

Norwegian University of Life Sciences

Master's Thesis 2018 60 ECTS

Faculty of Science and Technology Main Supervisor: Odd-Ivar Lekang

Effluent Water Quality of Fish Laboratory at Norwegian University of Life Science (NMBU)

Weijian Dong Master of Science in Aquaculture Department of Animal and Aquaculture Sciences Faculty of Biosciences



Effluent Water Quality of Fish Laboratory at Norwegian University

of Life Science (NMBU)



Master Thesis 2018

Weijian Dong

Department of Animal and Aquaculture Sciences Faculty of Biosciences Faculty of Science and Technology Norwegian University of Life Sciences (NMBU)

Table of content

Ac	knowledgement	1
Ab	stract	2
Ab	breviations	3
1.	Introduction	4
2.	Literature review	5
	2.1 The introduction of aquaculture effluent	5
	2.2 The main content of aquaculture effluent	6
	2.2.1 Nutrient waste	6
	2.2.2 Particle waste	7
	2.3 Effluent regulation	8
	2.4 Water treatment in RAS	10
	2.4.1 Ammonia removal	10
	Biofilters	10
	Nitrification process	12
	The factors that affect nitrification	13
	Denitrification	14
	2.4.2 Particle management	14
	2.4.3 Disinfection	16
	2.5 Alternative treatment methods	17
3.	Fish laboratory in NMBU	
	3.1 Location of fish laboratory in NMBU	
	3.2 Water treatment system of fish laboratory in NMBU	
	3.2.1 Drum filter	20
	3.2.2 MBBR (Moving Bed Biofilm Reactor)	20
	3.2.3 Fixed bed	22
	3.2.4 Aeration	22
	3.2.5 Pump chamber	22
	3.2.6 The UV treatment	23

3.3 Effluent water and license25
4. Materials and methods
4.1 Routine records during the experiment period
4.2 Sampling of water
4.3 Measuring of water flow
4.4 Measurement of TN, TP, COD and TSS
5. Result and discussion
5.1 Water quality variation through the day
5.1.1 The result of experiment 1
5.1.2 The result of experiment 2
5.2 The effluent water quality comparison of experiment 1 and experiment 237
5.3 Estimated discharge amount from fish laboratory
5.4 The evaluation of TN, TP, COD and TSS concentration from effluent water
5.5 The evaluation of the effluent water quality from NMBU fish laboratory44
5.5.1 Water quality compare with old fish-lab44
5.5.3 Water quality compared with outlet of municipal plant45
5.5.2 Water quality compare with receiving water body
6. Conclusion
7. Reference
8. Appendix

List of figures

Figure 1: Particle removal in different removal methods	16
Figure 2: The model of RAS in fish laboratory of NMBU	18
Figure 3: The water flow model of RAS	19
Figure 4: The volume of RAS1 and 2	19
Figure 5: The volume of RAS 3	20
Figure 6: Mutag BioChip ™	21
Figure 7: RK BioElements Light	21
Figure 8: The model of pump chamber	23
Figure 9: UV reactor	24
Figure 10: Screen of UV reactor	25
Figure 11: Three types of effluent in fish-lab	25
Figure 12: The end of UV, sampling site	29
Figure 13: 1liter measuring cup	30
Figure 14: 5 liters container	30
Figure 15: Outlet pipe of drum filter	30
Figure 16: Graduated bucket	30
Figure 17: Photometer NOVA 60	31
Figure 18: Thermoreactor CR 3200	31
Figure 19: Pipettes and test-tube rack	32
Figure 20: Moisture Analyzer	33
Figure 21: Microfiber filter	33
Figure 22: Filter with vacuum gas pump	33
Figure 23: The comparison of TN, TP, TSS and COD concentration in	experiment1
and experiment2 at different time	
Figure 24: COD, TN, TP and SS in main effluent produced by 1kg feed	1 in old and
new fish lab. The amount of nutrient in old fish lab was showed as	s blue and new
fish lab was white	45

List of tables

Table 1: Nutrients excretion and retention rates (as percentages of the constituent
present in the feed consumed) in different species6
Table 2: A suggestion for aquaculture effluent concentration
Table 3: A suggestion concentration from International Finance Corporation
Table 4: General overview of the average TAN areal removal rate for frequently used
biofilters in aquaculture systems)11
Table 5: Technical specifications for RK (RK Plast A/S, Skive, Denmark)22
Table 6: Technical specifications for RK BioElement Heavy (RK Plast A/S, Skive,
Denmark)
Table 7: Technical specifications for UV reactor (WEDECO BX80e FAN)24
Table 8: Average system data for period 14.01-18.01 and 10.02-15.02 (The sum of
three RAS system)
Table 9: Feed consumption
Table 10: Standard accuracy of a measurement for three water parameters (mg/l)
(Merck KGaA, Darmstadt, Germany
(
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1
Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1

Table 18: Steams draining of Årungen and water and TP concentration	48
Table 19: The comparison between Brønnerudbekken and fish lab	48

Acknowledgement

First of all, I would like to express my sincere gratitude to my main supervisor, Odd-Ivar Lekang, and co-supervisor, Bjørn Frode Eriksen, for their guidance support, comments and encouragement in the process of completing my master thesis.

I would also express my heartfelt thanks to fish laboratory of NMBU and Department of Mathematical Sciences and Technology (IMT) for providing experiment laboratory and equipment. Writing and data analysis was done at the Department of Animal and Aquaculture Sciences (IHA)).

I would express my sincere thanks Mr. Bjørn Reidar Hansen in Fish Laboratory at Norwegian University of Life Sciences for his help in experiment stage and discussion of result.

I would like to express my gratitude to Yang Jing for his help in writing and my classmate Stian Gitmark and other friends who helped me.

Finally, a lot thanks to my family for supporting and encouragement.

Ås, May. 2018 Weijian Dong

Abstract

The fish laboratory at Norwegian University of Life Science (NMBU) is a modern center for fish experiment with a recirculation aquaculture system. The main effluent water is overflow from recirculation aquaculture system (RAS), the quality of overflow is equal to system water. However, the main effluent water (overflow) is required to be pump to the municipal water treatment plant. The objective of this study is to understand the water quality of the main effluent water by measuring concentration total nitrogen (TN), total phosphorus (TP), total suspended solid (TSS) and chemical oxygen demand (COD). Samples was taken every 2 hours from 7:00 to 19:00 at January 18th and February 15th2018. The result showed that the average TN, TP, TSS and COD concentration at January 18th was 5.59 mg/l, 0.21 mg/l, 0.61 mg/l and 16.03 mg/l respectively. The TN, TP, TSS and COD concentration at February 15th was 5.2 mg/l, 0.152 mg/l, 0.472 mg/l and 5.83 mg/l. The concentration of these parameters was lower compare with the outlet water of municipal waste treatment plant (Søndre Follo) in Vestby. This suggests that the treatment in Søndre Follo does not significantly increase the water quality of the main effluent water from fish laboratory at NMBU. However, it will load the municipal plant with water that is cleaner than the outlet. The amount of TP, TN TSS and COD produced by 1kg feed supplied was 17.17 g, 0.56 g, 1.71 g and 33.2 g, respectively. In addition, the amount of TP, TN, TSS and COD discharged with main effluent water was estimated at 23.9 kg, 0.79 kg, 2.37 kg and 46.1 kg per year. One possible solution is to set the effluent water to lake in close area called Årungen. The amount of TP discharge to Årungen with stream will be increased with 0.025%.

Abbreviations

AB: Autotrophic Nitrifying Bacteria

- BOD₅: 5-day Biochemical Oxygen Demand
- COD: Chemical Oxygen Demand
- C/N: Carbon to Nitrogen Ration
- DOC: Dissolved Organic Carbon
- FCR: Feed Conversion Ratios
- HB: Heterotrophic Bacteria
- MBBR: Moving Bed Biological Reactor
- RAS: Recirculation Aquaculture System
- **RBC:** Rotating Biological Contactor
- TAN: Total Ammonia Nitrogen
- TKN: Total Kjeldahl Nitrogen
- TN: Total Nitrogen
- **TP:** Total Phosphorus
- TSS: Total Suspended Solid

1. Introduction

In 2014, there were 73.8 million tons of fish produced by aquaculture, which accounted for 44.1% of world food fish production (FAO, 2016). As the resources of capture fisheries are limited, aquaculture will become more important for fish supply. Aquaculture produces large amount of effluent water while producing fish, which can have significant impacts on the environment (Buschmann et al., 2006; Dierberg & Kiattisimkul, 1996; Iwama, 1991; Sapkota et al., 2008; Wu, 1995). To meet the challenge from aquaculture effluent water, regulations of effluent are imposed by governments. The regulations are often strict, especially in developed countries like Norway (Asbjorn Bergheim & Brinker, 2003).

Recirculation aquaculture systems (RAS) is a system that reuses water by water treatment. RASs can not only reduce water consumption (Verdegem, Bosma, & Verreth, 2006), but also achieve a better environmental control on the aquaculture system (Ebeling & Timmons, 2012). However, most of the water treatment in RASs cannot achieve an "overall reduction in discharge", the waste materials are only relocated (Piedrahita, 2003). So, the effluent from RASs are generally high concentrated with waste materials. The waste components (constituents) are often divided into organic matters, TSS, nutrients etc. (Piedrahita, 2003).

Fish laboratory of Norwegian University of Life Sciences (NMBU) has 3 effluent water flows, overflow, backwashing of drum filter and tank flushing. Fish lab is required to pump all effluent water to municipal water treatment plant. However, the water quality of main effluent water (overflow) is equal to system water. Pump the cleaner overflow water with highly concentrated backwashing of drum filter and tank flushing might be inefficient and unnecessary. The objective of this study is to know the water quality of main effluent water (overflow) by measuring TN, TP, TSS and COD to know the concentration of nutrients, TSS and organic matters.

2. Literature review

2.1 The introduction of aquaculture effluent

The environmental concerns about aquaculture has been concluded as water pollution, destruction of sensitive aquatic habitat and agriculture land, negative impact of non-native species escape, disease spreading and salinization of water and land (Claude E Boyd, 2003). Effluent water is one of the most important consideration in the environmental impact of aquaculture.

Effluent water from aquaculture could cause negative impact to receiving water environment. One of the most common negative influence is eutrophication pollution result due to the high level of nutrient such as nitrogen, phosphorous compounds and carbon-based organic matters in the aquaculture effluent water. The high nutrient level can cause the blooms of phytoplankton in receiving water. The bacterial degradation of large amount of dead phytoplankton would consumes the oxygen in the water, which can cause the hypoxia of fish (Goldburg, Fund, & Triplett, 1997). Furthermore, there are many potential risks to human health should also be considered in aquaculture practices: antibiotic residues, antibiotic-resistant bacteria, metals, persistent organic pollutants etc. (Sapkota et al., 2008).

The main content that caused the environmental problem were identified as chemicals, biological pollutants and nutrient waste. Specifically, the waste material in aquaculture effluent water were also summarized as the following categories: dissolved and particulate organic matter, TSS, nutrients and some specific compounds (Crites & Technobanoglous, 1998; Piedrahita, 2003). These pollutants are mainly from uneaten feed, metabolic wastes, chemicals and therapeutics during aquaculture operation (Ackefors & Enell, 1990; Braaten, 1992).

2.2 The main content of aquaculture effluent

2.2.1 Nutrient waste

Not all of the nutrient in the feed can be used by fish because the limited digestion of fish (Amirkolaie, 2005). These uneaten and undigested nutrients is the main source of aquaculture nutrient waste. Feed conversion ratios (FCR) can be used to determined nutrient discharge from fish farm, good management to access maximum growth rate and minimum FCR to control the discharge of nutrient (Einen, Holmefjord, Åsgård, & Talbot, 1995).

Nitrogen and phosphorus was the main nutrient components in aquaculture effluent. The nutrient retention and excretion are various from different species and feed is showed in table 1 (Piedrahita, 2003).

Numerous of study indicated that most N is excreted in the dissolved form and most P is in particulate form (Bureau & Cho, 1999; Skonberg, Yogev, Hardy, & Dong, 1997; Sugiura, Raboy, Young, Dong, & Hardy, 1999). Furthermore, van Rijn (2013) has concluded from several studies about different species fish that 60-90% of nitrogen waste is dissolved in the water. And 25-85% of phosphorus is excreted in the fecal waste. One study (Dalsgaard, Larsen, & Pedersen, 2015) on rainbow trout Nitrogen waste has indicated that 81.6% of TN waste was dissolved nitrogen.

Table 1: Nutrients excretion and retention rates (as percentages of the constituent present in the feed consumed) in different species (Piedrahita, 2003)

Retained		In feces (particulate)		Excreted (dissolved)		Type of fish	Reference
N	Р	N	Р	N	Р		
49	36	14	55	37	9	A. salmon	Johnsen et al., 1993; Bergheim and Åsgård, 1996
	17 - 19		48 - 54		28 - 34	A. salmon	Holby and Hall, 1991
11	32					Carp	Avnimelech and Lacher, 1979
27	30					Channel catfish	Boyd, 1985
10	40	35	15	55	45	Sea bass	Lemarié et al., 1998
30		10		60		Sea bream	Porter et al., 1987
19-26						Sea bream	Krom et al., 1995
30		13		57		Rainbow trout	Beveridge et al., 1991
25	30	15	70	60	0	Rainbow trout	Håkanson, 1988; Pillay, 1992
21-22	18.8	3.6 - 5.4	19 - 22	59-72	60 - 62	Tilapia hybrid	Siddiqui and Al-Harbi, 1999

Phosphorus (P) waste is a major concern in aquaculture (Bureau & Cho, 1999). The reason is that phosphorus is the limiting nutrient for the growth of aquatic plants in fresh water, high level of phosphorus could result in eutrophication and algal bloom (Talbot & Hole, 1994). Dissolved phosphorus could be taken up rapidly (within minutes) by Bacteria and phytoplankton (Levine, Stainton, & Schindler, 1986) Short-term leaching rates of P from feeds and feces were reported by Phillips et al (Phillips, Clarke, & Mowat, 1993). They reported that up to 10% of TP may be leached from feces and feed in a 30m deep water column. Higher leaching rates was showed in feces compared with feed in this study.

The nutrient waste can be reduced by improvement of diet formulation. A review concluded that Nitrogen waste could be controlled by reducing digestible protein to digestible energy rate and Phosphorus waste could be reduced through by increase the digestible phosphorus content of phosphorus (C. Y. Cho & Bureau, 2001).

2.2.2 Particle waste

Solids can not only clog the gills of fish but also provide habitat for micro-organisms. Accumulation of suspended solids has significant negative impact on nitrification activity, which can reduce the TAN removal in the system (Andersson, Aspegren, Parker, & Lutz, 1994; Michaud, Blancheton, Bruni, & Piedrahita, 2006).

There are many factors affect the solids in culture water: type of fish, feeding factor, feed management, variation in solid load and flow management (Shulin Chen, Timmons, Aneshansley, & Bisogni, 1993). Different type of fish has different feeding ability, which can lead to different amount of uneaten feed. Factor of feed is also important, good feed can produce less uneaten feed. Feeding in good quality can improve the efficiency of nutrient utilization. For example, improved modern diet formulations could produce less than 150 kg solid waste for one metric ton of salmonid fish production (C Young Cho & Bureau, 1997).

One study (Shulin Chen et al., 1993) about suspended solids characteristic showed an average particle weight of 10.6*10⁻⁷ mg constituted 40-70% of TSS by weight. More than 95% of suspended solids in RAS was in low diameter (<20µm). The possible factors can affect particle size distribution has been identified: feed pellet integrity, dust content and physical characteristic and daily management such as tank washing (Kelly, Bergheim, & Stellwagen, 1997; Patterson, Watts, & Timmons, 1999). However, the particle size distribution was not directly affected by feed regime in flow through system. The water could be break down by turbulence in the system. For example, a waterfall at end of fish farm has been reported can reduce the particle size and higher removal performance in drum filter after removing the water fall (Brinker & Rösch, 2005). Furthermore, biofilter used in aquaculture system can also affect the particle distribution. A study (Fernandes, Pedersen, & Pedersen, 2017) showed a 10% reduction of particle concentration, particle surface area and particle volume in water sample through FBBR. One the other hand a 10% increase of total particle area and particle concentration was showed in water through MBBR, but on effect on particle volume. In other words, MBBR can increase number of fine particles but cannot remove particles. That might because the particle disintegration result from vigorous aeration and mixing process in the moving bed. On the other hand, FBBR can reduce particle concentration, particle surface area and particle volume. Because the fixed bed can catch the solid in the water.

A review (C. Y. Cho & Bureau, 2001) about formulation strategies and feeding system to reduce excretory and feed wastes indicate that reduction of solid waste can be achieved by careful selection of the ingredients and the nutrient balance of the feed.

2.3 Effluent regulation

Environmental regulations for aquaculture effluent varies greatly from country to country. It might because of the various differences in environment, aquaculture technology, species and the water quality of the natural water bodies. Most of legislation for the aquaculture effluent control in land-based farms still obey the rule from a Germany review (Asbjorn Bergheim & Brinker, 2003; Rosenthal, 1994).

Asbjorn Bergheim and Brinker (2003) reviewed the environmental regulation of several countries in EU (Germany, Denmark and UK) and Norway. the Denmark regulation has been described that required suspended solids less than 3 mg/l, TP less than 0.05 mg/l, TN less than 0.6 mg/l, BOD5 less than 1mg/l and the oxygen saturation should be more than 60% saturation. The regulation also includes a rule for sampling and feed composition, which required nitrogen less than 9% and phosphorus less than 1% in feed (Rosenthal, 1994).

Although the regulation of aquaculture effluent depends on different situation. There was still some suggestion for aquaculture effluent concentration can be found (Table 2 and Table 3). A target standard was reported that TP should be less than 0.3mg/l, TAN and TSS should be less than 3mg/l and 50mg/l (C. E. Boyd & Gautier, 2000). The maximum concentration for TN, TP, TSS and BOD₅ has been suggested at 10 mg/l, 2 mg/l, 50 mg/l and 50 mg/l respectively.

Table 2: A suggestion for aquaculture effluent concentration (C. E. Boyd & Gautier,2000)

Variable	Initial standard	Target standard
pH (standard units)	6.0–9.5	6.0–9.0
Total suspended solids (mg/l)	100 or less	50 or less
Total phosphorus (mg/l)	0.5 or less	0.3 or less
Total ammonia nitrogen (mg/l)	5 or less	3 or less
5-Day biochemical oxygen demand	50 or less	30 or less
(mg/l)		
Dissolved oxygen (mg/l)	4 or more	5 or more

Parameter/pollutant	Maximum value
рН	6 to 9
BOD5	50 mg/l
Oil and grease	10 mg/l
Total suspended solids	50 mg/l
Total phosphorus	2 mg/l
Total nitrogen	10 mg/l

Table 3: A suggestion concentration from International Finance Corporation (International Finance Corporation, 2007)

2.4 Water treatment in RAS

In indoor RAS system, the effluent treatment is often achieved within the recirculating loop. In RAS system, the basic treatments are ammonia removal, particle removal. Typical Recirculating aquaculture system (RAS) include waste solids removal, Ammonia and nitrite nitrogen control, dissolved gas management, and disinfection (Losordo, Masser, & Rakocy, 2000).

2.4.1 Ammonia removal

Biofilters

There are many methods could be utilized to remove ammonia nitrogen from water. Biological filtration is the widely used in RAS for ammonia nitrogen removal which can use nitrifying bacteria to oxidize ammonia into nitrate.

There are several types of biofilter to remove the ammonia nitrogen. Rotating biological contactor (RBC), tricking filters, expandable media filters, fluidized bed filters and mixed bed reactors have been used in RAS (Losordo et al., 2000). A review article (Crab, Avnimelech, Defoirdt, Bossier, & Verstraete, 2007) compared rotating biological contactors, trickling filters, bead filters and fluidized sand biofilters in RAS (Table 4). Rotating can achieve the highest TAN areal removal rate with highest while fluidized sand biofilter had the lowest removal rate with lowest cost.

RBC are widely used in aquaculture water treatment as biofilter (Brazil, 2006). The rotating biological contactor has low head requirements to move water through the

vessel. This advantage implies passive aeration and carbon dioxide removal, and low chance of clogging.

Table 4: General overview of the average TAN areal removal rate for frequently used biofilters in aquaculture systems (Crab, Avnimelech, Defoirdt, Bossier, & Verstraete, 2005)

Biofilter type	Average TAN areal removal rate	Cost	References
	(g TAN/m2 day)	(Euro/kg year)	
Rotating	0.19-0.79	1.143	Miller and Libey, 1985; Brazil,
biological			2006
contactor			
Trickling	0.24-0.64	1.036	Kamstra et al., 1998; Schnel et
filter			al., 2002; Eding et al., 2006;
			Lyssenko and Wheaton, 2006
Bead filter	0.30-0.60	0.503	Greiner and Timmons, 1998;
			Timmons et al., 2006a
Fluidized	0.24	0.198	Miller and Libey, 1985;
sand			Timmons and Summerfelt, 1998
biofilter			

Moving bed biofilters or moving bed biofilm reactor(MBBR) are quite popular in RAS. Timothy (Pfeiffer & Wills, 2011) has evaluated three types of plastic media in MBBR, the highest percent of TAN removal was 12.3% and 14.4% in different feed loads. MBBR was developed in Norway in the late 1980s and early 1990s (Odegaard, Rusten, & Siljudalen, 1999; Ødegaard, Rusten, & Westrum, 1994). MBBR is widely used in municipal and industrial waste water treatment. Application of MBBR in aquaculture has been successful in Atlantic salmon smolt production, brown trout, arctic char juveniles productions and etc. (Rusten, Eikebrokk, Ulgenes, & Lygren, 2006). The TAN removal rate of MBBR influenced by many factors such as temperature, organic loading, dissolved oxygen, TAN concentration, pH and alkalinity (Rusten et al., 2006). The advantage of MBBR are continuously operating

(no need for backwashing), no-clog biofilm, low head loss and high specific biofilm surface. The capacity of MBBR could be adjusted by degree of filling, maximum filling degree is around 70% (Ødegaard et al., 1994).

Anaerobic ammonium-oxidizing (Anammox) technology is a new technology which can transform TAN directly to nitrogen gas (Gut, Płaza, Trela, Hultman, & Bosander, 2006).

Nitrification process

There are two forms ammonia: NH3 and NH4+ (Ionized ammonia and unionized ammonia). The sum of the two forms called total ammonium nitrogen (TAN). The maximum safe concentration of un-ionized ammonia is unknown, but in many cases, it is not close to the 0.0125 mg/L value commonly accepted by fish culturists (Meade, 1985). Ionized ammonia and unionized ammonia are in equilibrium depending on the pH and the temperature (Timmons, Ebeling, Wheaton, Summerfelt, & Vinci, 2002). Both ionized ammonia and unionized ammonia may be toxic to fish. Unionized ammonia is more toxic form (Körner, Das, Veenstra, & Vermaat, 2001).

Nitrification was widely applied to control the amount of ammonia in RAS. The process of nitrification could be concluded as follow:

Ionized ammonia(NH_4^+) oxidized into nitrite(NO_2^-) by autotrophic bacteria, Nitrosomonas is the most important autotrophic bacteria 1). Nitrite is then oxidized to the much less toxic nitrate(NO_3^-) by several other bacteria, the most important of which is Nitrobacteria 2) (USEPA, 1984; WPCF, 1983).

$$NH4^{+} + 1.5O_2 \rightarrow 2H^{+} + H2O + NO_2^{-} \quad 1)$$

$$NO_2^- + 0.5 O2 \rightarrow NO_3^-$$
 2)

The complete nitrification process can be express as:

 $NH4^{+} + 1.83O_{2} + 1.98HCO_{3}^{-} \rightarrow 0.021C_{5}H_{7}O_{2}N + 1.041H_{2}O + 0.98NO_{3}^{-} + 1.88H_{2}CO_{3}^{-} 3)$

The factors that affect nitrification

Nitrification in the bacterial film of the biofilter is affected by a variety of parameters such as substrate and dissolved oxygen concentrations, organic matter, temperature, pH, alkalinity, salinity and turbulence level (Shulin Chen, Ling, & Blancheton, 2006). The growth of bacteria depend on the nutrient in the water. The most frequent limiting factor for heterotrophic bacteria has been indenticated to be carbon, whereas nitrogen and are seldom limiting (Leonard, Guiraud, Gasset, Cailleres, & Blancheton, 2002). The competition from heterotrophic bacteria is an important consideration in biofilter design and management. Heterotrophic bacteria (HB) have competition with autotrophic nitrifying bacteria (AB) for oxygen and space. Moreover, the by-products of metabolic of HB may cause the diseases of fish (Leonard et al., 2002; Nogueira, Melo, Purkhold, Wuertz, & Wagner, 2002). And the negative impact from heterotrophic bacteria should be controlled for a higher nitrification efficiency.

The most possible important factor to control heterotrophic bacteria population is the quantity of feces reaching the biofilter. So, the possible solution to control the population of heterotrophic bacteria is remove the feces as more as possible. Because the dissolved organic carbon (DOC) was not the limiting factor for HB growth (Leonard et al., 2002).

The organic carbon/inorganic nitrogen (C/N) ratio shows the availability and competition of organic carbon and ammonia in the water. Generally, heterotrophic bacteria out-compete nitrifying bacteria for oxygen and space when the C/N ratio is high. The occasion AB can out-compete HB is that the C/N ration is relative low in the biofilter water environment. However, the critical C/N ratio affecting the nitrification rate varies among systems and is related to the characteristics of the organic carbon available. One study for submerged biofilter found that TAN removal rate at 0.5 C/N ratio was 30% lower than that C/N ratio at 0 (Michaud et al., 2006). Another experiment on fixed film biofilter showed similar result, solution with C/N = 1.0 or 2.0 resulted in approximately a 70% reduction of TAN removal rate as

compared with the solution with C/N = 0 and have similar inorganic nitrogen amount. Moreover, one research demonstrated that extension of hydraulic retention time in biofilter with nitrification and organic carbon removal may not be effective (Nogueira et al., 2002).

Denitrification

Nitrate concerntration could be high in RAS when the recirculating degree was high. Nitrate is less toxic than nitrite. But in certain occasion, nitrate can be toxic to fishes. The toxicity of nitrate have been reported in variance, maximum concentration in freshwater was reported at 96hLC50s >1000mg/l nitrate nitrogen (Colt, 2006). The nitrate toxicity for marine species has been tested (Pierce, Weeks, & Prappas, 1993). Marine white spot disease has been linked to nitrate concentrations above 30 mg nitrogen per liter (Burgess, 1995). Denitrification process is a traditional way to reduce nitrogen pollution in agricultural, domestic and industrial wastewater streams that threaten eutrophication of surface waters. By means of denitrification, oxidized inorganic nitrogen compounds, such as nitrite and nitrate are reduced to elemental nitrogen (N2). The process is conducted by facultative anaerobic microorganisms with electron donors derived from either organic (heterotrophic denitrification) or inorganic sources (autotrophic denitrification). Due to the low efficiency in removal and high cost, the application of anaerobic denitrification is not wide in aquaculture water treatment. Generally, the nitrate in RAS system are removed by water exchange (Christianson, Lepine, Tsukuda, Saito, & Summerfelt, 2015; Menasveta et al., 2001; Singer, Parnes, Gross, Sagi, & Brenner, 2008; Zhu et al., 2015).

2.4.2 Particle management

The solids removal is to remove solids in high flow and low concentration aquaculture waste water. Many methods can be used to remove particles, but the size of the removed particles varies (Figure 1). The sedimentation can only remove solids size $>100\mu$ m, tube settle can remove solids size $>75\mu$ m and rotating micro screen can

remove the solids > $30\mu m$. The largest removal method was media filter, which can remove solids size >15 μm .

Mechanical filtration is widely used to remove the solids waste. The advantage is that minimal space was used to remove particles. Typical mechanical filter used in aquaculture are drum filter, disk filter and inclined belt filter (Timmons et al., 2002). All these three filters use microscreen to remove solids. Particle with size that larger than mesh size of screen could be removed by physical restriction when water go through the microscreen. The mesh size of filters determined the size of particle that can be removed. However, smaller size solids can also be captured when several small size particle bridges together (Ebeling & Timmons, 2012).

Twarowska, Westerman, and Losordo (1997) reported a 41% suspended solids removal efficiency on a rotating drum filter with 60 µm screen mesh size.

The mesh size is not as small as possible in practical treatment, because too small mesh size can limit water quality by breaking down large particles. Drum filter have been indicated that could result in the fine particles ($<20 \mu m$) dominates (Shulin Chen et al., 1993). Another reason is the higher investment and low and cost are caused by larger pressure loss and more frequently backwashing (Cripps & Bergheim, 2000; Dolan, Oliver, Murphy, & O'Hehir, 2011).

The removal of solids can also reduce the particle-bond nutrients and organic matters. One study (Sindilariu, Brinker, & Reiter, 2009) has analyzed treat efficiency of two drum filters with 80µm and 63µm mesh size in a partial aquaculture reuse system showed that both two microgreens had a statistical significant treatment effect on particulate matter TSS, BOD₅, COD and TP. An average treatment efficiency of 60-µm mesh size drum filter has been reported: SS (67–97%), TP (21–86%) and TN (4–89%) (Cripps & Bergheim, 2000). However, mechanical filtration has low efficiency in the reduction of dissolved nutrients (Cao et al., 2007; Schulz, Gelbrecht, & Rennert, 2003). A removal of 95.8-97.3% TSS, 64.1-73.8% of COD, 49%-68.5% of TP and 20.6%-41.8% TN was reported in another study (Schulz et al., 2003). In addition, Continuous backwashing can be used to ensures an unblocked screen to achieve a maximum flow rates (Dolan et al., 2011).



Figure 1: Particle removal in different removal methods (St Chen, Stechey, & Malone, 1994; Cripps & Bergheim, 2000)

2.4.3 Disinfection

In RAS, particle removal could reduce the organic load such as TSS, BOD and COD in the water but pathogenic and other micro-organisms cannot be removed efficiently (Hassen et al., 2000). Ozone are mainly used in RAS to disinfect, remove organic carbon for improving water quality. The advantage is that ozonation has rapid reaction and few harmful reaction by-products. In addition, the end-product is oxygen which can supply supersaturated levels of dissolved oxygen that will increase the culture tank carrying capacity (S. T. Summerfelt, 2003). Ultraviolet (UV) irradiation is considered as a credible alternative to chemical disinfection, because of the UV irradiation does not produce toxic by-products (Hassen et al., 2000). In RAS, UV irradiation can be used to destroy ozone residual and to denature the DNA of microorganisms to make them die or lose their function (Rodriguez & Gagnon, 1991)

Ozone residuals are destroyed at UV light wavelengths ranging from 250 to 260 nm, while microorganism inactivation can be achieved at UV wavelengths ranging from 100 to 400 nm, although a wavelength of 254 nm is most effective.

2.5 Alternative treatment methods

There are some techniques for treating effluent water from aquaculture system. For example, using the food crops to clean aquaculture effluents. It requires the plants to remove nutrients to low levels without a reduction in productivity and quality. Treatment of fishery effluent using hydroponic crop production represents a potentially profitable secondary enterprise for the aquaculture producer (Adler, Harper, Takeda, Wade, & Summerfelt, 2000). A pilot unit was constructed in the existing wastewater treatment plant at El Mansoura governorate located in north Egypt. The optimum dose of coagulants used in the combined unit gives removal efficiencies for COD, BOD, and TP as 65%, 55%, and 83%, respectively (Ismail, Fawzy, Abdel-Monem, Mahmoud, & El-Halwany, 2012).

Constructed wet land are wildly used in treatment of aquaculture effluents. This treatment method showed good performance on the nutrient fractions containing particulate matter (Schulz et al., 2003; Sindilariu, Schulz, & Reiter, 2007; S. T. Summerfelt, Adler, Glenn, & Kretschmann, 1999). One study (S. T. Summerfelt et al., 1999) reported that in vertical flow and horizontal flow wetlands removed 98% and 96% TSS, 91 and 72% total COD, and 81and 30% dissolved COD. Both types of wetland cell removed most (82-93%) of TKN, phosphorus and dissolved phosphate.

3. Fish laboratory in NMBU

3.1 Location of fish laboratory in NMBU

The study site was in fish laboratory of Norwegian University of Life Sciences (NMBU). The fish laboratory has three fresh water RAS for treating the water.

3.2 Water treatment system of fish laboratory in NMBU

There are two main parts: fish tank and water treatment. The water in the fish tank will flow to the water treatment units then pump back to fish tank.

The water treatment system includes drum filter, MBBR, fixed bed and UV treatment unit. This system is to remove the particle, ammonia and micro-organism of the outlet water from fish tank to make water can be reused.

The model of RAS is showed in figure 2 and figure 3. Water from fish tank treated by the Drum filter, MBBR 1 and 2 and Fixed bed in turn. Water flow in drum filter was from the top to bottom. Water in MBBR was up flow and in fixed bed was down flow.



Figure 2: The model of RAS in fish laboratory of NMBU(Sterner AS)



Figure 3: The water flow model of RAS

The volume of each chamber in RAS 1and 2 is showed in figure 4, the volume of drum filter was 2.4 m³, the volume of biofilter chamber 1 and chamber 2 was 1.8m² and 1.6m² respectively, and the fixed bed volume was 1.8m². The whole volume of one treatment unit was 10.3 m³. The volume of each chamber in RAS 3 is showed in figure 5, the volume of drum filter was 2.4 m³, the volume of biofilter chamber 1 and chamber 2 was 0.9 m² and 0.7m² respectively, and the fixed bed volume was 1.8m². The difference between RAS 1, RAS 2 and RAS 3 in volume was that RAS 1and 2 has about 2 times larger volume for MBBR.



Figure 4: The volume of RAS1 and 2



Figure 5: The volume of RAS 3

3.2.1 Drum filter

Water from fish tank mix with the new inlet water and then flow into the water treatment unit from drum filter. Drum filter is one type of mechanical filter. The function is to remove the organic particles inside the RAS system by the screen, which can secure a low and stable concentration of organic matter for keeping the biofilter have an optimal performance. The removed particle discharged by back-flushing water of drum filter.

The water filtered by a 40μ m-mesh drum filter (NP Innovation AB, F802) before going to the biofilter.

Well-designed filter can remove 60-80 percent of organic matter such as BOD₅. Transmission is also important, because turbulence can help to preserve organic matters.

3.2.2 MBBR (Moving Bed Biofilm Reactor)

In RAS system, the function of the biofilter is nitrification, which can remove ammonia. Nitrification process can oxidize the ammonia into nitrate. Nitrifying bacteria were established on the filter media and growing in the biofilm. And it shows in both water and filter media. Denitrification are used in high intensity RAS to remove nitrate. In fish laboratory, moving bed biofilter are used, which were heavily aerated by air pump to provide air for the bacteria growing. Furthermore, there are 2MBBR chambers (1.8m³ and 1.6m³) in one RAS. Both chambers were fill with two type of filter medias: Mutag BiochipTM (Umwelttechnologie AG, Germany) (Figure 6) and RK Bioelements (RK Plast A/S, Skive, Denmark) (Figure 7). The bacteria Nitrosomonas established biofilm on the filter medias. The MBBR only function with nitrification, nitrate controlled by water exchange.

Mutag BioChip [™] is sheet and round. Because of its fine pore structure in the surface, active growth area is more than 3000 m²/m³. These chips provide an optimal condition for the bacteria.



Figure 6: Mutag BioChip TM



Figure 7: RK BioElements Light

As showed in table 5, RK BioElements Light have a density of 0.93 g/cm3 can be used in the moving bed biofilter. In addition, the specific surface area is 750 m² per m³ and the volume weight is 158 kg/m3.

Volume weight (kg/m3)	158
Number (pcs/m3)	255.000
Specific surface area (m2/m3)	750

Table 5: Technical specifications for RK (RK Plast A/S, Skive, Denmark)

3.2.3 Fixed bed

After the MBBR treatment, the water flows into the fixed bed biofilm reactor which is filled with filter media (RK BioElements Heavy). The fixed bed is one kind of down-flow fixed-bed. The aim of the fixed bed is to remove the peeled biofilm and particles. Water from MBBR flow from top of fixed bed chamber to the bottom. Filter media can catch the fine particles and peeled biofilm efficiently.

RK BioElements Heavy have a density of 1.20 g/cm3 and are used primarily in "down-flow fixed-bed" filters.

Table 6: Technical specifications for RK BioElement Heavy (RK Plast A/S, Skive, Denmark)

Volume weight (kg/m3)	210
Number (stk/m3)	255.000
Specific surface area (m2/m3)	750

3.2.4 Aeration

After fixed bed, water flows to a single chamber for aeration. The aim is effective convection of water.

3.2.5 Pump chamber

Pump chamber (Figure 8) located after the aeration chamber. There are two ways that water can flow out of the treatment unit. Water flow in this chamber is up-flow. Most of water can be pump out from the bottom of the chamber and a small percent of water goes to out from overflow pipe (main effluent).



Figure 8: The model of pump chamber (Sterner AS)

3.2.6 The UV treatment

Water was pumped into UV reactor (WEDECO BX80e FAN) after fixed bed (Figure 9). The UV reactor can kill the micro-organisms by disrupt their DNA structure. The aim is to control the micro-organisms and Pathogens.

Table 7 shows the specification of the UV reactor. Maximum flow rate, minimum UV dose, reactor volume and etc. was list on the table to show the capacity of this reactor. Parameters can be read on a monitor connected to UV (Figure 10).

After the treatment of UV reactor, water will be pump to the fish tank.

Max. flow rate	30m3/h
Min. UV dose	400J/m2
Min. UV transmission	50%
Operating pressure	0-16bar
Water temperature	+5-45
Reactor volume	51 liters
Rating	65

 Table 7: Technical specifications for UV reactor (WEDECO BX80e FAN)



Figure 9: UV reactor (WEDECO BX80e FAN)



Figure 10: Screen of UV reactor (WEDECO BX80e FAN)

3.3 Effluent water and license

There are 3 types of effluent water flow in fish lab (Figure 11):

- 1. Main effluent water: discharged in the end of RAS as overflow.
- 2. Back-flushing water from the drum filter.
- 3. Tank flushing water



Figure 11: Three types of effluent in fish-lab

Fish laboratory in NMBU has license A A 0001 (appendix 1). This license requires fish-lab to transport all the effluent water to municipal waste water treatment plant. It costs around 20nok to treat $1m^2$ water and around 240000nok per year. This is expensive and means a lot for the fish labs economy.

4. Materials and methods

4.1 Routine records during the experiment period

The fish tanks were regularly cleaned by brushing and flushing during the routine work of fish lab. The average tank flushing flow was 1.2 l/min.

The average system data was from the routine record of fish lab. Total biomass, daily feeding and water flow during the experimental period was showed in table 8. In the period of experiment 1, the average total biomass was 405 kg, the average daily feeding was 8.1 kg, the average total flow was 730 l/min, the average make up flow was 18 l/min and the average back flushing flow was 0.7 l/min. In experiment 2, the average total biomass was 380 kg, the average daily feeding was 5.8 kg, the average total flow was 650 l/min, the average make up flow was 16 l/min and the average back flushing flow was 16 l/min and the average back flushing flow was 16 l/min and the average back flushing flow was 16 l/min and the average back flushing flow was 16 l/min and the average back flushing flow was 16 l/min and the average back flushing flow was 0.6 l/min.

Obviously, there are more fish in fish-lab during experiment 1 than that During experiment 2. It is reasonable that more feed consumption and higher water flow in the system. In addition, the recirculation degree for the sum of three RAS during the experiment was calculated as follow:

 $Recirculation \ degree = (Total \ flow - Make \ up \ flow)/(Total \ flow)$

Substituting the corresponding value into the formula gives the result that recirculation degree during experiment 1 was 97.534% and the degree during experiment 2 was 97.538%. So, the average recirculation degree of two experiment was 97.536%.

Main effluent flow from fish lab of NMBU was calculated as follow:

Main effluent flow

= Make up flow – Backfluwsh flow – Tank flushing flow

In experiment 1, the make-up water flow was 18 l/min, backflush flow of drum filters was 0.7 l/min and tank flushing flow were 1.2 l/min. These number were average data for period 14th January to 18th January, and these number were the sum of three RASs. So, the effluent flow was 16.1 l/min (966 l/h, 23184 liter/day)

In experiment 2, the make-up water flow was 16 l/ min, backflush flow of drum filters was 0.6 l/min and tank flushing flow were 1.2 l/min. These number were average data for period 10th February to 15th January, and these number were the sum of three RASs. So, the effluent flow was 14.2 l/min (852 l/h, 20448 liter/day)

The feed used in fish lab were various due to the different feed are used for different experiment. But, the percentages of phosphorous and nitrogen by weight could be assumed as 1.2% and 6.5%, respectively. The feed composition was showed in table 9.

	Experiment 1	Experiment 2	Mean
Total biomass	405 kg	380 kg	392.5 kg
Daily feeding	8.1 kg	5.8 kg	6.95 kg
Total flow	730 l/min	650 l/min	690 l/min
Make-up water	18 l/min	16 l/min	17 l/min
Backflush of drum filters	0.7 l/min	0.6 l/min	0.65 l/min
Flushing fish tank flow	1.2 l/min	1.2 l/min	1.2 l/min

Table 8: Average system data for period 14.01-18.01 and 10.02-15.02 (The sum of three RAS system).

Table 9: Feed consumption

Year	Feed amount (kg)
2016	1325
2017	1450

4.2 Sampling of water

There were 2 experiments in this study:

Experiment 1 (Jan.18): Samples was taken every 2 hours from 9:00 to 19:00, the TN, TP, TSS and COD was measured after sampling. Every parameters were measured with 3 replicates per sample.

Experiment2 (Feb.15): The repetition of experiment 1.

After the treatment of RAS, the treated water was divided into two parts. Most of the water were transferred to UV treatment unit and then pumped to fish tank. The rest water was the main effluent water, which was connected with the pipe to municipal plants. The main effluent water is difficult to collect because the water was pumped to municipal plant. Hence, water samples were taken from outlet of UV treatment unit (Figure 12). The aim of this study is to analyze the main effluent water quality in fish lab. The water sample was taken from 3 sampling sites by a 11iter measuring cup (Figure 13). In experiment 1, 1.5L water samples were taken from samplings, and mix in a 5L container (Figure 14) to get a 4.5L mixed water sample. In experiment 2, water samples were mix in two 5L container to get 9L (4.5L per container) mixed sample by the same way as experiment1. Because the TSS measurement in experiment 1 cost 1L water per test and in experiment 2 cost 2L water per measurement.

The 3 sites were from different RAS system:

• Site1: Overflow of RAS1, at end of UV.

- Site2: Overflow of RAS2, at end of UV.
- Site3: Overflow of RAS3, at end of UV

Samples were taken after the water flowing out for a while to remove the solid accumulated in the outlet when it takes off. The 5L container and measuring cup was washed by the water from UV. And the water samples were mixed well before every single measurement.



Figure 12: The end of UV, sampling site





Figure 13: 1liter measuring cup

Figure 14: 5 liters container

4.3 Measuring of water flow

The water flow was measured in every sampling day. The make up flow was measured directly by flowmeter. The average water flow of drum filter back-flushing water is measured by collecting the back-flushing water in an outlet pipe (Figure 15) by a graduated bucket (Figure 16) in a certain time (1 hour). The tank flushing water flow was average number from routine record of fish lab.





Figure 15: Outlet pipe of drum Figure 16: Graduated bucket filter

4.4 Measurement of TN, TP, COD and TSS

TN, TP ad COD were analyzed immediately after sampling. All these three parameters determination were measured by Spectroquant® Photometer NOVA 60(Merck KGaA, Darmstadt, Germany) (Figure 17) following the standard procedure (appendix 2, 3 and 4) on three different test kits. During these three measurements, two thermo-reactors (Figure 18) is required to heat the cells at a certain temperature for a certain time in guidance of the standard procedure. COD measurement required 148°C for 2 hours, TN and TP measurement need 100°C (120°C) for 1hour and 0.5hour respectively. Other equipment was also necessarily used during analyzing period, including pipettes, test-tube racks (Figure 19), gloves etc. In addition, the standard test kit for TN is determined by measuring the parameters ammonium, nitrite and nitrate nitrogen. The organic nitrogen requires the additional decomposition of the sample. Therefore, organic nitrogen was not measured in this study.



Figure 17: Photometer NOVA 60



Figure 18: Thermoreactor CR 3200



Figure 19: Pipettes and test-tube rack

According to documents given by the producer of test kits, all measurements had technical accuracy and measuring range (Table 10).

Table 10: Standard accuracy of a measurement for three water parameters (mg/l)(Merck KGaA, Darmstadt, Germany

Test kits	ТР	TN	COD
Measuring range	0.05-5	0.050-15.0	4-40
Accuracy of measurement	Max.±0.08	Max.±0.50	Max.±1.5

TSS was measured by the standard method (Federation & Association, 2005). A well-mixed sample is filtered through a weighed standard glass-fiber filter, the residue retained on the filter is dried to a constant weight at 103 to 105 degrees. The increase in the weight of the filter represents the total suspended solids. The filter paper in this study was glass microfiber filter (Whatman®, grade GF/A) with diameter 47 mm (Figure 21). The water sample was filtered by vacuum filter (Figure 22) and weighed by a moisture analyzer (Figure 20).



Figure 20: Moisture Analyzer

Figure 21: Microfiber filter



Figure 22: Filter with vacuum gas pump

5. Result and discussion

5.1 Water quality variation through the day

5.1.1 The result of experiment 1

After taking six sample from 09:00 to 18:00 with three repetitions per sample at January 18th2018. The result with average number and standard deviation of four parameters were expressed in table 11 to show the water quality of main effluent water from fish lab. All results of TN, TP and COD were in the measuring range and accuracy according to standard accuracy of a measurement (See table 10).

The TN concentration was 4.40 ± 0.35 mg/l (9:00), 6.07 ± 0.12 mg/l (11:00), 5.73 ± 0.25 mg/l (13:00), 5.80 ± 0.10 mg/l (15:00), 5.90 ± 0.36 mg/l (17:00) and 5.67 ± 0.12 mg/l (19:00) respectively. The average TN of these 6 samples was 5.59 ± 0.60 mg/l. The concentration of TN at 9:00 was obviously lower than the other results. Then the number increased to 6.07 at 11:00, which is highest in these 6 results. The amount stayed nearly stable around from 13:00 to 17:00.

The TP concentration was 0.197 ± 0.006 mg/l (9:00), 0.203 ± 0.006 mg/l (11:00), 0.203 ± 0.006 mg/l (13:00), 0.207 ± 0.006 mg/l (15:00), 0.213 ± 0.023 mg/l (17:00) and 0.233 ± 0.006 mg/l (19:00) respectively. The average TP of these 6 samples was 0.21 ± 0.015 mg/l. The concentration of TP was staying in a low level during the experiment period compared with other parameters. As showed in table 11, the highest concentration was 0.233 mg/l at 19:00 while the lowest concentration was 0.197 mg/l at 9:00.

The result of COD was 17.43 ± 0.55 mg/l (9:00), 18.17 ± 0.59 mg/l (11:00), 18.27 ± 0.59 mg/l (13:00), 17.67 ± 0.67 mg/l (15:00), 11.87 ± 1.21 mg/l (17:00) and 12.80 ± 0.28 mg/l (19:00) respectively. The average COD of these 6 samples was 16.03 ± 2.75 mg/l. The concentration from 9:00 to 15:00 was nearly stable. However, after 15:00, the number of COD concentration drop from 17.67 (15:00) to 11.87(17:00), and then increased to 12.80(19:00).

The TSS concentration was 0.33 ± 0.58 mg/l (9:00), 0.67 ± 0.58 mg/l (11:00), 1.33 ± 0.58 mg/l (13:00), 0.33 ± 0.58 mg/l (15:00), 0.33 ± 0.58 mg/l (17:00) and 0.67 ± 0.58 mg/l (19:00) respectively. The weight of suspended solids was stable, and the concentration in 13:00 was higher than other time points.

The water quality during the experiment was quite stable in terms of TN and TP. The standard deviations were around 10 percent of average numbers. Regarding of COD, the concentration at 17:00 and 19:00 was much lower than another sample. By analyzing the variation of these six samples, the main effluent variation through the day can be roughly inferred that TN and TP concentration is in a quite stable state and the standard deviation is at a lower level. These two parameters were lower at 9:00, this might be affected by routine tank flushing in the morning. The standard deviation of TSS was very high, this probably because of the inconsistent distribution of TSS in the water in small quantities. TSS content was so low that it was difficult to get accurate concentration.

Time	TN (mg/l) ^a	TP (mg/l) ^a	COD (mg/l) ^a	TSS (mg/l) ^a
9:00	4.40 ± 0.35	0.197 ± 0.006	17.43 ± 0.55	0.33 ± 0.58
11:00	6.07 ± 0.12	0.203 ± 0.006	18.17 ± 0.59	0.67 ± 0.58
13:00	5.73 ± 0.25	0.203 ± 0.006	18.27 ± 0.59	1.33 ± 0.58
15:00	5.80 ± 0.10	0.207 ± 0.006	17.67 ± 0.67	0.33 ± 0.58
17:00	5.90 ± 0.36	0.213 ± 0.023	11.87 ± 1.21	0.33 ± 0.58
19:00	5.67 ± 0.12	0.233 ± 0.006	12.80 ± 0.28	0.67 ± 0.58
Average value ^b	5.59 ± 0.60	0.210 ± 0.015	16.03 ± 2.75	0.61 ± 0.61

Table 11: The TN, TP, TSS and COD concentration of water samples in experiment 1

a: Mean \pm Standard deviation (mg/l)

b: The average value was calculated from all of the replicates in the 6 samples during the experiment 1.

5.1.2 The result of experiment 2

The experiment 2 was repetition of experiment 1. The date was February 15th2018. All the measurement was same except suspended solids (2L water was filtered in one measurement). The water samples were taken at 9:00, 11:00, 13:00, 15:00, 17:00 and 19:00. The result of four parameters was showed as mean number and standard deviation in table 12 to show the water quality variance during 9:00 to 19:00. All the results of measurement of TN, TP and COD were in the measuring range and accuracy according to standard accuracy of a measurement (See table 10).

The TN concentration was expressed at 5.00 ± 0.36 mg/l (9:00), 4.33 ± 1.68 mg/l (11:00), 4.97 mg/l ±0.76 (13:00), 5.43 ± 0.23 mg/l (15:00), 5.57 ± 0.25 mg/l (17:00) and 5.90 ± 0.35 mg/l (19:00) respectively. The average TN concentration of these 6 samples was 5.20 ± 0.82 mg/l. The concentration of TN was varied from 4.33 mg/l to 5.90 mg/l (11:00 and 19:00) in these samples. Three replicates in TN at 11:00 were 5.4 mg/l, 5.2 mg/l and 2.4 mg/l. One of the results was 2.4 mg/l, which is 2 times smaller than another two repetitions. This number resulted in larger standard deviation at 11:00. So, this repetition was reasonable to be remove out of the analysis. If only consider two repetitions at 9:00, the average number will be 5.3 mg/l and the variance of theses 6 sample will be 0.1.

The average TP concentration was 0.157 ± 0.006 mg/l (9:00), 0.157 ± 0.006 mg/l (11:00), 0.147 ± 0.006 mg/l (13:00), 0.150 ± 0.000 mg/l (15:00), 0.153 ± 0.006 mg/l (17:00) and 0.153 ± 0.000 mg/l (19:00) respectively. The average TP concentration of these 6 samples was 0.152 ± 0.005 mg/l. The TP concentration was stable during the experiment period.

The result of COD was 5.53 ± 0.67 mg/l (9:00), 5.80 ± 0.10 mg/l (11:00), 6.03 ± 0.35 mg/l (13:00), 6.03 ± 0.49 mg/l (15:00), 5.93 ± 0.50 mg/l (17:00) and 5.20 ± 0.29 mg/l (19:00) respectively. In addition, the average COD of these 6 samples was 5.83 ± 0.42 mg/l.

The result of TSS measurement was $0.33\pm0.29 \text{ mg/l}$ (9:00), $0.67\pm0.29 \text{ mg/l}$ (11:00), $0.17\pm0.29 \text{ mg/l}$ (13:00), $0.50\pm0.50 \text{ mg/l}$ (15:00), $0.33\pm0.29 \text{ mg/l}$ (17:00) and $0.83\pm0.76 \text{ mg/l}$ (19:00) respectively. The average TSS concentration of these 6 samples was $0.47\pm0.44 \text{ mg/l}$.

Compare with experiment 1, smaller variation through the day was showed in experiment 2. After removing the abnormal replicate of TN at 11:00, TN, TP and COD were in a stable state, the variation among the samples was small. Concentration about TSS was discussed in experiment 1 that it was difficult to get high accuracy concentration in low TSS concentration. Combining the results of two experiments, it can be inferred that the main effluent water quality of fish lab was stable through day. The average concentration of all replicates for TN, TP, COD and TSS could be regard as the average value of the whole day.

	TN (mg/l)	TP(mg/l)	COD(mg/l)	TSS(mg/l)
9:00	5.00 ± 0.36	0.157 ± 0.006	5.53 ± 0.67	0.33 ± 0.29
11:00	4.33 ± 1.68	0.157 ± 0.006	5.80 ± 0.10	0.67 ± 0.29
13:00	4.97 ± 0.76	0.147 ± 0.006	6.03 ± 0.35	0.17 ± 0.29
15:00	5.43 ± 0.23	0.150 ± 0.000	6.03 ± 0.49	0.50 ± 0.50
17:00	5.57 ± 0.25	0.153 ± 0.006	5.93 ± 0.50	0.33 ± 0.29
19:00	5.90 ± 0.35	0.150 ± 0.000	5.63 ± 0.29	0.83 ± 0.29
Average value ^a	5.20 ± 0.82	0.152 ± 0.005	5.83 ± 0.42	0.47 ± 0.44

Table 12: The TN, TP, TSS and COD concentration of water samples in experiment 2

a: Mean \pm Standard deviation (mg/l)

b: The average value was calculated from all of the replicates in the 6 samples during the experiment 2.

5.2 The effluent water quality comparison of experiment 1 and experiment 2

As showed in Figure 23, TN concentration in experiment 1 was higher than experiment 2, at 11:00, 13:00, 15:00 and 17:00, but lower than experiment at 9:00 and 19:00. The average concentration experiment 1 was 5.59 mg/l while the concentration of experiment 2 was 5.2 mg/l. It is reasonable that experiment 1 had a higher concentration in TN because of higher feed amount and biomass. However, experiment 1 had about 40% higher feed amount than experiment 2 (8.1kg in experiment1 and 5.8kg in experiment2). The reason might be different nutrient concentration in the feed, the feed used in experiment 1 may have lower concentration of TN.

The TP concentration in experiment 1 was around 33% higher than that in experiment 2 while 40% higher feeding amount was added. It may also because of the higher feed amount and biomass.

The COD concentration in experiment1 was 3 times higher than experiment 2 at 9:00, 11:00 and 13:00 and 2 times higher than experiment 2 at 15:00 and 17:00. The reason of large difference at only 40% more feeding amount could be one feed experiment was running in one of the RASs in fish lab during experiment 1, the feed in this experiment were easier to be dissolved. The fixed bed of RAS 3 was cleaned at 17th January, which could be another reason for the much higher COD concentration in experiment 1 (Hansen, 2018).

Regarding for TSS, experiment 1 has the same concentration as experiment 2 at 9:00, 11:00 and 17:00. The TSS concentration in experiment 1 was higher than experiment 2 at 13:00 while the concentration was lower at 15:00 and 19:00. The average concentration in experiment 1 are a bit higher than experiment 2.





9:0011:0013:0015:0017:0019:00

Figure 23: The comparison of TN, TP, TSS and COD concentration in experiment1 and experiment2 at different time.

5.3 Estimated discharge amount from fish laboratory

The amount of constituents discharged with the main effluent was showed in table 13. The number was calculated from average number of parameters produced by 1kg feed and the feed consumption per year. In experiment 1, around 0.016 kg TN was produced per kg feed supplied, while slightly more TN (0.018 kg) was produced in experiment 2. Similarly, more TSS was generated in experiment 2 (0.00175kg) than in experiment 1 (0.00166kg). On the other hand, slightly less TP was produced in experiment 2 than experiment 1, which were 0.00054 kg and 0.00060kg respectively. The amount of COD in effluent was over 2 times higher in experiment1 than in expriment2, which was0.46kg and 0.021kg respectively per kg feed. The constituents amount provided by inlet flow were assumed as 0, because the amount of inlet flow only occupied about 2.5% of total flow and the concentration of constituents is very low (Hansen, 2018). To know the effluent loading (constituents discharged per kg fish production) from fish lab, FCR was assumed as 1.25. The hypothetical effluent loading would be 25% higher than the amount of constituent's amount produced by 1 kg feed. This amount is in range of that Norwegian RAS effluent loading that reported by Asbjørn Bergheim (2013). The average of discharge of TN, TP, TSS and COD was estimated at 0.12kg/day, 0.004 kg/day, 0.012kg/day and 0.245kg/day, respectively. And the amount discharged by 1 kg biomass for TN, TP, TSS and COD was 300.03 mg/day, 10.1 mg/day, 30.2 mg/day and 615 mg/day, respectively.

	The amount discharged (kg/day)		
	Experiment 1 ^a	Experiment 2 ^a	Averaged
COD	0.37172	0.11917	0.24544
TN	0.12971	0.10633	0.11802
ТР	0.00486	0.00311	0.00398
TSS	0.01417	0.00966	0.01191
	The amount prod	uced per kg feed supplied	(kg)
	Experiment 1 ^b	Experiment 2 ^b	Averaged
COD	0.04589	0.02055	0.03322
TN	0.01601	0.01833	0.01717
ТР	0.00060	0.00054	0.00057
TSS	0.00175	0.00166	0.00171
	The amount prod	uced by 1kg biomass (mg/	day)
	Experiment 1 ^c	Experiment 2 ^c	Averaged
COD	917.81900	313.59579	615.70739
TN	320.25090	279.81474	300.03282
ТР	11.98953	8.19116	10.09034
TSS	34.98272	25.41053	30.19662

Table 13: The estimate amount of TN, TP, TSS and COD produced in fish lab per day

a: The discharge amount per day $(kg/day) = average \ concentration \ (mg/l) * effluent amount (l/day) *1000000 \ (mg/kg)$

b: The amount produced by 1kg feed supplied (*kg/kg*) = (*discharge amount per day* (*kg/day*)) / (*feed supplied per day* (*kg/day*))

c: The amount produced by 1kg biomass (kg/(kg*day)) = ((discharge amount per day (kg/day) / (total biomass(kg))/1000000

Furthermore, the amount of feed consumption per year was 1325 kg in 2016 and 1450kg in 2017. Based on the feed consumption per year and the average amount of constituent in main effluent water produced by 1 kg feed, a predicted TN, TP, TSS and COD discharge amount was calculated. The predicted amount discharge per year was 23.82kg TN, 0.791kg TP, 2.37kg TSS and 46.09kg COD (Table 14).

Parameters	Amount(kg) 2016 ^a	Amount(kg) 2017 ^a	Predicted
			amount(kg) ^b
COD	44.0165	48.169	46.09275
TN	22.75025	24.8965	23.82338
ТР	0.75525	0.8265	0.790875
TSS	2.259125	2.47225	2.365688

Table 14: The estimate amount of TN, TP, TSS and COD discharged with main effluent per year based on the amount produced by 1 kg feed supply

a: The discharge amount was calculated as: The amount produced by 1kg feed supplied (kg)* The feed consumption per year (kg).
b: The predicted amount was the average amount of 2016 and 2017.

5.4 The evaluation of TN, TP, COD and TSS concentration from effluent water

The average concentration of COD, TN, TP and SS during the day is showed in table 15. The average concentrations were calculated based on concentrations in experiment 1 and experiment 2. These average number were regard as the average of the whole day.

The average concentration of TN was 5.59 ± 0.60 mg/l and 5.2 ± 0.55 mg/l, in experiment 1 and 2 respectively. The TN concentration was lower than the hypothetical effluent nitrogen concentration of fully recirculating system without treatment, and a bit lower than the hypothetical effluent nitrogen concentration for partial reuse system that calculated by Piedrahita (2003). It is reasonable that the TN concentration of main effluent is lower than the hypothetical concentration without treatment even the hypothetical concentration was calculated from different FCR and feed content, because the main effluent water was treated by RAS in fish lab and the water quality is good enough to be reused in aquaculture system. In fish lab, the reduction of TN is mainly achieved by solids removal. However, most of TN is in dissolved form (Bureau & Cho, 1999; Skonberg et al., 1997; Sugiura et al., 1999). Therefore, TN can be accumulated in RASs in forms of nitrate nitrogen. The TN

concentration was low compared with two RASs described by Martins, Pistrin, Ende, Eding, and Verreth (2009). This may because of lower biomass loading and feed loading of fish lab.

The TP concentration in fish lab was in a low level (0.21 mg/l and 0.152 mg/l in experiment 1 and 2), which was much lower than the hypothetical effluent phosphorus concentration (Piedrahita, 2003). Most of TP was in particulate form (van Rijn, 2013), which can be removed efficiently by microscreen of drum filter (Sindilariu et al., 2009). Therefore, the leftover TP in main effluent was mostly in forms of dissolved phosphorus (usually as orthophosphate) that from feed leaching and excretion. Orthophosphates is the form of phosphorus that is most readily utilized by biota (Phytoplankton and bacteria) (Claude E Boyd & Musig, 1981). Although the TP concentration was at a low level, the environmental impact still needs to be considered because the phosphorus is the limiting nutrient for growth of phytoplankton in fresh water (Talbot & Hole, 1994).

Although both TN and TP in fish lab were concentrated, the concentration was still in range suggested by International Finance Corporation (2007) and C. E. Boyd and Gautier (2000), and also lower than the outlet concentration of one intensive flow-through system with biofilter reported by Asbjorn Bergheim and Brinker (2003). Daily feed amount was 8.1 kg and 5.8 kg during period of experiment 1 and 2. Percentages of nitrogen in the feed was assumed to be 6.5% as the commercial feed. Percentages of TP in the feed was assumed to be 1.2% due to the various feed utilized in fish lab for different experiment. This percentage was higher than typical dietary phosphorus requirements in most fish and crustacean feeds (0.3-0.8%) (Peñaflorida, 1999). The percentage of TN and TP left in the main effluent water from 1kg feed was calculated at 24.6% and 5% in experiment1, 28.2 % and 4.5% in experiment 2 and 26.4% and 4.75% in average. This percentage means around 26.4% of nitrogen and 4.75% of phosphorus in 1kg feed were discharged with main effluent water from RAS. The percentage of TN was higher than TP, because of most of Nitrogen waste

was dissolved nitrogen which cannot be removed by RASs (Dalsgaard et al., 2015; van Rijn, 2013). Furthermore, the nitrification in MBBR cannot remove the dissolved TN. It is reasonable that a higher percentage of TN left in the main effluent water. The percentage for TP is in the range 0 to 62% (the percentage of dissolved P in 1 kg feed consumed), this percentage of N is less than the range 37% to 72% (the percentage of dissolved N in 1 kg feed consumed) that reported by Piedrahita (2003).

The COD concentration is the parameter to see how much oxygen consumed to chemical oxidize the organic compounds in the main effluent water (Kim et al., 2001). The high level of organic matter can consume the dissolved oxygen in the receiving water bodies and could cause the negative impact on environment (Goldburg et al., 1997). The average concentration of COD in fish lab was 16.03 mg/l in experiment 1 and 5.83 mg/l in experiment 2. The COD concentration was in a low level compare with the effluent from flow through systems and RASs (Asbjorn Bergheim & Brinker, 2003; Suhr & Pedersen, 2010; Suresh & Lin, 1992).

A large percentage of TSS was removed by the 40µm mesh size drum filter in the system. That is the reason why the TSS concentration in main effluent was 0.61 mg/l and 0.472 mg/l which was in a very low level (Asbjorn Bergheim & Brinker, 2003; Piedrahita, 2003; S. T. Summerfelt et al., 1999). Moreover, the TSS had a similar concentration with make up water reported by R. C. Summerfelt and Penne (2005)

In addition, the TP concentration was in a normal range, while the TN concentration was much higher and TSS concentration was much lower, after comparing with the Norwegian flow through farms (Asbjorn Bergheim & Brinker, 2003). The lower TSS concentration is easy to explain by great solid removal in RAS of fish lab. Even higher percent of TP was removed with TSS, the concentration of TP was similar with flow through system. The higher TN and TP in fish lab may be resulted from accumulation in RAS. In addition, the TN and TP concentration is still much higher than the strict Danish environmental regulation (Asbjorn Bergheim & Brinker, 2003).

However, removal of nutrients in low concentration would require high cost and investment. The method that use the food crops to clean effluents could be a possible solution to reduce the TN and TP in the main effluent water (Adler et al., 2000).

	Experiment 1 ^a	Experiment 2 ^a	Average number ^b
COD (mg/l)	16.03 ± 2.75	5.83 ±0.42	10.93
TN (mg/l)	5.59 ± 0.60	5.2 ± 0.82	5.395
TP (mg/l)	0.21 ±0.015	0.152 ± 0.005	0.181
TSS (mg/l)	0.61 ± 0.61	$0.472\pm\!\!0.44$	0.541

Table 15: The average concentration of experiment 1 and experiment2

a: Mean \pm standard deviation, calculated from all measurements in experiment 1 and 2.

b: The average number was calculated from mean of experiment 1 and 2.

5.5 The evaluation of the effluent water quality from NMBU fish laboratory

5.5.1 Water quality compare with old fish-lab

Compared with old fish-lab, the new fish-lab has better capacity in effluent treatment. The quantity amount of constituents in main effluent produced by 1kg feed in old fish lab is much higher than new fish lab. As showed in Figure 24, the amount of COD from 1kg feed in old fish lab was almost 3 times than that in new fish lab. TP was 5 times more in old fish lab than new lab. And the performance of TN has 50% higher amount in old fish-lab. This indicates that new fish lab has better design than old one. For example, new fish-lab has a fixed bed after MBBR, the new fish lab may have higher capacity due to larger volume in RAS (3 RAS in new fish-lab compare with 1 RAS in old fish-lab).

The average TP concentration in fish lab was lower than that in old fish lab. However, the number of TN and COD was higher. That could because of the different recirculation. Recirculation degree in old fish-lab has been calculated at 94%. And the degree in new fish-lab during the experiment period was around 97.5%. Furthermore,

old fish lab has higher amount of effluent. The volume of overflow from RAS was 34 m^3 /day in old fish-lab and around 21.8 m^3 /day (during experiment period), even there were more fish in new lab.

The estimated discharge of COD, TN, TP and TSS was 46.10kg, 23.82kg, 0.79kg and 2.37 kg per year. The amount in old fish-lab was much higher at same feed consumption, which means that if the feed consumption is the same, old fish-lab would discharge more pollutants.

Table 16: COD, TN, TP and SS in main effluent produced by 1kg feed in old and new fish lab.

	New fish lab	Old fish lab ^a
COD (kg)	0.0332	0.0883
TN (kg)	0.0172	0.0287
TP (kg)	0.00057	0.00327
TSS (kg)	0.00171	/

a: From Tran (2014)





5.5.3 Water quality compared with outlet of municipal plant

The license requires fish lab to pump all of the effluent water to the municipal plant. However, the main effluent water of fish lab has much lower concentration of TN, TP and COD compared with the outlet of municipal waste water treatment plant (Søndre Follo). The concentration of TN, TP and COD was 41.57mg/l, 0.3mg/l and 10.93mg/l in the outlet water of Søndre Follo (Table 17), which was around 7.7 times higher in TN concentration, around 66 percent higher in TP concentration and almost 8 times higher in COD than main effluent water concentration of fish lab (Figure 25).

The main effluent pumped to and treated in Søndre Follo could not improve the water quality. By contrast, the main effluent water could dilute the backwashing water from drum filter and tank flushing water, which with high sludge and pollutants concentration (Shulin Chen, Coffin, & Malone, 1997). The treatment efficiency of municipal plants may be reduced.

The pumping of main effluent would also raise the pressure of pipe lines due to the limitation and high investment of new pipe lines. Pipes should be given priority to more polluted wastewater.

	Søndre Follo	Fish lab	
TP (mg/l)	0.3	0.181	
TN (mg/l)	41.57	5.395	
COD (mg/l)	82	10.93	

Table 17: The comparison between main effluent water of fish lab and outlet water of Søndre Follo in TN, TP and COD



Figure 25: The comparison of water quality between Søndre Follo outlet water and main effluent water of fish lab. Søndre Follo showed around 7.7 times higher TN concentration, around 66 percent higher TP concentration and almost 8 times higher COD than effluent water of fish lab.

5.5.2 Water quality compare with receiving water body

One possible solution is to set the main effluent water to the lake in the close area called Årungen through the small steam "Brønnerudbekken". As showed in Table 18, The water average TP concentration was 0.065 mg/l in this steam. This steam draining 400000m³ water with 26kg phosphorus into Årungen per year (Borch, Yri, Løvstad, & Turtumøygard, 2007). In addition, Brønnerudbekken was the water steam with lowest phosphorus concentration in all steams draining into Årungen.

Assuming discharge all the main effluent water into Brønnerudbekken, the concentration of phosphorus in Brønnerudbekken would not change much. Discharging 7962 m³ effluent water with 0.790875kg phosphorus into Brønnerudbekken would only increase the average TP from 0.065 mg/l to 0.06567 mg/l. The change of TP in Brønnerudbekken would not be obvious. The change in Årungen would be less obvious. The TP discharge to Årungen with steam draining will be increased with 0.025%. Therefore, new fish lab will apply for license for release main effluent water directly into Årungen through Brønnerudbekken could be a possible solution.

Locality	Discharge (mill	Average TP (µg/l)	Kg P/year
	m3/year)		
Bølstadbekken	12.2	115	1430
Storgrava	4	138	552
Smedbølbekken	3.5	85	298
Vollebekken	1	437	437
Norderåsbekken	1.3	158	205
Brønnerudbekken	0.4	65	26
Others	2	100	200
Sum	24.4		3121

Table 18: Steams draining of Årungen and water and TP concentration (Borch et al., 2007)

Table 19: The comparison between Brønnerudbekken and fish lab

	Brønnerudbekken	Fish lab
Discharge (mill m3/year)	0.4	0.007
Average TP (µg/l)	65	181
Kg P/year	26	0.79

6. Conclusion

The water quality of the main effluent water in fish laboratory have been measured in concentration of TP, TN, TSS and COD. The result showed that the average TN, TP, TSS and COD concentration at January 18th was 5.59 mg/l, 0.21 mg/l, 0.61 mg/l and 16.03mg/l respectively. The TN, TP, TSS and COD concentration at February 15th was 5.2 mg/l, 0.152 mg/l, 0.472 mg/l and 5.83mg/l. These concentrations were in a low level as the culturing water. Based on the result of measurement, the amount of TN, TP, TSS and COD produced by 1kg feed supplied was 17.17 g, 0.56 g, 1.71 g and 33.2 g, respectively. In addition, the amount of TN, TP, TSS and COD discharged with main effluent water was estimated at 23.9 kg, 0.79 kg, 2.37 kg and 46.1 kg when 1387 kg feed was consumed.

Compared with the outlet water of municipal waste treatment plant in the 4 parameters that was measured in this study, the water quality in main effluent water of fish lab was better. This means the treatment in Søndre Follo could not improve the water quality of the main effluent water from fish lab. By contrast, it will load the municipal plant with water that is cleaner than the outlet. One possible solution is to set the effluent water to lake in close area called Årungen. The amount of TP discharge to Årungen with stream will be increased with 0.025%.

7. Reference

- Ackefors, H., & Enell, M. (1990). Discharge of nutrients from Swedish fish farming to adjacent sea areas. *Ambio*, 28-35.
- Adler, P. R., Harper, J. K., Takeda, F., Wade, E. M., & Summerfelt, S. T. (2000). Economic evaluation of hydroponics and other treatment options for phosphorus removal in aquaculture effluent. *HortScience*, 35(6), 993-999.
- Amirkolaie, A. K. (2005). *Dietary carbohydrate and faecal waste in the Nile tilapia* (*Oreochromis niloticus L.*).
- Andersson, B., Aspegren, H., Parker, D., & Lutz, M. (1994). High rate nitrifying trickling filters. *Water science and technology*, 29(10-11), 47-52.
- Bergheim, A. (2013). Comparing water consumption and waste load in RAS and FTS farms in Canada Iceland Norway.
- Bergheim, A., & Brinker, A. (2003). Effluent treatment for flow through systems and European environmental regulations. *Aquacultural Engineering*, 27(1), 61-77.
- Borch, H., Yri, A., Løvstad, Ø., & Turtumøygard, S. (2007). Tiltaksplan for Årungen. *Bioforsk Rapport*, 2, 52-56.
- Boyd, C. E. (2003). Guidelines for aquaculture effluent management at the farm-level. *Aquaculture*, 226(1-4), 101-112.
- Boyd, C. E., & Gautier, D. (2000). Effluent composition and water quality standards. *Global Aquaculture Advocate*, *3*(5), 61-66.
- Boyd, C. E., & Musig, Y. (1981). Orthophosphate uptake by phytoplankton and sediment. *Aquaculture*, 22, 165-173.
- Braaten, B. (1992). Impact of pollution from aquaculture in six Nordic countries. Release of nutrients, effects, and wastewater treatment. Special Publication, European Aquaculture Society[SPEC. PUBL. EUR. AQUACULT. SOC.]. 1992.
- Brazil, B. L. (2006). Performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. *Aquacultural Engineering*, 34(3), 261-274.
- Brinker, A., & Rösch, R. (2005). Factors determining the size of suspended solids in a flow-through fish farm. *Aquacultural Engineering*, *33*(1), 1-19.
- Bureau, D., & Cho, C. (1999). Phosphorus utilization by rainbow trout (Oncorhynchus mykiss): estimation of dissolved phosphorus waste output. *Aquaculture*, 179(1-4), 127-140.
- Buschmann, A. H., Riquelme, V. A., Hernández-González, M. C., Varela, D., Jiménez, J. E., Henríquez, L. A., . . . Filún, L. (2006). A review of the impacts of salmonid farming on marine coastal ecosystems in the southeast Pacific. *ICES Journal of Marine Science*, 63(7), 1338-1345.
- Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., & Diana, J. (2007). Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environmental Science and Pollution Research -International*, 14(7), 452-462. doi:10.1065/espr2007.05.426

- Chen, S., Coffin, D. E., & Malone, R. F. (1997). Sludge production and management for recirculating aquacultural systems. *Journal of the World Aquaculture Society*, 28(4), 303-315.
- Chen, S., Ling, J., & Blancheton, J.-P. (2006). Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural engineering*, *34*(3), 179-197.
- Chen, S., Stechey, D., & Malone, R. (1994). Suspended solids control in recirculating aquaculture systems. *Developments in aquaculture and fisheries science*, 27, 61-61.
- Chen, S., Timmons, M. B., Aneshansley, D. J., & Bisogni, J. J. (1993). Suspended solids characteristics from recirculating aquacultural systems and design implications. *Aquaculture*, 112(2-3), 143-155.
- Cho, C. Y., & Bureau, D. P. (1997). Reduction of waste output from salmonid aquaculture through feeds and feeding. *The Progressive Fish-Culturist*, 59(2), 155-160.
- Cho, C. Y., & Bureau, D. P. (2001). A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research*, *32*, 349-360. doi:10.1046/j.1355-557x.2001.00027.x
- Christianson, L., Lepine, C., Tsukuda, S., Saito, K., & Summerfelt, S. (2015). Nitrate removal effectiveness of fluidized sulfur-based autotrophic denitrification biofilters for recirculating aquaculture systems. *Aquacultural Engineering*, 68, 10-18.
- Colt, J. (2006). Water quality requirements for reuse systems. Aquacultural Engineering, 34(3), 143-156.
- International Finance Corporation. (2007). *Environmental, health, and safety guidelines for fish processing* Retrieved from http://documents.worldbank.org/curated/en/959521484218191411/Environme ntal-health-and-safety-guidelines-for-fish-processing
- Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., & Verstraete, W. (2005). *Nitrogen removal techniques in aquaculture for a sustainable production* (Vol. 270).
- Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., & Verstraete, W. (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270(1-4), 1-14.
- Cripps, S. J., & Bergheim, A. (2000). Solids management and removal for intensive land-based aquaculture production systems. *Aquacultural Engineering*, 22(1), 33-56.
- Crites, R., & Technobanoglous, G. (1998). Small and decentralized wastewater management systems: McGraw-Hill.
- Dalsgaard, J., Larsen, B. K., & Pedersen, P. B. (2015). Nitrogen waste from rainbow trout (Oncorhynchus mykiss) with particular focus on urea. *Aquacultural Engineering*, 65, 2-9.
- Dierberg, F. E., & Kiattisimkul, W. (1996). Issues, impacts, and implications of shrimp aquaculture in Thailand. *Environmental Management*, 20(5), 649-666.

- Dolan, E., Oliver, R., Murphy, N., & O'Hehir, M. (2011). A Test Method for Optimal Micro-screen Drum Filter Selection.
- Ebeling, J. M., & Timmons, M. B. (2012). Recirculating aquaculture systems. *Aquaculture Production Systems*, 245-277.
- Einen, O., Holmefjord, I., Åsgård, T., & Talbot, C. (1995). Auditing nutrient discharges from fish farms: theoretical and practical considerations. *Aquaculture Research*, 26(9), 701-713.
- FAO, U. (2016). The State of World Fisheries and Aquaculture, 2016. 3.
- Federation, W. E., & Association, A. P. H. (2005). Standard methods for the examination of water and wastewater. *American Public Health Association* (APHA): Washington, DC, USA.
- Fernandes, P. M., Pedersen, L.-F., & Pedersen, P. B. (2017). Influence of fixed and moving bed biofilters on micro particle dynamics in a recirculating aquaculture system. *Aquacultural Engineering*, 78, 32-41.
- Goldburg, R., Fund, E. D., & Triplett, T. (1997). Murky waters: Environmental effects of aquaculture in the United States.
- Gut, L., Płaza, E., Trela, J., Hultman, B., & Bosander, J. (2006). Combined partial nitritation/Anammox system for treatment of digester supernatant. *Water science and technology*, 53(12), 149-159.
- Hansen, B. R. (2018, May 2018). [Personal communication].
- Hassen, A., Mahrouk, M., Ouzari, H., Cherif, M., Boudabous, A., & Damelincourt, J. J. (2000). UV disinfection of treated wastewater in a large-scale pilot plant and inactivation of selected bacteria in a laboratory UV device. *Bioresource technology*, 74(2), 141-150.
- Ismail, I. M., Fawzy, A. S., Abdel-Monem, N. M., Mahmoud, M. H., & El-Halwany, M. A. (2012). Combined coagulation flocculation pre treatment unit for municipal wastewater. *Journal of Advanced Research*, 3(4), 331-336.
- Iwama, G. K. (1991). Interactions between aquaculture and the environment. *Critical Reviews in Environmental Science and Technology*, 21(2), 177-216.
- Kelly, L., Bergheim, A., & Stellwagen, J. (1997). Particle size distribution of wastes from freshwater fish farms. *Aquaculture International*, *5*(1), 65-78.
- Kim, Y.-C., Sasaki, S., Yano, K., Ikebukuro, K., Hashimoto, K., & Karube, I. (2001). Photocatalytic sensor for the determination of chemical oxygen demand using flow injection analysis. *Analytica Chimica Acta*, 432(1), 59-66.
- Körner, S., Das, S. K., Veenstra, S., & Vermaat, J. E. (2001). The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to Lemna gibba. *Aquatic botany*, 71(1), 71-78.
- Leonard, N., Guiraud, J., Gasset, E., Cailleres, J., & Blancheton, J. (2002). Bacteria and nutrients—nitrogen and carbon—in a recirculating system for sea bass production. *Aquacultural Engineering*, 26(2), 111-127.

- Levine, S., Stainton, M., & Schindler, D. (1986). A radiotracer study of phosphorus cycling in a eutrophic Canadian Shield lake, Lake 227, northwestern Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 43(2), 366-378.
- Losordo, T. M., Masser, M. P., & Rakocy, J. (2000). *Recirculating aquaculture tank production systems*: Southern Regional Aquaculture Center.
- Martins, C. I., Pistrin, M. G., Ende, S. S., Eding, E. H., & Verreth, J. A. (2009). The accumulation of substances in Recirculating Aquaculture Systems (RAS) affects embryonic and larval development in common carp Cyprinus carpio. *Aquaculture*, 291(1-2), 65-73.
- Meade, J. W. (1985). Allowable ammonia for fish culture. *The Progressive Fish-Culturist*, 47(3), 135-145.
- Menasveta, P., Panritdam, T., Sihanonth, P., Powtongsook, S., Chuntapa, B., & Lee, P. (2001). Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock. *Aquacultural Engineering*, 25(1), 35-49.
- Michaud, L., Blancheton, J.-P., Bruni, V., & Piedrahita, R. (2006). Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquacultural Engineering*, 34(3), 224-233.
- Nogueira, R., Melo, L. s. F., Purkhold, U., Wuertz, S., & Wagner, M. (2002). Nitrifying and heterotrophic population dynamics in biofilm reactors: effects of hydraulic retention time and the presence of organic carbon. *Water research*, 36(2), 469-481.
- Odegaard, H., Rusten, B., & Siljudalen, J. (1999). The development of the moving bed biofilm process- from idea to commercial product. *European water management*, 2(3), 36-43.
- Ødegaard, H., Rusten, B., & Westrum, T. (1994). A new moving bed biofilm reactor-applications and results. *Water science and technology*, 29(10-11), 157-165.
- Patterson, R. N., Watts, K. C., & Timmons, M. B. (1999). The power law in particle size analysis for aquacultural facilities. *Aquacultural Engineering*, 19(4), 259-273.
- Peñaflorida, V. D. (1999). Interaction between dietary levels of calcium and phosphorus on growth of juvenile shrimp, Penaeus monodon. *Aquaculture*, 172(3-4), 281-289.
- Pfeiffer, T. J., & Wills, P. S. (2011). Evaluation of three types of structured floating plastic media in moving bed biofilters for total ammonia nitrogen removal in a low salinity hatchery recirculating aquaculture system. Aquacultural Engineering, 45(2), 51-59.
- Phillips, M., Clarke, R., & Mowat, A. (1993). Phosphorus leaching from Atlantic salmon diets. *Aquacultural Engineering*, *12*(1), 47-54.

- Piedrahita, R. H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, 226(1-4), 35-44.
- Pierce, R. H., Weeks, J. M., & Prappas, J. M. (1993). Nitrate toxicity to five species of marine fish. *Journal of the World Aquaculture Society*, 24(1), 105-107.
- Rodriguez, J., & Gagnon, S. (1991). Liquid purification by UV radiation, and its many applications. *Ultrapure Water*, 8(6), 22-24.
- Rosenthal, H. (1994). Fish farm effluents and their control in EC countries: summary of a workshop. *Journal of Applied Ichthyology*, *10*(4), 215-224.
- Rusten, B., Eikebrokk, B., Ulgenes, Y., & Lygren, E. (2006). Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacultural Engineering*, *34*(3), 322-331.
- Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., & Lawrence, R. (2008). Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environment international*, *34*(8), 1215-1226.
- Schulz, C., Gelbrecht, J., & Rennert, B. (2003). Treatment of rainbow trout farm effluents in constructed wetland with emergent plants and subsurface horizontal water flow. *Aquaculture*, 217(1-4), 207-221.
- Sindilariu, P.-D., Brinker, A., & Reiter, R. (2009). Waste and particle management in a commercial, partially recirculating trout farm. *Aquacultural Engineering*, *41*(2), 127-135.
- Sindilariu, P.-D., Schulz, C., & Reiter, R. (2007). Treatment of flow-through trout aquaculture effluents in a constructed wetland. *Aquaculture*, 270(1-4), 92-104.
- Singer, A., Parnes, S., Gross, A., Sagi, A., & Brenner, A. (2008). A novel approach to denitrification processes in a zero-discharge recirculating system for small-scale urban aquaculture. *Aquacultural Engineering*, 39(2-3), 72-77.
- Skonberg, D. I., Yogev, L., Hardy, R. W., & Dong, F. M. (1997). Metabolic response to dietary phosphorus intake in rainbow trout (Oncorhynchus mykiss). *Aquaculture*, 157(1-2), 11-24.
- Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M., & Hardy, R. W. (1999). Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (Oncorhynchus mykiss). *Aquaculture*, 170(3-4), 285-296.
- Suhr, K., & Pedersen, P. B. (2010). Nitrification in moving bed and fixed bed biofilters treating effluent water from a large commercial outdoor rainbow trout RAS. Aquacultural Engineering, 42(1), 31-37.
- Summerfelt, R. C., & Penne, C. R. (2005). Solids removal in a recirculating aquaculture system where the majority of flow bypasses the microscreen filter. *Aquacultural Engineering*, *33*(3), 214-224.
- Summerfelt, S. T. (2003). Ozonation and UV irradiation—an introduction and examples of current applications. *Aquacultural Engineering*, 28(1-2), 21-36.

- Summerfelt, S. T., Adler, P. R., Glenn, D. M., & Kretschmann, R. N. (1999). Aquaculture sludge removal and stabilization within created wetlands. *Aquacultural Engineering*, 19(2), 81-92.
- Suresh, A. V., & Lin, C. K. (1992). Effect of stocking density on water quality and production of red tilapia in a recirculated water system. *Aquacultural Engineering*, 11(1), 1-22.
- Talbot, C., & Hole, R. (1994). Fish diets and the control of eutrophication resulting from aquaculture. *Journal of Applied Ichthyology*, *10*(4), 258-270.
- Timmons, M. B., Ebeling, J. M., Wheaton, F. W., Summerfelt, S. T., & Vinci, B. J. (2002). Recirculating aquaculture systems, 2nd edition. NRAC Publication, 01-002.
- Tran, T. T. P. (2014). Outlet of nutrients from the fish laboratory at Norwegian University of Life Sciences (NMBU). Norwegian University of Life Sciences, Ås.
- Twarowska, J. G., Westerman, P. W., & Losordo, T. M. (1997). Water treatment and waste characterization evaluation of an intensive recirculating fish production system. *Aquacultural Engineering*, 16(3), 133-147. doi:10.1016/s0144-8609(96)01022-9
- van Rijn, J. (2013). Waste treatment in recirculating aquaculture systems. Aquacultural Engineering, 53, 49-56.
- Verdegem, M., Bosma, R., & Verreth, J. (2006). Reducing water use for animal production through aquaculture. Water Resources Development, 22(1), 101-113.
- Wu, R. (1995). The environmental impact of marine fish culture: towards a sustainable future. *Marine pollution bulletin*, 31(4-12), 159-166.
- Zhu, S.-M., Deng, Y.-L., Ruan, Y.-J., Guo, X.-S., Shi, M.-M., & Shen, J.-Z. (2015). Biological denitrification using poly (butylene succinate) as carbon source and biofilm carrier for recirculating aquaculture system effluent treatment. *Bioresource technology*, 192, 603-610.

8. Appendix

Appendix 1: license of fish laboratory of NMBU

FYLKESMANNEN I OSLO OG AKERSHUS Postboks 8111 DEP. -- 0032 Oslo 1 -1 Г Norges Landbrukshøgskole, Institutt for Tekniske fag, Boks 65, 1432 Ås-NLH ____ Vår ref. (bes oppgitt ved svar) Dalo Deres ref. 3. oktober 1991 1892/91 jh J A 881.0 NORGES LANDBRUKSHØGSKOLE, 1432 ÅS – OPPDRETTSKONSESJON TIL FORSKNING - OG UNDERVISNINGSFORMÅL. Fylkesmannen i Oslo og Akershus gir med hjemmel i Lov av 14 juni 1985 om oppdrett av fisk, skalldyr mv., tillatelse til Norges Landbrukshøgskole, Institutt for tekniske fag å kunne drive oppdrett av ørret og laks til forsknings- og undervisningsformål. Anlegget er plassert på NLH's område. Det er søkt om en gjennomsnittlig bestand på 1 tonn. Avløpet skal føres inn på kommunal pumpeledning med overføring til Søndre Follo Kloakkrenseanlegg. Vi viser til mottatt søknad fra Fiskerisjefen for Skagerrakkysten i brev derfra den 27.3.90. Følgende dokumenter er vurdert i saken : - Brev fra NLH av 22.8.90 vedrørende behandling av søknaden. - Brev fra NLH av 26.10.90 vedrørende alternative rensemetoder. - Brev fra miljøvernavdelingen ved Fylkesmannen pr. 30.8.90 til NLH vedrørende renseløsninger. - Brev av 12.4.91 fra Fylkesveterinæren med tillatelse til oppdrett etter §7 i midlertidig lov om tiltak mot sykdommer hos akvatiske organismer (vedlegg 1). - Brev fra Ås kommune til NLH av 3.7.91 vedr. tilkobling til pumpeledning (vedlegg 2). - Brev fra NLH pr. 27.8.91 til Akershus fylkeskommune vedrørende avslag på utslipp til lokal resipient, samt forslag til utslipp til kommunal ledning. - Anbudsdokument O.Nr. 607.001 "NLH, Sanering overløp" (fra Østlandskonsult A/S) oversendt til Akershus fylkeskommune 12.9.91. Teleks: Besøksadresse: Telefon: Telefax: H.Heycrdahlsgt. 1 Akcrsgt. 41 21588 fboa a Fylkesmannen i Oslo og Akershus Miljøvernavdelingen (02) 42 21 22 (02) 42 22 65 (02) * 42 90 85 (02) * 42 90 85

Søknaden gjelder konsesjon for oppdrett av laks (salmo salar) og ørret (salmo gairdneri) til forsknings- og undervisningsformål. Det er søkt om et oppdrettsvolum på 100 m3 med antatt förforbruk 3 tonn pr år. Akvarievirksomheten vil foregå i eksisterende bygninger (et tidligere forsøksfjøs; Nerfjøset). Kartblad 114. III, NM 991160.

Generelt / Hjemmelsgrunnlag

Fylkesmannen i Oslo og Akershus er etter rundskriv T-3/86 fra miljøverndepartementet (1.3.1986) delegert myndighet til å avgjøre oppdrettssøknader etter forurensningsloven, samt å avgi uttalelser i forhold til naturvern,fisk, vilt og friluftsinteresser.

Myndighet etter forurensningsloven er som ledd i frifylkeprosjektet overdratt til Akershus fylkeskommune. Behandling av søknad etter forurensningsloven har derfor skjedd under fylkeskommunal myndighet etter 1.1.91.

Fylkesmannen i Oslo og Akershus er av Fiskeridirektoratet den 12.1.1989 delegert myndighet etter oppdrettsloven av 14.juni 1985, nr. 68. Delegasjonen gjelder kompetanse til å treffe enkeltvedtak innen sitt distrikt etter :

- Forskrifter om klekking av rogn og for produksjon av settefisk av 7.desember 1985 med unntak for oppdrett med utslipp i sjø.

- Forskrifter om oppdrett av fisk på ferskvannslokalitet av 20. desember 1985.

<u>Vurdering av utslipp i forhold til naturforvaltning og</u> forurensninger :

I den opprinnelige søknaden fra NLH søkes det om utslipp av avsilt/renset vann til resipienten Vollebekken, mens sedimentert/filtrert slam skulle kjøres inn på høgskolens eget nett. En slik løsning ble avslått av Akershus Fylkeskommune med hjemmel i Forurensningsloven, jfr brev av 21.3.91. Det ble imidlertid i avslaget åpnet for tillatelse etter F-loven under forutsetning av at avløpet ikke ble ført til lokal resipient, men ført inn på kommunal pumpeledning.

Det er nå inngått avtale mellom NLH v/Teknisk kontor og Ås kommune v/teknisk etat om at avløpet fra anlegget tilkobles eksisterende pumpeledning til Søndre Follo kloakkverk. Vilkår for denne tilknytning framgår av vedlegg nr. 2. Anleggelse av separat fordrøyningstank på 300 m3 samt egen pumpeledning fra denne inn på kommunal ledning sør for eksisterende pumpestasjon er beskrevet i anbudsdokument O.Nr. 607.001 fra Østlandskonsult A/S. Gjennomsnittlig bestand av fisk vil etter opplysninger i søknaden være ca 1 tonn. Når det gjelder krav til desinfisering / filtrering av vann fra anlegget vises det til de krav som er satt i brev fra Fylkesveterinæren pr. 12.4.91 (vedlegg 1). Vilkår for utslipp og for konsesjon etter oppdrettsloven er gitt i avsnittet under. VILKÅR FOR KONSESJON ETTER OPPDRETTSLOVEN : Alt avløpsvann skal samles opp og føres i lukket ledning r. til kommunal pumpeledning. Det skal monteres en fordrøyningstank med 300 m3 II. Det skal monteres en fordrøyningstank med 300 m3 kapasitet mellom akvariet og pumpeledningen for å hindre at avløpsvann vil kunne gå i overløp. Den forlengede oppholdstid skal sikre at parasitten Gyrodactylus salaris ikke overlever og kan nå vassdrag eller sjøresipient. Vilkår for oppsamlingstank og videre tilførselsledning til pumpeledning settes av Ås kommune. III. Mengden avløpsvann skal registreres og målekum e.l. skal være plassert slik at avløpsmålinger kan gjennomføres/ kontrolleres. Oppdrettsanlegget skal drives slik at forurensningene IV. blir minst mulig. Anleggets eier plikter å sørge for og bekoste V. undersøkelser av forurensningseffekten ved anlegget i henhold til følgende Fiskeavfall, død fisk, innmat, oppsamlet forspill, fett, slam o.l. skal håndteres slik at sjø eller vassdrag ikke forurenses. Dumping i sjøen tillates ikke. Det skal utarbeides en plan for hvordan avfall som nevnt i dette VI. punkt håndteres. Planen skal leveres konsesjonsmyndighet innen 3 måneder fra konsesjonen trer i kraft. VII. Det skal føres driftjournal som viser mengde avløpsvann sluppet ut. Anvendt mengde för og type skal angis, samt mengden fisk i anlegget. Arsrapport med ovennevnte resultater samt utdrag av driftjournalen sendes Fylkesmannen/Fylkeskommunen senest 1 måned etter årets utgang. Vilkår satt av Fylkesveterinæren for Oslo, Akershus og Østfold i brev av 12.4.91 skal følges. VIII.

GENERELT

Anlegges eier plikter å la representanter for Fylkesmannen/Fylkesrådmannen og de etater og institusjoner som dette forvaltningsnivå bemyndiger, inspisere anlegget til enhver tid.

Dersom Fylkesmannen/Fylkesrådmannen finner det påkrevet, kan ytterligere tiltak kreves gjennomført for å hindre eller redusere forurensning fra oppdrettsanlegg og tilknyttet virksomhet. Eventuelt kan anlegget kreves flyttet eller nedlagt.

Klageadgang

6)

Konsesjon etter Oppdrettsloven kan påklages til Fiskeridepartementet av sakens parter eller andre med særlig klageinteresse innen 6 uker fra det tidspunkt underretning om avgjørelsen er kommet fram til vedkommende part. Klagen sendes via Fylkesmannen.

Anders Omholt (e.f) Fylkesmiljøvernsjef

Jens Hertzberg overingeniør

.

Kopi av konsesjon sendt :

Fylkesveterinæren for Oslo, Akershus og Østfold, Postboks 8156 DEP, 0033 Oslo 1.

Fiskeridirektøren, Postboks 185, 5002 BERGEN

Ås kommune, teknisk etat, 1430 ÅS.

Akershus Fylkeskommune, Fylkesrådmannen.



Appendix 3: The operation of TN test kits



Appendix 2 : The operation of COD test kits



Appendix 4: The operation of TP test kits



Norges miljø- og biovitenskapelige universitet Noregs miljø- og biovitskapelege universitet Norwegian University of Life Sciences Postboks 5003 NO-1432 Ås Norway