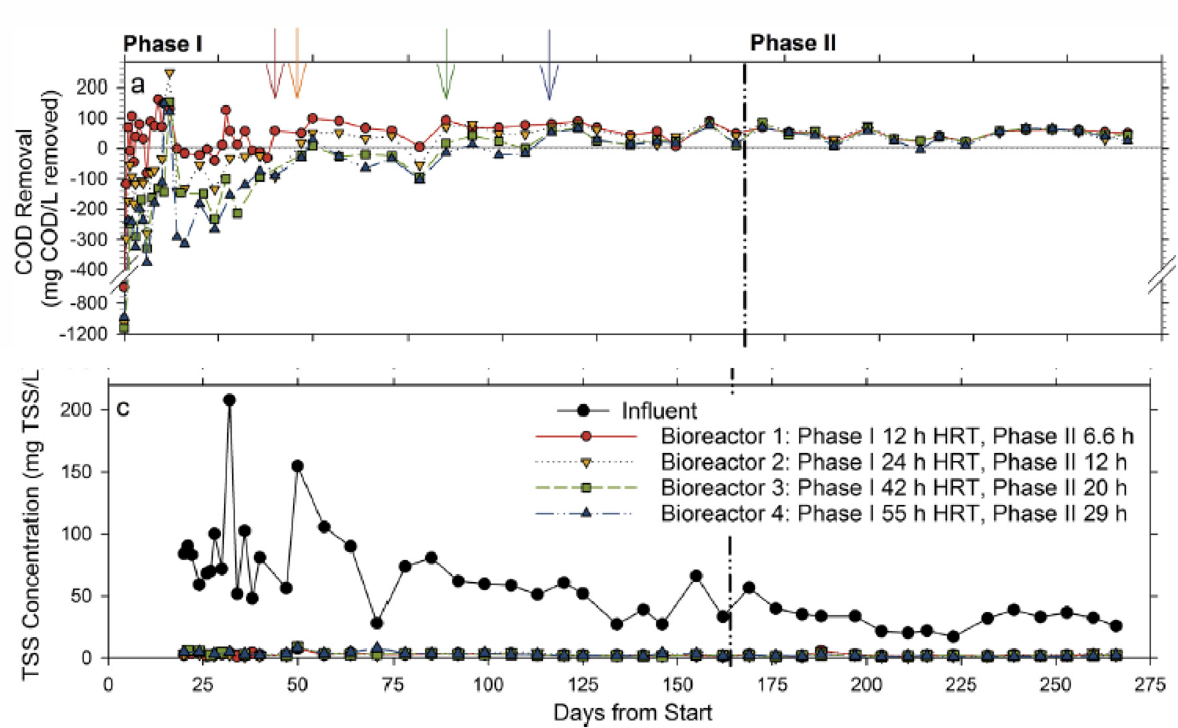

Figure 2.12: Diagrams over COD removal and TSS concentration for four bioreactors with different retention times. (Christianson et al., 2016).

As we can see in figure 2.13 the removal of COD is positive for all the systems 100 days into the experiment and for some the bioreactors earlier than 100 days. From the bottom figure, we can see that the removal of TSS is very effective for all the bioreactors through the entire study period.

Pilot scale woodchip denitrification comparison

In a different study on denitrification, (Saliling et al., 2007) compared denitrification of woodchips, wheat straws, and Kaldnes plastic media. The wastewater used were synthetically made, and methanol was used as a carbon source for the denitrification process. Their study was performed with nine lab-scale bioreactors, 40 cm in height and 10 cm in diameter. Constant flow rate was used, and the influent NO_3-N concentration was set to 50, 120 and 200 mg/L.

The study found that woodchips and wheat straw showed comparable denitrification rates compared to plastic media. The removal rate of 99 % for the 200 mg/L influent concentration was achieved. Their conclusion was that both woodchips and wheat straw are ideal for biological denitrification, but considerations must be made based on the time limitations for the life of both materials (Saliling et al., 2007).

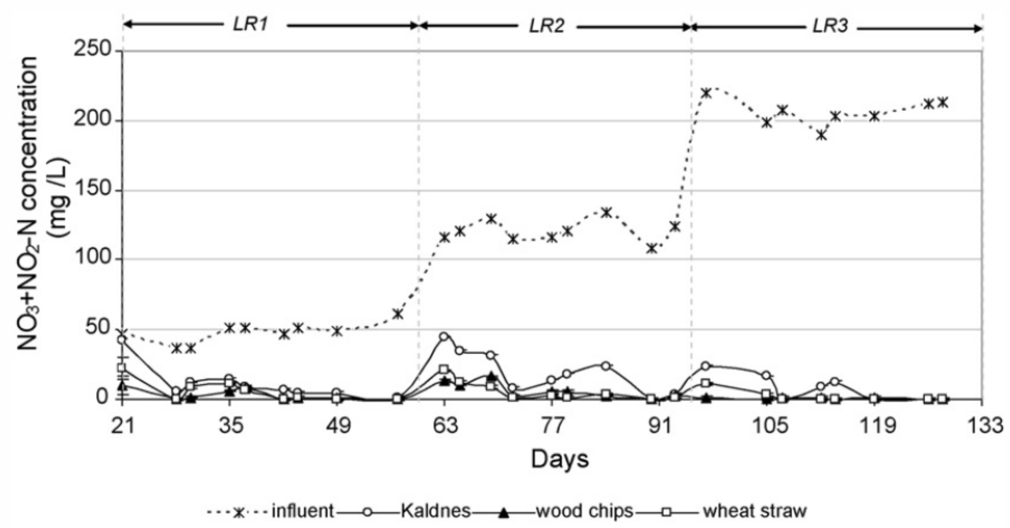

Figure 2.13: Diagram over concentration of nitrate and nitrite-nitrogen. LR1, LR2 and LR3 stands for loading rate of influent nitrate and nitrite-nitrogen concentrations, respectively 50, 120 and 200 mg/L (Saliling et al., 2007).

Table 2.4: Summary of denitrification performance for three filtration medias tested for different influent concentrations of nitrate (Saliling et al., 2007).

Influent $NO_3 + NO_2$–N concentration (mg/L)	Reactor media type	Effluent $NO_3 + NO_2$–N concentration (mg/L)	Percentage reduction (%)	Denitrification rates (g N/(m^3 d)
51.8 ± 5.8 (LR1)	Kaldnes	5.13 ± 2.80	89.7 ± 5.8	313 ± 45[a]
	Wood chips	2.12 ± 3.33	95.9 ± 6.5	333 ± 32[a]
	Wheat straw	1.73 ± 2.86	96.6 ± 5.6	328 ± 42[a]
120.4 ± 9.6 (LR2)	Kaldnes	11.74 ± 9.66	90.6 ± 7.5	743 ± 36[b]
	Wood chips	3.42 ± 2.39	97.2 ± 2.0	760 ± 46[b]
	Wheat straw	1.76 ± 1.29	98.6 ± 1.0	806 ± 42[c]
203.6 ± 10.4 (LR3)	Kaldnes	4.45 ± 5.83	97.8 ± 2.9	1326 ± 74[d]
	Wood chips	0.58 ± 0.30	99.7 ± 0.2	1365 ± 39[d]
	Wheat straw	0.14 ± 0.09	99.9 ± 0.05	1361 ± 80[d]

Note: Similar letters indicate that values are not significantly different.

Figure 2.14 over concentration of nitrate and nitrite-nitrogen shows that all the filtration media are effective at removing nitrate and nitrite for all three loading rates. Table 2.4 over the denitrification performance shows that the systems are not significantly different from each other. The denitrification rate is shown to have a positive correlation with influent nitrate concentration.

Table 2.5: Average COD removal performance for Kaldnes media, wood chips and wheat straw for three different loading rates of COD (Saliling et al., 2007).

Influent COD Concentrations (mg/L)	Reactor Media type	COD removed (mg/L)	Residual COD (mg/L)	COD removed/per $NO_3 + NO_2$–N reduced	Residual Methanol* (mg CH_3OH/L)
232 ± 34 (LR1)	Kaldnes	186.9 ± 21.4 a	45.4 ± 6.5	3.95 ± 0.50 a	25
	Wood chips	187.0 ± 19.0 a	45.3 ± 4.0	3.64 ± 0.36 b	25
	Wheat straw	178.9 ± 19.6 a	53.4 ± 5.2	3.46 ± 0.32 b	31
483 ± 78 (LR2)	Kaldnes	364.9 ± 5.3 b	118.4 ± 21.9	3.41 ± 0.13 c	57
	Wood chips	387.0 ± 25.3 b	96.3 ± 12.5	3.34 ± 0.16 c	42
	Wheat straw	381.2 ± 38.1 b	102.1 ± 1.6	3.26 ± 0.18 c	46
799 ± 52 (LR3)	Kaldnes	694.3 ± 31.9 c	104.7 ± 18.5	3.50 ± 0.17 d	37
	Wood chips	696.8 ± 21.8 c	102.2 ± 3.8	3.46 ± 0.13 d,e	35
	Wheat straw	685.3 ± 14.5 c	113.7 ± 12.4	3.40 ± 0.14 e	43

Note: Similar letters indicate that values in the same columns are not significantly different.

* Estimated residual methanol assuming ratio of 1.5 for COD reduction to methanol reduction.

Table 2.5 over COD shows that the three media show comparable removal of organic material, which indicates that there is no leaching of organic material as experienced in the studies by (Ahnen et al., 2018) and (Christianson et al., 2016).

3. Materials and method

3.1 Laboratory scale microbiological filtration test

The filtration media experiment took place in the period from 15 of January – 18 of March 2019. A total of 63 days. Four different types of media were tested in separate working filtration systems of lab scale, using water from a smolt aquaculture facility, and placed alongside an operating recirculating aquaculture system, RAS.

3.1.1 The Centre for Fish Research at NMBU

The filtration reactors were placed alongside the recirculating aquaculture water system at the smolt aquaculture facility at the Norwegian University of Life Science. The facility has three RAS systems, and this study used the smallest of these systems. This system had the highest fish load. The system is built to treat 300 liters of water from the culture chambers per minute but was operated at 100 liters per minute. The recirculating rate was 93,5 % where 6,5 liters/min of new water were taken in from an intake pool. Figure 3.1 is a simple illustration of the used system, and the components are explained under the figure.

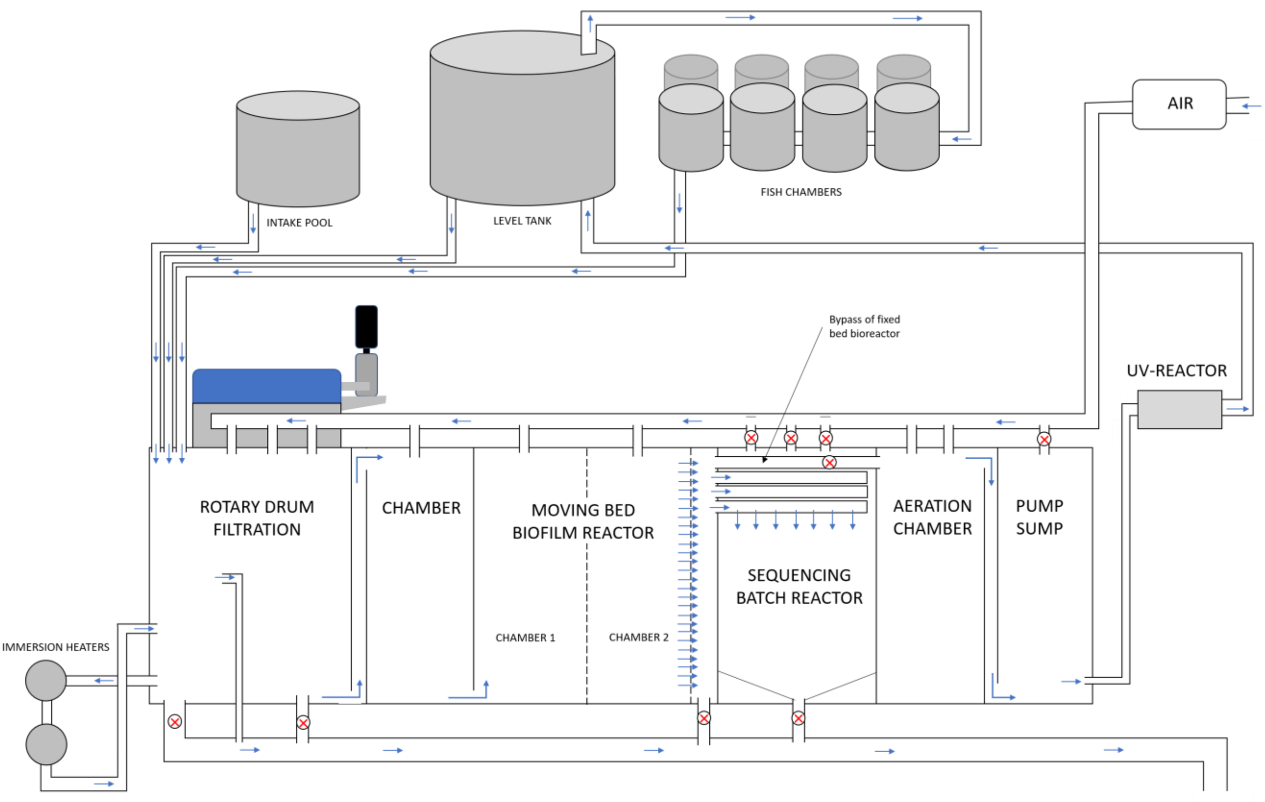

Figure 3.1: Illustration of the recirculating aquaculture system the lab testing units were operated alongside. Blue arrows indicate flow direction. Red crosses show closed valves during normal operation, which can be opened during maintenance.

The RAS system is delivered by Sterner AS and consists of several different treatment-chambers. The water that flows into the recirculating system comes directly from the fish chambers, but some also from an intake tank and some from a level tank where the water is pumped into after the RAS. The water is added in a chamber with a rotary drum filter with screen filters for particle removal. This is an automatic system with frequent backwashes to avoid clogging. The backwash water is taken from the chamber and exits with the particles removed to the drain. The rotary drum system has the potential to remove up to 60-80 % of the organic material (given as BOD_5) (Sterner BioTek AS, 2019).

Due to the loss of water in backwashing, and spill in other parts of the system, new water is added from an intake pool. This is both mains water and groundwater run through a charcoal filter before entering the intake pool and pumped to the mechanical filter chamber. Water is added with a flowrate of 6,5 liters per minute, giving a recirculating rate of 93,5 % for the system.

Air is added at the bottom of the rotary drum chamber through three diffusers. After the rotary drum, there is an aeration chamber. Here the water enters at the top and exits at the bottom, this means that the air and the water have opposite directions, and this ensures good contact and mixing with the air. From the rotary drum filter chamber, water is also pumped out of the tank to two immersion heaters and pumped back, to heat up the water.

The two next chambers are biological nitrification removal chambers and are of the type moving bed, where the chamber is filled with two types of plastic media in a mix. The RK BioElements Light with a density of 0,93 g/cm^3, and Mutag BioChip with a density of 0,95 g/cm^3. The mix is fifty-fifty and fills up 60 % of the chamber volumes, which is 1,91 m^2 each. The two chambers are separated by a grating. For these two chambers, the air is added at the bottom to keep the filtration media in continuous movement. This prevents overgrowing of the media. The amount of air can be adjusted to achieve optimal biofilm thickness. The reason for having two biochambers in series is due to the organic material which is left after the mechanical filter. Heterotopic bacteria will establish as biofilm in the moving bed reactor and are faster growing than the nitrification bacteria (Sterner BioTek AS, 2019).

After the moving bed biofilm reactor, the water enters a fixed bed filter. This is mainly to filtrate out loosened excess biofilm that occurs in the biofilm chamber and other fine particles. The sequencing batch reactor is also filled with plastic media, but this is of the type RK BioElements Heavy which has a density of 1,20 g/cm^3. The particle filter clogs over time and must be washed once a month to keep the flow at the wanted rate.

After the particle filter the water enters an aeration chamber, here the air diffusers are placed higher up in the chambers, and the effect of the chamber is to remove $CO_{2,}$ this is necessary as the fish uses oxygen when it breathes.

After the aeration chamber, the water enters a chamber with still water called the pump sump, where the water is pumped out to a UV-reactor for disinfection, and the cleaned water is pumped further to a level tank where it is mixed with the water from the two other recirculating systems at the Centre for Fish Research. The fish chambers are then supplied with water from the level tank.

The recirculating water system does not include a biological denitrification reactor for reducing NO_3 and NO_2 to nitrogen gas, but some water is separated out, mainly through backwash. To compensate, new water is added. This prevents the accumulation of NO_3 to dangerous levels.

Data for the fish chambers and full-scale RAS

Table 3.1: Fish data measurements taken early January 2019

Chamber	Number of fishes	Biomass [kg]	Feed [g/day]	Size	Avg. biomass per fish [kg]
1	120	11	220	3 mm	0,09
2	182	18,9	250	3 mm	0,10
3	172	18,1	250	3 mm	0,11
4	127	11,5	240	3 mm	0,09
7	20	2,5	80	3 mm	0,13
8	26	1,3	250	3 mm	0,05
9	28	13	250	3 mm	0,46
SUM	675	76,3	1540	AVG	0,15

Table 3.2: Fish data measurements taken 19.03.2019

Chamber	Number of fishes	Biomass [kg]	Avg. biomass per fish [kg]
1	77	19,3	0,25
2	23	20,5	0,89
3	20	20,7	1,04
4	85	19,3	0,23
7	100	20,9	0,21
8	26	24,2	0,93
9	28	19,6	0,70
SUM	359	144,5 AVG	0,61

Table 3.3: Recirculating aquaculture system performance

New water	6,5 L/min
RAS 3 flow rate	100 L/min
Temperature	12,8 °C
Recirculation rate	93,5 %
pH	7,7-8,0

For the system, the total number of fishes decreased during the study, but overall there was an increase in biomass. While the feed rate was kept stable through the testing period. Because of increased biomass in the fish chambers, there is a reason to expect increased levels of ammonium in the effluent water from the fish chambers.

3.1.2 Filtration media

Beech woodchips

Woodchips of beech were used in two of the filtration systems. They were of different sizes, to test the effect of different particle sizes. The wood was bought heat-treated to avoid any bacteria or insects. The beech woodchips were not uniform in size, and the range of the particles was measured to be from 5-35mm. To make filtration substrate of different sizes a grinder, sieves and a laboratory sieve shaker was used. The small chips were made to the size 2,80-4,76 mm and the big chips to the size 4,76-35 mm.

Figure 3.2: A Retch AS 200 control laboratory sieve shaker were used to separate the particles in different sizes.

Plastic biofiltration media

The woodchip was compared with plastic media which is commonly used in the process today. At the Centre for Fish Research at NMBU, the filtration media consists of a mix of half Mutag BioChip white plastic flakes of a density of 950 kg/m^3 and half black RK BioElements Light plastic with a density of 930 kg/m^3. The same mixture of plastic media was used in the lab-scale filtration reactors.

Two reactors were filled with plastic media. One was filled with new plastic media, and the other one was filled with plastic media with biofilm established. This was taken from the operating RAS in the Centre for Fish Research. New media was chosen to compare the time for the biofilm to establish, and the biofilm media was chosen as a reference for what rates to achieve.

Technical specifications of the plastic media are listed in table 4.3, and the four filtration reactors with the filtration media are pictured in figure 3.3.

Figure 3.3: The four different media used in the experiment; Small woodchips, big woodchips, new plastic and already culturized plastic media.

Table 3.4: Technical specifications for filtration media

RK BioElements Light	Technical specifications (RK-Plast A/S, 2019).	
	Density (kg/m^3)	930
	Bulk weight (kg/m^3)	158
	Number (pcs/m^3)	255.000
	Specific surface area (m^2/m^3)	750
	Material	PP
Mutag BioChip	Technical specifications (Multi Umwelttechnologie AG, 2019).	
	Density (kg/m^3)	950
	Bulk weight (kg/m^3)	165 kg/m^3 ± 2 %
	Specific surface area (m^2/m^3)	3000 (up to 5,500)
	Material	PE

3.1.3 Filtration reactor setup

The filtration media was tested in external filtration systems made for household aquariums. The model used was EHEIM eXperience 150, made for aquariums from 80-150 liters. The maximum pumping capacity is given to be 500 L/h (8,33 L/min), and pumping head max is 1,30 meter (EHEIM GmbH & Co KG, 2018). The filtration system contains two baskets and comes with filter pads. For the study, the baskets were filled with the woodchips and plastic media, as seen in figure 3.4. The top filter pad included in the filter system kit was used to avoid the substrate from entering the pump and slow down or stop the system. The volume for each filter basket was measured to be 1120 ml. The filtration reactor is completely sealed, so no air entered the system except for minor bubbles in the water.

The water was taken from the second chamber in the RAS, an aeration chamber, as illustrated in figure 3.5. The hoses were placed along the side of the chamber to avoid the bubbles from the aeration.

Figure 3.4: Setup of the filtration units (Eheim GmbH & CO. KG, 2019) (modified).

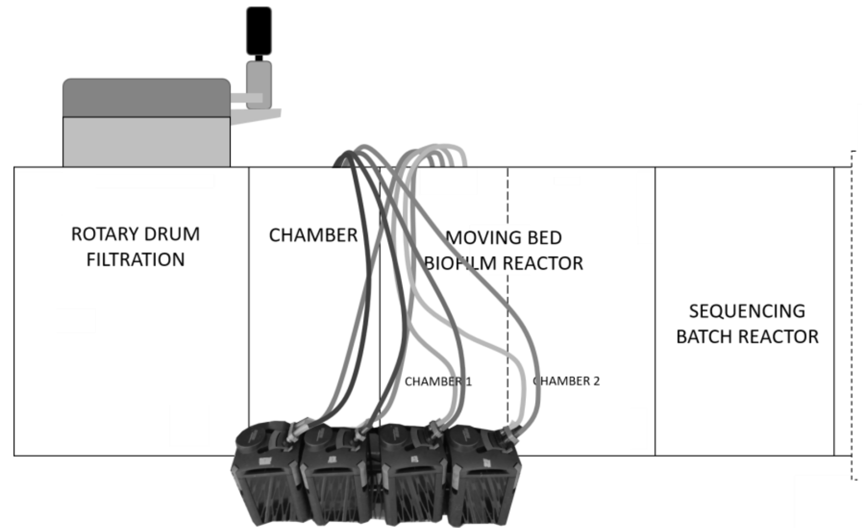

Figure 3.6: The set up for the systems alongside the operating RAS.

Figure 3.5: Flow in the filtration systems (Eheim GmbH & CO. KG, 2019) (modified).

The four systems were connected to the RAS in the facility. The inlet hose was placed in the second tank after particle removal by a mechanical drum and the outlet hoses in the bioreactor of the system. The water was pumped into the four filtration reactors by centrifugal pumps inside each reactor. As shown in figure 3.6, the water flows through the filtration media from the bottom up.

At the start of the testing phase, the flowrate was set to 4 L/min for the four filtration reactors. The flow was not regulated during the test, and the flow was measured at each sample day to visualize the clogging of the systems.

The water used was only from the fish chambers, and no additional substances were added to achieve higher concentrations of nitrogen or other nutrients. The water loss of pumping the water to waste after the filtration in the reactors was considered too high for the RAS, so the reactors had to deliver the water back into the RAS. Therefore, there was not an option to add nutrients, as the experiment were not to affect other experiments on smolt production in the facility.

3.1.4 Sampling

For the test period samples was taken each day the first week. While in the rest of the period water samples were taken for analysis twice a week. The samples were taken of the intake water and of the outlets on the four filtration reactors. The sample containers were flushed and shaken with the sample water before sampling to minimize pollution. The three samples were taken with 1,5-minute separation for each sample point.

Figure 3.7: Setup for measuring flowrate of the filtration units.

The volume flow of each system was measured by using a timer and a measuring jug of 2L, with three repetitions for each reactor, and mean value calculated.

At the end of the study there was performed a control of the RAS, compare the filtration reactors against the operating RAS. Samples were taken from the intake chamber, the bioreactor, the sequencing batch reactor, and the pump sump.

There was also gathered data on the flow of the RAS, temperature, new water added to the system, feeding amount and fish biomass. This data was collected and provided by Bjørn Reidar Hansen and Harald Støkken at the Centre for Fish Research at NMBU and can be studied further in chapter 3.1.1.

3.1.5 Analysis of water samples

The water samples were tested for total nitrogen (Tot N), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), total oxygen demand (TOD) and pH. The flow rate was measured, and the retention time was also calculated based on a test of porosity for the different media.

For analyzing the content of total nitrogen, ammonium, nitrite, and nitrate a machine called Systea EasyChem Plus was used. The EasyChem plus is a laboratory analyzing machine. The machine uses colorimetric analysis to determine the concentration of a chemical compound. This is done by the help of color reagents which vary for what chemical compound you want to determine. Recipe for these components can be found in Attachment A1. The water samples were filled in cuvettes and placed in the machine. For the determination of ammonium, nitrite, and nitrate the samples could be placed directly into the colorimetric analyzer. This machine analyzed the ammonium, nitrite and nitrate in a form where the components are called ammonium-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N). This is a measurement method where only the amount of nitrogen of the ions is calculated as the concentration.

Figure 3.8: Systea EasyChem Plus used for nitrogen analysis.

These were measured in ppm (parts per million) and converted to mg/L (where the density of water is set to 1 kg/L for simplicity). For the ions then one measurement of ammonium-nitrogen of 1 ppm, equals 1 mg/L nitrogen in the sample, which gives an amount of mol, by the mol we can find the mass of the whole ion, and not only the amount of nitrogen in the ion.

Table 3.5: Conversion table for conversion from ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen to ammonium, nitrite and nitrate.

	Conc. [ppm N]	Conc. mg/L N	n [mol]	Mm [g/mol]	M [mg/L]
NH_4^+-N	1	1	0,00007139	18,04	1,29
NO_2-N	1	1	0,00007139	46,00	3,28
NO_3-N	1	1	0,00007139	62,00	4,43

Ammonium = Ammonium Nitrogen x 1,29 [18]

Nitrite = Nitrite Nitrogen x 3,28 [19]

Nitrate = Nitrate Nitrogen x 4,43 [20]

The samples that were analyzed for total nitrogen had to be digested in 100 degrees Celsius for 60 minutes before the analysis. For these samples, 5 ml of each sample were extracted to a reagent bottle, and 0,5 ml (5g/100ml) potassiumperoxidsulphate and 100 µL concentrated sulphuric acid (2M NaOH) were added and then digested. This oxidized all the nitrogen components in the samples to NO3-N.

In studies regarding nitrification and denitrification, it is normal to use the nitrogen-concentration when presenting the data for ammonium, nitrite and nitrate. Thus, this will also be used for the data in the results of this thesis. Conversion between the concentration of nitrogen to the concentration of the ions can be done by using the formulas 18-20.

For measuring the pH, a pH-meter from WTW, model pH3110 was used. For the first two sample days, a faulty pH-meter was used. The measured values did not correspond with the values measured by the Centre for Fish Research staff, and the pH-meter was therefore replaced.

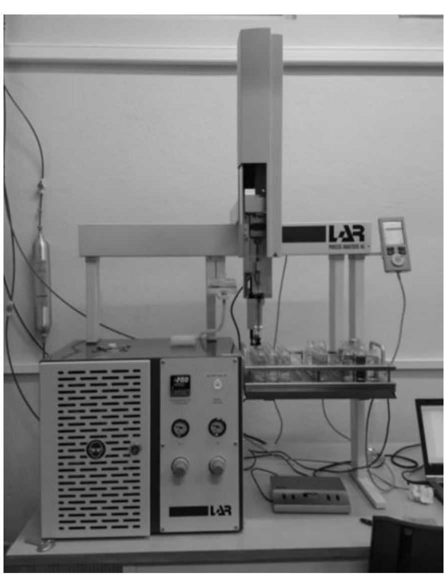

Figure 3.9: LAR QuickCODlab analyser was used to determine the total oxygen demand in the samples.

For analyzing the total oxygen demand, a COD analyzer from LAR Process Analysers was used. The machine is called QuickCOD$_{lab}$ and uses combustion at 1200°C to oxidize the sample. An oxygen detector determines the amount of oxygen consumed by the combustion. This method uses no toxic chemicals and is relatively fast compared to BOD methods. The oxygen demand of all oxidizable material in the water is calculated (LAR, 2019). Each sample was run three conbustions on, and mean values, and the standard deviation was found.

3.2 Physical parameters of the filtration media.

3.2.1 Finding the porosity

The porosity of the filtration media was found by testing. The porosity, for the media in water, is defined as the void volume of the pores inside the media, and space between the particles. The procedure for finding the total porosity (%) for the filtration media was done by filling a bottle up to a known volume and measuring how much water that could be added before reaching the marking point this over a couple of days. First, an empty bottle was filled with 0,75-liter water, and the point on the bottle was marked. The bottle was then emptied and filled to the mark with filtration media. The packing of the media was the same as in the filtration reactors. Water was filled in a measurement jug and poured from this into the bottle with the media, up to the marked point. The volume used was calculated from the volume remaining in the measuring jug. The cap was placed on the bottle, and the media were let to adsorb water. The bottle was filled up with water to the 0,75L mark after 24 hours and after 48 hours, and the amount waster added was noted. The total porosity for the filtration media was found as the total volume water added (which is the volume of the void space), divided by the total volume of the material, as seen in formula [21]. The experiment was done after the method described by (Christianson et al., 2010).

Figure 3.10: Porosity experiment set up. Bottle with volume marking, filled with media and water to determine void volume.

$$\emptyset = \frac{V_V}{V_T} \qquad [21]$$

Where $\emptyset$ is the porosity, V_V is the void volume, and V_T is the total volume of the material.

3.2.2 Finding densities of the medias

Several methods were tested to determine the density of the filtration media. The problems faced, were high variance between the measurements which gave standard deviations. This was particularly difficult achieving when using only a single particle. For the measurements of the used woodchips and plastic particle, three small weights with holders for 6-7 particles were made. These were used with a small measurement jug, and a precise scale for small weights. This gave results with some variance. The procedure was therefore later further developed to give more consisting results. The improved method was used for the woodchip density test. This was a simple design where a net that could hold many particles and was used, and larger measurement jugs were used, as seen in figure 3.4. This method gave less variance between the measurements, as sources of errors were lowered.

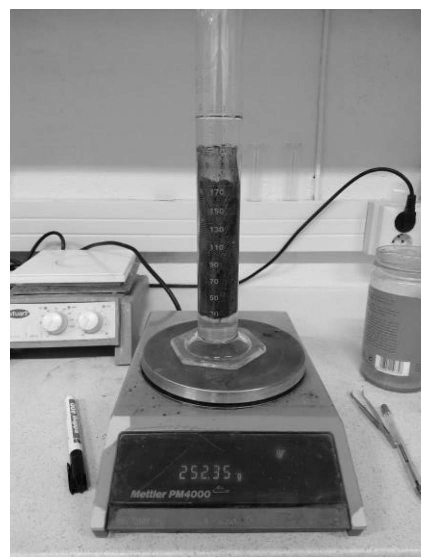

Figure 3.11: Density measurement of woodchips, three cylindric nets were created to contain the particles and keep them under water.

The density of the wood particles and plastic particles in the dry condition is lower than the density of water. Which means that to measure the density by using the displaced water method, a weight must be used to sink the particles under water. The method to find the volume is to use a measurement jug, with water with a known density. The weight used to make the particle sink must first be measured for mass and volume. The volume of the particle can then be found as the volume of displaced water.

The method used for measuring the density of the different media can be divided into these steps;

Preparations:

- Find the density of the water, by using a measuring jug and a scale. Density is found as the measured mass of the water divided by the volume of the water.
- Find the mass of the weight, then find the volume of the net by submerging it in water in a jug. Measure the total volume and the total mass. The mass of the water is found as the total mass minus the mass of the weight. The volume of the water can be found as the mass divided by the density of the water. Then the volume of the weight can be found as the total volume minus the water volume.

Particle density:

- Measure the mass of the particles and weight, the mass of the particles is found as the total mass minus the known mass of the weight.
- Put a measuring jug on a scale and press tare.
- Put the weight with particles in the jug and note the mass.
- Fill up with water to a known volume and note the total mass on the scale.
- The mass of water is found as the total mass minus the mass of the weight and the particle.
- The volume of the water can be found as the mass of the water divided on the density of the water.
- The volume of the particles is then found as the total volume minus the volume of the water and the weight.
- The density of the particles can finally be found as the mass of the particle divided on the volume of the particle.

The test was done with dry particles in the first measurement, and the particles were kept in water for it to soak up. For the more comprehensive woodchip density test, the measurements were done three times a day the first two days, then one-two times for eight days. For the test, a measuring jug with a volume of 250 mL was used. The measuring jug had an error of +- 1 ml in 20^oC. For all the density tests deionized water was used, and the density was measured. The experiment was done in room temperature (20-25^oC).

For finding the bulk densities of the different media, both in new and dry condition and in the used and wet condition the procedure is straight forward. Placing a measuring jug on a scale and using the tare function and then filling the jug with the media up to a specific volume and noting the weight on the scale. The bulk density of the media is then found as the weight divided by the volume.

3.2.3 Specific surface area

The specific surface area of filtration media tells how much surface area that it is available for biofilm to grow on per volume. It is easy to find the specific area of a perfectly squared box with smooth surfaces, but more complex to find the specific surface area of particles with irregular forms and rough surface, woodchips are such particles. In a study on woodchips (Lungulesasa et al., 2009) have developed a method for finding the specific area of the chips based on the thickness of the chips.

Their formula is as follows:

$$S_s = \frac{2 \cdot m_{ch}}{\rho_w \cdot g} \quad [22]$$

Where M_{ch} is the mass of the chip, S_s is the specific area of the chips, in m^2/g, ρ_w is the density of the wood from where the chips were obtained, in kg/m^3 and g is the thickness if the chips in mm.

The formula is made by treating the sum of the woodchips as a block of woodchips with no gap. The mass of such a block is equal;

$$M_{ch} = V_w \cdot \rho_w \quad [23]$$

The volume of this block V_w can also be described as;

$$V_w = g \cdot S_s/2 \quad [24]$$

where g is the thickness of the chips and $S_s/2$ is half the area of the woodchips. Then the formula [22] is found by combining these equations.

In the experiment, ten wet woodchips of rectangular shapes were measured for the area, volume, and weight. For these, the average density, specific area, and specific surface area are also found. A summary of this is found in chapter 4.3.3.

The average thickness and the density found for wet woodchips was used in the formula by (Lungulesasa et al., 2009) to find the specific area of the chips. The specific surface area of the woodchips in a volume was found by taking the bulk density for wet woodchips times the specific area [25].

$$S_a = \frac{2 \cdot m_{ch}}{\rho_w \cdot g} \cdot \rho_{bulk} \quad [25]$$

3.3 Test of pressure drop.

For a better understanding of the head loss over the reactor due to filtration media, a system for measuring the pressure drop was designed.

A test setup was put together by using standard pipe parts for water and waste transportation. The system consisted of a Ø110 mm transparent ABS pipe, Ø110 mm pipe ends were inserted, and netting of 2 mm mesh was placed inside these endcaps, holes were drilled through the caps and connectors for connecting the pipe to a 12 mm hose system was fastened. The planned hose system included a small centrifugal pump, a pressure gauge, a ball valve to adjust the flow rate and a flow meter to read the flowrate. This setup can be seen in figure 3.12.

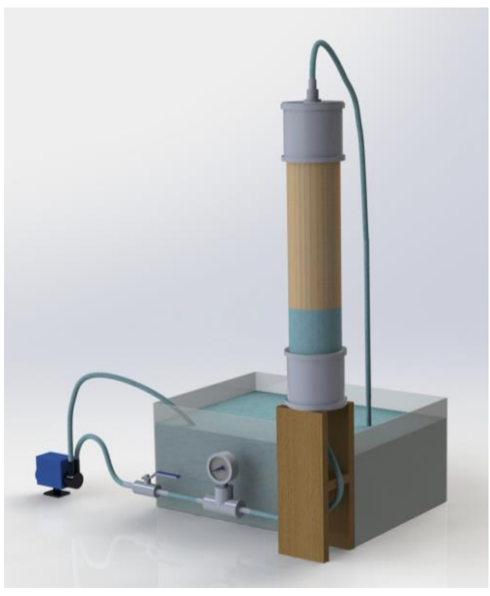

Figure 3.12: The pressure drop system was designed in Solidworks before the unit was built.

The pressure drop caused by the filtration media was too low to be registered with a manometer. The manometer was in range 0-2,5 bar, while the measured pressure drop with the final configuration was between 0-700 Pa, equal to 0-0,007 bar. The system was therefore redesigned, and the new configuration can be seen in figure 3.17.

This design is based on reading the pressure by meter water column, where the resistance in the media raises the pressure before the column. This pressure can be read as the height the water gets raised in the vertical pipe, connected to the hose between the pump and the pipe. The meter water column is a pressure unit that can be converted to more common units as bar or Pa. The pressure is measured with and without media, at the same flowrate, to find the pressure head loss caused to the media resistance. The configuration for the water column measurement is seen in figure 3.13, where a T-connection is used to connect the vertical hose to the system.

The system is run by a constant centrifugal pump, and the water flow is regulated by a ball valve placed at the outlet of the pump. The reason for choosing this side to regulate the flow is that by regulating the flow on the suction side of the pump, the chances of starving the pump will increase. This happens if the level of water entering the pump is less than what the pump is trying to deliver. This is not good for magnetic drive pumps. The distance from the pump outlet is also of interest. March pumps, a manufacturer of centrifugal magnetic drive pumps recommend that for a ½” pump outlet, the valve should be placed 5” (127 mm) from the pump (Marchpumps, 2014).

Figure 3.13: T-connection connecting the water column hose to the system.

A device was developed in Solidworks to break up the flow from the inlet in the chamber. This design was developed based on testing in the Solidworks Flow Simulation module, and several configurations were tested, with different mesh sizes. The change of flow for the chosen unit is shown in the figures 3.14 and 3.15. We can see that the flow is more uniform with the flow device inserted. The device was made to fit in the lid of the chamber for easy maintenance as seen in figure 27. The part was made by 3D-printing.

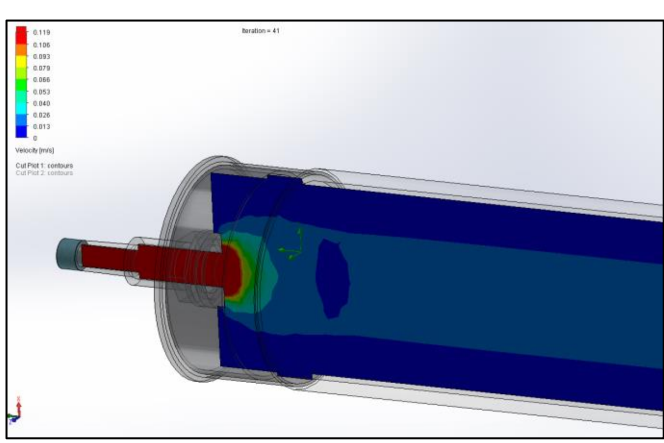

Figure 3.15: Cut plot of unit without flow device, showing velocity of the water. Here the distribution of the water is low.

Figure 3.14: Flow break-up device. Designed in Solidworks Flow Simulation and 3D-printed.

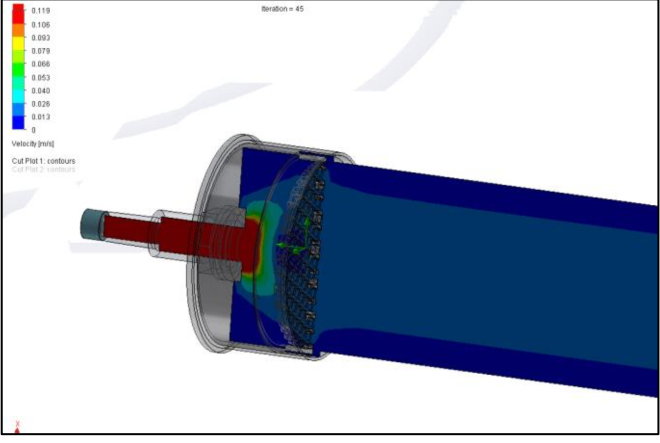

Figure 3.16: Cut plot of unit with designed flow device, showing velocity of the water. Here the flow of the water is distributed evenly in the cylinder.

The system was used to test the media both unused and with biofilm. The tests on media with biofilm were not as successful as the biofilm from the media clogged the netting in the endcaps instantly. The pressure drop unit was cleaned, and measurements taken, but consistent results were not achieved.

For the unused media, it was done a test with four different volumes of media, ¼ full, ½ full, ¾ full and full (1,06 L, 2,12 L, 3,17 L, and 4,23 L). For the used media it was only done tests with two volumes, ¼ full and ½ full. Due to limiting volume of the filtration reactors used in the biofiltration test. For each series, the pressure for the system was measured without media, and with media for 13 flowrates from 2 L/min – 5 L/min. This limited by the flowmeter that was used. Measurements were taken for each 0,25 L/min.

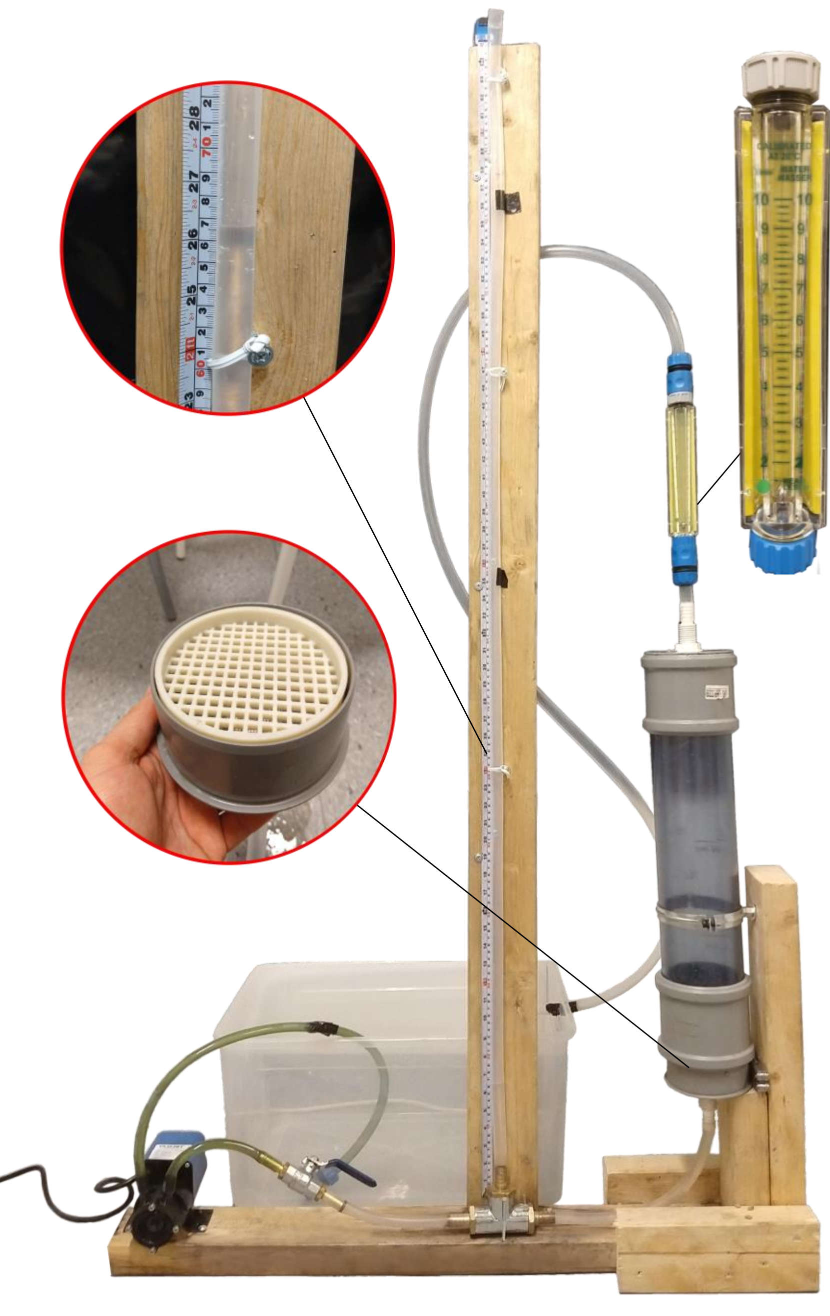

Figure 3.17: Final configuration for the pressure drop testing system. Centrifugal pump run the system. The flow is regulated by a valve, the pressure is read as meter water column, while the flowrate is measured by the flow meter.

The unused woodchips were placed in water and let swell out for 72 hours before the testing was performed.

3.4 Data treatment

3.4.1 Analysis tools

The Pearson correlation coefficient, denoted r, is a measurement of the linear correlativity between two variables. The method attempts to draw the best fit line through the data of two variables, and the coefficient indicates how far away the data points are to the line. The coefficient varies from -1 to +1, where -1 indicate a strong negative relationship. Which means that when one variable increases, the other variable decreases. 0 indicates that there is no connection between the two variables, and +1 indicates a strong positive relationship between the two variables. When one increases, the other one also increases (Lærd Statistics, 2019b). The following guidelines are proposed:

Table 3.6: Guidelines for correlation strength indicated by the Pearson coefficient (Lærd Statistics, 2019b).

	Coefficient, r	
Strength of correlation	Positive	Negative
No correlation	0,0 - 0,1	-0,0 – 0,1
Small	0,1 - 0,3	-0,1 - -0,3
Medium	0,3 - 0,5	-0,3 - -0,5
Large	0,5 to 1,0	-0,5 to 1,0

One-way ANOVA test and Tukey's range test

The one-way ANOVA is an analysis of variance. The analysis is used to compare independent groups and calculates whether any of the means of the groups are statistically significantly different from each other. This is done by testing a null hypothesis (Lærd Statistics, 2019a).

$$H_0: \mu_1 = \mu_2 = \mu_3 = \cdots = \mu_k \quad [26]$$

Where μ = group mean, k = number of groups tested.

The analysis returns a probability (p-value). Here the hypothesis is that the means of the groups are not statistically significantly different, and the alternative hypothesis is that they are not. If the analysis returns a p-value higher than 0,05, then is hypothesis is confirmed. If the analysis returns a p-value lower than 0,05 then the alternative hypothesis, $H_{A,}$ is accepted, and we can say that at least means of two groups that statistically different from each other (Lærd Statistics, 2019a).

The test does not tell which specific groups are statistically different from each other. To find this, a test called post hoc test must be performed (Lærd Statistics, 2019a).

There are many post hoc tests that can be performed, but an often-used test is the Tukey`s range test. It is a single step multiple comparison statistical test. The test uses the difference between the two means for the two groups that you want to compare, and standard error for the sum of the means, as shown in equation [26]. This equation gives a value q_s, to compare with a value Q which is collected from a probability-table based on the significance level wanted, the numbers of groups compared, and the total number degrees freedom in the groups. If the q_s value is lower than the Q value, then the H_0 hypothesis is accepted. If it is higher than the Q value, then the H_A hypothesis is accepted (Schlegel, 2018).

$$q_s = \frac{Y_A - Y_B}{SE} \quad [27]$$

In the result chapter, statistically significantly different groups are marked with different letters, while not statistically significant different groups have the same letters.

One-Sample T-Test

For comparing the mean of one sample group against a specified constant a one-sample t-test can be performed. This is useful for controlling whether the mean change in concentrations is significantly different from zero. For the t-test, it is common to make a null hypothesis and a one-tailed or a two-tailed alternative hypothesis (Kent State University, 2019). As the most interesting for this study is whether the sample is equal to zero or not a two-tail hypothesis is chosen. The two hypothesizes are formulated;

Null hypothesises
$H_0: \mu = 0$ [28]

Alternative hypothesises
$H_A: \mu \neq 0$ [29]

The t-test calculates a t-value based on the specified mean to test, the sample group mean, the sample group standard deviation and the sample size. As shown in equation [30].

$$t = \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}} \quad [30]$$

For testing the hypothesis, the calculated t-value is compared to a t-critical value taken from a t-table, which contains t-critical values, these are chosen based on the number degrees of freedom for the group (df = n-1) after the significance level chosen for the study ($\alpha > 0,05$) and based on whether it is a one or two tail t-test (Kent State University, 2019). For a left tailed hypothesis, the null hypothesis is accepted if the negative t-critical value is higher than the t-value. While for a right-tailed hypothesis the null hypothesis is accepted if the positive

t-critical value is higher than the t-value. Left or right tale hypostasis is based on whether the sample means is smaller or higher than the specified constant.

Standard deviation

Standard deviation indicates how accurately the mean represents the sample data and measures the variability for a set of data from the mean (Investopedia, 2019).

The standard deviation for samples can be found with the following equation:

$$SD = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}} \quad [31]$$

For the tables with concentration change, the inlet concentration is subtracted. The total standard deviation for both the reactor and the inlet is found by taking the square root of the sum of the variance of each, and the variance is found as the root of the standard deviation.

$$Total\ Standard\ Deviation = \sqrt{SD^2 + SD^2} \quad [32]$$

3.4.2 Error from Systea analysis

For the measurements of ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and total nitrogen, the Systea Colorimetric analysis each series of measurements, and a control sample, and a blank sample was also done. The blank sample was distilled water, and for an analysis where all reagents were of correct concentrations, and the machine was correctly calibrated, the concentration registered in the blank will come out as zero. In addition, there was a control sample with a known concentration, where the same conditions will give the same value as the known concentration. The errors registered for the blank and the samples are systematic errors which are occurring for all the samples while variation in concentration between the samples is treated as random errors, which can be presented with standard deviation.

Systematic errors remain constant unaffected by how many measurements that are made. It can only be reduced by selecting a different method or eliminating the problem causing a systematic error. Systematic errors should not be treated using probability theory, and there are no general procedures for this. There must usually be done a case by case analysis. The systematic error should as a general rule be kept separate from random errors (Leo, 1994).

The following method was used for treating the systematic errors from the method. The error in the blank was subtracted from the measurement values to compensate for error in

the reagents and the machine, while the calibration error seen for the control sample was treated as a percentage misread, and the measurements were divided by the registered value of the control sample divided by the actual concentration of the control sample. The random error in the measurements are presented as standard deviation and was calculated of the three measurements of each sample.

3.4.3 Error from TOD analysis

For the TOD analysis, no blank samples were used for controlling the accuracy of the method, but the machine had been calibrated to work with the TOD method before the study started. Each sample was tested with three repetitions, and there were three samples for each measurement. Standard deviation was used to calculate the error of the measurements by using the equation of standard deviation for samples [31].

3.4.4 Error in density tests

For the measurements of densities and bulk density, variation is a significant factor when it comes to the measurements. For the bulk densities, a large measurement jug was used to bring down the variation, while for practical uses smaller jugs were used for the density measurements, for these the measurements varied more. The density measurements are presented with standard deviation as three different tests were done for each density measurement. Variance due to small measurement jugs and particles gave high standard deviation, indicating that the accuracy of the measurement is not too good. The variance was lowered for the density of the woodchip test, where a net for measuring many particles and a larger measurement jug was used.

4. Results and discussion

In the results and discussion chapter, the results of the experiments will be presented and discussed in detail. This includes the measurements taken connected to biological filtration, but also testing and analysis of flowrate, pressure drop and clogging. As well as tests to determine the physical properties of the wood and the filtration media. Finally, appliances for woodchip in aquaculture filtration systems will be discussed based on the results of this study and what earlier research have found out about the topic.

4.1 Nitrification and denitrification performance of the reactors

4.1.1 pH measurements

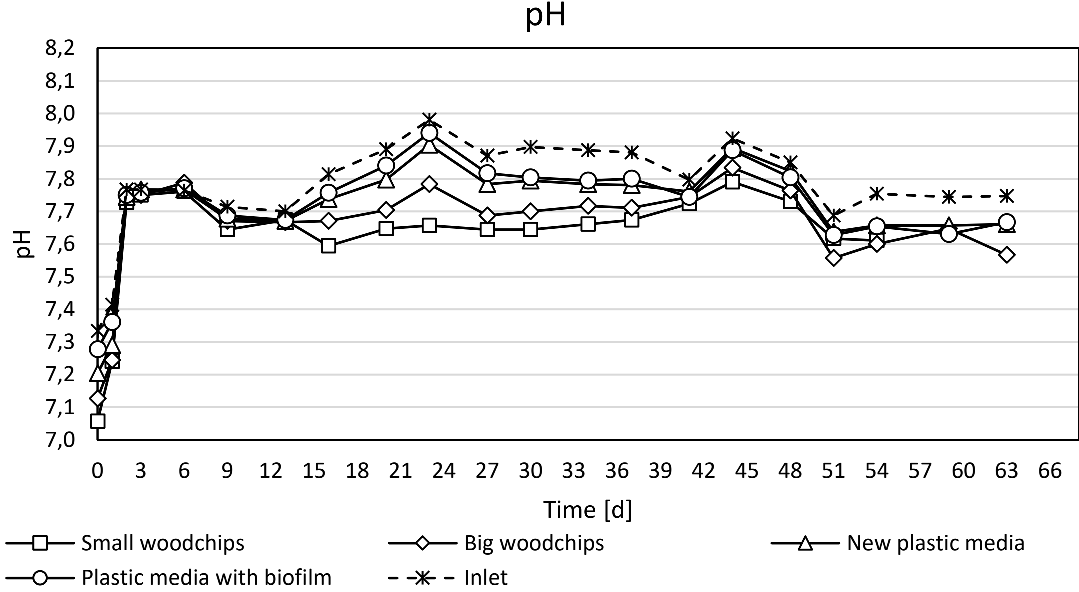

Figure 4.1: Diagram over pH in the filtration reactors and the inlet. First two measurements are systematic errors from a faulty pH-meter.

Table 4.1: Average pH for the filtration reactors.

Average pH			(value ± S.D.) (day 3-63).	
Filtration reactor	In	Out	Change	t-test
Small woodchips	7,81 ± 0,08	7,67 ± 0,06	-0,15 ± 0,09 a	μ ≠ 0
Big woodchips	7,81 ± 0,08	7,70 ± 0,07	-0,12 ± 0,07 a	μ ≠ 0
New plastic	7,81 ± 0,08	7,75 ± 0,08	-0,06 ± 0,04 b	μ ≠ 0
Old plastic	7,81 ± 0,08	7,76 ± 0,09	-0,06 ± 0,03 b	μ ≠ 0

Note: Same letters indicate that values are not significantly different. The t-test is done on the means for change in pH.

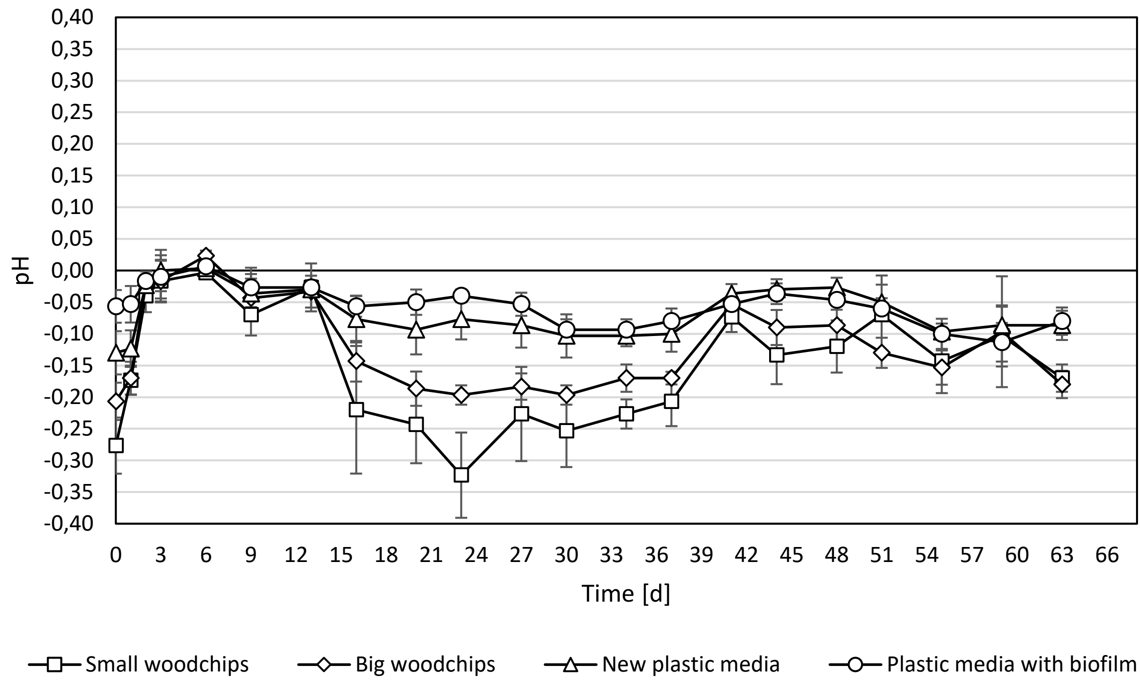

Figure 4.2: Diagram over the change in pH due to reactions in the filtration reactors.

Both nitrifying and denitrifying bacteria affect pH-balance when they grow. For the nitrification bacteria, the pH decreases as they produce H^+ ions. Denitrification bacteria on the other hand produce raises the pH, as OH^- ions are one of the products of the reactions (Tehobanoglous et al., 2003). The pH-measurements for the four systems show a decrease in pH. This is a good indication of nitrification, but the reason can also be other reactions happening where H^+-ions are produced.

If there are heterotrophic bacteria present and there is enough dissolved oxygen, the organic material will not be oxidized by use of nitrogen components as in denitrification, but of dissolved oxygen as shown in the equation [5]. Here carbon dioxide is produced. The increased amount of CO_2 can cause a right shift in the balance with hydrogen ions, as seen in equation [4]. This will give more H^+ ions and give reduced pH.

For the stoichiometry of denitrification, the first step is the reduction of nitrate, shown in equation [15]. Where organic material from wastewater is used as an electron donor, this shows that of 1 mol NO_3^-, 1 mol OH^- ions produced, but also 1 mol CO_2 molecules are produced. The correlation between pH and CO_2, given in equation [4], indicates that 1 mol CO_2 molecules have the potential to produce 1 mol H^+ ions and eliminate any pH changes. This means that the first step of denitrification can be happening even though it is not necessarily measurable when looking at pH.

The reason for the odd measurements seen in the diagram the two first days, is due to the use of a faulty pH-meter. The measurements around day 41 show a reduction of pH change as seen in figure 4.2. This was when the reactors were restarted after a flow stop. The low difference between the pH-values in the start corresponds with the expected low changes of ammonium and nitrite concentration experienced in the beginning.

ANOVA comparison states that the average pH change for two woodchip reactors are not significantly different ($p > 0,05$), but both woodchip reactors have changes in pH significantly different from the two plastic reactors. This is indicating that the nitrification in the woodchip reactors are higher than in the plastic reactors and/or that aerobic breakdown of the woodchip media is decreasing the pH. The t-test shows that the mean change is different from zero ($p < 0,05$) for all the reactors.

4.1.2 Ammonium oxidation and nitrification rate

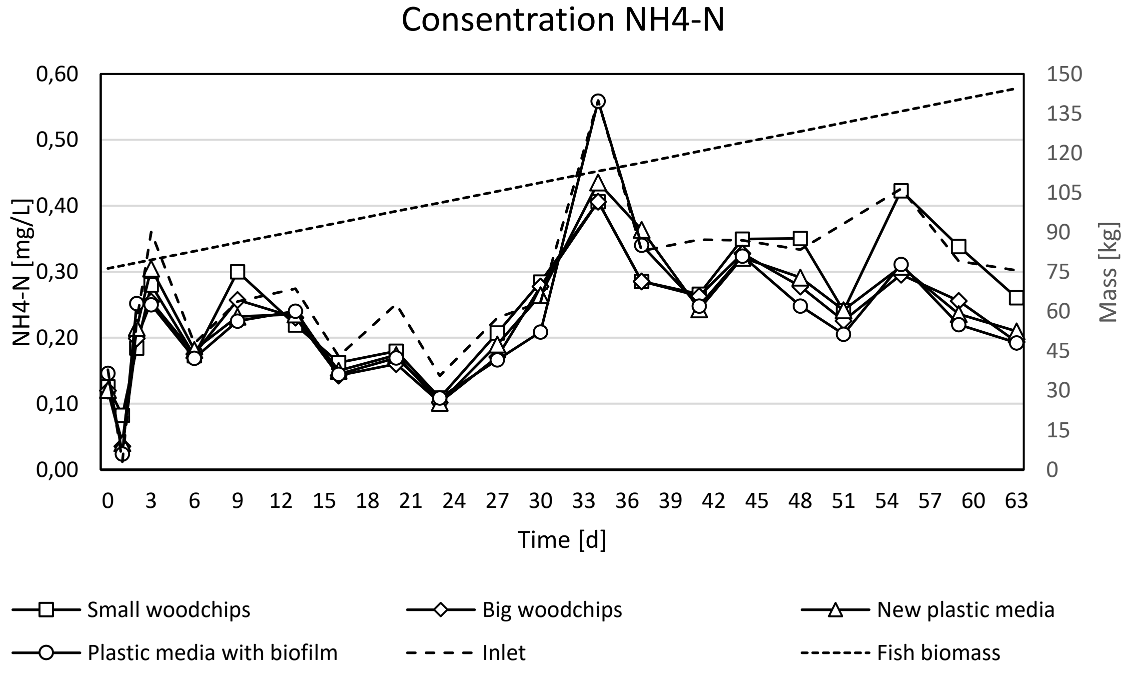

Figure 4.3: Diagram over the concentration of ammonium-nitrogen [mg/L] and change in fish biomass [kg].

The levels of ammonium-nitrogen concentration in the influent water were measured to an average of 0,31 ± 0,12 mg/L. This is a very low value. The highest acceptable limits for ammonia NH_3-N in aquaculture is set to 3-5 µg/L (Bjerknes, 2007), and in the Centre for Fish Research RAS the pH is in the range 7,7-8,0, and the average water temperature is 12,8 °C. When using figure 2.1 over the concentration vs. pH for different water temperature, we can read that the amount is 1-1,5 % of NH_3 and 98,5-99 % of NH_4^+. Based on this we can

calculate the average concentration of NH_3-N to be approximately 3,1-4,7 µg/L. This means that the levels of ammonia are within the limits before any biological filtration is done.

Compared to the full-scale RAS in the study by (Ahnen et a., 2018) the registered effluent concentration of TAN for the smallest facility was measured to be 1,0 ± 0,12 mg/L, if we assume the same pH and temperature, they have about 20 µg/L NH_3-N in the effluent water before biological filtration.

Table 4.2: Pearson comparisons, average concentrations for ammonium-nitrogen.

Pearson comparison	r
NH_4^+-N concentration in influent water vs. time	0,527
NH_4^+-N concentration in influent water vs. biomass change	0,527
NH_4^+-N concentration change in small woodchip reactor vs. time	0,046
NH_4^+-N concentration change in big woodchip reactor vs. time	-0,318
NH_4^+-N concentration change in new plastic reactor vs. time	-0,353
NH_4^+-N concentration change in old plastic reactor vs. time	-0,398

Average concentration NH_4^+-N			**[**mg/L] (value ± S.D.)	
Filtration reactor	In	Out	Change	t-test
Small woodchips	0,279 ± 0,115	0,249 ± 0,094	-0,030 ± 0,054 a	µ ≠ 0
Big woodchips	0,279 ± 0,115	0,222 ± 0,084	-0,057 ± 0,050 a	µ ≠ 0
New plastic	0,279 ± 0,115	0,232 ± 0,091	-0,047 ± 0,047 a	µ ≠ 0
Old plastic	0,279 ± 0,115	0,227 ± 0,106	-0,053 ± 0,050 a	µ ≠ 0

Average nitrification rate	[mg NH_4^+ / (m^2 min)] (value ± S.D.)	t-test
Small woodchip reactor	-0,068 ± 0,141 ab	µ ≠ 0
Big woodchip reactor	-0,102 ± 0,110 a	µ ≠ 0
New plastic reactor	-0,030 ± 0,032 b	µ ≠ 0
Old plastic reactor	-0,026 ± 0,034 b	µ ≠ 0

Note: Same letters indicate that values are not significantly different. The t-test is done on the mean values of change in concentration and for nitrification rate.

In figure 4.3, we can see the concentration diagram for ammonium. From this, we can see that the concentration for the reactors are similar for most of the study, while there is a clear difference between the reactors and the concentration of ammonium in the inlet. For the table 4.2, we can see the average change in concentration for each reactor, this equals an average change in ammonium-nitrogen concentration for the small woodchip reactor, big woodchip reactor, new plastic reactor and old plastic reactor of respectively of 9,7 %, 18,5 %, 15,4 %, and 17,0 %.

The figure 4.4 shows the change in ammonium-nitrogen concentration through the reactors, and here it is also clear to see that the filtration reactors show comparable performance. The average means of the reactors are not significantly different from each other, as stated with an ANOVA test (p > 0,05) as and marked in table 4.2. While the t-test for the concentration change indicates that the mean change is different from zero, indicating that oxidation is occurring.

For the single diagrams of the reactors, the changes in concentration can be studied more in detail. We can see that oxidation of ammonia occurs early. The reactor with plastic media and biofilm shows the clearest oxidation of ammonium initially. For the filtration reactors, we can see that the nitrification is stable for the first 27-30 days, and where peaks are seen, the variation between the measurements are also high, indicating that the peaks could be random errors.

All the reactors cross the x-axis around day 30-37, where the flow rate of the reactors was very low, this must be seen in correlation with low flowrate and low concentration.

The similarity of the reactors is striking, and it’s clear that the nitrification for all of them are related. This is likely to be connected to ammonium concentration varying in the water. When the concentration change diagram is compared with the concentration diagram for ammonium, we can see that the highest oxidation was achieved

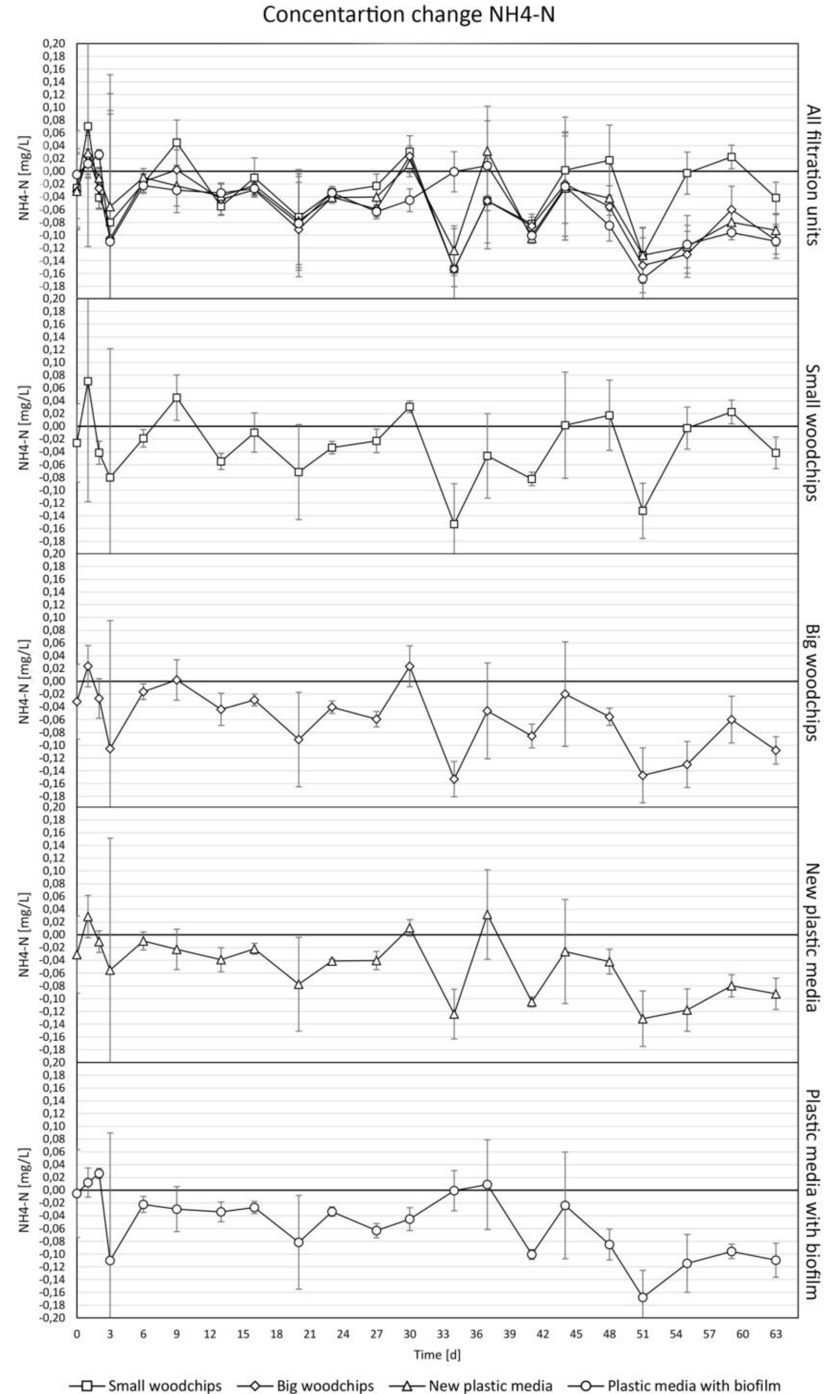

Figure 4.4: Diagrams over change in concentration of ammonium-nitrogen [mg/L] in the filtration reactors (mean ± S.D.).

when the influent concentration of ammonium also was high. This corresponds well with the literature, which tells that the nitrification effect is dependent on ammonium concentration (Lekang & Fjæra, 1997).

As seen from the table 4.2, the average concentration change is measured highest for the big woodchip reactor, second best for the biofilm plastic reactor, third best for the plastic reactor, and worst for the small woodchip reactor. ANOVA test places them not significantly different as mentioned, but from the diagrams in figure 4.4, we can see that the small woodchip reactor differs in behavior from the three other reactors for the measurements after day 43.

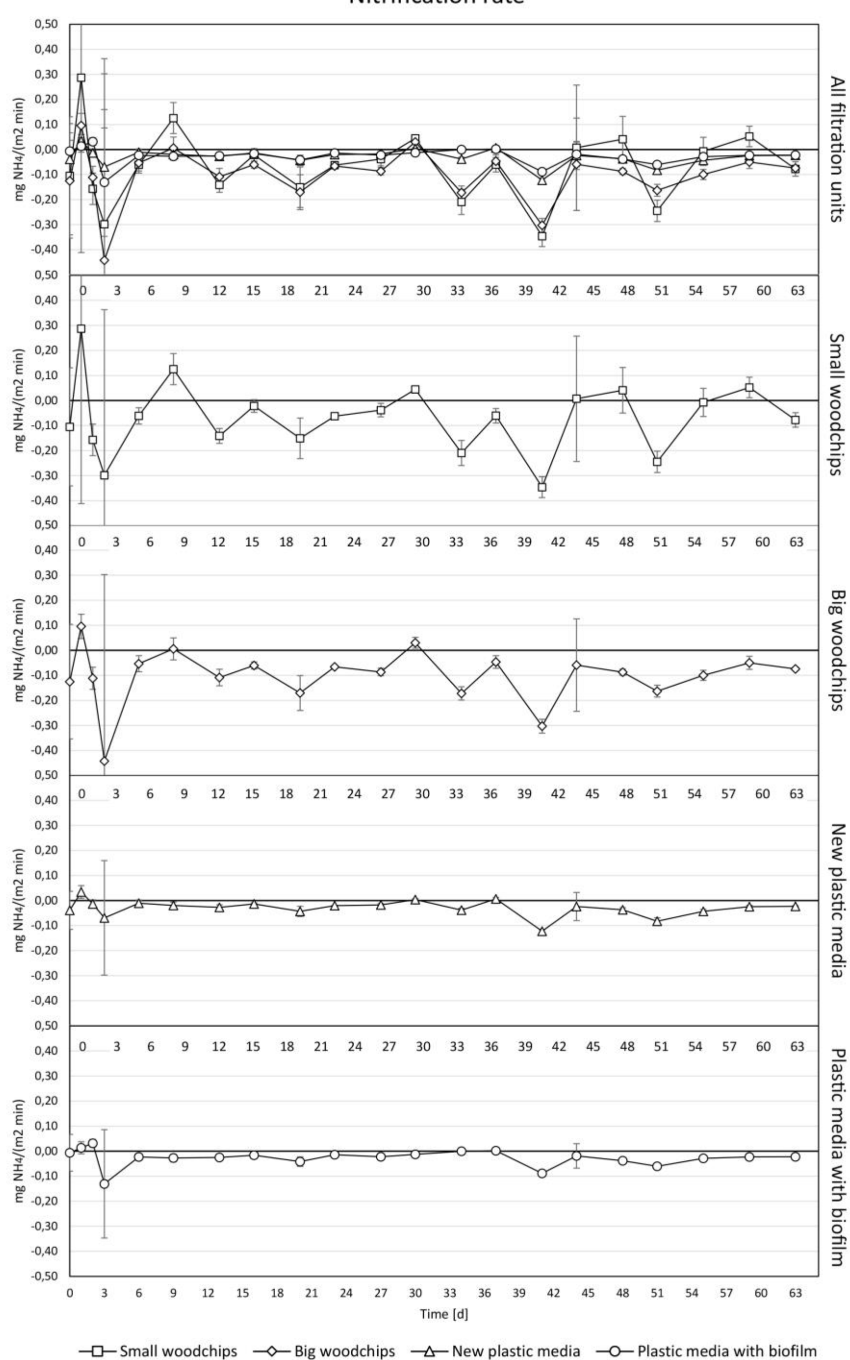

Figure 4.5: Diagrams over nitrification rate for filtration media [mg NH_4^+/m^2 min]. Nitrification rate is amount of ammonium oxidized per litre times flowrate, divided by the total surface area of the filtration media in the reactor.

This is likely to be because the reactor was affected of the stop between day 38-41 and it can also be seen in correlation with higher flowrate for the small woodchip reactor compared to the other reactors, as seen in figure 4.12.

From the diagrams, we can see that the oxidation of ammonium seems to increase with time. This relationship is confirmed with a Pearson comparison as seen in table 4.2, indicating a strong negative relationship for the big woodchip reactor and the two plastic reactors, while the correlation is none for the small woodchip reactor.

The increase in nitrification can be seen in connection with the concentration increase of ammonium in the water over time. The increase in biomass is marked in figure 4.3, and the increase in ammonium corresponds well with this change. For the relationship between NH_4-N concentration in the intake water and the biomass increase, the Pearson value is 0,527 indicating a strong positive relationship.

For the figure 4.5 of nitrification rate, we can see that the nitrification rate is higher for the woodchip reactors than for the plastic reactors, this is due to the high specific area of the plastic media. Because the surface area is higher for the plastic media, the oxidation of ammonium should be higher as the biofilm have more surface area to grow on. An explanation to why this is not occurring might be that the concentration of ammonium in limiting for growth. As (Lekang & Fjæra, 1997) states the concentration of ammonium affects the efficiency of the nitrification process. Another theory can be that the biofilm on woodchips is more active than on plastic. By ANOVA comparison the small woodchip reactor and the plastic reactors are shown not significantly different ($p > 0,05$), while the big woodchip reactor is significantly different from the plastic reactors ($p < 0,05$). A t-test of the concentration change shows that the mean nitrification rate for the reactors is different from zero.

4.1.3 Nitrite oxidation

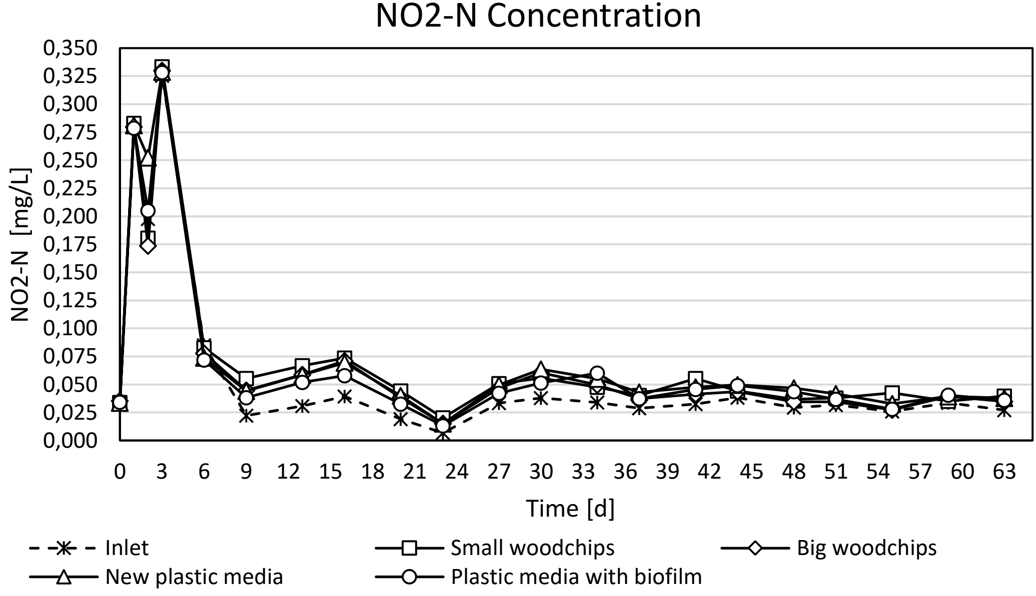

Figure 4.6: Diagram over the concentration of nitrite-nitrogen [mg/L] in the influent water and the four reactors.

Table 4.3: Pearson comparison and average nitrite-nitrogen concentration change (for days 6-63).

Pearson comparison	r
NO2-N concentration in influent water vs. time	-0,238

Average concentrations NO_2-N			[mg/L] (value ± S.D.)	
Filtration reactor	In	Out	Change	t-test
Small woodchips	0,033 ± 0,015	0,049 ± 0,015	0,016 ± 0,011 a	$\mu \neq 0$
Big woodchips	0,033 ± 0,015	0,045 ± 0,015	0,012 ± 0,010 a	$\mu \neq 0$
New plastic	0,033 ± 0,015	0,048 ± 0,014	0,015 ± 0,010 a	$\mu \neq 0$
Old plastic	0,033 ± 0,015	0,043 ± 0,013	0,011 ± 0,009 a	$\mu \neq 0$

Note: Same letters indicate that values are not significantly different. The t-test is done on the mean values of change in concentration.

In figure 4.6 showing average nitrite-nitrogen concentration, there is a visible positive difference between inlet and outlet concentration for the four reactors from day nine. This is indicating that there is an accumulation of nitrite. There is no significant difference between the reactors. The overall concentration of nitrite seems to decrease with time, and a Pearson comparison indicate a small negative correlation between time and nitrite-nitrogen concentration (r = -0,238).

For the first measurements of nitrite-nitrogen, the levels are high compared to the other measurements in the study. The influent concentration of nitrite-nitrogen is also high, indicating that this is not due to high oxidation of ammonium. A plausible explanation can be that the reagents used in the analysis were bad or instrument error.

Figure 4.7 of nitrite-nitrogen concentration changes shows a positive concentration increase for the measurements taken after day nine. The concentration of nitrite-nitrogen is measured at under 0,075 mg/L for the samples from day 6, which is very low levels. While the change in concentration varies from 0-0,035 mg/L as seen in figure 4.6. The increase is likely to be due to oxidation of ammonium, which we can see that are occurring from the diagram over concentration change in ammonium-nitrogen.

If we consider the stoichiometric equations for the nitrification process, [9] and [10]. We can see that 1 mol of ammonium produces 1 mol of nitrite which produces one mol of nitrate. If we take the average oxidized ammonium-nitrogen of big woodchips, 0,045 mg/L, this can give 0,045 mg/L nitrite-ammonium. While the average concentration increase of nitrite-nitrogen for big woodchips is only 0,012 mg/L, which indicates that most of the ammonium-nitrogen oxidized to nitrite-nitrogen is missing, which indicate that there is most likely oxidation of nitrite occurring

A different theory could be that denitrification bacteria are reducing nitrite to nitrogen gas. The weakness of this theory is the presence of dissolved oxygen in the water, which will cause the bacteria to use oxygen as electron acceptors instead of nitrite and nitrate (Lu et al., 2014). Gayle et al., (1989), also writes that when there are cycles of aerobic and anaerobic conditions, there is a phase with lag, before denitrification occurs. Based on this it is most likely to believe that oxidation of nitrite is the explanation for the missing nitrite.

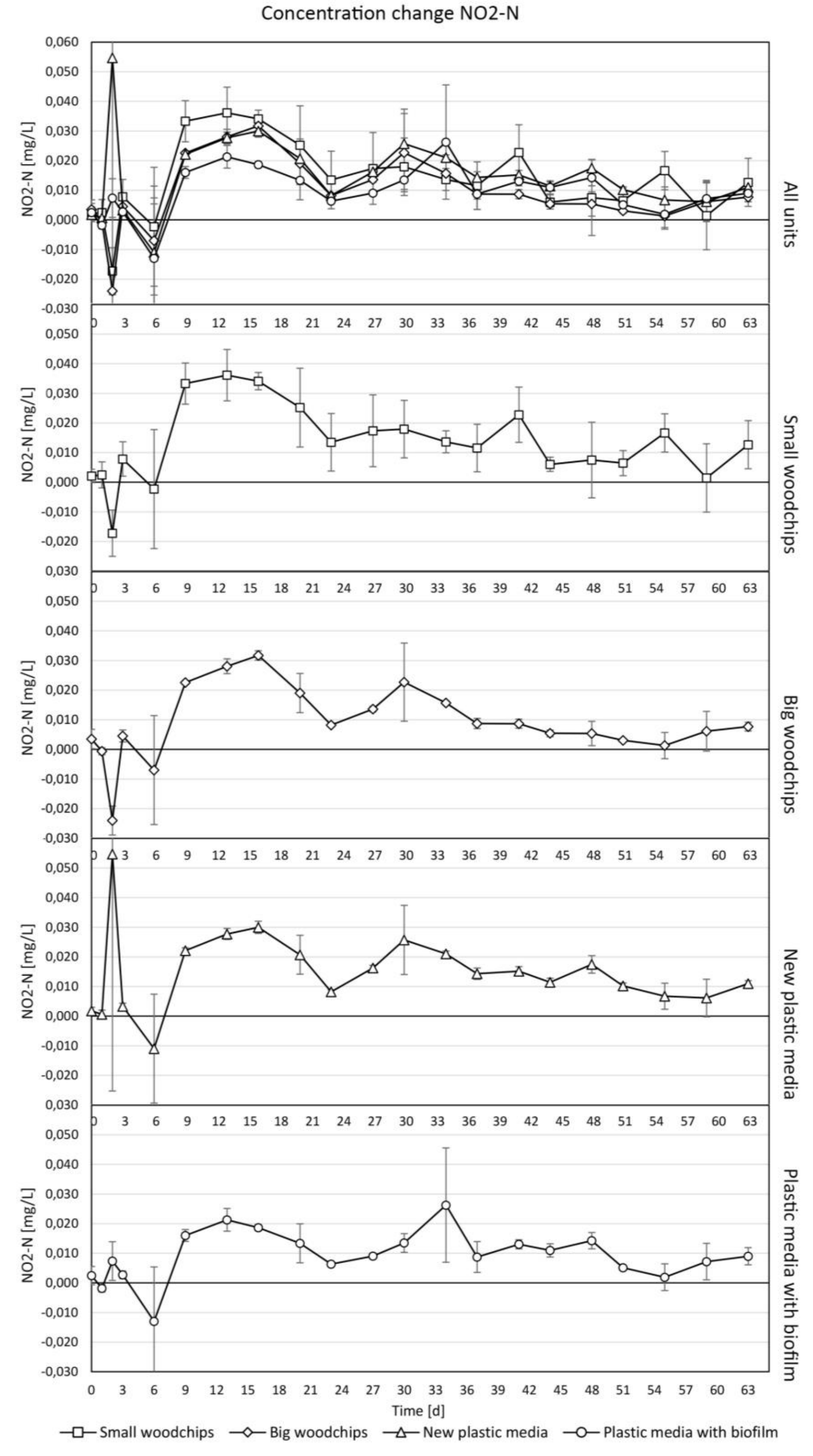

Figure 4.7: Diagrams over change in nitrite-nitrogen concentration [mg/L] for the filtration reactors.

The lowest concentration increase of nitrite is registered for the plastic with biofilm reactor, this can be an indication of a more well-established *Nitrobacter* bacteria culture. The plastic reactor with biofilm had the lowest nitrite concentration change in the beginning, which can be due to the biofilm that was on the media from the beginning, which is likely to be that Nitrobacter bacteria already were established.

The concentration changes of nitrite for the reactors decrease with time, while the oxidation of ammonium increases with time, seen in effect with increased ammonium concentration in the influent water. Both indicate that the oxidation of nitrite must have increased, indicating an increased growth of *Nitrobacter* bacteria.

Stickney, 2000, writes that the *Nitrobacter* achieves colonization and nitrification at a later state than *Nitrosomonas,* which can describe that the reduction in concentration change happens from day 13. There is a peak for concentration increase from day 9-15. This can be in correlation to the oxidation of ammonium starting to occur at the same time, as seen in figure 4.4.

4.1.4 Nitrate concentration

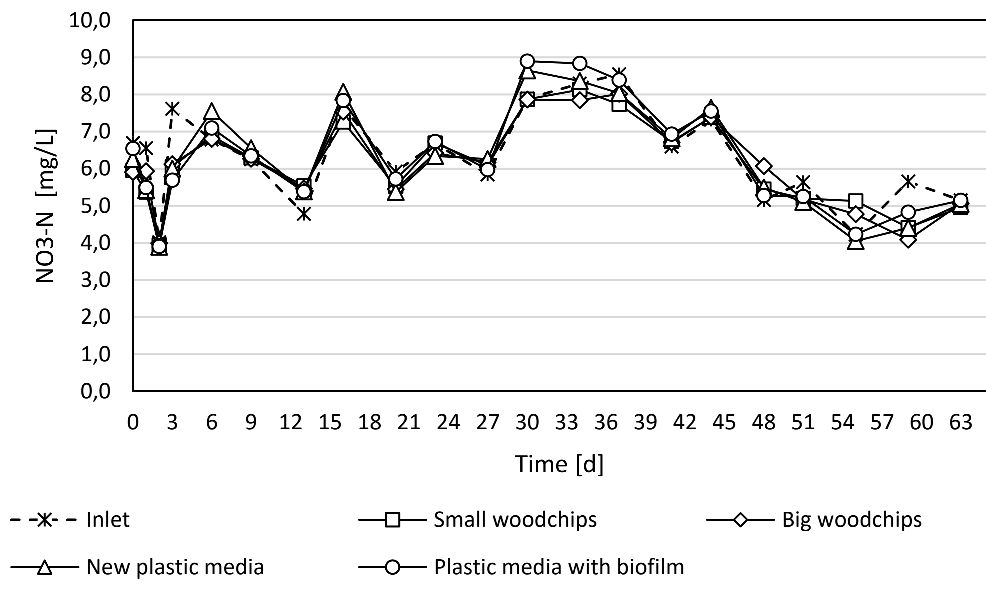

Figure 4.8 Diagram over nitrate-nitrogen concentration [mg/L] in the inlet water and the for reactors.

Table 4.4: Pearson comparison and average nitrate-nitrogen concentration change (value ± S.D.).

Pearson comparison				r
NO_3-N concentration in influent water vs. time				-0,174
Average concentration NO_3-N (day 6-63)				mg/L
Filtration reactor	In	Out	Change	t-test
Small woodchip	6,372 ± 1,220	6,308 ± 1,100	-0,065 ± 0,502 a	μ = 0
Big woodchip	6,372 ± 1,220	6,297 ± 1,148	-0,075 ± 0,548 a	μ = 0
New plastic	6,372 ± 1,220	6,419 ± 1,401	-0,047 ± 0,530 a	μ = 0
Old plastic	6,372 ± 1,220	6,494 ± 1,399	-0,122 ± 0,395 a	μ = 0

Note: Same letters indicate that values are not significantly different. The t-test is done on the mean values of change in concentration.

The concentration of nitrate in the water is much higher than the concentrations of ammonium and nitrite. The variation between the reactors is small as seen in figure 4.8 over concentration. Doing an ANOVA test of the concentration changes shows that there is no significant difference between the mean values of the reactors ($p > 0,05$), and a t-test shows that there is no significant difference from zero. From the measurements of ammonium-nitrogen and nitrite-nitrogen, we know that there is nitrification happening, but the concentrations are low. From the consideration of the stoichiometric equations that were done for nitrite for the big woodchip reactor, we found that oxidation of ammonium-nitrogen gave 0,045 mg/L nitrite-nitrogen. Nitrite can oxidize further and give 0,045 mg/L nitrate-nitrogen. As seen in figure 4.9 the variation between the samples varies at higher rates. Thus it is not possible to see the effect of nitrite oxidation.

Figure 4.9: Diagrams over change in nitrate-nitrogen concentration [mg/L] for the four filtration reactors.

For the big woodchip reactor and the new plastic reactors, there are a few points where the variation between the samples are low and the measurements differ from zero, but these points indicate both increasing and decreasing concentrations for the reactors. Due to the varying positive and negative results, and high standard deviation, either nitrification or denitrification can be proved by studying the nitrate diagrams. For the overall nitrate concentration, a Pearson comparison shows a small negative correlation between the nitrate concentration in the inlet and time. Indicating that the overall concentration of nitrate is decreasing.

4.1.5 Total Nitrogen

Figure 4.10: Diagram over concentration of total nitrogen [mg/L] in the inlet and the four reactors.

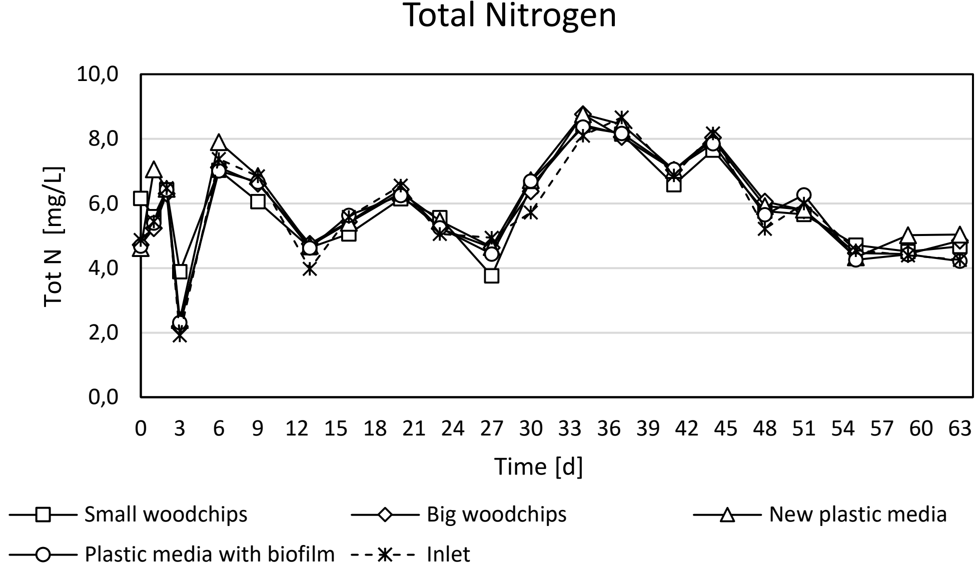

Table 4.5: Pearson comparisons and average Tot N concentration change.

Pearson comparison			r	
Tot N concentration in influent water vs. time			-0,06	
Average concentration Tot N			[mg/L] (value ± S.D.)	
Filtration reactor	In	Out	Avg. change	t-test
Small woodchips	5,760 ± 1,572	5,855 ± 1,256	0,095 ± 0,710 a	μ = 0
Big woodchips	5,760 ± 1,572	5,834 ± 1,477	0,074 ± 0,397 a	μ = 0
New plastic	5,760 ± 1,572	6,042 ± 1,521	0,281 ± 0,511 a	μ ≠ 0
Old plastic	5,760 ± 1,572	5,790 ± 1,477	0,030 ± 0,370 a	μ = 0

Note: Same letters indicate that values are not significantly different. The t-test is done on the mean values of change in concentration.

The total nitrogen concentration is variable useful for measuring if denitrification is appearing in the reactors. Nitrification will not affect the total nitrogen concentration, as all the inorganic nitrogen components are included in the measurement (in addition to organic bound nitrogen), while denitrification will reduce the concentration if occurring, as nitrate and nitrite are reduced to nitrogen gas.

For the experiment, we can see that figure 4.10 over total nitrogen concentration is almost identical to the diagram over NO_3-N concentration. This is because the concentration of nitrogen in nitrate is much higher than the concentrations of nitrogen in ammonium and nitrite.

What also can be noted is that the average concentration of total nitrogen is lower than the concentration of nitrate-nitrogen. This is strange as nitrate-nitrogen is included in the parameter. The laboratory noted that this also was the case for other experiments using the same procedure. Thus, the results of the total nitrogen measurements can only be used to show a trend and not the exact values in the samples.

The diagram for change in concentration for total nitrogen does not show a clear reduction or increase of concentration, as the curves vary both over and under the x-axis. The ANOVA test reveals that there is no significant difference in mean concentration change between the reactors and the inlet concentration ($p > 0,05$). This means that there are no significant changes in the average nitrogen content of the water, and if there is denitrification occurring, this effect is not measurable with the method used. T-tests for the reactors show that the new-plastic reactor is different from zero, indicating that the concentration of total nitrogen is increasing.

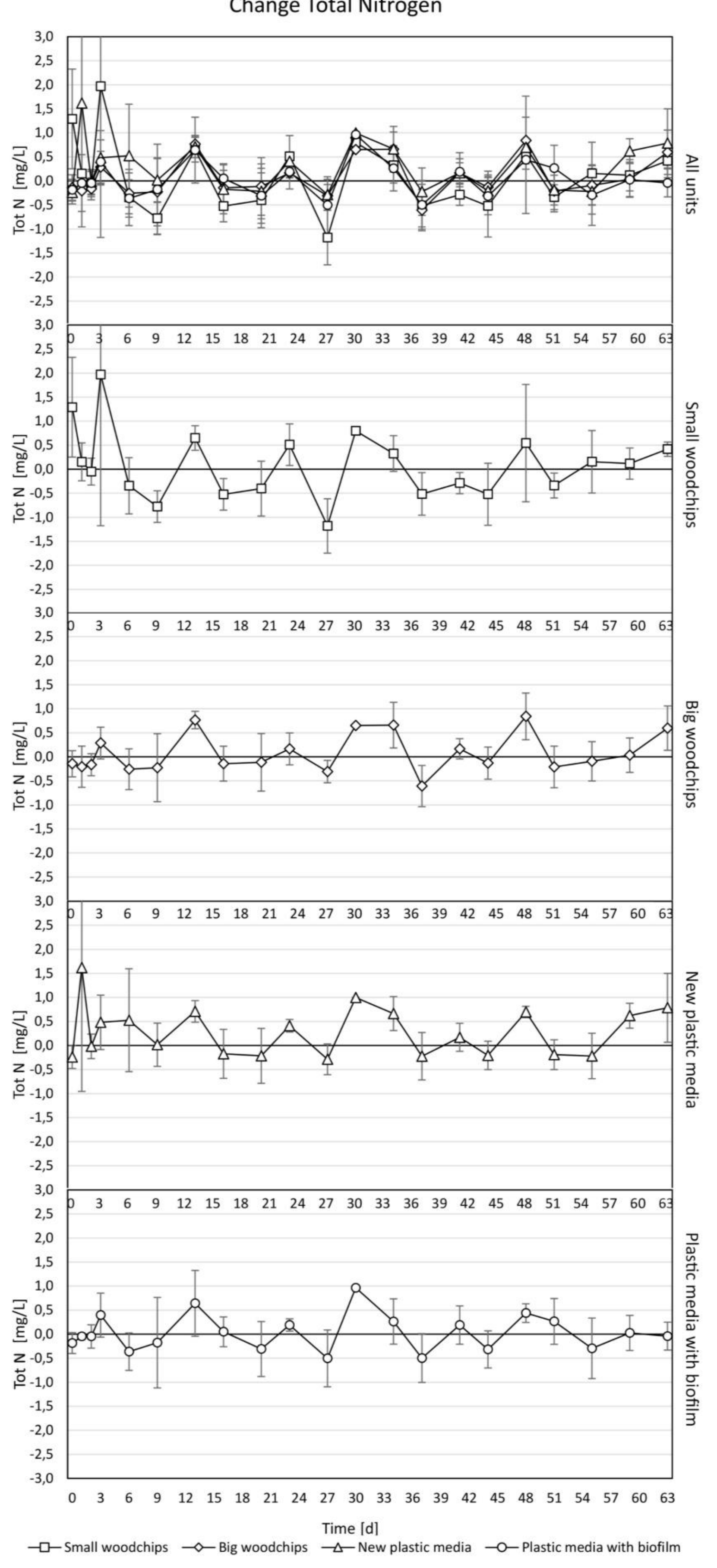

Figure 4.11: Diagrams over change in total nitrogen concentration [mg/L] for the filtration reactors.

4.1.6 Comparing with the biological reactor in the full-scale RAS

At the final sampling day, samples were taken of the filtration reactors, but also from several chambers in the full-scale recirculating system. Based on these measurements the performance of the reactors was compared with the RAS.

pH

Table 4.6: pH measurements were taken last sample day.

pH			(value ± S.D.)
Filtration reactor/sample	In	Out	Change
Inlet	7,75 ± 0,02	7,75 ± 0,02 e	-
Small woodchips	7,75 ± 0,02	7,58 ± 0,02 d	-0,17 ± 0,02
Big woodchips	7,75 ± 0,02	7,57 ± 0,02 d	-0,18 ± 0,02
New plastic media	7,75 ± 0,02	7,66 ± 0,02 c	-0,09 ± 0,02
Plastic media with biofilm	7,75 ± 0,02	7,67 ± 0,02 c	-0,08 ± 0,02
RAS3 - Biochamber	7,75 ± 0,02	7,89 ± 0,02 b	0,14 ± 0,02
RAS3 - Sludge chamber	7,75 ± 0,02	7,91 ± 0,02 b	0,16 ± 0,02
RAS3 - Pump sump	7,75 ± 0,02	8,00 ± 0,01 a	0,25 ± 0,02

Note: Same letters indicate that values are not significantly different.

For the measurements in table 4.6, we can see that the pH decreases from the influent water to the effluent of each of the filtration reactors, indicating that there may be some nitrification occurring. As noted for the main experiment, the production of CO_2 from the decomposition of the wood can give reduced pH. As seen in the ANOVA test the systems have significantly different pH from the inlet ($p < 0,05$). The woodchip reactors are also shown to have significantly lower pH than the plastic reactors. This can be an indication of higher nitrification effects for the woodchip reactors, but it can also be due to the decomposition of wood. If we compare the results from the ammonium-nitrogen measurements in table 4.7, the difference in pH between the woodchip and plastic reactors are not corresponding with the oxidation levels of ammonium, where the oxidation in the woodchip reactors are lower or equal to the plastic reactors. This strengthens the theory that the difference in pH, may be affected by a secondary source, which can be the decomposition of woodchips.

The pH increases for the chambers in the recirculating system. As stated, the pH decreases with increased dissolved CO_2 in the water but will thus increase when CO_2 is removed. This effect occurs in the aeration of the water, where CO_2 is removed when the concentration is high (Patel & Majmundar, 2018). This is a reasonable theory for why the pH increases through the RAS. A different theory can be that denitrification increases the pH, but as seen for the results of ammonium-nitrogen and nitrate-nitrogen measurements below there is no indication of denitrification.

Ammonium

Table 4.7: Concentration of ammonium-nitrogen (value ± S.D.) in the reactors and RAS3 taken last sample day.

Concentration ammonium-nitrogen			[mg/L] (value ± S.D.)
Filtration reactor/sample	In	Out	Change
Inlet	0,302 ± 0,021	0,302 ± 0,021 a	-
Small woodchips	0,302 ± 0,021	0,261 ± 0,014 b	-0,042 ± 0,025
Big woodchips	0,302 ± 0,021	0,194 ± 0,006 df	-0,108 ± 0,022
New plastic media	0,302 ± 0,021	0,210 ± 0,013 cde	-0,092 ± 0,025
Plastic media with biofilm	0,302 ± 0,021	0,193 ± 0,017 de	-0,110 ± 0,027
RAS3 - Biochamber	0,302 ± 0,021	0,218 ± 0,011 cd	-0,085 ± 0,023
RAS3 - Sludge chamber	0,302 ± 0,021	0,244 ± 0,008 bc	-0,058 ± 0,022
RAS3 - Pump sump	0,302 ± 0,021	0,177 ± 0,002 ef	-0,126 ± 0,021

Note: Same letters indicate that values are not significantly different.

ANOVA analysis indicates that there is a significant difference between the measurements in concentration ($p < 0,05$). As seen in table 4.7 the highest concentration change is read for the pump sump, which is the last chamber in the RAS. That the concentration change is higher for the pump sump than the biochamber and the sludge chamber, indicates that it is likely that there is some nitrification occurring in the sludge camber in addition to the biochamber. This is not unreasonable as the sludge chamber contains filtration media, while the water likely has high dissolved oxygen concentration from areation in the previous chambers. For the filtration reactors, the highest concentration change is experienced for the big woodchip reactor and the two plastic media reactors, while these are indicated as not significantly different from each other ($p > 0,05$).

As written in the chapter about nitrification filtration systems, the effectiveness of these systems are often measured in nitrification rate (mg $NH_4^+/(m^2$ min)) (Lekang, 2007). Under are these rates calculated for the reactors and the RAS. The rate is calculated based on the specific area given by the producers of the filtration media. While the specific area for woodchips is found based on a method developed by (Lungulesasa et al., 2009), further described in chapter 4.4.3. The measurement in the pump sump is used for the nitrification rate for the RAS. The nitrification rate is calculated for the last sample day, and the flow-rates are based on the individual measured flowrates for the reactors and the flow given for the RAS.

Table 4.8: Nitrification rate of filtration reactors and RAS.

Filtration reactor	Change NH_4^+ [mg/L]	Flow [L/min]	Volume Reactor [L]	Specific area [m^2/m^3]	Nitrification rate [mg NH_4^+/(m^2 min)]
Small woodchips	-0,054 ± 0,032	1,82	2,24 L	561	-0,078 ± 0,046 a
Big woodchips	-0,139 ± 0,028	0,68	2,24 L	571	-0,074 ± 0,015 a
New plastic media	-0,119 ± 0,032	0,82	2,24 L	750 / 3000	-0,023 ± 0,006 b
Old plastic media	-0,142 ± 0,034	0,66	2,24 L	750 / 3000	-0,022 ± 0,005 b
RAS Biochamber	-0,163 ± 0,027	100	3825 L*	750 / 3000	-0,004 ± 0,001 c

*Note: Same letters indicate that values are not significantly different. * 60 % of the reactor volume is filled with filtration media, while the filtration reactors are filled 100 % with filtration media.*

Based on the calculated nitrification rates for the last sample day, the filtration reactors show better performance compared to the RAS, and the woodchip reactors significantly better performance than the plastic media. As seen the change in mg/L for the RAS is highest, but the specific area of the plastic media and retention time is higher for this reactor, making the nitrification rate lower. As pointed out in chapter 4.1.2 the concentration of ammonium may be limiting for nitrification. Where if the concentration of ammonium was higher, the results of Table 4.8 might have shown the opposite, as the plastic media have a higher surface for the biofilm to grow on.

Nitrite

Table 4.9: Concentration of nitrite-nitrogen in the reactors and RAS3, taken the last sample day.

Concentration nitrite-nitrogen			[mg/L] (value ± S.D.)
Filtration reactor/sample	In	Out	Change
Inlet	0,026 ± 0,001	0,026 ± 0,001 bc	-
Small woodchips	0,026 ± 0,001	0,038 ± 0,008 a	0,012 ± 0,008
Big woodchips	0,026 ± 0,001	0,034 ± 0,001 ab	0,007 ± 0,001
New plastic media	0,026 ± 0,001	0,037 ± 0,000 a	0,011 ± 0,001
Plastic media with biofilm	0,026 ± 0,001	0,035 ± 0,003 ab	0,009 ± 0,003
RAS3 - Biochamber	0,026 ± 0,001	0,026 ± 0,002 bc	0,0003 ± 0,002
RAS3 - Sludge chamber	0,026 ± 0,001	0,026 ± 0,001 bc	-0,0003 ± 0,001
RAS3 - Pump sump	0,026 ± 0,001	0,017 ± 0,000 c	-0,009 ± 0,001

Note: Same letters indicate that values are not significantly different.

ANOVA test marks the measurements as significantly different ($p < 0,05$). As seen in the table the concentration of nitrate-nitrogen is increasing for the big woodchip and the plastic filtration reactors, it is unchanged for the small woodchip filtration reactor, biochamber and the sludge chamber, while for the pump sump there is a decrease in nitrite-nitrate concentration. As discussed for the nitrite results in the main experiment there is likely oxidation of nitrite occurring, and from the measurements in the RAS we can see that the

oxidation of nitrite is more effective than in the filtration reactors, indicating that the Nitrobacter bacteria is less active in the filtration reactors.

This can be a result of several factors, as inhibition by heterotrophic bacteria, or limiting concentration of dissolved oxygen which is proven to be more inhibiting for *Nitrobacter* than *Nitrosomonas* bacteria (Tehobanoglous et al., 2003).

Nitrate

Table 4.10: Concentration of nitrate-nitrogen in the reactors and RAS3, taken the last sample day.

Concentration nitrate-nitrogen			[mg/L] (value ± S.D.)
Filtration reactor/sample	In	Out	Change
Inlet	5,143 ± 0,262	5,143 ± 0,262 a	-
Small woodchips	5,143 ± 0,262	4,954 ± 0,234 a	-0,189 ± 0,351
Big woodchips	5,143 ± 0,262	5,061 ± 0,372 a	-0,082 ± 0,445
New plastic media	5,143 ± 0,262	5,049 ± 0,018 a	-0,094 ± 0,263
Plastic media with biofilm	5,143 ± 0,262	5,141 ± 0,287 a	-0,002 ± 0,389
RAS3 - Biochamber	5,143 ± 0,262	5,028 ± 0,165 a	-0,115 ± 0,309
RAS3 - Sludge chamber	5,143 ± 0,262	5,360 ± 0,110 a	0,217 ± 0,284
RAS3 - Pump sump	5,143 ± 0,262	5,315 ± 0,260 a	0,171 ± 0,369

Note: Same letters indicate that values are not significantly different.

The ANOVA comparison of the measurements shows that they are not significantly different ($p > 0,05$). Which means that the difference in concentration can’t be stated with certainty. The average increase in nitrate-nitrogen concentration for the big woodchip reactor, is expected to come from oxidation of 0,108 mg/L ammonium-nitrogen, which can give 0,108 mg/L nitrite-nitrogen. From table 4.9 we know that there is an increase of 0,007 mg/L nitrite-nitrogen for the big woodchips, the rest of the nitrite-nitrogen from oxidized ammonium must be oxidized further to nitrate. This gives 0,101 mg/L nitrate-nitrogen, as we can see the change in nitrate-nitrogen concentration for the big woodchip reactor is -0,082 ± 0,445 mg/L. The results show that the expected increase can be occurring, but the measurements of the nitrate-nitrogen concentrations have too much variance to show changes occurring

Total Nitrogen

Table 4.11: Concentration of total nitrogen in the reactors and RAS3.

Concentration total nitrogen			[mg/L] (value ± S.D.)
Filtration reactor/sample	In	Out	Change
Inlet	4,252 ± 0,143	4,252 ± 0,143 a	-
Small woodchips	4,252 ± 0,143	4,669 ± 0,041 a	0,417 ± 0,149
Big woodchips	4,252 ± 0,143	4,848 ± 0,439 a	0,596 ± 0,461
New plastic media	4,252 ± 0,143	5,036 ± 0,702 a	0,784 ± 0,716
Plastic media with biofilm	4,252 ± 0,143	4,221 ± 0,253 a	-0,041 ± 0,290
RAS3 - Biochamber	4,252 ± 0,143	4,400 ± 0,094 a	0,148 ± 0,171
RAS3 - Sludge chamber	4,252 ± 0,143	4,427 ± 0,189 a	0,175 ± 0,237
RAS3 - Pump sump	4,252 ± 0,143	4,720 ± 0,289 a	0,468 ± 0,322

Note: Same letters indicate that values are not significantly different.

For the total nitrogen samples, the measurements there is no significant difference occurring ($p > 0,05$). As for the study, the concentration of Tot N was measured to be lower than what expected.

4.1.7 Nitrification and limiting factors in the experiment

Nitrification was quickly established in the reactors. The oxidation of ammonium was seen to have a correspondence with the amount of ammonium in the influent water. Through the study the oxidation of nitrite is also believed to increase, indicating increased growth of nitrite-oxidizing bacteria. The performance of the filtration systems showed comparable results to the full-scale RAS. The nitrification rate of the systems revealed that the woodchip reactors were performing significantly better than the plastic reactors and the RAS. This is due to the surface area of the filtration media. The RAS had the lowest nitrification rate, this is as for the plastic reactors due to the higher surface area of the media, but also higher retention time in the reactor. The measurements of pH indicated that the woodchip reactors had a significantly higher impact on the pH, while the nitrification of the systems was comparable, indicating that other reactions could be contributing to the pH-decrease. The stoichiometric equations for the breakdown of organic material by use of oxygen as an electron donor, show that CO_2 can be produced, which will give increased h^+-concentration, thus lower pH in the water.

There are several factors that are known to be limiting for the nitrifying bacteria. Presence of organic material is one of them, known to give the formation of faster growing heterotrophic bacteria giving competition in the biochamber (Lekang & Fjæra, 1997). Stickney, (2000), writes that accumulation of organic material in biological filtration could inhibit and annihilate the nitrifying bacteria. The use of woodchips has the potential to limit the nitrification. This effect of this was not noticeable in the study, where the woodchip

media showed comparable nitrification results with the plastic media, so the woodchips cannot be said to have a significant effect on the nitrification.

The temperature is known to be a limiting factor. The ideal temperature for the nitrification bacteria is as high as 30°C, while temperatures below 10°C are known to give low biofilm growth (Lekang & Fjæra, 1997). The temperature in the water was as low as 12,8^{o}C. Thus the temperature is not supporting high growth of bacteria and can be a good explanation for the low nitrification effects measured in the reactors and in the full-scale RAS.

The nitrification bacteria are also known to be limited by dissolved oxygen concentration. Nitrification bacteria need dissolved oxygen present to be able to live and oxidize nitrogen components. When deprived of oxygen, even only for a short period, the bacteria will die, and the biofilter can start producing high levels of ammonium and nitrite (Stickney, 2000). The effect of low oxygen concentrations is shown to inhibit nitrification bacteria (Haug & McCarty, 1972). The *Nitrobacter* bacteria oxidizing nitrite to nitrate is shown to be more inhibited than *Nitrosomonas* which is oxidizing ammonium to nitrite (Tehobanoglous et al., 2003). Because the *Nitrobacter* bacteria is more inhibited by low dissolved oxygen levels than *Nitrosomonas*, it should be possible to see the effect of low dissolved oxygen as an accumulation of nitrite. The filtration reactors showed an increase in nitrite concentration through the study, this can be an indication that oxygen concentration was limiting for *Nitrobacter* bacteria. The RAS showed no accumulation of nitrite in the samples the last sample day, which strengthens the theory that the *Nitrobacter* bacteria is inhibited in the filtration reactors. Since the increase in nitrite concentration is similar for both the woodchip and the plastic reactors, it is no reason to believe that the effect is created by the woodchips.

Retention time is a criterion for achieving desired nitrification levels (Tehobanoglous et al., 2003). The retention times for the reactors used in the experiment were varying from 25 to 160 seconds, while the retention time calculated for the nitrification chamber in the recirculating system at the Centre for Fish Research was around 38 minutes. Higher retention times means that the bacteria have longer time to react with the water. The amount ammonium oxidized in the RAS was comparable with the oxidation of ammonium in the filtration reactors, this tells us that the retention time was probably not a limiting factor for this study in terms of nitrification.

The reactors have a design where the filtration media are static. This contributes to the clogging of the system. Excess biofilm is not removed which allows the nitrifying bacteria to produce a thick biofilm. This is known to give lower nitrification rates than thin biofilm layer (Lekang, 2007) (Sterner BioTek AS, 2019).

4.1.8 Denitrification and limiting factors in the experiment

For the study, there were no clear indications of denitrification occurring. The concentration of nitrate-nitrogen and nitrite-nitrogen gave no indication of being reduced. There are many factors that limit denitrification, the most significant of those is the presence of dissolved oxygen.

The water used in the reactors is taken from a chamber with aeration, which means that the water will have a concentration of oxygen close to saturation. The reactors are closed systems, and there is no additional oxygen added to the water. Gayle et al., (1989), writes that most denitrifying bacteria are facultative anaerobic organisms, which can use oxygen as electron-acceptors when it is present, while when oxygen is absence the bacteria can use nitrate or nitrite as electron-acceptors. Tehobanoglous et al., (2003), writes that in established denitrification processes it is found that low concentrations of dissolved oxygen can inhibit denitrification bacteria. Gayle et al., (1989), also writes that when there are cycles of aerobic and anaerobic conditions, there is a phase with lag, before denitrification occurs. The literature on the subject gives reason to believe that the denitrification in the filtration reactors is likely to be heavily if not completely inhibited by water rich on dissolved oxygen. This can explain why there were no signs of denitrification in the filtration reactors.

Organic material is as stated in the literature review a source of carbon for denitrifying bacteria (Tehobanoglous et al., 2003). As woodchips is an organic material, containing carbon, there is a reason to believe that heterotrophic bacteria will grow on the woodchips. Woodchips have been stated as a viable carbon source for denitrifying bacteria in studies by (Ahnen et al., 2018), (Christianson et al., 2016) and (Saliling et al., 2007). Even though no denitrifying can be stated for the filtration reactors, there were signs of decomposing of the woodchips as described further in chapter 4.2.4. The measurements of pH were also indicating that the reduction of pH in the woodchips might have help from other reactions than nitrifying bacteria. Breakdown of organic material by heterotrophic bacteria using dissolved oxygen has the potential to produce CO_2, thus increase the H^+-ions in the water and lower the pH.

4.2 Flow performance of the filtration systems

4.2.1 Flow measurements

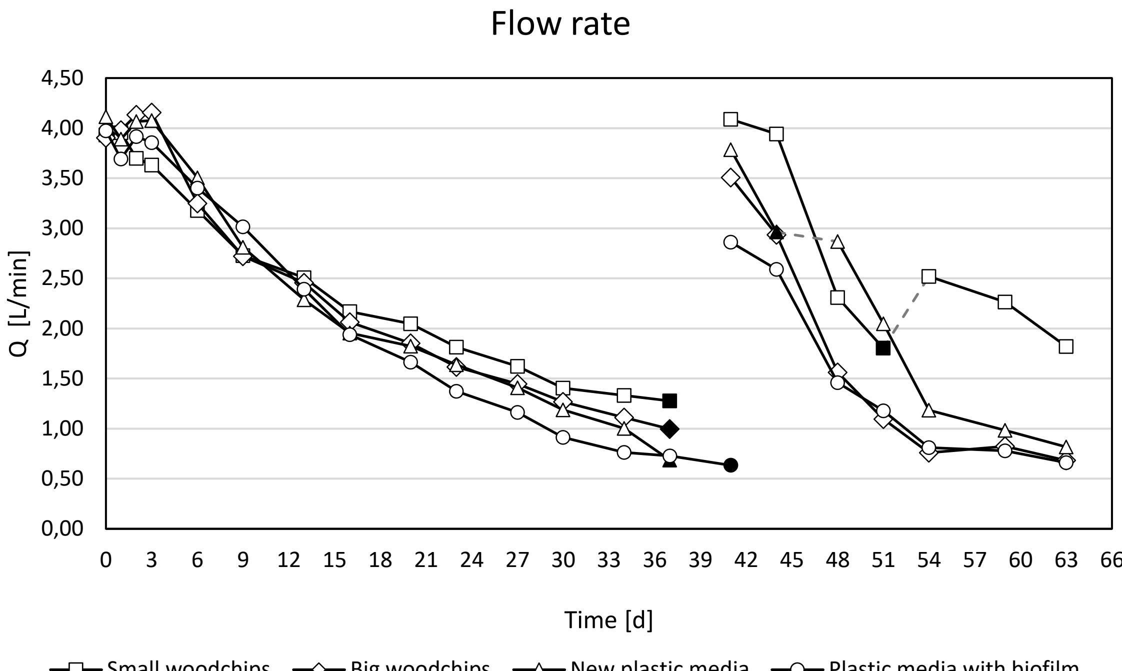

Figure 4.12: Flow rate for the four filtration units [L/min] over the course of the experiment. Black symbols indicate system stop due to clogging or air. All the systems were started at flowrate 4 L/min with media, after restart the systems were put on maximum flow (initially 6 L/min without media).

The flow rate for the four different reactors was regulated to be 4 L/min the starting date and was not adjusted during the study. The change of flowrate for the four reactors followed the same behavior through the course of the experiment. The flowrate falls drastically at a slightly decreasing rate. After 37 days three of the systems stopped. The stopping points are marked in black. The three systems that stopped between day 37 and 41 were measured to the flow rates 1,28 L/min for the small woodchips, 1,00 L/min for the big woodchips and 0,69 L/min for the new plastic media, while the reactor with plastic with biofilm in the beginning, had not stopped, and were measured to the flowrate of 0,73 L/min at day 37. That three of the filters stopped at the same time, with very different flowrates is suspicious, there could be several reasons, under are a couple of theories why this occurred.

First, the stop may have been caused by varying water level in the recirculating system. The inlet hoses were placed under water in the second chamber of the RAS, and the hoses were placed about 15 cm or more under the water surface. A reduction in flowrate from the fish chambers could have caused such a lowering in the water level, but the fact that it didn`t occur before 37 days into the experiment is odd. The staff at the Centre for Fish Research also stated that they were very careful not to do changes that would affect the water level in the RAS scientifically.

Secondly, an explanation could be that the stops were caused by air bubbles from aeration in the chamber the water was taken from, or a combination of low flowrate in the reactors and bubbles. If the reactors were taking in too much air through the inlet hose, it is possible that the centrifugal pump was not able to sustain the pressure difference necessary for the water to enter the pump. Third, the stop may be caused because of too high resistance in the filtration media and clogging of the system. The filtration material covered with biofilm and the inlet pipes were found to be significantly clogged, as shown in figure 4.16.

As seen in figure 4.12 these were not the only stops. The reactor with the new plastic media stopped between day 44 and 48, and the flow rate was 2,96 L/min at day 44. The reactor with small woodchips also stopped, between day 48 and 51, and the flow rate was 1,80 L/min at day 48. These stops occurred after the restart and increase of pump capacity of all the reactors. The reason for these stops is suspected to be due to modifying the inlet hoses after the stop. As the inlet hoses were considered a great contributor to the clogging, the green plastic cover and a green pipe with smaller dimensions than the rest of the hose were taken away to see the effect. As a result of this, the hoses were shorter and was placed only 10 cm or more under the water level. The stops might of this have been caused by varying water levels. At the same time, one will expect more than one reactor would have stopped if this was the cause. A different explanation can be the air bubbles as described. The pumps of the reactors were found to be very sensitive for stops of water flow, so this seems as a reliable explanation.

Taking the measurements before the first stop into consideration, the reactors show comparable results of flowrate and clogging. An ANOVA test of the flow-rate the last day before the stop (day 37), shows that small woodchip and the big woodchip reactor are significantly different from each other and the two plastic reactors ($p < 0,05$), while the two plastic reactors are not significantly different from each other ($p > 0,05$). From the results we can state that the filters clog rapidly when used in such a configuration, but that the woodchip does not show any worse effect on the clogging intensity when compared to the plastic filtration media, rather better performance.

After the restart, we can see that the clogging occurs at a much higher rate. This is likely to be connected to increased biomass levels compared to the beginning of the experiment. But it can also be due to the removal of the green fish blocker as seen in picture 4.16, which when fully covered of algae and microorganisms might have worked as a filter. It can also be that the restarting of the reactors, caused the media to loosen and that the high clogging rate is due to the media settling again, or a combination of the factors mentioned.

4.2.2 Hydraulic retention time

Figure 4.13: Hydraulic retention time for the filtration units. Black symbols indicate system stop due to clogging or air. Hydraulic retention time calculated as void volume in the reactors, found by the porosity of the filtration medias, divided by the flow rate.

The retention time for the system was calculated as the porosity of the media times the volume of the filtration media containers divided by the flowrate. The porosities of the media were found by experiments, which are explained in the method section. These porosities were found to be close for the three media, 74,1 % for small woodchips, 72,0 % for big woodchips and 75,6 % for the plastic media. The curves for the HRT for the four systems is therefore very similar to the opposite curves of the flow rates.

The retention times vary from 25 seconds to 160 seconds. The highest retention times are experienced for the two reactors with plastic media for the first period.

4.2.3 Total Oxygen Demand

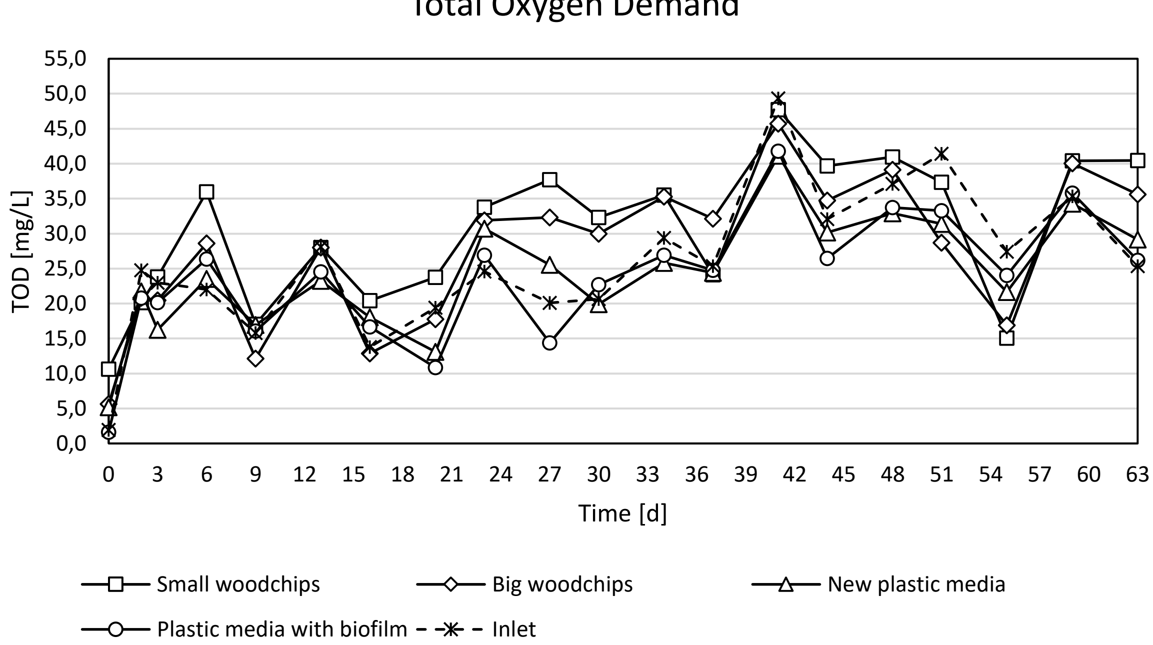

Figure 4.14: Diagram over concentration of Total Oxygen Demand [mg/L] in the inlet and the filtration reactors.

Table 4.12: Pearson comparisons and average TOD concentration change.

Pearson comparison			r	
TOD concentration in influent water vs. time			0,64	
Average concentration TOD			[mg/L] (value + S.D.)	
Filtration reactor	In	Out	Change	t-test
Small woodchips	33,6 ± 10,0	30,5 ± 10,3	4,4 ± 7,2 a	μ ≠ 0
Big woodchips	33,6 ± 10,0	27,0 ± 8,0	1,6 ± 6,5 ab	μ = 0
New plastic media	33,6 ± 10,0	26,3 ± 8,9	-1,6 ± 4,6 b	μ = 0
Plastic media with biofilm	33,6 ± 10,0	28,7 ± 10,0	-2,1 ± 3,7 b	μ ≠ 0

Note: Same letters indicate that values are not significantly different.

The measurements of total oxygen demand indicate the amount of organic material in the water. For the two filtration reactors with wood, one expected outcome would be an increase in organic material due to leaching of the woodchip. The filtration reactors are also expected to filtrate out organic material from the influent water. Both outcomes have been experienced in other studies on woodchips in biological reactors (Ahnen et al., 2018; Christianson et al., 2016).

For figure 4.14 over Total Oxygen Demand, we can see that the measurements indicate some difference between the filtration reactors, but as seen in figure 4.15 over concentration change the standard deviation is high and overlap each other. ANOVA test reveals that the small woodchip reactor is different from the plastic reactors, but the big woodchip reactor is

not different (p > 0,05) from the plastic reactors. T-test shows that the average change for the small woodchip reactor and the reactor with plastic media with biofilm is different from zero. The average indicates that the TOD concentration is increasing for the small woodchips, which is indicating leaching of organic material from the media. While for the plastic media with biofilm the reactor is decreasing, indicating that organic material is filtrated out.

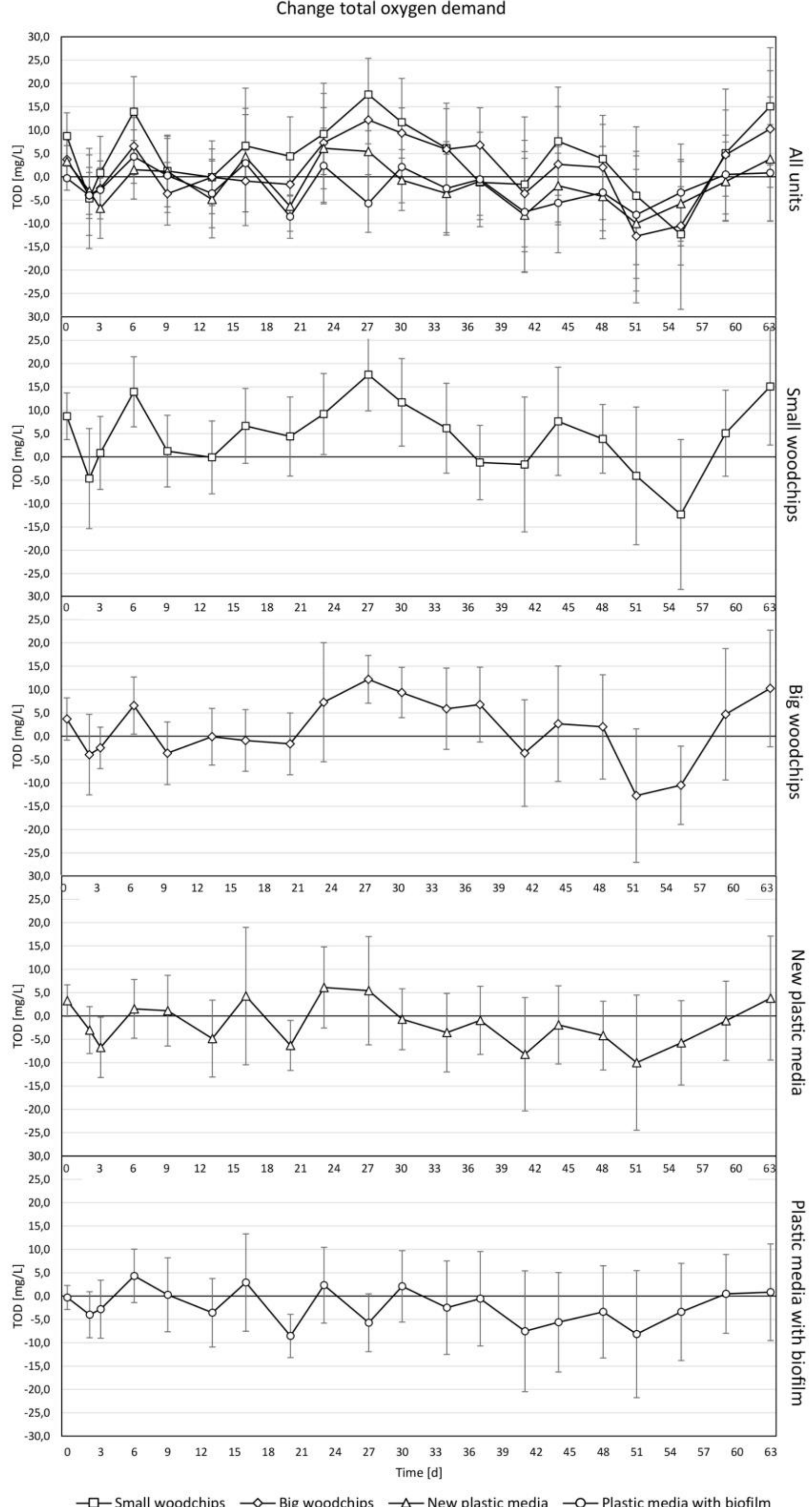

Figure 4.15: Diagrams over change in TOD concentration [mg/L] for the filtration reactors.

As seen in the individual diagrams for the reactors in figure 4.15, The woodchip reactors differ from the plastic reactors, but as seen the variation in the measurements are high, as indicated with the standard deviation error bars.

The results indicate that the woodchip reactors are increasing the TOD and the plastic reactors are decreasing the TOD, but as mentioned the ANOVA test only indicate that the small woodchip reactor is significantly different from the plastic reactors. Based on this it is likely to believe that the leaching is connected to the woodchip size, but the results are not nailed in stone because of the uncertainty connected to the high variance for the results compared to the mean values.

There can be seen a trend in increasing concentration for the inlet overall, as seen in figure 4.14. This corresponds well with increasing fish biomass.

Total Oxygen Demand of the reactors compared with the full-scale RAS

Table 4.13: Measurements for total oxygen demand (value ± S.D.).

Sample taken	TOD [mg/L]	Change TOD [mg/L]
RAS3 - Inlet	25,3 ± 7,7 a	-
Small woodchips	35,9 ± 10,5 a	10,6 ± 13,0
Big woodchips	31,6 ± 10,4 a	6,3 ± 13,0
New plastic media	29,2 ± 11,4 a	3,8 ± 13,7
Plastic media with biofilm	26,2 ± 7,8 a	0,8 ± 10,9
RAS3 - Biochamber	25,4 ± 6,9 a	-0,1 ± 10,3
RAS3 - Sludge chamber	28,7 ± 5,6 a	3,3 ± 9,5
RAS3 - Pump sump	23,7 ± 7,0 a	-1,7 ± 10,4

Note: Same letters indicate that values are not significantly different.

ANOVA analysis indicates that there is no significant difference between the filtration reactors, the RAS or the inlet ($p > 0,05$), as marked with the same letter in the table. No significant reduction or increase in TOD can be stated, but the trend where a change in TOD concentration is higher for the woodchip reactors than the plastic media is seen. The RAS show a low change in concentration. The variance in the results is high for the reactors and the RAS, as seen in the main experiment. While the woodchip material seems to leach organic material, the results are weak because the variance of the measurements in the same samples is so high.

4.2.4 Flow tests on filtration reactors

To understand why the reactors were clogging so rapidly visual inspections were done. Tests were also performed on the reactors with and without the different components.

Table 4.14: Flow test after the first full stop.

Flow at ½ of max	Flow before stop [L/min]	Empty* [L/min]	Only Bio* [L/min]	Only Filter* [L/min]	Restart* [L/min]
Small woodchips	0	3,38	3,31	3,40	2,74
Big woodchips	0	3,43	3,06	3,41	2,16
New plastic	0	3,70	2,91	3,56	2,88
Plastic with biofilm	0,63	2,81	1,56	2,63	2,30

** The clogged green fish blocker and inlet pipe was removed.*

The testing of the reactors after the first stop revealed that the clogging of the reactors was not only due to one significant source but due to clogging of all the different components in the system. The white filter pad was found not to have a great impact on the filtration effect. By restarting the system and doing the tests, the flow was increased significantly.

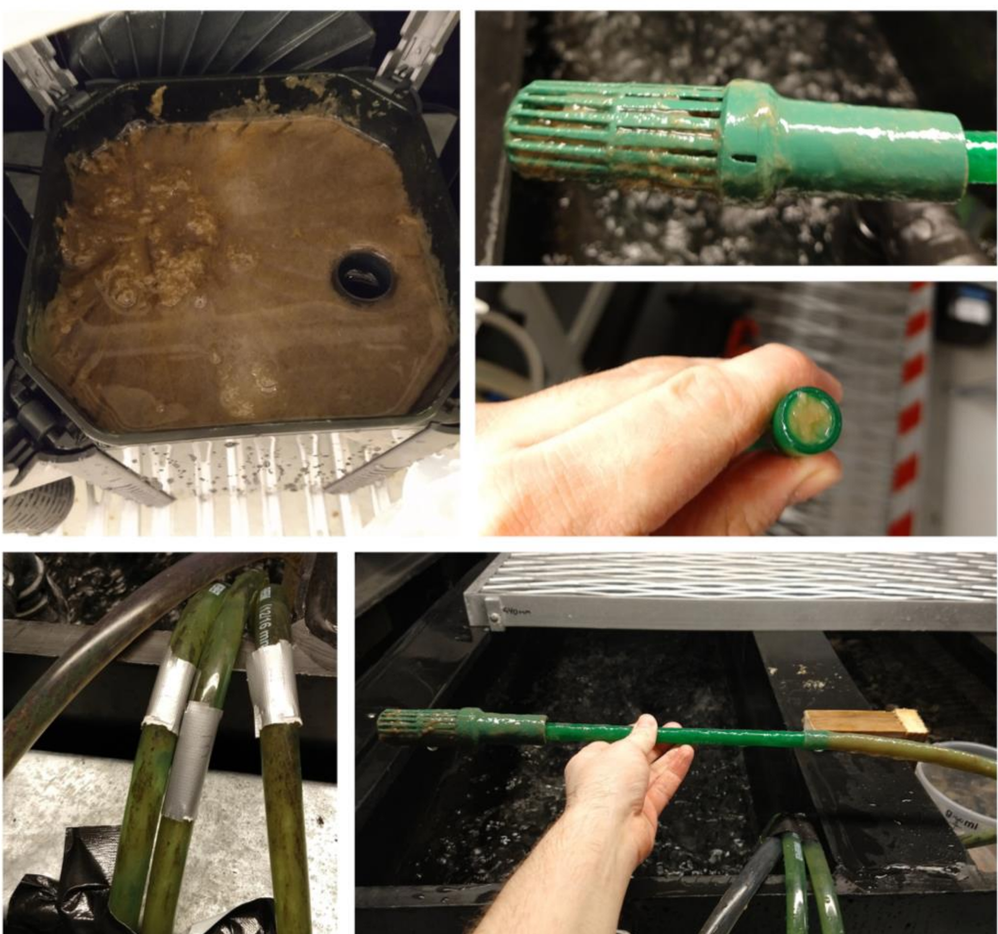

Figure 4.16: Visual inspections of the units after first stop. The different components showed clear growth and clogging.

The visual inspections of the system revealed that the inlet hose and the filter at the inlet that was meant to keep out to big particles (fish in an actual aquarium), were clogging due to the growth of microorganisms and algae. Some of the inlet pipes were completely clogged at the entrance. As seen in the picture at the bottom left the hoses leading water in and out of the filtration reactors were subjected to massive microorganism growth. The same was seen on the inside of the filtration reactors, where the filtration media and the white filtration pad was covered in microorganisms.

Clogged material from the filter and other components leached during testing. Therefore, the flow measurements for the different components might not represent the actual clogging effect as experienced before the system were tested.

The flow of the systems was increased significantly after performing tests on the different components, indicating that the clogged material does not attach firmly to the components.

What also can be concluded from the visual inspection and the tests are that the clogging is occurring in the entire reactor, and not only due to clogging of the filtration media. After the first stop, the clogged green fish blocker and the thin green pipe was removed from the inlet pipes, as they were believed to affect the flow.

Table 4.15: Test of flow at the end of the experiment

Flow at max	Flow before stop [L/min]	Restart [L/min]	Only Bio. filter [L/min]	Only Filter pad [L/min]	Empty [L/min]	Empty w. new pipes [L/min]
Small woodchips	1,82	2,20	3,37	3,61	4,30	6,33
Big woodchips	0,68	x	1,78	2,48	2,88	6,04
New plastic	0,82	0,82	3,39	2,89	3,71	6,47
Plastic with biofilm	0,66	2,01	4,72	x	x	x

Note: X indicates that there were no measurements, this due to one unsuccessful restart and due to one pump that stopped working.

Figure 4.17: Visual inspections of the units after finishing the experiment. Clear accumulation of organic material in the reactors and signs of decomposing of the woodchip media.

When opening the reactors after the study, it was discovered that the reactors were filled with more microorganism material than at the first stop. In the pictures above, the left picture shows that a significant amount of material growth and organic material have occurred on the filter pad. The second picture shows the small woodchip material, which shows clear signs of degradation. The picture to the right show material trapped in the bottom compartment of the reactor.

After finishing the experiment, the reactors were tested again. First, the effect of stopping and restarting the reactors was performed. The effect of restarting the systems was a small increase in flow for the small woodchip reactor and a significant increase in flow for the reactor with plastic media with biofilm from the beginning. For the reactor with new plastic media, the flow was the same, while for the reactor with big woodchips there was no success restarting the system.

The reactors were tested with only filtration media, and afterward only the white filter pad, completely empty and empty with new hoses. The effect of each is calculated and described in figure 4.18. Due to clogging material that loosens during testing, these values need to be treated as an indication only. The reactor with plastic with biofilm from the beginning stopped working during testing. Therefore these measurements are marked with x in the table 4.15, and not shown in figure 4.18.

From the graph, we can see that the highest single contributor to flowrate loss is the hoses, while the rest of the loss can be described as a combination of clogging occurring in the filter material and the filter pad. The field “other sources” are loss in flowrate that is not explained by the measurements for the loss in the filter material and the white filter pad. This loss may be caused to microorganism material clogging under the filter media baskets, and media loosening during testing. This material can be seen in the right picture in figure 4.17.

Figure 4.18: Percentage flow loss due to different components in the reactors. Other sources can be material loose in the system.

4.2.5 Pressure-drop test on filtration media

Pressure-drop tests on new material

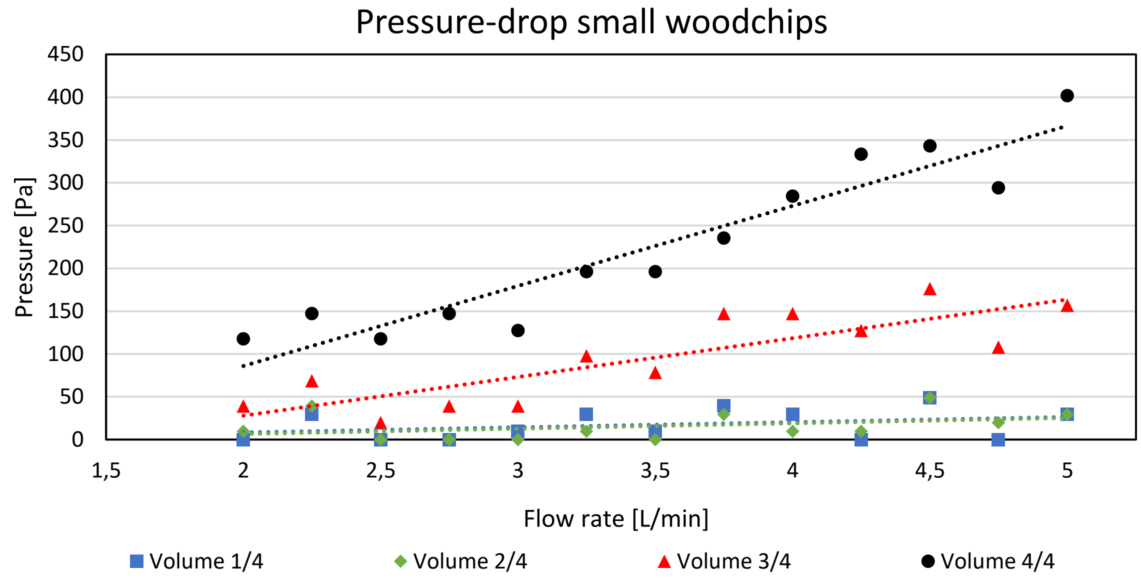

Figure 4.20: Pressure drop measurements for the small woodchip volumes 1,06 L, 2,12 L, 3,17 L and 4,23 L. Trend lines are linear.

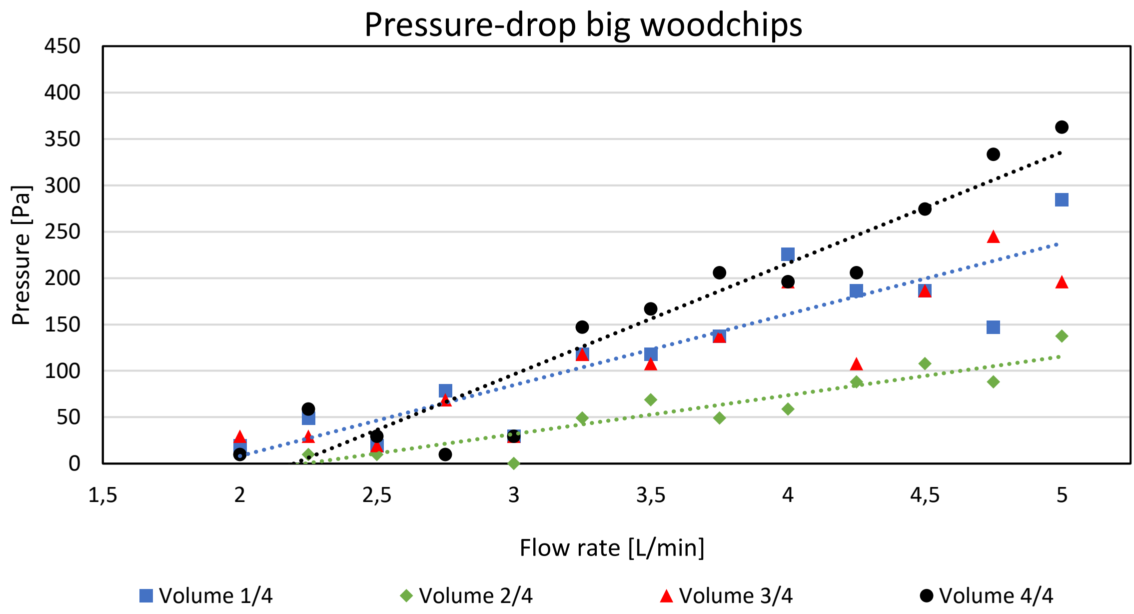

Figure 4.19: Pressure drop measurements for the big woodchip volumes 1,06 L, 2,12 L, 3,17 L and 4,23 L. Trend lines are linear.

Pressure-drop plastic

Pressure [Pa]

Flow rate [L/min]

Volume 1/4 Volume 2/4 Volume 3/4 Volume 4/4

Figure 4.21: Pressure drop measurements for the plastic media volumes 1,06 L, 2,12 L, 3,17 L and 4,23 L. Trend lines are linear.

For the pressure-drop caused due to the small woodchips, we can see that there is a connection between volume and pressure. Pascal (Pa) is a small pressure-unit, 1 Pa is equal to one newton per m^2. For the volumes 1/4 and 2/4 (1,06 L and 2,12 L) we can see that the pressure drop is almost none, but for the volumes 3/4 and 4/4 the pressure-drop is higher.

The big woodchips have about the same pressure drop as the small woodchips for the volume 4/4, but the measured pressure-drop is higher for the other volumes. The pressure drop for the volume 1/4 is the second highest, which is not as expected.

For the plastic media, the pressure-drop measurements for the different volumes are closer to each other and do not increase as much with volume as for the small woodchips, or the big woodchips. The pressure-drop overall is higher for the plastic media than measured for the woodchip media.

From the testing of pressure-drop on new media we can state that the plastic media causes higher head loss. This is reasonable when considering the shapes of the filtration media. While woodchips have an organic and aerodynamic shape, the plastic filtration media is not as organic in shape. The Mutag BioChip comes in the form of round flakes, in the pressure-drop cylinder the flakes will create a wide obstacle for the water to cross, as the diameter of the particles is 3 cm. The same for the RK BioElements Light which is made for breaking up the flow.

Pressure-drop test on used media

The measurements of pressure-drop of the material used in the filtration reactors were not successful. The biofilm on the media was quickly washed off and clogged the system. Stable measurements were not achieved before the system was cleaned, then most of the biofilm was washed off and the measurements are therefore not describing the full pressure drop due with the biofilm. The volume of the media was only enough to test the pressure-drop for the volume 1/4 and 2/4 of the pressure-drop column.

Pressure-drop small woodchips with biofilm

Pressure [Pa]

Flow rate[L/min]

Volume 1/4 Volume 2/4

Figure 4.22: Pressure drop for used small woodchips for two volumes 1,06 L and 2,12 L. Trend lines are linear.

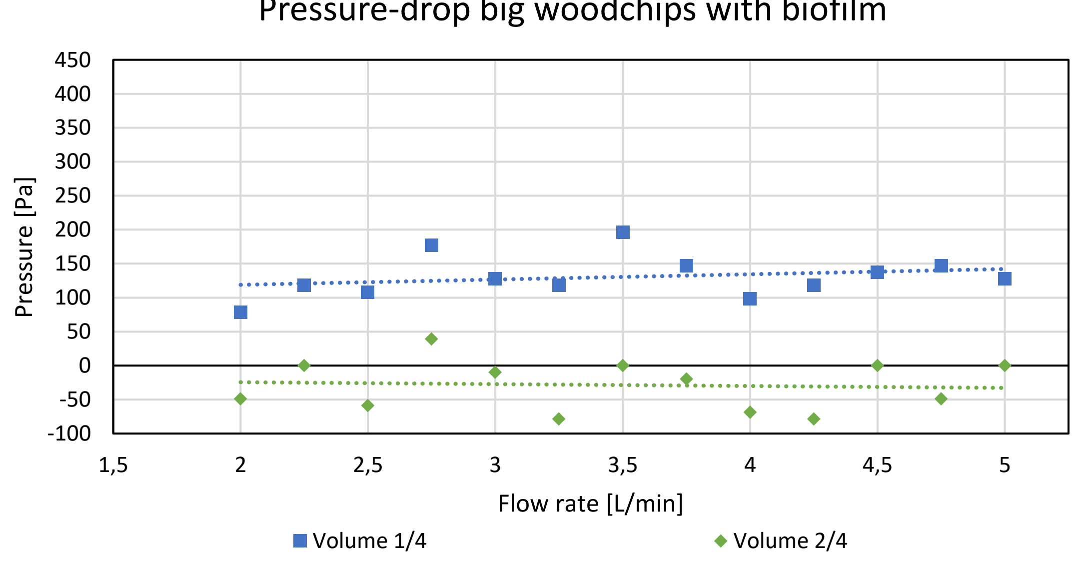

Figure 4.23: Pressure drop for used big woodchips for volumes 1,06 L and 2,12 L. Trend lines are linear.

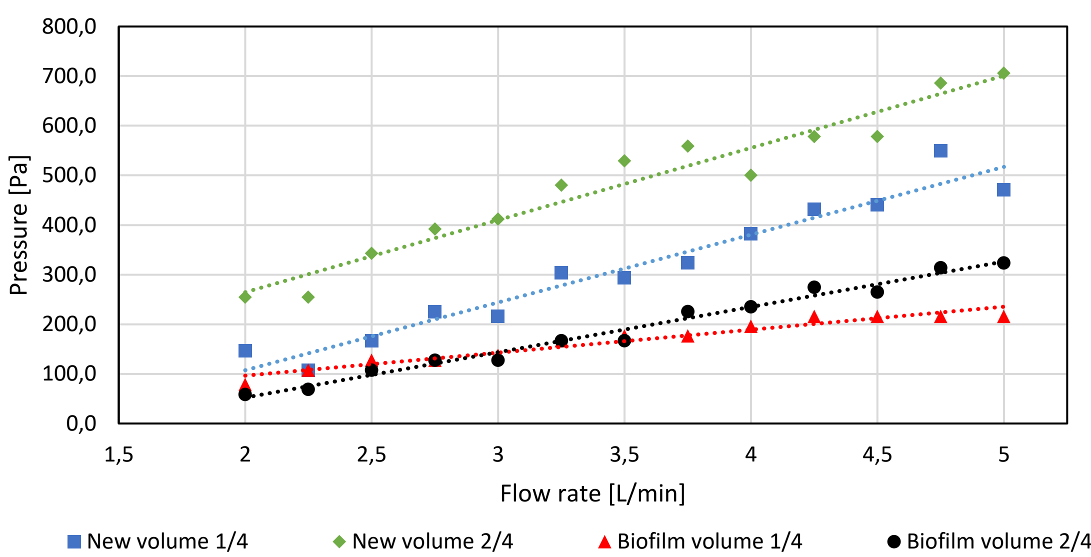

Figure 4.24: Pressure drop for used plastic, new and biofilm, for two volumes 1,06 L and 2,12 L.

The measured pressure-drop for the small woodchip with biofilm is higher than experienced for volume 1/4 and 2/4 than with the new small woodchips. We can also see that the volume 1/4 have higher pressure-drop than 2/4 which is not expected and weakens the results of the measurements. The measurements for the big woodchips with biofilm are not as expected, as the pressure-drop for volume 2/4 are measured under zero. The pressure drop for the volume 1/4 full of big woodchips with biofilm are a little less than measured for the small woodchips with biofilm, which is expected as the small woodchips are likely to settle more. The woodchip media with biofilm show higher pressure-drop than the new woodchip media did for the same volumes, which is indicating that the biofilm increases the pressure drop for the flow.

The measurements for the plastic media are higher than for the new plastic tested. As seen, there is a difference in pressure for the two volumes tested for the reactor with new plastic initially, while for the reactor with biofilm initially the pressure-drop is measured about the same for both. The measurements from the reactor with new plastic initially were also higher than for the reactor with old plastic initially. As noted earlier the results of the testing were not consistent, and the pressure-drop is probably around the same for each.

From the measurements of the used media, the same trend can be drawn as for the tests with new media. The plastic seems to create a higher pressure drop. For the used media the biofilm seems to be increasing the pressure drop.

4.2.6 Flow and clogging

For the flowrate in the filtration systems, the decrease in flowrate during the experiment were comparable. This indicates that woodchips as a filtration material do not have a higher clogging potential than plastic material when applied in a static filter. For the pressure-drop tests done the results were indicating that the woodchips were causing less pressure drop than the plastic media. The filtration media caused higher pressure drop when covered with biofilm.

From testing of the reactors after the stop, it was found that the reason for clogging of the filtration reactors was due to several contributors. Clogging and growth of material in the pipes were a factor, but also loose media trapped in the reactor, the filter pad along with other reasons. This shows the importance of the design of the reactor, as also experienced in studies on woodchips by (Ahnen et al., 2018) and (Christianson et al., 2016). The moving bed bioreactor is common to use for nitrification, and one of the effects achieved by having the particles in constant movement is keeping the biofilm thin, by removal of excess biofilm on the filtration media (Sterner BioTek AS, 2019). This does that the filter does not clog as in a static filtration system. Because of this, it is a reason to believe that using woodchips in a moving bed filter would not cause as much clogging as in the laboratory scale reactors used in the experiment.

The retention time was short in the reactors compared to the reactor at the Centre for Fish Research. This means that the load per volume filtration media are higher in the reactors tested than in the recirculating water system. The flowrate of the RAS was 100 L/min in two bioreactors of total volume about 3825 L, 60 % of this volume was filter-material. This makes up 0,044 L effluents to treat per liter filter material per minute. While for the reactors tested the initial flow was 4 L/min in a reactor of volume 2,24 L, this makes up 1,79 L effluents to treat per liter filter material per minute. This shows that the load rate of the filtration reactors initially was 41 times higher than in the full-scale RAS, which can explain why the filters were clogging at such a rapid rate.

4.3 Physical properties of woodchips and cost

4.3.1 Porosity

Table 4.16: Porosity in water

Media	Initial volume [ml]	Water added over 48h [ml]	Porosity [m^3/m^3]
Plastic mix	750	567	0,756
Small woodchips	750	556	0,741
Big woodchips	750	540	0,720

The porosity of the media gives us information about how much void space there is in a volume filled with media. The porosity is found to be close for the three media. The plastic mix did not absorb water over time, but the Mutag BioChips are designed with small gaps in the particles, and the RK BioElements a lot of room between within the particle, and the gaps between the particles are larger, than the woodchips, due to bigger particle sizes. By using the porosity, the volume of the container and flowrate of the water, it is possible to calculate the hydraulic retention time which is an important design factor for nitrification and denitrification reactors.

4.3.2 Density

Table 4.17: Density of media [kg/m3] (value ± S.D.).

Media	Density of media [kg/m^3]
Distilled water	990,1 ± 1,4
Used wet woodchips	1097 ± 48
Used wet Mutag BioChips (new at the start)	1288 ± 124
Used wet Mutag BioChips (biofilm at the start)	1110 ± 45
Used wet RK BioElements (new at start)	914 ± 128
Used wet RK BioElements (biofilm at the start)	988 ± 92
New wet Mutag BioChips	963 ± 97
New dry Mutag BioChips	533 ± 31
New dry RK BioElements plastic	803 ± 52
New dry woodchips	719 ± 12,4
New wet woodchips	1075 ± 13,0

The producers list the density of the media to be 930 kg/m^3 for RK BioElements Light and 950 kg/m^3 for Mutag BioChip - without biofilm.

The density measurements can be used to tell whether the filtration media particles will sink in the water or not. The tested plastic media are used in a moving bed filter and are thus chosen after a density close to the density of water in order to get the best movement, by using the least energy in the water. If the density is high, then the aeration intensity must be higher to keep it in motion. To high aeration will cause more of the biofilm to loosen, which will reduce the capacity of the biofilter (Sterner BioTek AS, 2019).

As seen from the experimental determination of the densities the different particles show comparable densities in wet condition with biofilm, and this is a little over the density of water. In dry condition, the densities of the particles differ. The Mutag BioChip is listed to have a density of 950 kg/m^3 without biofilm, and this corresponds well with the wet measured value. The RK BioElements Light is listed at 930 kg/m^3. This value corresponds well with the density found in the test of the used filtration media with biofilm. In dry condition, the Mutag BioChips have the lowest density, then the woodchips, and then the RK BioElements. In water with biofilm, the RK BioElements have the lowest density, and the woodchips and the Mutag BioChips show comparable densities in water. The reason why

the density of Mutag BioChips increases so much is all the pores in the media that can be filled with biofilm.

Table 4.18: Bulk density of media [kg/m3] (value ± S.D.).

Media	Bulk density of media [kg/m^3]
New dry woodchips (mix)	297,6 ± 8,3
New dry small woodchips	284,7 ± 9,5
New dry big woodchips	300,8 ± 2,2
New dry Mutag BioChips	159,2 ± 3,3
New dry RK BioElements	158,9 ± 1,8
Used wet small woodchips	662,4 ± 6,8
Used wet big woodchips	675,0 ± 29,7
Used wet plastic mix (new)	303,6 ± 10,5
Used wet plastic mix (biofilm)	301,8 ± 1,8

Tests on bulk density were also done, this is important when considering the transport of the media, both new and used. The new woodchips have a bulk density of about 300 kg/m^3, while the bulk density of the plastic media was both measured to about 159 kg/m^3, the producers list the bulk densities at 158 kg/m^3 (RK BioElements Light) and Mutag BioChip at 165 kg/m^2 ± 2 %. This shows that the bulk measurements taken are accurate. For the measurements of used wet filter material, the two used woodchips sizes have about the same density (about 660-680 kg/m^3), while the plastic mix shows a bulk density of under half of this (about 300 kg/m^3). Based on the bulk densities measured the density of woodchips are higher in both dry and wet condition than the plastic media.

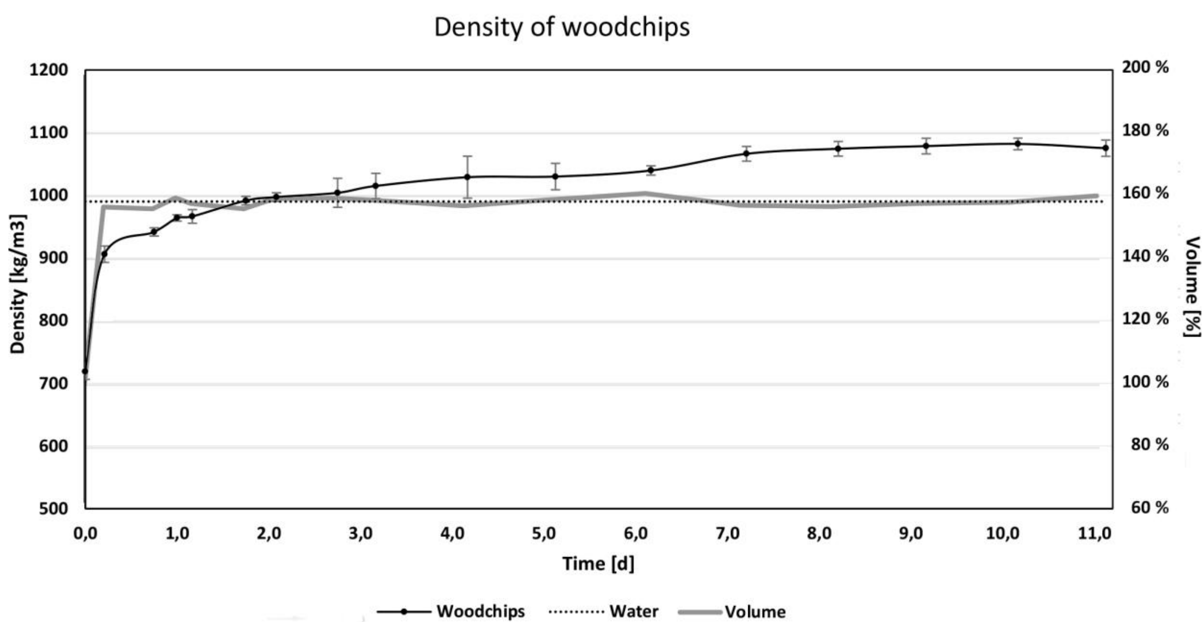

Figure 4.25: Diagram that shows the density and volume increase of woodchips when placed in water.

For the woodchips, the test on dry media showed a dry density (dry weight on dry volume) of 719 ± 12,4 kg/m^3. Beech is listed to have a raw density for sapwood of 1060 kg/m^3 while

the basis density (dry weight on raw volume) is given 570 kg/m^3 (Moen et al., 1998). To compare the densities, we can convert the basis density, by using the shrinkage volume for beech, which is given 17,9 %. This gives a dry density of 694 kg/m^3, which is close to our measured dry density.

The wood particles showed a density of 1075 ± 11 kg/m^3 after being soaked in water for eleven days; this is comparable to the raw density of sapwood. The volume of the woodchips increased with 60,2 % over the test period. 95 % of the volume increase happened within the first five hours.

For the measurement of the woodchips in wet condition with biofilm, the density was 1097 ± 48 kg/m^3, which shows that the density of wet woodchips is close to the same as the density of wet woodchips with biofilm.

As seen from table 4.17, the density of the Mutag BioChips increased after being kept in water for some days. This is because of small pockets in the plastic where water can enter. The measurements of the Mutag BioChips in new condition compared to the measurements of the Mutag BioChips with biofilm shows a further increase of density. The density of the plastic between the reactors are similar for the RK BioElements, but a little higher for Mutag BioChips in the reactor with new plastic media initially than the reactor with biofilm from the beginning.

4.3.3 The specific surface area of woodchips

The specific area is as mentioned used for calculating the nitrification rate of the filtration media. Thus, the specific area for woodchips was determined. This was as described done by measuring ten woodchip particles and using the method by (Lungulesasa et al., 2009) as described in chapter 3.2.3.

Table 4.19: Measurements of ten rectangular shaped woodchips (value ± S.D.).

Cond.	Thickness [mm]	Height [mm]	Length [mm]	Volume [mm^3]	Areal [mm^2]
Dry	2,22 ± 0,28	9,20 ± 0,84	13,98 ± 3,66	278,3 ± 47,0	357,1 ± 67,7
Wet	2,43 ± 0,28	9,30 ± 0,72	14,71 ± 3,52	327,7 ± 69,6	388,0 ± 72,8
Cond.	**Weight [g]**	**Density [kg/m^3]**	**Specific area [m^2/g]**	**Specific surface area [m^2/m^3] (one particle)**	
Dry	0,157 ± 0,039	560,8 ± 98,8	0,00235 ± 0,0004	1282,6 ± 91,4	
Wet	0,280 ± 0,072	851,3 ± 121,4	0,00143 ± 0,0002	1191,7 ± 89,3	

The method from (Lungulesasa et al., 2009) gives a specific area of 0,000846 m^2/g when using the density 1075 kg/m^3 found for the wet woodchips and the average thickness (2,43 mm) found for the wet particles. This gives a specific area lower than the one found for the

wet particles in table 4.19, by taking the average area of the woodchips divided by the average volume of the woodchips. Due to higher accuracy (lower standard deviation), and longer soaking time for the woodchip density test described in chapter 4.3.2, the specific area found by the method from (Lungulesasa et al., 2009) was used.

The bulk density of the used woodchip media was found to be 662,4 ± 6,8 kg/m^3 for the small woodchips and 675,0 ± 29,7 kg/m^3 for the big woodchips. Based on these densities we can find the specific surface area of woodchips filling a volume. These densities are shown in table 4.20.

Specific surface area	[m^2/m^3]
Small woodchips	561,0
Big woodchips	570,8

Table 4.20: Specific surface area for woodchips

4.3.4 Cost and expected lifetime

The cost of the filtration media will be a factor when considering the viability for use in filtration. In a study on the use of woodchip in denitrification by (Saliling et al., 2007), there was done a simple price comparison, with a plastic filtration media called Kaldnes, based on initial cost. Labour cost of changing the media or shipping was not included in the cost. A summary of this comparison can be seen in table 4.21, and price estimates for the plastic media used in this experiment are also added to the table.

Table 4.21: Comparison of cost of filtration media (Saliling et al.,2007)

Reactor media	Expected lifetime [years]	Initial cost [US$/m^3]	Total 10-year cost [US$/m^3]
Kaldnes	10,0	953	953
Wood chips	1,2	19	158
Wheat straw	0,5	6	120
Mutag BioChips	10*	1300[1]	1300
RK BioElements Light	10*	486[2]	485

**Listed as long lifetime, 10 years used for calculation.*
[1]Average for the price listed as US$ 698-1,969 (Shanghai Ecopro Environmental Engineering, 2019).
[2]converted from 434 euro/m^3, cost received in email correspondence with RK Plast, May 2019.

The cost comparison shows that woodchips have a lower cost. Thus it has benefits in an economic perspective, but factors as labor cost regarding the change of the media after ended use must be considered to get a full overview of the cost. The price listed for the Mutag biochips was taken from a Chinese supplier webpage and must be treated as a rough estimate.

4.4 Appliances for woodchip in filtration systems

4.4.1 Nitrification

The literature review reveilles that when choosing filtration media for nitrification, there must be shown caution to particle size and configuration of the biochamber (Lekang & Fjæra, 1997). Mechanical filters with media as sand and gravel are found to clog rapidly, and channeling can occur, which creates low contact with the filtration media, thus low filtration effect.

The results for the study show that the oxidation of ammonium and nitrite is occurring. The nitrification rate for the woodchips was found to be higher than for the plastic reactors and the full-scale RAS. The reason for this was that thus the much higher specific surface area for the plastic filtration media, the oxidation of ammonia was not equally high. This can be an indication that the nitrification bacteria grow better on a natural surface than on the artificial plastic surface, but the reason can also be that the concentration of ammonium is limiting the growth of nitrifying bacteria. This is listed as a factor for the efficiency of the nitrification process (Lekang & Fjæra, 1997).

From the results, it can be stated that nitrification still was occurring in the static bioreactors after 63 days with the presence of organic material, and the inhibiting effect of organic material was not noticeable during this study. As no clear inhibition effects occurred, there is likely to believe that heterotrophic bacteria were not dominating in the chamber. It could be that the oxygen concentration limited the growth of heterotrophic bacteria, as no additional oxygen was added. Earlier studies have found that organic material does inhibit the nitrifying effect (Strauss & Lamberti, 2000). Lekang & Fjæra, (1997), states that the growth of heterotrophic bacteria can outcompete nitrifying bacteria as the growth is faster than the denitrifying bacteria. Thus, the risk of inhibition is present when adding organic material to the water. Woodchip has been shown to work as a sufficient source of organic carbon in earlier studies (Saliling et al., 2007) (Christianson et al., 2016). This is also strengthened by the signs of decay of the woodchips seen after the experiment. For these reasons, there is a reason to believe that systems open to air will not limit the establishment of heterotrophic bacteria that can inhibit the nitrification.

The results achieved for nitrification in the filtration reactors are interesting. The reason for using several moving bed nitrification chambers in a recirculating system is to reduce the inhibiting effect of the growth of heterotrophic bacteria (Sterner BioTek AS, 2019). Then the design of the filtration is appealing, as there were not experienced any inhibiting effect, even though there was a carbon source available. Because of these results, it would be interesting to look closer into woodchips and the use of such closed filtration reactors for nitrification, where the water is aerated before the chamber. The limiting factor of these systems is as

described in chapter 4.2 the high clogging rate, which will need backwashing at a frequent level to maintain the flowrate within acceptable levels. The biofilm might be damaged by such treatment. As seen after the first stop of the bioreactors, the nitrification effect of the small woodchips showed signs of being damaged of the stop and the tests on flowrate.

4.4.2 Denitrification

The experiment did not indicate that denitrification was present in the filtration reactors. Therefore, reactors with the same configurations used in the filtration reactors are not usable in denitrification, unless some of the inhibiting factors are eliminated. The main factor for achieving denitrification is the removal of dissolved oxygen. If the dissolved oxygen is removed, which can be done by adding methanol, as described by (Lekang, 2007), it is likely that the reactors used in this experiment can be used for denitrification. The submerged reactor is the most common design for denitrifying, as these support absence of oxygen (Lekang, 2007).

The woodchips showed signs of degradation when the reactors were inspected after the experiment, which indicates that there were bacteria in the chamber breaking down the woodchips. If these were bacteria capable of denitrification is hard to say, but the experiment supports the theory that woodchips could be used as a carbon source for bacteria. If oxygen is removed, it is a reason to believe that heterotrophic denitrifying bacteria will establish and use the nitrogen components in the water as electron acceptors, and woodchips as an electron donor and carbon source. This is supported by the study on full scale denitrifying by (Ahnen et al., 2018) which shows that the use of woodchips is a viable carbon source for denitrifying bacteria. This is also supported in studies by (Christianson et al., 2016) and (Saliling et al., 2007).

As experienced in the study the reactors with static media clogging occurred at a high rate until the systems stopped. No significant difference was experienced between clogging in the reactors of plastic or woodchips, but in the pressure drop tests the pressure drop was higher for the plastic material than the woodchips. This experience of clogging in static media is also pointed out by (Stickney, 2000). While the studies on denitrification presented in the literature, stated the clogging potential of woodchips as a concern, and that the design of the bioreactors was important. In moving bed reactors, the loading rate of media is usually 2/3 of the volume, and in nitrification use of aeration removes excess biofilm from the filtration particles. Thus a thin and effective layer is achieved (Bjerknes, 2007) (Sterner BioTek AS, 2019). Selecting a system with moving filtration media will lower the maintenance cost as the media does not need to be cleaned as often. The risk of channeling will also be reduced with a moving bed reactor. Water pumped through diffusers can be used instead of air to keep the particles in movement to create the same biofilm effect.

4.4.3 Particle removal

Particle removal in recirculating aquaculture system is often placed before nitrification bioreactors to remove most organic material as possible. Particle filters using filtration media in the reactors are types of depth filtration reactors. These filters require to be cleaned, as they clog over time. The size of the filtration media particles determines the size of the particles that are removed (Lekang & Fjæra, 1997), often quartz sand is used in these filters, but gravel is also used (Bjerknes, 2007).

As experienced in our system the clogging potential is high in closed filters. As Stickney, (2000), have written, clogging in a mechanical filter can make the filter anaerobic and decomposing of organic material can give a release of ammonia, hydrogen sulfide, in addition to low oxygen concentrations. As Tehobanoglous et al., (2003), states organic material are decomposed by aerobic bacteria, and there will be decomposing present as long there is material to degrade and enough oxygen. Therefore such a particle removal system using woodchips with claim absence of oxygen not to degrade.

The experiences with the full-scale experiment on nitrification by (Christianson et al., 2016) was that the woodchip media were leaching organic material for half a year, which is not ideal for a filter with the intention of removing organic material in the water. From the TOD-measurements, we found that the small woodchips were leaching organic material at a measurable level. The increase in TOD could not be stated to be significantly different from zero for the big woodchips. The leaching might be higher at higher retention time. Thus more experiments should be performed.

Because of the leaching potential of woodchips, the use of woodchips in particle filtration cannot be recommended in systems where the chamber subsequent is nitrification chambers. This can still be viable if several nitrification chambers are used in series to reduce the effect of organic material as recommended by Sterner BioTek AS, (2019).

4.4.4 Sequencing batch reactor

After a moving bed nitrification biochamber, it is normal to have a particle filtration for filtrating out the biofilm that loosens from filtration media in a moving bed chamber (Sterner BioTek AS, 2019). In the system at the Centre for Fish Research, a sequencing batch reactor is used. This chamber has a static filtration media, and in the Centre for Fish Research the BioElements Heavy is used, with a density of 1200 kg/m^3. The particles are entering the chambers from the top and are allowed to settle in the chamber or are caught by the filtration media. The chamber clogs due to the material from the nitrification chamber, algae, and other particles. Due to this, the chamber is washed once a month, where the sludge is discharged to the outlet.

The use of woodchips in this chamber could be viable if the chamber is washed frequently to avoid the problems with clogging as experienced in the study, and the problems with decomposing as described by (Stickney, 2000) which could give a release of unwanted substances. The woodchips in water were measured to a density of about 1075 ± 11 kg/m^3, this means that the woodchips will sink in a static chamber, avoiding contact with the air above the chamber. As stated by (Moen et al., 1998), beech can last long under water.

The bacteria in the sequencing batch chamber that flows with the water, can be removed by having a disinfection process after the chamber. As for particle removal before biological filtration, the negative sides of using woodchips in filtration systems is the risk of leaching organic material as there was a small indication of in the study, but also experienced in other studies, as in the study on full-scale denitrification system by Christianson et al., (2016) described in the literature review.

4.5 Environment and reuse

After the use as filtration media, the woodchips can be used for further purposes. From filtrating wastewater of aquaculture, the woodchips will absorb nutrients. As seen in a study by (Christianson et al., 2016). For this study, the content of carbon, nitrogen, phosphorus pentoxide, and cellulose increased in the woodchips, while the content of lignin decreased.

While regular wastewater is a source of pollution, the water in a recirculating water system are easier controlled, and the main components added to water are increased amounts of phosphorus and nitrogen from fish feed and feces. Because of this, the biomass is likely to be safe to use as a fertilizer for the growth of crops or other purposes.

The main question of this is whether it is viable or not. The amounts of woodchips used, if replacing a regular biofiltration chamber are not very significant, if we use the recirculating system at the Centre for Fish Research as a reference. With a filling rate of 60 %, then 2295 L of woodchips is needed for the biochamber of volume 3825 L. Saliling et al., (2007), estimated a lifetime of 1,2 years for the woodchips. If we assume that a large smolt facility uses ten RAS of the same size, then 19,1 m^3 fertilizer will be produced each year. For a crop of corn in Norway, the average use of nitrogen in fertilizer is 11 kg N/daa (Riley, 2016). From the study by (Saliling et al., 2007) the highest nitrogen content in woodchips was found to be 0,51 N% after use in filtration. The bulk density for the used big woodchips were found to be 675,0 ± 29,7 kg/m^3. From ten RAS systems with 3825 L bioreactors, we can then get 65,8 kg N, which is enough to cover 6 daa. The average size of agricultural areas in farming is 235 daa in Norway (Syverud et al., 2019). This simple comparison shows that the yield of used woodchips as a fertiliser product to earn money is not viable, but woodchips can be delivered to local farms this purpose and environmental disposal.

Microplastics can have a great impact on living creatures, as microplastic easily can be ingested. Plastics can be mistaken to be food, but also ingested because they are so small that they are a part of the food or water. As Tyree & Morrison, (2017), writes it is even found in our drinking water. The long-term effects of plastics have yet to be discovered, but studies have shown changes in gene regulation of fish (Nerland et al., 2014). In a narrow perspective, there should be at least be taken precautions regarding the food we eat. Thus sources of microplastics should be reduced in food production.

One of these sources is plastic filtration media, especially when applied in bioreactors with moving particles. Some types of plastic filtration media will break down over time and create microplastics that can be digested by fish or caught in particle filters and leaving the system with the wastewater and ends up in recipient waters.

Scientists believe that microplastics can act as a vessel for the transport of chemicals adsorbed on or contained in plastic particles (Viršek et al., 2017), but also transport organisms outside of their native ranges. This can cause the transfer of potentially harmful organisms (Gregory, 2009). Viršek et al., 2017, identified the bacteria *A. salmonicida* on microplastics in water samples from the North Adriatic. This bacteria is known to cause fish illness.

The fact that microplastics can transport bacteria's dangerous for fish raises the demand for effective disinfection taking in water containing microplastics. Another concern is releasing water from aquaculture where diseases have occurred if the water contains microplastics from the degradation of plastic filtration media. This can pose a great danger for wild fish in recipient waters. Thus, disinfection is important for effluent water, but will not remove the plastic particles.

5 Conclusion

The study found that woodchips showed comparable results to plastic filtration media for nitrification. This when applied in a closed submerged bioreactor with still filtration media. Low concentrations of ammonium and low temperatures in the water treated are believed to have limited the nitrification effectivity, but oxidation of both ammonium and nitrite was occurring. The comparable nitrification results for the filtration media show that nitrification bacteria can produce active biofilm on woodchips. The nitrification rates were significantly higher for the woodchips than for the plastic media in the lab-scale filtration reactors and compared to a full-scale RAS. The plastic media have a higher specific surface area than the woodchips, but did not show any higher nitrification, thus the difference in nitrification rate. The reason can be growth limitation because of low ammonium concentrations and temperature, or that biofilm is more productive on woodchips.

Visual signs of decomposing of the woodchips were found, supporting that woodchips can be used as a carbon source for bacteria. Therefore, it is likely that woodchips can be applied in denitrification processes, where it will act as both a carbon source and as a surface area for denitrification bacteria, provided that dissolved oxygen is removed. Other uses of woodchips can be in particle filtration, if the number of nitrifying chambers is increased to cope with the inhibition of organic material. From the TOD-measurements, it was found that the small woodchips were leaching organic material at a measurable level, and it is suspected that the leaching can be measured more significant at lower retention times. Earlier studies also support the fact that there is a risk of leaching of organic material. This raises doubt about the use of woodchips for purposes where organic material is unwanted, which can have unwanted effects on nitrification and reduce the oxygen content in the water.

The inhibition effect of organic material in nitrification reactors was not experienced in the closed submerged reactor system with the use of static media. There was an establishment of nitrification bacteria in the reactors with woodchips. Thus, the design is believed to inhibit the establishment of heterotrophic bacteria. More research should be done on such closed submerged reactors for the use of woodchips in nitrification.

The potential of reduced flowrate and clogging were showed to be high for all the reactors. The filtration media showed comparable clogging and reduction in flowrate. Thus, there is no basis for concluding that woodchips have any higher clogging potential than plastic media for a bioreactor with static media. The reason for the rapid clogging is believed to be a combination of the reactor design, where access biofilm is not flushed away as in a moving bed filter, and because of high load due to the small volume and the high flowrate. This shows that the reactor design is crucial for the flow and clogging potential.

6 References

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Attachments

A.1 Mixing color reagents

Component to determine	Reagent		Dilute to	Stability
NO_2 Nitrite	R1	Sulfanilamide 2,0 g Conc HCL 42 ml N-1-Naphthylenediamine 0,2g	200 mL	1 month
Fill bottle with approx 150 mL DI water. Add HCL, then adjust to 200mL and allow to cool Dissolve 2g Sulfanilamide Dissolve 0,2g of N-1-Napthylenediamine				

Component to determine	Reagent		Dilute to	Stability
NO_3 Nitrate and TOT N	R1	5 mL $CuSO_4$ stock	100 mL	Stable
	R2	Hydrazine 0,12 g Sodium Hydroxide 0,4N	100 mL	1 day
	R3	Sulfanilamide 2,0 g Conc HCL 42 ml N-1-Naphthylenediamine 0,2g	200 mL	1 Month
Stock 0,4N NaOH	8 g NaOH		500 mL	Stable
Stock 0,5% $CuSO_4$	0,05 g $CuSO_4$		100 mL	1 month
R2: Weigh ca 0,12g hydrazine in R2 bottle. Fill up to 100mL with 0,4N NaOH R3: Add HCL to 250mL water and allow to cool. Add 2g sulphanilamide and then add 0,2g diamine. Fill up to 200mL with DI water Store all solutions in refrigerator If internal control is way off, make new R2				

Component to determine	Reagent		Dilute to	Stability
NH_4 Ammonium	R1	7,5 mL stock A 7,5 mL stock B 10 mL stock C	Mix in reagent bottle	1-2 days in instrument
	R2	Dilute 8,8 mL 14% to ca 40 mL		1 day
Stock A:	30g Sodium Phenoxide 5,5g Sodium hydrogen carbonate		200 mL	At least 2 weeks
Stock B:	10g EDTA		200 mL	At least 2 weeks
Stock C:	0,1g Nitroferricyanide		200 mL	At least 1 week
Prepare R1 in fume cupboard Make R1 in the reagent bottle, wear gloves and use finger as stopper when mixing chemicals Phenixide waste in a waste bottle in fume cupboard All reagents are sensitive to light so make reagents and then place stocks in fridge If internal control is way off make new R2				

Table A1: Recipe for reagents used in colormetric analysis. By: (Högfeldt, 2019)

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway