



Figure 0.1: Clarias Gariepinus, Illustrations of the Zoology of South Africa, 1838

# Analysis of a Recirculating Aquaculture System

#### An analysis at Lantfisk

Master's thesis in Innovative and Sustainable Chemical Engineering

#### Amanda Andersson and Måns Gerdtsson

MASTER'S THESIS ACEX30-18-91

#### Analysis of a Recirculating Acuaculture System

An analysis at Lantfisk

Amanda Andersson

Måns Gerdtsson



Department of Architecture and Civil Engineering Division of Water Environment Engineering CHALMERS UNIVERSITY OF TECHNOLOGY Gothenburg, Sweden 2018 Analysis of a recirculating aquaculture system An analysis at Lantfisk AMANDA ANDERSSON AND MÅNS GERDTSSON

#### © AMANDA ANDERSSON AND MÅNS GERDTSSON, 2018.

Supervisor: Torsten Wik, Department of electrical engineering Examiner: Britt-Marie Wilén, Department of Water Environment Technology

Master's Thesis ACEX30-18-91 Department of Arcitechure and Civil Engineering Division of Water Environment Technology Chalmers University of Technology SE-412 96 Gothenburg Telephone +46 31 772 1000

Cover: Clarias Gariepinus

Typeset in  $\[\] ETEX$ Gothenburg, Sweden 2018 Analysis of a recirculating aquaculture system An analysis at Lantfisk Master's Thesis in the Master's programme Innovative and Sustainable Chemical Engineering AMANDA ANDERSSON MÅNS GERDTSSON Department of Civil and Environmental Engineering Division of Water Environment Technology Chalmers University of Technology

#### Abstract

The water treatment in a commercial RAS used for production of *Clarias Gariepinus* was studied in order to gain understanding of the efficiency of the process. In order to evaluate the capacity of the water treatment several methods were used such as; analysis of nitrogen compounds with ion chromatography, analysis of total organic carbon, microscopic investigation of sludge, analysis of COD and BOD and activity tests of nitrifying and denitrifying bacteria. It was found that the concentration difference of the nitrogen compounds between the incoming and outgoing flow of the treatment process were small due to low activity and short retention times. No concentrations of the nitrogen compounds exceeded the limit values for what the fish can withstand. However, the water has high COD and very low BOD. Carbon should be removed in order to improve nitrification while the denitrification is limited by the low amount of biodegradable carbon. It was also found that the sludge in the pump sumps performed better in the activity test than the sludge from the denitrification tanks. Although the water treatment process of the RAS has some areas of improvements, the process has shown to be insensitive to disruptions and able to recover from interference.

Keywords: Recirculating aquaculture system, RAS, Clarias Gariepinus, nitrification, denitrification.

#### Acknowledgements

Thanks to our examiner Britt-Marie Wilén and our supervisor Torsten Wik for their support throughout the project.

Thanks to Diana Olsson Waage at Lantfisk for letting us use their facility for the purpose of this study. Also thanks to Robin Ek and Kalle Larsson for their assistance during the work at lantfisk.

Special thanks to Mona Pålsson for her assistance during laboratory work at the Environmental Chemistry Laboratory at Chalmers university of technology.

Amanda Andersson and Måns Gerdtsson, Gothenburg, June 2018

## Contents

Li	List of Figures xi						
Li	st of	Tables xii	i				
1	<b>Intr</b> 1.1 1.2 1.3	Foduction       Fish production       Fish production         1.1.1       Recirculating aquaculture systems RAS       Fish production         Lantfisk       Fish production       Fish production         1.2.1       RAS at lantfisk       Fish production         1.3.1       What is the nitrogen removal rate?       Fish production         1.3.2       Are there daily variations of nitrogen compounds in the system?       Fish production         1.3.3       What is the amount of dissolved carbon in the system and how much of it is biodegradable?       Fish production         1.3.4       Is it viable to operate a RAS without a dedicated sludge removal unit?       Fish production	L 1 2 2 4 4 5 5 5				
2	<b>The</b> 2.1 2.2 2.3 2.4 2.5	water treatment in RAS       9         Ion chromatography       9         Carbon removal       10         Flow       11         Excretion       11	7 7 9 0 1				
3	Met 3.1 3.2 3.3 3.4 3.5	Mapping of recirculating system       13         Analysis of nitrogen compounds       13         3.2.1       Activity test       14         3.2.1.1       Nitrification test       14         3.2.1.2       Denitrification test       14         Carbon removal       14         Microscopic investigation of process water and sludge       14         Analysis of metals       14	<b>3</b> 3 3 4 4 5 6				
4	<b>Res</b> 4.1	ults and Discussion       17         Nitrogen removal       17         4.1.1       Nitrogen excretion       17	<b>7</b> 7 7				

		4.1.2	Ammonium		17		
		4.1.3	Nitrate		19		
	4.2	Activit	ty test		20		
		4.2.1	Nitrification test		21		
		4.2.2	Denitrification test		23		
	4.3	Carbor	n removal		27		
	4.4	Micros	scopic investigation		29		
		4.4.1	Characteristics of flocks		29		
		4.4.2	Characteristics of process water		30		
		4.4.3	Characteristics of sludge		30		
	4.5	Metal a	analysis		34		
	~						
5	Con	clusion	n		37		
Bi	Bibliography						

## List of Figures

~

c 0

. . .

0.1	Clarias Gariepinus, Illustrations of the Zoology of South Africa, 1838	1
$2.1 \\ 2.2$	Theoretical RAS setup	7
	monium peaks.	10
4.1	Ammonium concentration over the nitrification unit.	18
4.2	Ammonium concentration into the denitrification and out of the OCR	10
	unit over 24 hours.	18
4.3	Denitrification and OCR unit.	19
4.4	Series 1: Nitrate concentration as $mgNO_3 - N/l$ in and out of the	
	nitrification unit over 24 hours.	20
4.5	Series 1: Nitrate concentration as $mgNO_3 - N/l$ into the denitrifica-	
	tion and out of the OCR unit over 24 hours	20
4.6	Nitrification test of the first nitrification tank.	21
4.7	Nitrification test of the last nitrification tank.	22
4.8	Nitrification test of the OCR tank.	22
4.9	Pictures taken of the biofilter media from aerobic tanks	23
4.10	Denitrification test of the denitrification tank with biofilter media	24
4.11	Picture of biofilter media from the denitrification tank	24
4.12	Denitrification test of sludge from the denitrification tank	25
4.13	Denitrification test of sludge from the pump sump	26
4.14	Denitrification test of thick sludge from the pump sump	26
4.15	The sum of nitrate and nitrite from the denitrification tests of the	
	pump sump	27
4.16	Total organic carbon concentration as mg/l over 24 hours	28
4.17	COD	28
4.18	BOD	29
4.19	Common structure of the flocks in the water going to the fish	30
4.20	Characteristics of process water	30
4.21	Higher organisms in the flocks	31
4.22	The pictures shows how the same amoebae changes its form	32
4.23	Two different specimen of actinopodas	32
4.24	Two different examples of rotifiers.	33
4.25	Unknown structures found in the sludge samples	34

## List of Tables

1.1	Dimensions of tanks	3
1.2	Components of studied RAS at Lantfisk	4
1.3	Average retention times. Since there are three parallel lines for DN and OCR the total retention time is shown for a single line. Fish	
	tanks are also connected in parallel and retention time is given as the	
	average for a single tank	4
4.1	Nitrogen excretion based on feed rate during the series. The concen-	
	tration increase is based on the volume of the entire system	17
4.2	Rate of nitrification, NF1: First nitrification tank, NF5: last nitrifi-	
	cation tank, L14: First OCR tank.	23
4.3	Rate of denitrification.	27
4.4	Concentrations of metals (mg/kg dry matter)	35
4.5	Weights of dry sludge samples	35

## 1 Introduction

#### 1.1 Fish production

The fish production, both aquaculture and capture production, has grown significantly since the 1950's and must grow further to satisfy the increasing global population and consumption. In 2013, the total fish production reached a number of 162.9 million tonnes of which 141.5 million tonnes was used for human consumption. The estimated global annual fish consumption per capita has increased from 9.9 kg in the 1960s to 14.4 kg in the 1990s to 19.7 kg in 2013. This is partly because of increasing production of fish but also due to better distribution to consumers and better utilization of the product, which reduces waste. This fast increase of fish production demands sustainable strategies and techniques for fishing that take social, economical and environmental aspects into consideration[2].

At some places around the world, the capture fisheries production has reached a point where it risks extinction of local fish stocks. This could in turn lead to disruption of ecosystems and devastate the subsistence for people who depend on fishing. However, improvements of the of the fisheries management has led to small amends in the state of some fish stocks. The increased growth in aquaculture stands for almost half of the human consumption of fish. The most common method for traditional fish farming is in open cage systems in the ocean or in lakes. Large fish cages are placed in already existent lakes or in the ocean where they utilize the surrounding ecosystem for water flow into the system and transport of faeces and food waste out of the system. This is a cost effective and well established method but it also causes strain on the ecosystem because of the nutrients and particles spread to the local environment. There is also a risk of spreading disease and escape of fish, which could disrupt the already existing ecosystem [2].

#### 1.1.1 Recirculating aquaculture systems RAS

Semi-closed or closed systems have been developed to reduce the environmental impact of the open cage systems. This technique for fish farming can also be placed in lakes or in the ocean. Water is pumped into a closed container with fish, which can be a "moving bag" or a solid tank, and the water flows out from the container at specific outlets. The water is then processed in a water treatment plant and can be returned to surrounding water or a closed container for the fish[1]. One method of fish farming that gives better control of the water treatment process is RAS, recirculated aquaculture system. It is a land based process that implements biological water treatment processes that removes nitrogen, biological matter and phosphorus. This enables a high degree of water to be recirculated and reused in the fish tanks. Nitrogen removal is important since the fish excrete ammonia from their gills and ammonia is toxic to the fish at high concentrations. Nitrogen removal is achieved by the processes called nitrification and denitrification, which is further explained in section 2.1. The sludge produced in the process can be removed and sent to sewage treatment or used as fertilizer. Compared to open cage system, RAS has many advantages, such as reduction of pathogenic bacteria and disease, low water use and high control of operational parameters. It also enables fish farming in areas where the access to water is poor. On the other hand, it is an expensive process, both in investment cost and in operational cost. It also requires close control by experienced staff since the system is sensitive to changes in process parameters[1].

#### 1.2 Lantfisk

Lantfisk is a small but expanding company on the outskirts of Gothenburg that utilizes the RAS technique to farm *Clarias gariepinus*, also calles African sharptooth catfish. They started their business in 2013 at a very small scale and in 2017 they produced 24 tonnes of fish. In 2018 they are planning on expanding their production even further and expect to produce 40 tonnes of fish. Since Lantfisk aims at continuous expansion of their production they wish to gain further knowledge about their RAS.

#### 1.2.1 RAS at lantfisk

The flow chart of the RAS at Lantfisk is shown in Figure 1.1a. Floating feed is provided with automatic feeders from 06:00 to 17:00. The tanks labeled NF and OCR are aerated with pressurized air, which also cause agitation. The process is a closed system, which means that all the water is recirculating within the system. It is only refilled with water that corresponds to the loss of evaporation. More loops than expected were found in the system. The loops have been introduced in order to increase operating safety. Mainly the risk of overflow has decreased according to Lantfisk. As is shown in Figure 1.1a water leaving Pump 2 can either pass through the anoxic denitrification tanks (DN) and the aerated organic carbon removal tanks (OCR), or pass directly to the OCR tanks. This bypass is introduced in order to avoid overflow in the DN tanks while maintaining a high flow through the OCR tanks in order to aerate the water to provide sufficient oxygen to the fish.



(b) Flow through pumps

deni-		
rbon		Flow (l/
The	Pump 1	2.0
each	Pump 2	2.1
	Pump 3	8.2

(a) RAS flow chart. DN=anaerobic tanks for denitrification, OCR=aerated tanks for organic carbon removal, NF=aerated tanks for nitrification. The number of tanks in series is also indicated for each unit.

The bioreactors used for the water treatment are filled with Kaldnes bio carriers in order to provide sufficient area for microorganisms to grow. In the tanks labeled NF and OCR in Figure 1.1a the bio carriers are moving around in the water as a result of the aeration. The bio carriers in the tanks labeled DN are stationary because the these tanks are filled with more carriers than the others, the flow is lower and there is no aeration. This effectively turns these tanks into fixed bed bio reactors. The water treatment of RAS is discussed further in section 2.1

Since Pump 3 has a higher flow than Pump 2 most of the water from the fish is recirculated back through the pump sumps and does not reach the treatment. All the tanks, including the pump sumps has the same dimensions, see Table 1.1. In Table 1.2 the components of the system is listed.

Table 1.1: Dimensions of tanks

Height (m)	Length (m)	Width (m)	Volume $(m^3)$
1	1.2	1	1.2

	Fish tanks	Anaerobic tanks	Aerobic tanks	Pump sump	Total RAS
Number of units $\binom{n}{2}$	20	9	11	4	44
Average water level					
in units $(m)$	0.81	0.75	0.69	0.58	-
Total volume	24	10.8	13.2	4.8	52.8
in units $(m^3)$		10.0	10.2	4.0	02.0
Ratio of					
component to	45.5	20.5	25.0	9.1	100
entire system $(\%)$					

Table 1.2: Components of studied RAS at Lantfisk

Retention times in different tanks are calculated according to Equation 2.4. The retention time vary between the units and is shown as averages in Table 1.3. Since the denitrifying tanks are not agitated the hydraulic retention time is not a good approximation of the residence time. However, the flow rate is 3-4 times lower into the denitrifying tanks than into the OCR tanks.

**Table 1.3:** Average retention times. Since there are three parallel lines for DN and OCR the total retention time is shown for a single line. Fish tanks are also connected in parallel and retention time is given as the average for a single tank

	Individual tanks (min)	Total (min)
Nitrification	5,9	30,0
Organic carbon removal	13,2	26,4
Fish tanks	33,5	

There is no dedicated unit for removal of solids and most of the solids are trapped in the denitrification tanks where the flow is lowest and there is no agitation. These tanks fill up with solids and are therefore emptied approximately once a month. Solids also settle in the pump sumps. This creates anoxic environments where denitrification can occur both in the denitrification tanks and in the pump sumps.

#### 1.3 Research questions

The following are research questions that this project was aiming to answer.

#### 1.3.1 What is the nitrogen removal rate?

The fish excrete ammonium which is toxic and has to be removed in a recirculating system. The removal rates of ammonium and nitrate have therefore been studied.

## 1.3.2 Are there daily variations of nitrogen compounds in the system?

The fish is only fed during parts of the day. This could for example result in lower concentrations of waste in the morning than at night.

## 1.3.3 What is the amount of dissolved carbon in the system and how much of it is biodegradable?

The amount of dissolved carbon in the water was expected to be high in the entire system because the water has a brown colour. The majority of the dissolved carbon is also expected to not be digestible by the microorganisms. An aim has therefore been to determine the amount of carbon in the system, and if it is biodegradable.

## 1.3.4 Is it viable to operate a RAS without a dedicated sludge removal unit?

The system has no dedicated sludge removal unit. Instead sludge builds up in the denitrification tanks where the flow is low and there is no agitation. When there is too much sludge in the denitrification tanks they are emptied and are therefore used for both sludge removal and denitrification.

#### 1. Introduction

## 2

### Theory

#### 2.1 Water treatment in RAS

An efficient water treatment process is crucial for RAS. Ammonium should be kept at a level below 45  $mgNH_4 - N/l$  and nitrate below 140  $mgNO_3 - N/l$  in order to avoid disturbances in physiology, growth and feed intake [16][8]. There are several different RAS setups for fish production and the one that Lantfisk based their system on is shown in Figure 2.1.



Figure 2.1: Theoretical RAS setup.

The conventional RAS configuration uses nitrifying biofilters to reduce ammonia and nitrite concentrations by oxidizing them into nitrate. This is combined with organic carbon removal where organic matter remaining after denitrification is removed by heterotrophic bacteria in aerobic tanks. The sludge created in this process can be removed by sedimentation or mechanical filtration [3]. The nitrification is carried out by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in aerobic tanks according to the following reactions 2.1 and 2.2. These bacteria are autotrophic and can be outcompeted by heterotrophs. The presence of organic carbon can therefore reduce the effectiveness of the nitrification units[7]. The ratio of carbon to nitrogen will affect which species are favoured. Especially the amount of biodegradable carbon is of interest. The ratio of biological oxygen demand (BOD) to total ammonia nitrogen (TAN) is used in this report. In order to avoid negative effects on the nitrification rate the nitrification unit is placed after the organic carbon removal unit.

Nitrification:

$$2NH_4^+ + 3O_2 \rightleftharpoons 2NO_2^- + 4H^+ + 4H_2O \tag{2.1}$$

$$2NO_2^- + O_2 \rightleftharpoons 2NO_3^- \tag{2.2}$$

Ammonia and nitrite are toxic for aquatic animals while nitrate is much less harmful. Consequently, priorities have been on removal of ammonia and nitrite. The nitrate produced is normally removed in two ways, by dilution with water exchange or by denitrification. In the denitrification, nitrate is reduced to nitrogen gas by oxidation of organic matter and is emitted to the surrounding air according to equation 2.3. Denitrification was introduced in order to increase the nitrate control and lower the water exchange rate. In cases when the denitrification does not match the nitrification the maximum allowed nitrate concentration steers the external water exchange rate in the system. The conventional semi-closed RASs have a varying external water exchange rate between  $0.1-1m^3/kg$  feed to avoid accumulation of nitrate. This corresponds to a water renewal of 5-10% of the system volume [3, 4]. Denitrification:

$$NO_3^- \to NO_2^- \to NO \to N_2O \to N_2$$
 (2.3)

The denitrification occurs at anaerobic conditions by facultative bacteria. The facultative bacteria are using electron donors originated from organic or inorganic sources. In RAS and traditional wastewater treatment plants, heterotrophic denitrification is the most commonly applied method. It uses organic electron donors from a carbon source (e.g. carbohydrates, organic alcohols) that can be added externally to the system or originate from the fish feed or faeces. If the process has limited access to a biodegradable carbon source, accumulations of intermediate products, such as NO<sub>2</sub> and N<sub>2</sub>O, can occur. If the process has an excess of carbon, the concentration of ammonia could increase due to AOB being outcompeted by heterotropic bacteria[3].

By reducing the concentration of nitrate, the need for water exchange will be lowered and thus decrease the water use of the process. Apart from the direct toxic effect from high nitrate concentrations on aquatic animals, there are regulations on how much nitrate that is allowed to be discharged. Since the denitrification reduces the nitrate levels and thereby the water use, these restrictions are more easily attained and increase the sustainability of the RAS [3]. Another positive effect of denitrification is improved alkalinity. The intensive nitrification of RAS leads to a decreased alkalinity and a resulting drop in pH. Acidic conditions negatively affects the performance of the biofilter and the environment for the aquatic organisms. Alkalinity supplements, usually sodium bicarbonate, are commonly added to stabilize the alkalinity and pH. By incorporating heterotrophic denitrification the alkalinity will be increased and thus the need for alkalinity supplement will be reduced or even eliminated[3]. There is also a risk with a low water exchange rate. When much of the same water is used in the process, accumulation of growth inhibiting substances may occur. These substances come from the fish, bacteria or the food and cannot be degraded by the water treatment processes. Examples of these substances are cortisol, a stress hormone from the fish, or metals that are brought to the process by the feed [4].

After the denitrifying units the water is transported to aerated tanks for organic carbon removal. In these tanks organic material is consumed by bacteria and carbon dioxide is released. The tanks for denitrification and organic carbon removal are connected in series.

#### 2.2 Ion chromatography

Ion chromatography was used in order to determine concentrations of the nitrogen containing ions in the system. However, there are disproportionate concentrations of ammonium and sodium in this system and since they have similar retention times that causes interference. In Figure 2.2a and 2.2b there is an example of a chromatogram where this can be seen. This is common when there are disproportionate concentrations of sodium and ammonium, but by using different equipment better separation of the peaks can be achieved[10]. This was not in the scope of the project and this source of error in determining ammonium concentration could not be avoided.



Figure 2.2: Example of chromatogram with interference between sodium and ammonium peaks.

#### 2.3 Carbon removal

As mentioned in Section 2.1, carbon is required for denitrification but undesirable in nitrification. No external carbon source apart from the fish feed is used in the studied RAS. In order to determine the amount of carbon present in the system the total organic carbon (TOC) was measured. Samples were taken so that the change in concentration over the different treatment units could be determined. In order to find out how much of that carbon that could be utilized by the microorganisms biological oxygen demand (BOD) and chemical oxygen demand (COD) were analyzed. BOD is a measurement of how much oxygen is consumed by microorganisms in a sample over a specified time. BOD7, for example is the consumption over seven days which was used in this case. This can be compared to COD which is the oxygen consumption when the content of a sample is oxidized chemically[15].

#### **2.4** Flow

In order to estimate the residence time in the bioreactors the hydraulic retention time (HRT) was calculated using the relation:

$$HRT = Volume \, of \, tank/Inlet \, flow \, rate \tag{2.4}$$

Using the residence time along with concentrations from the flow to and out of the reactor the reaction rate can be estimated using:

$$Reaction \, rate = (C_{in} - C_{out})/HRT \tag{2.5}$$

#### 2.5 Excretion

As mentioned in section 1.1.1 fish excrete ammonium. However they only do this when they have been fed. When they are being fed the excretion rate increase and when the feeding stops the excretion decline over time. Approximately five hours after feeding ceased the ammonia production was undetectable in a study by Bovendeur et al.[9]. The excretion rate of total ammonia nitrogen is estimated to be 3% of the daily feeding rate[7].

#### 2. Theory

### Methods

#### 3.1 Mapping of recirculating system

No complete overview or map of the system was available beforehand and therefore one was made to gain understanding of the system. All connecting pipes and valves were mapped in order to understand the flows in the system. The complete map was then deconstructed so that a more comprehensive overview of the system could be made. Flows were determined by diverging the flow into a vessel and measuring the time it took to fill a certain volume. All tanks in the system have the same dimensions and by measuring water level the volumes could be determined. Volumes in tubes are neglected. The variation in water level over time due to evaporation, removal of sludge and addition of fresh water was also neglected.

#### 3.2 Analysis of nitrogen compounds

Ammonium, nitrite and nitrate concentrations in the system were measured to be used in combination with flows to calculate removal rates. In order to do that water samples were taken and filtered through 45  $\mu$ m polyethersulfone syringe filters to remove particles. Then the samples were frozen before transport and finally analyzed using ion chromatography with a ICS-900 Dionex. Anionic and cationic standards were used to ensure accuracy of the analysis.

To evaluate how the concentrations of nitrogen compounds vary over a longer time period, samples were collected during a day. The difference in concentration over the treatment units varied significantly the hole day and no overall removal rate could be determined. In order to reduce variability, activity tests on removal rates for both nitrification and denitrification were therefore performed in lab-scale.

#### 3.2.1 Activity test

To measure the activity of the bacteria present in the process water several activity tests were performed. The capacity of denitrification and nitrification were tested for different process sites. The samples were collected the same day or the day before the tests to ensure they would be fresh. To keep most of the bacterial activity, the samples were kept in room temperature during the transport from Lantfisk to Chalmers. The experimental setup is partially the same for the nitrification and the denitrification tests. A glass bottle with a total volume of 2 litres was filled with approximately 400 ml of carriers and then filled completely with process water from the sample site that was to be tested. By having these proportions it will reflect the estimated volume ratio between the the carriers and the process water at Lantfisk. The glass bottle was placed in a water bath at a temperature around 25-27°C to keep the original process conditions. The temperature was measured regularly to make sure it did not decrease.

The test were run for 2-3 hours. During the first hour, samples were taken every 10 or 15 minute. After that, samples were taken every 30 minutes. All samples were filtered with 45  $\mu$ m polyethersulfone syringe filters and frozen in a freeze until analysis with an ion chromatograph.

#### 3.2.1.1 Nitrification test

In a nitrification test the capacity of the nitrifying bacteria are tested. In summary, the bacteria are supposed to turn ammonia into nitrate. A small reactor with similar process parameters as the real plant were created and samples were taken regularly for analysis of ammonia. The result was then used for calculating the rate of nitrification.

Nitrification tests were performed on samples from three different origins; one from an OCR tank, one from the first nitrification tank and one from the last nitrification tank (tank number five). All three experiments were prepared as described in the previous section. Air was added through a stone diffuser to create sufficient mixing in the bottle. To guarantee sufficiently high starting concentration when determining the removal rate ammonia was added to create a concentration of 2-3 times the normal concentration in the system. This corresponds to a starting concentration of 10-15  $mgNH_4 - N/l$ .

#### 3.2.1.2 Denitrification test

In a denitrification test the bacteria are tested for their ability to reduce the concentrations of nitrate. The nitrate is supposed be converted into nitrogen gas by several intermediate reactions. For the test, a smaller denitrification reactor was constructed and 95 % ethanol, was used as a carbon source. Samples were collected during the test for analysis of nitrate. Also here, the result was used for calculating the denitrification rate.

Four denitrification tests were performed; two from the denitrification tanks and two from a pump sump. From the denitrification tank, one sample was collected with carriers at the top of the reactor and one sample were collected as sludge from the bottom of the tank. From the pump sump, the first sample was taken from the middle of the reactor during normal process conditions. The second sample was taken from the bottom of the tank 2-3 days after a pump had stopped. The pump sump is an interesting tank to test for denitrification since it is anaerobic and thereby probably contains denitrifying bacteria.

The samples containing carriers were prepared as described previously while the sludge samples were pored directly into a 2 litres bottle. The second sample from the pump sump was diluted 2 times due to a very high content of suspended solids. To ensure similar concentrations of nitrate in the test as in the the real process, potassium nitrate was added to this sample. The other samples were assumed to have a sufficient concentration of nitrate based on earlier analysis. To achieve anaerobic conditions and mixing, nitrogen gas was added through a stone diffuser. 1.6 ml of 95% ethanol was added 30 minutes after the start of the tests, which corresponds to a COD/N ratio of 5,3[12]. The pH was checked regularly during the ongoing tests to ensure a stable value.

#### 3.3 Carbon removal

In order to determine how well the organic carbon removal units perform the TOC was determined. In combination with COD and BOD a better understanding on how carbon behaves in the system could be achieved. Samples were taken before denitrification and after organic carbon removal. Samples were also taken from the inlet and the outlet of the nitrification unit to determine if a low concentration of carbon is achieved there. The same samples used for ion chromatography was used and had therefore been filtered through  $45\mu$ m polyethersulfone syringe filters. COD was measured using a Hach DR/890 Colorimeter and Hach digestion solution for COD 0-150 ppm. Samples for BOD7 was sent to ALS for analysis in an accredited laboratory. Total carbon (TC) and total nitrogen (TN) was determined using Shimadzu Total Organic Carbon Analyzer (TOC-VCPH).

## **3.4** Microscopic investigation of process water and sludge

Microscopy is a very useful method to gather information about the quality of the biomass in a water treatment system. Information of many visually characteristics can be observed with aid from a microscope. However, other properties of the sludge, such as the activity or the quantity of the biomass, have to be determined other ways. In this study, the simple visible structure of the flocks and "higher organisms" where looked into. Lenses with a magnification of 10 and 20 times were used to accomplish this, and some photos were taken from the analysis that will be shown further in this report.

When analyzing biomass in a microscope, the sample has to be as fresh as possible and neither be cooled nor frozen. This is in order to keep the characteristics and ecosystem of the floc intact. For that reason, samples were taken and analyzed the same day and were kept at room temperature all the time. Since the average total hydraulic retention time is estimated to be relatively short, between one to two hours, it was assumed that the content of the process water would be the same all over the plant where the water is fully mixed. Therefore, only one sample of the process water was taken to represent the whole plant. The sample was collected from the water going to the fish. When the water sample was allowed to be still for some time, small sludge particles were formed and sedimented on the bottom of the sample vial. These small sludge particles were analyzed in the microscope. Two sludge samples were also collected, one from from the bottom of an anaerobic denitrifying tank and one from a pump sump.

#### 3.5 Analysis of metals

In order to receive more information about the constitution of the sludge and thereby more knowledge of the overall process, an analysis of metals were performed. The metals to be quantified were; magnesium, alumina, calcium, titanium, vanadium, manganese, chromium, iron, cobalt, nickel, copper, zinc, cadmium and lead. Three sludge samples were collected; one from the pump sump and two from the denitrification tanks. All the sludge originate from the feed and therefore similar results are expected in the three samples.

The samples were first dried completely in an oven for 24 hours at a temperature of 105 °C. Then approximately 0.5g of the dried samples were pored in tubes together with 10 ml of nitric acid and then run in a microwawe digestion system. This makes the metals more dissolved and available for detection in an inductively coupled plasma mass spectrometry ICP-MS. After the microwave digestion two solutions were made with samples diluted with nitric acid 1:10 and 1:50 respectively. The samples were then run in a ICP-MS. The model used was 3800 Varian gas chromatograph together with Saturn 2200 GC/MS.

4

### **Results and Discussion**

In this chapter the results are presented and discussed.

#### 4.1 Nitrogen removal

In this section concentrations of ammonium and nitrate before and after the different treatment units are presented. Samples were collected in two different series. Series 1 from 08:20 to 14:20 and series 2 from 07:45 to 15:45 and then 07:45 on the following day. Nitrite is not presented since it was seldom detected and close to zero when it was.

#### 4.1.1 Nitrogen excretion

During the series nitrogen is added with the feed. The expected amount of ammonium introduced in the process as a result of the fish metabolism is presented in Table 4.1.

**Table 4.1:** Nitrogen excretion based on feed rate during the series. The concentra-tion increase is based on the volume of the entire system.

Date	Feed (kg)	Nitrogen in feed (kg)	Excreted ammonium (g)	$NH_4$ concentration increase (mg/l)
Series 1	17,6	1,21	36,3	1,14
Series 2	13,6	0,94	28,1	0,88

#### 4.1.2 Ammonium

Ammonium concentrations from samples over the nitrification unit and the denitrification and OCR units are presented in Figure 4.1, 4.2 and 4.3. Figure 4.1 shows that there is no clear decrease in ammonium concentration over the nitrification unit. Over the denitrification and OCR unit in Figure 4.3 and 4.2 however there seems to be a decrease. This is probably due to nitrification in the aerated OCR tanks. Still the difference varies in the tests and no overall removal rate could be determined. The difference in concentration between the sample from 15:45 and 07:45 the next day in Figure 4.2 is small. The fact that the concentration is not reduced during the part of the day when the fish are not fed and ammonium excretion drops indicate that only limited nitrification takes place.



Figure 4.1: Ammonium concentration over the nitrification unit.



**Figure 4.2:** Ammonium concentration into the denitrification and out of the OCR unit over 24 hours.



Figure 4.3: Denitrification and OCR unit.

#### 4.1.3 Nitrate

Figure 4.4 and 4.5 contain nitrate concentrations for samples over the nitrification unit and the denitrification and OCR units. The concentrations are close to but never exceed 140  $mgNO_3 - N/l$  below which physiological and growth disturbance is avoided. This was true for all samples analyzed in this project. No clear difference in nitrate concentration over the different units was found. Samples were taken from water going in and out of the nitrification unit, into the denitrification unit and out of OCR unit. The concentration also seem to be stable over the course of the sample series. This could be interpreted as nitrate being added at the same rate as it is removed. However the concentration at all four sample points is very similar at 15:45 in the end of the feeding cycle and 07:45 the next day. This indicates that little to no nitrogen is removed during the night when no additional feed is put into the system.



Figure 4.4: Series 1: Nitrate concentration as  $mgNO_3 - N/l$  in and out of the nitrification unit over 24 hours.



Figure 4.5: Series 1: Nitrate concentration as  $mgNO_3 - N/l$  into the denitrification and out of the OCR unit over 24 hours.

As neither ammonium nor nitrate removal rates could be determined activity tests on lab scale were performed.

#### 4.2 Activity test

The results from the analysis of the activity tests are plotted in graphs in the following sections. Pictures of the biofilter media and estimated rates of nitrification and denitrification are also presented.

#### 4.2.1 Nitrification test

From the nitrification test of the first nitrification tank, Figure 4.6, the ammonium concentration is decreasing almost linearly the first 90 minutes. From this point, the concentration seem to level out. This indicates that initially there is bacterial activity in the tank. However as the ammonium concentration decrease, so does the activity. The nitrate levels were shifting randomly between 107-120 mg/l. The nitrate concentration is expected to rise as ammonium is oxidized but this is not observed. This indicate some sort of error in the sampling method or reactor set up. No nitrite levels were observed from this test.



Figure 4.6: Nitrification test of the first nitrification tank.

In the nitrification test of the last nitrification tank, Figure 4.7, the ammonium level did not decrease at the same extent as in the the test with the first tank. However, a small decrease might be assumed within the the first 45 minutes. Also in this test, the ammonium concentration seems to stabilize during the last hour of the experiment. The nitrate concentration varies in the beginning of the test but starts to increase after 45 minutes. No nitrite was detected in the analysis.



Figure 4.7: Nitrification test of the last nitrification tank.

The OCR tank also shows good capacity for nitrification just as the first nitrification tank. In Figure 4.8 it can be seen that the ammonium concentration is decreasing the first hour of the experiment. The curve then tends to flatten and even increase a little within the last 30 minutes. The nitrate concentration is shifting in the beginning and increasing at the end of this test as well. However this is not coupled with a corresponding decrease in ammonium concentration. The change in nitrate concentration is therefore probably the cause of some error in the same way as in the first nitrification tank in Figure 4.6. No nitrate levels were observed.



Figure 4.8: Nitrification test of the OCR tank.

Pictures of the biofilter media from the nitrification and the OCR tank are shown in

Figure 4.9. Both bio carriers have an evenly, distributed film of bacteria. The film is thin and firmly attached to the bio carriers. This indicates good flow of process water through the carriers and thereby sufficient substrate supply [13].



(a) Biofilter media from the nitrification tank.



(b) Biofilter media from the OCR tank.

Figure 4.9: Pictures taken of the biofilter media from aerobic tanks.

The nitrification rates for the different tanks are presented in Table 4.2. The rates are roughly estimated by assuming linearity between the initial ammonium value and the lowest ammonium value. From this table it can be concluded that the highest nitrification rate was found in the first nitrification tank.

**Table 4.2:** Rate of nitrification, NF1: First nitrification tank, NF5: last nitrification tank, L14: First OCR tank.

	NF1	NF5	L14
Time (min)	120	45	90
Start value $(mg/literNH_4^-)$	14.2	12.7	11.1
Lowest value $(mg/literNH_4^-)$	5.7	9.9	3.9
Rate of nitrification $(mgNH_4 - N/m^2h)$	26.5	15.2	20.0

#### 4.2.2 Denitrification test

In Figure 4.10 the result from the denitrification test of the anaerobic tank with biofilter media is shown. The nitrate concentration is increasing in the beginning of the test. The carbon source is added after 30 minutes but no immediate reduction of the nitrate level can be observed. 20 minutes after the carbon source is added, a sharp decline of both the nitrate and the ammonia concentrations occur. The levels then starts to increase again. From this test it is difficult to conclude if there is any

denitrification activity. The rapid decline in nitrate concentration a few minutes after the carbon source is added indicates that the denitrification could be more efficient if there were more biological degradable carbon available in the process. On the other hand this does not explain the simultaneous decrease in ammonium concentration.



Figure 4.10: Denitrification test of the denitrification tank with biofilter media.

A picture of two bio carriers from the anaerobic tank are displayed in Figure 4.11. The bio carrier to the right is filled with dark sludge-like material. When the carriers were exposed to agitation, the material was easily rinsed off. The bio carrier to the left is an example of what they look like after mild rinsing. The material was not as firmly attached to the bio media as the biofilm on the bio carrier from the nitrification and OCR tanks. This implies that the carriers don't receive sufficient amount of process water which supplies the bacteria on the carries with substrate. This results in poor growth of bacteria on the biomedia. [13]



Figure 4.11: Picture of biofilter media from the denitrification tank.

Another test was performed for the denitrification tank to establish whether there was any denitrification activity or not, this time on the bottom sludge. The result from this test is presented in Figure 4.12. When the sample was collected there had not been any flow through the tank for three days due to the break down of a pump. This generated very low initial values of nitrate and therefor the activity of denitrification couldn't be evaluated. Also, the initial concentration of ammonia, was very high compared to the normal concentrations in the process. The increased concentration is suspected to be the result of anaerobic hydrolysis of the sludge.



Figure 4.12: Denitrification test of sludge from the denitrification tank.

Two tests with sludge from a pump sump were performed. The result from the test with sludge collected during normal process conditions and test with thick sludge collected during the pump break down are presented in Figure 4.13 and 4.14. The thick sludge has a sharp increase of nitrate the first 15 minutes, this is because sodium nitrate was added to get a sufficient start concentration of nitrate. The nitrate concentration for both test are decreasing almost linearly and are not seemingly affected by the additional carbon source. From these tests it can be concluded that denitrification does occur.

The ammonium concentrations stay somewhat stable during the test. The ammonia concentration in the test of thick sludge was around 6 times higher than in the sludge during normal conditions. This sample was also diluted two times which means that the sludge from the pump sump contained around 12 times more ammonia than the process water. From this test it can also be concluded that ammonia is produced if the water flow through the tanks is very low. This ammonia could be produced by anaerobic bacteria during protein degradation[17].



Figure 4.13: Denitrification test of sludge from the pump sump.



Figure 4.14: Denitrification test of thick sludge from the pump sump.

From the tests it can also be seen that the nitrite concentrations are increasing. By adding together the nitrite and nitrate concentrations, Figure 4.15, it could be concluded that the total concentration are still decreasing. This implies that nitrate are converted into nitrite, which further forms other intermediate compounds and finally nitrogen gas according to Reaction 2.3. However, the transformation of nitrate into nitrite is somewhat faster than the subsequent part of the reaction. This causes accumulation of nitrite. If this levels of nitrite would to be spread to the rest of the system it could affect the health of the fish.



Figure 4.15: The sum of nitrate and nitrite from the denitrification tests of the pump sump

The rates of the denitrification are presented in Table 4.3. The rates are estimated by assuming linear correlation between the initial and the lowest value of nitrate. No clear coherent decrease of nitrate was detected for any of the two samples from the denitrification tank. Therefore, only the denitrification rates for the pump sump are represented in Table 4.3.

 Table 4.3:
 Rate of denitrification.

	PS3	PS3
Time difference (min)	140	150
Start value (mg/liter $NO_3^-$ )	53.1	120.0
Lowest value (mg/liter $NO_3^-$ )	31.6	73.2
Rate of denitrification $(gN0_3 - N/ \text{ kgSS h})$	1.31	0.47

#### 4.3 Carbon removal

Total organic carbon concentration in filtered water is very similar in the entire system. The concentration is also very stable over time as is shown in Figure 4.16. The fact that the TOC concentration does not change over the bioreactors indicates that most of the organic carbon is not easily biodegradable.



Figure 4.16: Total organic carbon concentration as mg/l over 24 hours

In order to determine how much of the organic carbon that is biodegradable COD and BOD was determined. The results are presented in Figure 4.17 and 4.18. BOD is much lower than COD which confirm that most of the organic carbon in the system is not biodegradable.



Figure 4.17: COD



Figure 4.18: BOD

As mentioned in chapter 2.1 the nitrification can be affected by carbon. In order to determine if the nitrification is affected by dissolved carbon in the system the BOD/TAN ratio was determined. The mean  $NH_4 - N$  concentration from Figure 4.1 was used to approximate the TAN, since less than one percent exists in the unionized form at pH 7 and 26°C which is maintained in the system [11]. The BOD7/TAN rato found was 3,6. This is similar to the BOD5/TAN ratio of 4 that was reported to reduce nitrification rate by 70% in a study by Songming Zhu and Shulin Chen[7]. This indicate that the dissolved carbon has a negative effect on the nitrification process even though BOD is much lower than COD. The heterotroph microrganisms used in denitrification rate when ethanol, which is easily biodegradable, was added during lab scale activity tests in Chapter 4.2.2. This suggests that the carbon present in the system is sufficient. If that was not the case there would have been an increase in nitrate removal rate when ethanol was added.

#### 4.4 Microscopic investigation

#### 4.4.1 Characteristics of flocks

Figure 4.19a and Figure 4.19b represent two common characteristics of the flocks in the process water. The shape of the flocks are mostly rounded as in Figure 4.19a but some flocks has more irregularly shaped flocks which can be seen in Figure 4.19b. The irregular shape is partly caused by filamentous bacteria, the black striped forms in Figure 4.19b. The filamentous bacteria forms a structure similar to a backbone on which the flocks are formed. Increasing load of the the plant can also result in more irregularly shaped flocks[5]. The flock contains both open and compact structures. The majority of the flocks are compact with very few open areas and has the same appearance as in Figure 4.19a. There are also a few flocks that have a more open structure as in Figure 4.19b. An open structure is often caused by filamentous bacteria as well [5]. Another characteristic of the flock is firmness. It was difficult to decide whether the flocks were weak or firm since the borderline between the flocks and the water is not sharply marked off and many cells may be free and not attached to the flock.



(a) Rounded and compact flocs.



(b) Irregular and open flocs.

Figure 4.19: Common structure of the flocks in the water going to the fish.

#### 4.4.2 Characteristics of process water

Many particles and bacteria are suspended in the process water of the treatment plant, which can be seen in Figures 4.20a and 4.20b. The water taken from the pump sump has much more free particles than the water going to the fish.



(a) Water going to the fish.



(b) Water from the pump sump.

Figure 4.20: Characteristics of process water.

#### 4.4.3 Characteristics of sludge

When looking at the sludge from the denitrification tanks, very little activity was observed and almost no larger organisms were present. When the samples were taken, the pump to the denitrification tanks (pump 2 in figure 1.1a) had been broken for 23 days and therefore there had been no flow through the tanks. This could be one reason for the low activity within the sludge. At regular conditions with a functioning pump, there is a water flow through the tanks but there is no mixing within them which causes the sludge to sink to the bottom. This regular conditions and the conditions at the time for the investigation are not that different which could also mean that there are little activity within the denitrification tanks at normal process conditions as well. The process water and the sludge from the pump sump on the other hand contained many "higher organisms" so called protozoa. The general perception when looking at the flocks was that it was very "alive". Many different organisms at a relatively high number were observed. Some of these organism will be presented next.

First protozoa to be presented is probably some species of free swimming ciliate, which is shown in Figure 4.21a. This ciliate was found in the pump sump but other specimens were found in the process water as well. This organism existed in large numbers and were rather quick swimmers, which unfortunately made them a little difficult to capture in a clear picture. They are characterized by a surface partly covered with cilia which play a part in the movement of the organism. The presence of ciliates indicates that the loading level of the plant is not too high and that the oxygen level is sufficient.[5].

Another large organism found in the process water is shown in Figure 4.21b. This one is a little more difficult to decide which species it is. The organism were sedentary in the matrix of the floc with small movement and has a spherical shape. Based on this observations it is supposedly a rhizopoda of some kind [5]. This organism did not occur to a large extent.



(a) Ciliate found in the pump sump.



(b) Unknown species, supposedly a rhizopoda of some kind.

Figure 4.21: Higher organisms in the flocks.

Figure 4.22a and 4.22b shows an amoebae which is an rhizopoda, it is the same amoebae in both pictures but it has different shape. The rhizopoda uses pseudopodia to create movement and mobility, it is hence very slow. The amoebae is unicellular organism with no rigid cell wall[5].



(a) Amoebae.

(b) Amoebae.

Figure 4.22: The pictures shows how the same amoebae changes its form.

The next two Figures (4.23a and 4.23b) show two different actinopdas. These organisms have a globular form and are also surrounded by psuedopodia in the form of thin needles. These needles are contractile and used for catching food[5]. The two different type of rhizopoda, amoeba and actinopoda, did not occur to a large extent in the process water and the sludge samples. When rhizopoda are found in water samples it is usually a sign that the plant is highly loaded and has a low or to low oxygen level[5].



(a) Actinopoda.



(b) Actinopoda.

Figure 4.23: Two different specimen of actinopodas.

Several rotifers (Figures 4.24a and 4.24b) were found in the process water and the sludge from the pump sump. Their body is surrounded by some shield like structure which creates movement by contractions of the whole body[5]. The existence of rotifiers in the process is an indication of a functioning activated sludge process. They enhance the flock formulation by secrete a material that stick the flock together[14].





(a) Rotifier.

(b) Rotifier.

Figure 4.24: Two different examples of rotifiers.

Another observation of the sludge samples were these unknown structures, shown in Figures 4.25a and 4.25b. These unknown materials could be found in all three sample sites and occurred to a large extent. Figure 4.25a and 4.25b have an almost straw like structure and could be waste from the fish feed or pieces of skin from the fish. In Figure 4.25c and 4.25d a dark brown material can be seen. It has more of a cellular constitution and could be guessed to be skin from the fish or a residual from the fish feed



(a) Straw-like structure of the flocks. (b) Straw-like structure of the flocks.



(c) Unknown material.

(d) Magnification of unknown material.

Figure 4.25: Unknown structures found in the sludge samples.

#### 4.5 Metal analysis

The concentrations of metals are shown in Table 4.4. PS represent the sludge sample from the pump sump and L21 and L23 are names of the denitrification tanks were the sludge samples were collected. Chromium, zinc, cadmium, lead, copper and nickel all have limit values that should not be exceeded if the sludge is intended to be used as a fertilizer in Swedish agriculture [6]. These limit values are also shown in table 4.4. The rest of the analyzed metals don't have limit values and are marked as N.E, which stands for Not existing.

From the table it can be seen that no values from the sludge samples, except for copper, exceeds the limit values. It was assumed that the result would be almost the same regardless of where the samples were collected. However, considering some metals, the results from L21 stand out more if compared with the result from L23 and PS. Especially prominent are the result for cadmium and copper. Why this result is obtained is unknown. On one hand, the results could be true, there are divergent concentrations of metals in L21. On the other hand, the odd results could have been caused by mistakes in the preparation or the analysis of the samples. The dried samples was supposed to weigh around 0.5g but the weighed sample for L21

was much lower which can be seen in table 4.5. This might have affected the final result from the ICP-MS.

	PS	L21	L23	Limit values [6]
Chromium	$3.5 \times 10^{-4}$	$2.7 \times 10^{-3}$	$4.3 \times 10^{-3}$	100
Zinc	0.50	0.32	0.46	800
Cadmium	0.50	1.86	0.53	2
Lead	0.70	0.21	0.67	100
Copper	238	763	190	600
Nickel	0.063	0.032	0.066	50
Cobalt	28	136	27	N.E
Iron	$5.0 \times 10^{-3}$	$2.0 \times 10^{-3}$	$3.7 \times 10^{-3}$	N.E
Manganese	$6.9 \times 10^{4}$	$2.8 \times 10^5$	$6.4 \times 10^4$	N.E
Vanadium	$2.5 \times 10^3$	$8.3 \times 10^3$	$4.6 \times 10^3$	N.E
Titanium	$2.4 \times 10^{-4}$	$1.2 \times 10^{-4}$	$1.1 \times 10^{-4}$	N.E
Calcium	$3.8 \times 10^4$	$1.3 \times 10^5$	$5.9 \times 10^4$	N.E
Alumina	0.014	$8.2 \times 10^{-3}$	$7.3 \times 10^{-3}$	N.E
Magnesium	$5.1 \times 10^3$	$2.4 \times 10^4$	$9.1 \times 10^3$	N.E

Table 4.4: Concentrations of metals (mg/kg dry matter)

 Table 4.5:
 Weights of dry sludge samples

Sample sites	PS	L21	L23
Weight (g)	0.30	0.16	0.30

#### 4. Results and Discussion

### Conclusion

Due to limitations in the measuring instruments the removal rates at the ammonia concentrations in the system could not be determined. However, there is ammonium oxidation in all aerated tanks which were confirmed during labscale activity tests with higher ammonia concentrations.

The amount of biodegradable carbon in the aerated tanks is enough to negatively impact nitrification and could be lowered with a particle trap and longer retention times in the OCR units.

The denitrification units fill up with sludge and the surface of the bio carriers is not utilized. The nitrate concentration in the system is close to the limit where negative effects on growth and feed intake appear. No clear denitrification could be observed from the activity test of the denitrification tanks. However, the activity test from the pump sump showed great capacity for denitrification. The efficiency of the denitrification tanks should be increased in order to achieve lower nitrate concentrations. A suggested improvement is agitation in the denitrification units in order to facilitate mass transfer.

The process contains high concentrations of organic carbon and generates a lot of sludge. All these particles reduces the water flow through pipes and tanks with poor mixing and accumulates within the system. This causes the pipes and tanks to clog which, in turn, demands more operational supervision. When the tanks clog the water and sludge becomes stationary and ammonia, which is toxic to the fish, is formed. To avoid these issues, some sort of particle trap for sludge removal is suggested. A drum filter would be a suitable particle trap due to small size but a simpler sedimentation unit might be preferred due to low cost.

The sludge contains metal levels that are far below the limit values for communal sludge. In this regard, the sludge is suitable for use in agriculture.

The microscopic investigation of the sludge from the pump sump and the process water showed contradictory results. The flocks had a varying structure and some higher organisms that are indications of high load of the process were present. On the other hand, the sludge was very active and organisms that indicate normal load and sufficient oxygen levels did occur to a large extent. The sludge from the denitrification tank showed very little activity. This confirms the result from the activity test and that the denitrification tanks are ineffective.

#### 5. Conclusion

### Bibliography

- Ungfors, A. Björnsson, T. Lindegarth, S. Eriksson, S. Wik, T och Sundell, S.K. (2015) Marin fisk odling på den svenska västkusten: Tekninska lösningar. Rapport från Vattenbrukscentrum Väst, Götebrg.
- [2] FAO. (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp. ISBN 978-92-5-109185-2
- [3] van Rijn, Jaap; Tal, Yossi; Schreier, Harold J. (2006) Denitrification in recirculating systems: Theory and applications, Aquacultural Engineering, Volume 34, Issue 3
- [4] C.I.M. Martins, E.H. Edinga, M.C.J. Verdegema, L.T.N. Heinsbroeka, O. Schneider, J.P. Blanchetond, E. Roque d'Orbcastel, J.A.J. Verretha. 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquacultural Engineering volume 43, 83–93.
- [5] Eikelboom, D.H. van Buijsen, H.J.J. (1981) Microscopic sludge investigation manual. Second edition. The Netherlands.
- [6] Henrik Tideström, HT. (2008) Slamregler i korthet. Svenskt vatten.se. http://www.svensktvatten.se/globalassets/avlopp-och-miljo/uppstromsarbeteoch-kretslopp/slamregler-i-korthet—kommentarer.pdf. (2018-05-14).
- Songming Zhu, Shulin Chen, Effects of organic carbon on nitrification rate in fixed film biofilters, Aquacultural Engineering, Volume 25, Issue 1, 2001, Pages 1-11, ISSN 0144-8609, https://doi.org/10.1016/S0144-8609(01)00071-1. (http://www.sciencedirect.com/science/article/pii/S0144860901000711)
- [8] Schram, E., Roques, J. A., Abbink, W., Yokohama, Y., Spanings, T., Vries, P., Bierman, S., Vis, H. and Flik, G. (2014), The impact of elevated water nitrate concentration on physiology, growth and feed intake of African catfish Clarias gariepinus (Burchell 1822). Aquac Res, 45: 1499-1511. doi:10.1111/are.12098
- [9] J. BOVENDEUR, E.H. EDING and A.M. HENKEN (1987), Design and Performance of a Water Recirculation System for High-Density Culture of the African Catfish, Clarias gariepinus (Burchell 1822), Aquaculture, 63 (1987) 329-353
- [10] Christopher Pohl, Maria Rey, Detlef Jensen, Jutta Kerth, Determination of sodium and ammonium ions in disproportionate concentration ratios by ion chromatography, Journal of Chromatography A, Volume 850, Issues 1–2, 1999, Pages 239-245, ISSN 0021-9673, https://doi.org/10.1016/S0021-9673(99)00002-3. (http://www.sciencedirect.com/science/article/pii/S0021967399000023)
- [11] Sabine Körner, Sanjeev K. Das, Siemen Veenstra, Jan E. Vermaat, The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to Lemna gibba, Aquatic Botany, Volume 71, Issue 1, 2001,

Pages 71-78, ISSN 0304-3770, https://doi.org/10.1016/S0304-3770(01)00158-9. (http://www.sciencedirect.com/science/article/pii/S0304377001001589)

- [12] Johanna Skogsberg Denitrifikationsförsök i laboratorieskala, GRYYAB, 1998, Page 10
- [13] Lustig, G (2012) Moving Bed Biofilm Reactors (MBBR) i Sverige Dimensionering och funktion, University of Lund
- [14] J. Lapinski, A. Tunnacliffe\* (2002) Reduction of suspended biomass in municipal wastewater using bdelloid rotifers. Water Research 37 (2003) 2027–2034
- [15] Wastewater treatment and reuse, (kolla upp rätt)
- [16] Edward Schram, Jonathan A.C. Roques, Wout Abbink, Tom Spanings, Pepijn de Vries, Stijn Bierman, Hans van de Vis, Gert Flik, The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus), Aquaculture, Volume 306, Issues 1–4, 2010, Pages 108-115, ISSN 0044-8486, https://doi.org/10.1016/j.aquaculture.2010.06.005. (http://www.sciencedirect.com/science/article/pii/S0044848610003704)
- [17] Ramsay, Ian R. and Pullammanappallil, Pratap C. Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry, Biodegradation, Volume 12, number 4, 2001, Pages 247-256 ISSN 1572-9729, https://doi.org/10.1023/A:1013116728817"