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Evolution of procedure for exchanging biofilm media

Case study in a running warm water system

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Evolution of procedure for exchanging biofilm media

Case study in a running warm water system

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Ås, Dec. 2012

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Summary

Moving bed biofilm reactor (MBBR) is a technology based on the biofilm theory, with biofilm attached on the suspended medium. Because of the low growth rate of nitrifying bacteria, the start-up period is quite long. Furthermore, nitrifying bacteria are very sensitive to water quality variations. Since the RAS is highly depend on biofilter, it poses a big challenge for the RAS fish farms that plan to exchange biofilm media with fish rearing in the system. This case study evaluated the procedure of exchanging biofilm media in a running warm water system. It also followed the developmental process of the "new" Anox K5 and BiofilmChip M (Krüger Kaldnes AS, Sandefjord, Norway) in a warm water system.

This case study focused on the tilapia warm water RAS of Fish laboratory, Norwegian University of Life Science (UMB, Ås, Norway). The MBBR includes three chambers. Chamber 1 (C1) was without biofilm media. Chamber 2 (C2) and chamber 3 (C3) contained a mixture of Kaldnes K1 (Krüger Kaldnes AS, Sandefjord, Norway) and 1" plastic Pall Rings (Vereinigte Füllkörper-Fabrikenj GmbH & Co, D-56235 Ransbach-Baumbach).

Because TAN and NO_2 levels sometimes exceeded the optimal concentration for Nile tilapia (*Oreochromis niloticus*), the fish laboratory decided to exchange the "old" biofilm media in chamber 2 and 3 (chamber 1 was empty) with Anox K5 and BiofilmChip M. According to the suggestions given by Krüger Kaldnes AS, chamber 2 (see overview of the chambers in Figure 3.1) of the MBBR should be filled with Anox K5, chamber 3 should be filled with BiofilmChip M and chamber 1 should be empty. To keep the stability of the system, the following plan was carried out:

1: "Old" media in chamber 2 were moved to chamber 1 (empty) and chamber 2 was then filled with Anox K5;

2: "Old" media in chamber 3 were taken out gradually until empty and then replaced by BiofilmChip M;

3: "Old" media in chamber 1 were gradually taken out until it was empty.

"Old" media should not be taken out if the water quality was reduced in a way that could affect growth and welfare of the tilapia.

This case study was held between 23.10.2011 and 02.01.2012. The exchange process was divided into 10 periods according to the amount of "old" biofilm media left in the MBBR. The duration of each period was according to the stability of the water quality. The water quality parameters measured were pH, temperature, dissolved oxygen (DO), alkalinity, NH_4-N and NO_2-N.

Water quality parameters were kept within the range for optimum growth of Nile tilapia and also for the nitrifying bacteria. The poisonous nitrogens NH_3 and NO_2 were kept at very low levels. The highest NH_3-N and NO_2-N levels in the outlet of the MBBR during the exchange process (inlet of fish tanks) were 0.01 mg/l and 0.15 mg/l respectively. Furthermore, there was no TAN and NO_2 accumulation during the exchange process. The concentrations of TAN and NO_2 in outlet of MBBR were always lower than that of inlet. The "new" Anox K5 showed TAN reduction within 9 days after it had been filled in chamber 2. It had an area TAN removal rate of 0.04 g TAN m^{-2} d^{-1} after 9 days. The function of removing NO_2 started 14 days after chamber 2 was filled with Anox K5. No nitrification was observed in chamber 3, neither in the procedure of taking out old" media nor after adding BiofilmChip M.

The exchange process was a success. There were no signs of stress for the fish during the exchange process. The establishment process for Anox K5 and BiofilmChip M in this case was slow, most possibly because of low TAN loading level.

Abberevations

AOB	Ammonia Oxidizing Bacteria
ATR	Areal TAN removal Rate
ANR	Areal Nitrite removal Rate
APC	Aquaculture Protein Center
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization of the United Nations
FCR	Food Convertion Rate
MBBR	Moving Bed Biofilm Reactor
NH_4-N	Ammonia Nitrogen
NO_2-N	Nitrite Nitrogen
NO_3-N	Nitrate Nitrogen
NOB	Nitrite Oxidizing Bacteria
PE	Polyethylene
PVC	Polyvinyl Chloride
RAS	Recirculating Aquaculture Systems
SGR	Specific Growth Rate
TAN	Total Ammonia Nitrogen
UMB	Norwegian University of Life Sciences
VTR	Volume TAN removal Rate

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1. Introduction

As one of the fastest growing food-producing sector, aquaculture accounted for nearly 45.6% of the world's food fish in 2008. Production had reached about 52.5 million tons in 2008, compared with 32.4 million tons in 2000 (FAO, 2010). It is estimated that by the end of 2012, more than 50 percent of the global food fish consumption will originate from aquaculture (FAO, 2010). This will contribute a lot to solve the food crisis caused by the increasing population. However, the increasing production volume will unavoidably create many problems. Maximum utilization of limited fresh water resources and at the same time keep the aquaculture industry ecologically sustainable will be very important challenges.

The discharges of nutrients and organics from aquaculture units may exceed the capacity of the ecosystems if without treatment (Boyd and Tucke, 1998). There are three main types of pollutants from aquaculture facilities: chemicals for maintaining facility cleanliness, drugs used for disease control and metabolic products such as feces and uneaten feed (Mugg *et al.*, 2000). The first two types vary with different cases and the impact can be reduced if the fish farmers use the chemicals in a proper way. At the same time, many drugs used in fish farms have been found to have minimal (if any) deleterious effects on the aquatic environment (Costelloe *et al.*, 1998). However, the third type of pollutant poses a big challenge to the development of the aquaculture industry. These pollutants include total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), dissolved carbon dioxide (CO_2), suspended solids (SS), and non-biodegradable organic matter (Molleda, 2007). The effluent will easily result in build-up of anoxic sediments, changes in the benthic communities and eutrophication if without special treatment before discharging to the water recipients.

However, if we can reduce the amount of water discharged from the aquaculture units, the side effects will be reduced and easier to control. This can be achieved by reusing the water. This idea, which is known as recirculating aquaculture systems (RAS), has been adopted and increasingly used by the aquaculture industry today. Recirculating aquaculture system (RAS) is a type of intensive fish culture technology in which a high proportion of the water is reused after treatment (Summerfelt *et al.*, 2004). It

does not only reduce environmental impacts of aquaculture industry, but can also reduce the costs.

The key to a successful recirculating aquaculture system is the use of cost-effective water treatment system components (Losordo *et al*., 1998). It requires at least one or more of the following treatment processes, depending on the water reuse intensity and species-specific water quality requirements (Molleda, 2007): Aeration system to add oxygen or strip out CO_2 and N_2; particle removal systems, such as granular filters or mechanical filters; biofilters to oxidize ammonia and nitrite; desinfection systems (UV or ozone) to inactivate harmful microorganisms; pH control by adding chemicals to increase buffering capacity and compensate for the alkalinity-consuming nitrification reaction; heater, heat exchanger or heat pump to make the water to desired temperature. Of course, all of these units must work in conjunction to fulfill optimal water quality.

Biological filters use natural filtering system consisting of helpful bacteria colonies that convert ammonia to nitrite (*Nitrosomonas sp*.), and then convert the nitrite to the less harmful nitrate (*Nitrobacter sp*.) (Timmons *et al.,* 2002). Since ammonia is very toxic to fish, RAS is highly depended on the efficiency of the biofilter. There are many different types of biofilters used in RAS, e.g. submerged biofilters, trickling biofilters, rotating biological contactors (RBC), floating bead biofilters, dynamic bead biofilters and fluidized bed biofilters (Timmons *et al.,* 2002). They all have their advantages and disadvantages, so the proper selection and sizing of biofilters are critical to both the technical and economic success of RAS (Malone and Pfeiffer, 2006). Recent development in biofilters has led to the use of moving bed biofilm reactors (MBBR) widely around the word (Pfeiffer and Wills, 2011).

Moving bed biofilm reactor (MBBR) is a process based on the biofilm principle with an active biofilm growing on small specially designed plastic elements (carriers) that are suspended in the reactor. The biofilm medium is made of high density polyethylene, which has a density of approximate 0.95 g/cm^3 (Ødegaard *et al.,* 1999). There are many kinds of biofilm media with different sizes and shapes, providing many options regarding different cases. Taking one of the most famous companies that produce biofilm media, Anox Kaldnes company has developed a series of biofilm

media, such as Kaldnes K1, Kaldnes K2, Kaldnes K3, and Natrix-media. Media size and surface area are usually used to evaluate different kinds of biofilm media. MBBR can be used both for aerobic and anaerobic process (Rusten *et al.*, 2006). In an aerobic process, the media´ movement is caused by air from aeration diffusers, while in the anaerobic case, a mixer provides the energy to keep the media moving (Ødegaard *et al.,* 1999). As one of the advantages of MBBR, the fraction of media in the reactor can be subject to the preference. But it is recommended that the percentage should be below 70% of its volume capacity to make sure the media can move freely (Rusten *et al.*, 2006). Other advantages of MBBR include non-cloggable, no need for back flushing, lower head loss and higher specific area.

1.1 Background of the case

The tilapia RAS in the Fish laboratory at UMB (Norwegian University of Life Sciences, Ås, Norway) was established in 2009. The biofilter was only filled with 30% of its volume capacity. The biofilm media in the MBBR consisted of 20% Kaldnes K1 (KaldnesMiljøTeknologi AS, Tønsberg, Norway) and the rest were plastic 1” Pall Ring (VereinigteFüllkörper-Fabrikenj GmbH & Co, D-56235 Ransbach-Baumbach). Historical analysis for TAN and NO_2-N showed the water quality from the outlet of MBBR was not optimal and the concentration of NO_2-N exceeded 1 mg/l in periods.

The modification with “new” biofilm media from the Krüger Kaldnes (Krüger Kaldnes AS, Sandefjord, Norway) provided an opportunity for optimizing the water quality. According to the suggestions given by the Krüger Kaldnes, the MBBR should be filled with the Anox K5 and BiofilmChip M.

Because waste water from aquacultural units contains low TAN concentration, longer time is needed for the nitrifying bacteria to establish on the biofilm media (Rusten *et al.*, 2006). TAN and nitrite levels will elevate if the “old” biofilm media is replaced with a “new” media at once. To ensure the survival of the fish in the system, plans were made to replace the “old” biofilm media as following: 1) move “old” media in chamber 2 to chamber 1 (empty) and then fill chamber 2 with Anox K5; 2) take out

"old" media in chamber 3 gradually and then fill in chamber 3 with BiofilmChip M; 3) take out "old" media in chamber 1 gradually until it is empty. The exchange process should be slowed down if the TAN and NO_2-N level increase remarkably. We decided the maximum un-ionzed ammonia and NO_2-N levels are 0.07 mg/l and 1 mg/l repectively (El-Shafai *et al.,* 2004; Atwood *et al.,* 2001).

1.2 Objective

The purpose of this case study was to check out the feasibility and safety of the exchange plan by measuring water quality parameters. At the same time, describe the development of Anox K5 and BiofilmChip M in a warm water system. We also planed to evaluate the establishment of nitrifying bacteria via microbial community composition analysis. This case study can provide practical reference for the running fish farms that are adopting the MBBR, in case of exchanging biofilm media.

2. Literature Review

2.1 Water quality requirements for the Nile tilapia's culture

Nile tilapia (*Oreochromis niloticus*) can tolerate a wide range of environmental conditions including factors such as salinity, dissolved oxygen (DO), temperature, pH, ammonia and nitrite levels than most cultured fresh water fishes can (Mjoun *et al.*, 2010). For the temperature, the highest FCR (feed convertion ratio) was gotten at the temperature between 26 °C and 30 °C in the experiment done by El-Sayed and Kawanna (2008). According to the experiment done for the three strains of Nile tilapia (Li *et al*., 2002), mortality began to appear when the temperature dropped to 11 °C. 100% mortality appeared when the temperature was below 7 °C.

Nile tilapia has high ability to tolerate low DO concentration due to its ability to use atmospheric oxygen (Pullin and Lowe-McConnel, 1982). The lowest tolerance limitation of DO reported for Nile tilapia ranged from 0.1 to 0.3 mg/l under different environmental conditions (Magid and Mabiker, 1975). According to Tsadik and Kutty (1987) long-term oxygen level should be close to saturation level to achieve maximal growth.

El-Sherif and El-Feky (2009) reported that pH 7-8 was optimal for tilapia culture. For salinity, Nugon (1997) reported that juvenile Nile tilapia exposed to 10 ppt showed 100% survival, while exposed to 35 ppt, mortality was 100%.

Nile tilapia is less resistant to the toxic effects of un-ionized ammonia as compared with other tilapia species. According to the report published by the Evans *et al.* (2006), the median lethal concentration (LC50) was 1.46 mg/l NH_3-N at 24 and 48 hrs post-exposure, 1.33 mg/l at 72 hrs post-exposure and 0.98 mg/l at 96 hrs post-exposure. 93-100% mortality was observed within 24 hrs among fish exposed to 2.0, 3.0 or 4.0 mg/l un-ionized ammonia. No mortality was observed in Nile tilapia exposed to 0.5 mg/l NH_3-N (Evans *et al*., 2006). NH_3-N between 0.07 and 0.14 mg/l will reduce the growth rate and incerease the feed coversion rate in 20 g Nile tilapia (El-Shafai *et al.,* 2004).

Nitrite is very toxic for the Nile tilapia and the toxicity is dependent on the size of the fish and chloride concentration. Chloride can inhibit the uptake and toxity of nitrite (Atwood *et al.,* 2001). The same author found that 96-h median lethal concentration of NO_2-N was 81 mg/l for small Nile tilapia (ca. 4.4 g) and 8 mg/l for large Nile tilapia (ca. 90.7 g) in dechlorinated water.

2.2 Description of moving bed biofilm reactor - MBBR.

Moving bed biofilm reactor process was developed in Norway in late 1980s and early 1990s (Ødgaard *et al.,* 1999). The idea behind its development was to adopt the best from both the activated sludge process and the biofilter process without including the worst (Rusten *et al.,* 2006). In MBBR, the biofilm mainly grows on the surface of the medium that with different size, shape and surface area. Biofilm media are suspended and move in the entire water volume of the reactor and retained by a sieve placed at the reactor outlet. The movements of media are caused by the agitation set up by the air in the aerobic processes, while in anoxic processes a mixer keeps the media moving (Rusten *et al.,* 2006).

One of most important advantages of moving bed biofilm reactor is that the filling fraction of biofilm media in the reactor can be subject to preference (Rusten *et al.,* 2006). While in order to keep the media moving freely, the filling percentage of media should be less than 70% of the reactor volume on the bulk volume basis. The problems with high media filling percentage include easier clogging, lower transport of air from surface to the deeper part of biofilm and reduced water flow through the reactor (Lekang and Kleppe, 2002). However, the capacity of the reactor can also be adjusted by changing different biofilm media with various surface area, which is defined as the total suface area per unit volume. Since the biofilm grows primarily on the protected surface area inside the media, only the protected surface area is used to dimention the biofilter (Rusten *et al.,* 2006). The parameters used to evaluate and compare ammonia removal performance of media include (Pfeiffer and Wills, 2011): 1) volume TAN removal rate (g TAN m^{-3} d^{-1}); 2) areal TAN removal rate (g TAN m^{-2} d^{-1}); 3) first-order rate constant that presents the product of substrate utilization rate constant and the active microbial mass per unit volume of the reactor; 4) percent TAN removal efficiency.

2.3 Nitrogen pathway in the system

Fish mainly get nitrogen from feed as a form of amino acid. Fish can digest the dietary protein very efficiently (Dosdat *et al.*, 1996), which makes a major contribution to the total energy production of fish (Mommsen and Walsh, 1992). Fish expel nitrogenous waste products through gill diffusion, gill cation exchange, urine and feces excretion (Timmons *et al.,* 2002).

The main end nitrogenous product in teleost fish is ammonia (Mommsen and Walsh, 1992). It accounts 75-90% of the nitrogen loss. Appreciate amount of nitrogen waste is also excreted as urea (5%-15%) (Dosdat *et al.*, 1996).

Most production of ammonia in the fish is from the liver by the process of deamination of free amino acids (Mommsen and Walsh, 1992). Certain amount of ammonia can also originate from muscle, intestine and kidney (Mommsen and Walsh, 1992).

In the aqueous solution, ammonia exists in the form of unionized ammonia (NH_3) and ionized ammonia (NH_4^+) (Randall and Tsui, 2002). The equilibrium can be described by Equation 2.1. In most cases, we ascribe the two forms as the total ammonia nitrogen (TAN = NH_3-N + NH_4^+-N). The ratio of ionized ammonia and un-ionized ammonia varies with the different pH, temperature and salinity (Timmons *et al.,* 2002). An increase in pH, temperature or salinity increases the percentage of un-ionzed ammonia. The fraction of un-ionzed ammonia at different temperatures and pH is shown in Table 2.1.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad \text{……………………………………………Equation 2.1}$$

The side effect of high concentration of unionized ammonia includes the growth decrement, disruption of ionic balance, increased vulnerability to diseases, pathological changes in gill structure and disruption of ionic balance (Sinha *et al.*, 2012).

Table 2.1 The percentage (%) of NH_3-N in the TAN under different pH and temperatures (Kutty and Delince, 1987).

	pH							
T(°C)	**6.00**	**6.50**	**7.00**	**7.50**	**8.00**	**8.50**	**9.00**	**9.50**
22	0.046	0.145	0.457	1.43	4.39	12.7	31.5	59.2
23	0.050	0.156	0.491	1.54	4.70	13.5	33.0	60.9
24	0.053	0.167	0.527	1.65	5.03	14.4	34.6	62.6
25	0.057	0.180	0.566	1.77	5.38	15.3	36.3	64.3
26	0.061	0.193	0.607	1.89	5.75	16.2	37.9	65.9
27	0.650	0.207	0.651	2.03	6.15	17.2	39.6	67.4
28	0.700	0.221	0.697	2.17	6.56	18.2	41.2	68.9
29	0.075	0.237	0.747	2.32	7.00	19.2	42.9	70.4

2.4 Nitrifying bacteria

There are two groups of organisms involved in the nitrification process. They are ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (Figuerola and Erijman, 2010). AOB include Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus and Nitrosovibrio. NOB include Nitrobacter, Nitrococcus, Nitrospira, Nitrospina.

AOB oxidize ammonia to nitrite and full-fill the process shown in Equation 2.2. NOB carry out the process shown in Equation 2.3 (Koops and Pommerening-Roser, 2001). Both AOB and NOB get the energy from the conversion shown in Equation 2.2 and Equation 2.3 to drive their life process (Timmons *et al.,* 2002).

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- \text{ ....Equation 2.2}$$

$$NO_2^- + 1.5O_2 \rightarrow NO_3^- \text{ ....Equation 2.3}$$

Nitrifying bacteria grow very slowly and are sensitive to toxic shock, pH and temperature fluctuation (Aoi *et al.,* 2000). The optimum temperature for the growth of Nitrosomonas and Nitrobacter is 30 ºC and the growth rate decrease by 50% at 20 ºC and 40 ºC (Bhaskar and Charyulu, 2005). Bhaskar and Charyelu also found the

nitrifying bacteria showd maximum growth rate at pH 8.0. Nitrifying bacteria show quite low growth. In Sedlak's (1991) report, the growth rate ranges from 0.46 to 2.2 g/g cell.d. In the research done by Pollard (2006), in which the author managed to measure the growth of total bacterial community and also the autotrophic-nitrifying bacteria in the fixed film nitrifying and active sludge reactor. The result showed the growth rate of 0.4×10^{8} cell ml^{-1} d^{-1} and 0.01×10^{8} cell ml^{-1} d^{-1} respectively at the temperature of 21 ºC.

2.5 The structure of biofilm

Biofilm is defined as a layered structure with an inner layer which is formed by inert biomass near the surface of the media and with an outer layer which is overlain tightly by the nitrifying rich population, with heterotrophs dominating the outer layer (Malone and Pfeiffer, 2006). According to the growth pattern of bacteria, the biological nitrification can be divided into two groups: attached and suspended growth. Biofilters like moving bed biofilm reactors and rotating biofilters belong to the formal, in which the microorganisms are attached to the surface of the support medium (Timmons *et al.,* 2002). The active-sludge reactor belongs to the second case, in which the microorganisms suspend freely in the liquid leading to the direct contact between the bacteria and water.

The nitrification process occurs in the biofilm instead of in the liquid, so attention should be paid to the structure of the attached biofilm (Moreau *et al*., 1994). There is a resistance when the substrate (e.g. TAN) is transferred from water to the biofilm. The typical structure of the biofilm is shown in Figure 2.1.

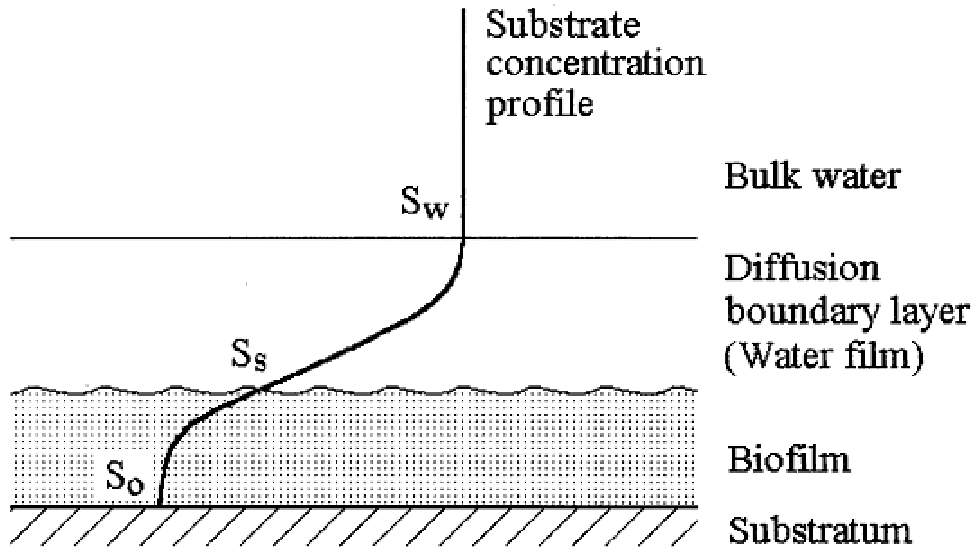


Figure 2.1 Biofilm' structure (Zhu and Chen, 2001b).

According to the report published by Zhang *et al.* (1995), most biofilm are heterogeneous, leading to the gradients of the chemical and physical parameters, especially the TAN and oxygen concentration in the case of nitrifying reactor. The above theory can be improved well by the experiment done by the same author, using the microelectrode technique and micro-slicing technique. The result is shown in Figure 2.2.

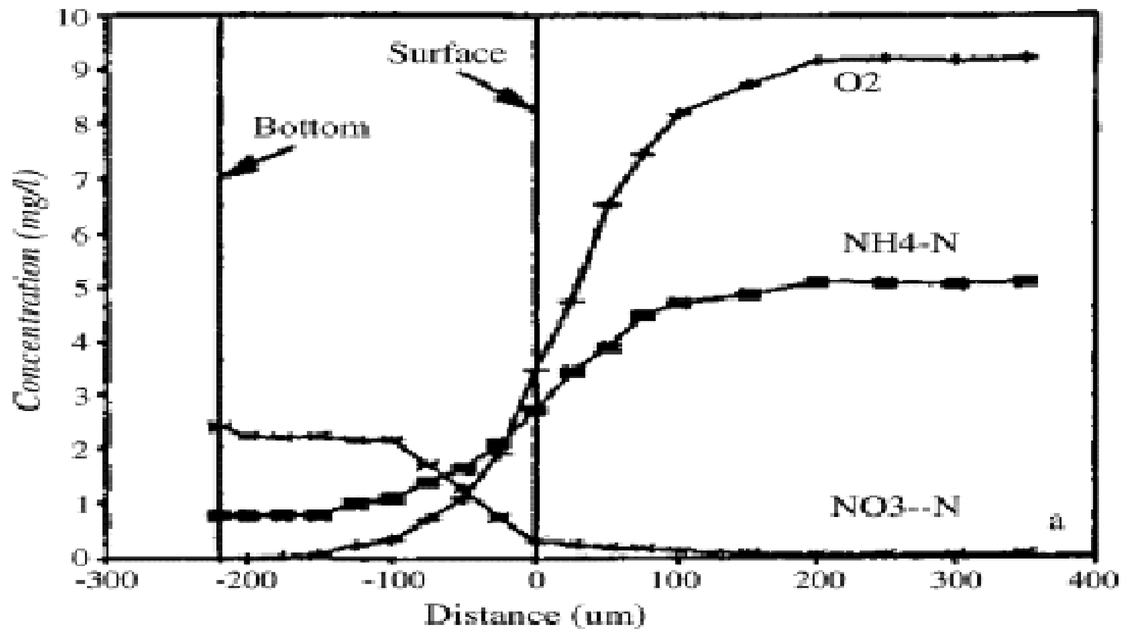


Figure 2.2 Concentration profile in the heterotrophic–autotrophic biofilm (Zhang *et al.*, 1995).

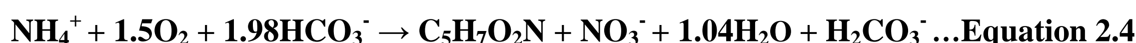
The depth of full substrate penetration is usually less than 100 μm (Rusten *et al.*, 2006). Horn (1994) reported that the nitrifying bacteria found at the bottom of the biofilm were maintained in the endogenous environment because of the limited oxygen. Furthermore, in the case of limited ammonia condition, nitrifying bacteria on the surface of the biofilm were the only survivor. So the ideal pattern of biofilm in the moving bed process is thin and evenly distributed (Rusten *et al.*, 2006). According to Zhang *et al.* (1995), the level of evenly distribution was determined by competition between the heterotrophic and autotrophic bacteria. Aeration of the biofilm media is also of great importance to maintain a thin biofilm on the media (Pfeiffer and Wills, 2011).

2.6 Nitrification process

Nitrification is a biological process, in which the ammonia is firstly oxidized to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB), then nitrite is oxidized to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB). The two steps in the reaction are normally carried out sequentially. Since the first step has a higher kinetic reaction rate than the second

step, the overall kinetics is usually controlled by ammonia oxidation and as a result there is no appreciable amount of nitrite accumulation (Timmons *et al.,* 2002).

As seen from Equation 2.4, the nitrification process consumes HCO_3^- which is expressed as the alkalinity. For every gram of TAN oxidized, it needs approximately 7.1 g of alkalinity (as $CaCO_3$) and 4.2 g oxygen (Chen *et al.*, 2006). The $C_5H_7O_2N$ produced is expressed as the cell mass constructed by the nitrifying bacteria. For every gram of TAN oxidized, 0.17 g of bacterial biomass is produced (Chen *et al.*, 2006).



$$\mathbf{NH_4^+ + 1.5O_2 + 1.98HCO_3^- \rightarrow C_5H_7O_2N + NO_3^- + 1.04H_2O + H_2CO_3^-} \text{ …Equation 2.4}$$

2.6.1 Nitrification kinetics

The nitrification rate depends strongly on the concentration of the substrate in the bulk liquid (Chen *et al.*, 2006). The Michaelis–Menten's type expression can be used to describe the relationship between the enzymatic reaction rate and the substrate concentration. It is expressed in Equation 2.5.

$$\mathbf{V = (V_m \times S)/(K_m + S)} \text{ ……………………………………………..Equation 2.5}$$

Where the V is the velocity of the reaction; V_m is the maximum reaction rate (g/day); S is the substrate concentration (g/m^3); K_m is the half saturation constant (g/m^3).

At a sufficient high substrate concentration, Equation 2.5 becomes the zero-order expression, which means the reaction rate does not increase with concentration of the substrate. When the substrate concentration is sufficiently low, the relationship becomes linear, following into the first-order (Chen *et al.*, 2006).

2.6.2 Nitrification rate

Nitrification rate in the fixed biofilm, like MBBR, can be decided by the substrate demand for the growth of nitrifying bacteria (Chen *et al*., 2006) and the diffusion rate of substrate in and out of the biofilm (Rusten *et al*., 2006). The above two parameters can be influenced by various factors, including the physical, chemical and biological factors. According to the report published by Chen *et al.* (2006), all these factors can be divided into three groups. The first group is the factors that can influence the biochemical process, such as temperature, pH and salinity. The second group includes the factors that affect the supply of nutrients to the biofilm, for example the substrate concentration, dissolved oxygen and the mixing regime. The third group can be described as the factors that affect the nitrifying bacteria' growth and nutrient supply, for example, the C/N ratio and alkalinity. Details of the main parameters involving in this study are described later.

2.6.2.1 Influence of TAN level on the nitrification rate

As the main function of MBBR is to remove TAN, the concentration of TAN is the most important factor to consider during the operation. On one side, the MBBR must be able to remove TAN at a sufficient rate to keep the TAN level under the toxic level for the fish. On the other hand, MBBR should have adequate nitrification rate to keep the sustainability of MBBR (Chen *et al., 2006*). Here presents two questions. What is the minimum TAN level that can keep the nitrification process going on? What is the relationship between TAN level and nitrification rate? A lot of researches have been done about these two questions.

Compared with industrial and municipal water, aquacultural waters have low TAN levels. In most cases, TAN level is so low that it becomes the rate-limiting factor of biological nitrification process (Zhu and Chen, 1999). The relation between the TAN level and nitrification always becomes linear (Chen *et al*., 2006). This theory is well proved in the experiment done by Zhu and Chen in 1999, in which the author used the series reactor system. They found that nitrification decreased with the dilution of TAN both at high and low feeding rate. The nitrification rate fell down to zero in the

last three reactors due to too low TAN concentration. The same situation was also shown in the article published by Rusten *et al.* (1995). The author set up the experiment at 15 ºC and low organic load with different DO levels. The results are shown in Figure 2.3. According to the results, Rusten *et al.* (1995) got a model that described the relation between nitrification rate and TAN level. It is shown in Equation 2.6.

$$\mathbf{R_n = k \times (S_N)^n} \qquad \textbf{Equation 2.6}$$

Where the R_n is the nitrification rate; k is the reaction rate constant, which depends on the waste water characteristics; S_N is the TAN concentration in the MBBR; n is the reaction order.

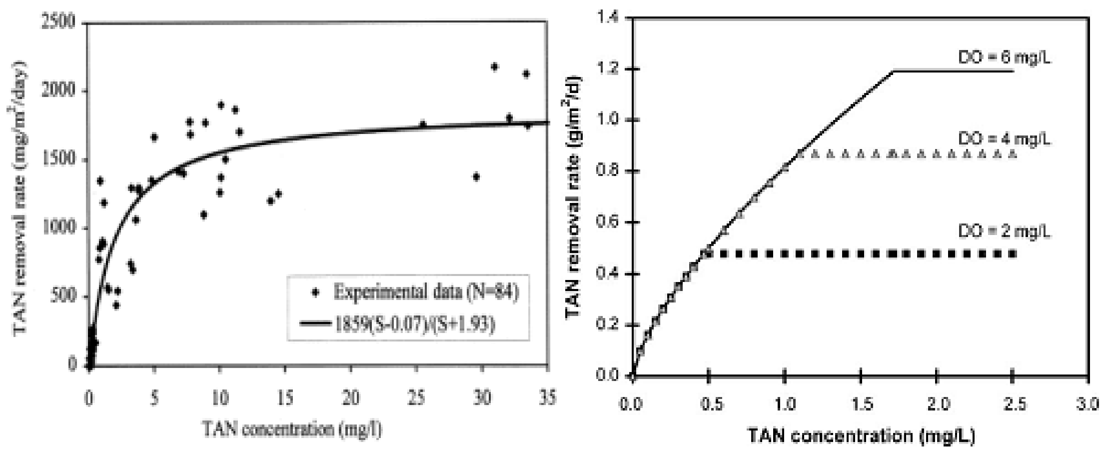


Figure 2.3 Relation between TAN concentration and TAN removal rate (Rusten *et al.,* 1995).

2.6.2.2 Influence of C/N ratio on the nitrification rate

The higher C/N ratio, the lower nitrification rate. This is mainly because of the nitrifying bacteria's competition with heterotrophic bacteria, which metabolize biologically degradable organic compounds. Increased organics provides substrate for the heterotrophic bacteria, which competes for the oxygen and space with nitrifying bacteria in the reactor (Chen *et al.,* 2006). However, nitrifying bacteria have lower competence compared with heterotrophic bacteria.

According to Zhu and Chen's experiment (2001a), experimental solution with C/N ratio = 1.0 or 2.0 resulted in approximately a 70% reduction of ATR when compared with a solution with similar nitrogen level, but without carbon (C/N = 0).

C/N rate should be kept low during the biofilter start-up period. According to the Okabe *et al*'s (1996) report, author found higher C/N rate retarded the accumulation of nitrifying bacteria, especially the NO_2 oxidizers. This resulted in longer start-up period for complete and stable nitrification process. So the water source for biofilter should be as clean as possible with minimal concentration of total solids (Timmons *et al.,* 2002).

2.6.2.3 Influence of oxygen on the nitrification rate

Seen from Equation 2.4, dissolved oxygen (DO) is a basic requirement for the nitrification process. 3.43 mg and 1.14 mg of DO are needed for the oxidation of 1 mg NH_4-N and NO_2-N respectively (Chen *et al*., 2006). Different from the suspended growth pattern nitrification reactor, the concentration of DO in the attached biofilm has a gradient. It is reported that the effective diffusivity ratio for DO decreased with the depth of biofilm (Zhang *et al*., 1995). Like the TAN concentration, DO can also be a rate limiting factor in the nitrifying process (Rusten *et al*., 2006). Although, there is no significant evidence about the optimum oxygen level for the most efficient nitrification process (Chen *et al*., 2006). Picioreanu *et al.* (1997) found that oxygen level less than 2 mg/l would lead to nitrite accumulation in the case of airlift biofilter by using the mathematic modeling.

However, in a practical view, the DO amount that is available for the nitrifying bacteria depends on the TAN level, turbulence in the reactor, organic load, temperature and pH (Chen *et al*., 2006). According to the Rusten *et al.*'s article (2006), oxygen will be the rate limiting factor at high TAN levels. While with high DO, TAN will become the rate limiting factor. The turning point is at ratio of 3.2 between the DO concentration (mg/l) and TAN (mg/l) level. But in the case like MBBR used in

aquaculture, which usually has low TAN levels (normally less than 1 mg/l NH_4^+-N), TAN will be the rate limiting factor.

2.6.2.4 Influence of temperature on the nitrification rate

Temperature is a major factor that affects the nitrification rate (Rusten *et al*., 2006). It promotes the growth rate of nitrifying bacteria. In general, the nitrification rate follows an Arrhenius relationship, i.e. a 10 ºC decrease in operating temperature will result in a 50 % reduction of removal rate (Timmons *et al.,* 2002).

However, there is a linear relationship between water temperature and oxygen content; the higher temperature the lower oxygen content. Based on this theory, Zhu and Chen (2002) found the temperature's effect on the nitrification rate was lower than that described in the Hoff-Arrhenius equation (Equation 2.7). It was proved by the experiment done by the same authors. With the temperature increasing from 14 ºC to 25 ºC, the nitrification rate had no significant change.

$$\mu = \mu_{20}\,\theta^{(T-20)} \qquad \text{Equation 2.7}$$

Where the μ is rate coefficient (d^{-1}); μ_{20} is the value of μ at the temperature of 20 ºC (d^{-1}); θ is the temperature coefficient (dimensionless); T is the temperature (ºC).

However, from the practical view, the temperature in RAS is normally determined by the requirements of the species being cultured, not by the needs of nitrifying bacteria (Timmons *et al.,* 2002). This posts a big challenge for the start-up of cold water MBBR.

2.6.2.5 Influence of pH on the nitrification rate

A great amount of researchs have been done to study the pH's influence on the nitrification rate. According to Villaverde et al. (1996), the influence of pH on nitrification can be divided into three aspects: activation and deactivation of nitrifying

bacteria; nutritional effect, connected with alkalinity; inhibition through free ammonia and free nitrous acid and through heavy metal.

The pH range for optimum nitrification reported is mainly within 7.2 to 7.8 (Timmons *et al.,* 2002). Within the pH range of 5.0-9.0, one unit increase in pH increase the nitrification rate by 13% (Villaverde *et al.,* 1996). However, the percentage of poisonous NH_3 increases with the pH. Timmons *et al.* recommend the pH should be maintained near the lower end of optimum pH for the nitrifying bacteria (7.0-7.5). Rapid pH variations will stress the bacteria and should be avoided.

2.6.2.6 Influence of alkalinity on the nitrification rate

As shown in Equation 2.4, the nitrification process produces H^+ and consumes alkalinity. Alkalinity plays two roles in the nitrification process. Firstly, it is a nutrient element for the nitrifying bacteria in the form of carbonate and bicarbonate (Chen *et al.*, 2006). Secondly, it increases the buffering capacity of the system to reduce pH variations. Alkalinity can be easily made up by adding sodium bicarbonate, such as baking soda ($NaHCO_3$) or other bicarbonate supplements (Timmons *et al.,* 2002). As a rule of thumb given by Timmons *et al.,* (2002), for every kilogram feed, 0.25 kg of sodium bicarbonate should be added to the water.

The alkalinity requirement is also related with the thickness of the biofilm. It is reported that higher alkalinity is required for the thick biofilm compared with the thinner one, because of less pH reduction in the thinner biofilm (Rusten *et al*., 1995). For the thin biofilm, the maximum nitrification rate was observed down to an alkalinity of 0.7 mmol/l (Rusten *et al.,* 2006).

2.7 Daily variation of ammonia production

There is a direct relationship between the ammonia excretion and protein intake (Mommsen and Walsh, 1992). Ammonia concentration increases after feeding. But the postprandial excretion pattern differs with diet, species and temperature (Wicks and

Randal, 2002). According to the experiment done by Wicks and Randal (2002) with rainbow trout, they found that the plasma ammonia increased significantly 30 minutes after feeding, changing from 11.1 (+/-1.5) in unfed fish to 15.5 (+/-1.5) µg /ml. But the concentration returns to the control level 2 hrs after feeding. However, the second significant peak 17.9 (+/-3.4) µg /ml appeared 8 hrs after feeding. In the article published by Leung *et al.* in 1998, two kinds of fish's ammonia excretion patterns with different weights and temperature were investigated. The peak rate of TAN excretion of *Lutjanus argentimaculatus* occurred 6 to 8 hrs after feeding at 15 ºC and 20 ºC and at 10 hrs after feeding at higher temperature. In the case of *Epinephelus areolatus*, the peak rate of TAN excretion appeared 12 hrs after feeding at 15 ºC, 4 to 8 hrs after at 20 ºC, 2 to 4 hrs after at 25 ºC and 6 to 8 hrs after at 30 ºC.

Furthermore, there is a significant relationship between feeding frequency and ammonia excretion fluctuation. In the experiment done by Zakes *et al*. in 2006, using three feeding frequency - once a day, three times per day and continuous feeding for the juvenile tench *Tinca tinca* under the water temperature of 23 ºC. For the case of feeding once, the excreted ammonia reached the peak 4 hrs after feeding. There were three maximum and three minimum corresponding to the three feeding routines in the case of feeding three times per day. The excreted ammonia kept constant after 6 hrs of continuous feeding.

Ammonia is also produced as a form of endogenous nitrogen excretion, which is the result of the catabolism and the turn-over of body proteins. It is irrespective of the nutritional status of the fish (Forsberg, 1997). According to the experiment conducted by the same author, the TAN excretion of starved post-smolt Atlantic salmon was 12 µg TAN kg^{-1} min^{-1}. However, for the fish fed with 0.59-0.62% body weight per day, the TAN excretion was 11.8-12.8 µg N kg^{-1}, which was approximately ten times of the starved fish. In the case of transferring Atlantic cod (starved for 24 hrs before transporting) by using closed well-boat, the TAN level was 0.01µg l^{-1} at the start and was between 0.08 µg l^{-1} and 0.22 ug l^{-1} after 24 hrs transportation with fish density from 10 to 20 kg m^{-3}. TAN level was lowest with a 24 hrs fast period when compared with 6 and 12 hrs fast period (before transporting), but the difference was not significant (Treasurer, 2010).

However, there is no published data about Nile tilapia's ammonia excretion pattern with different feeding routine.

2.8 Molecular techniques for microbial community composition analysis in MBBR

A better understanding of microbial ecology in the biofilm community is of great importance to improve reactor performance and have better control (Fu *et al.,* 2010). However, it is very difficult to characterize the biofilm's microbial community by just using conventional microbiological techniques, because it is not possible to get the pure culture of many important microorganisms (Sanz and Köchling, 2006). Fortunately, the appearance and development of molecular techniques in 1990s is of great success of solving this problem, which has been widely used in studying biofilter cases (Biswas and Turner, 2012; Egli *et al.,* 2003). Among all these techniques, cloning and the creation of a gene library (16 rRNA gene analyses), denaturant gradient gel electrophoresis (DGGE), fluorescent in *situ* hybridization with DNA (FISH) stand out (Sanz and Köchling, 2006;). Brief introduction about these techniques are described below.

2.8.1 16S rRNA gene analysis

16S rRNA gene is highly conserved between different species of bacteria and archaea. It is widely used for phylogenetic study of extremely fastidious or highly pathogenic bacteria species (Weisburg *et al.,* 1990). The general procedure is as follows (Weisburg *et al.,* 1990): a) DNA extraction; b) PCR (polymerase chain reaction) amplication and purification of product; c) cloning of PCR products; d) sequencing of the cloned gene and creating a clone library; d) determinating for the phylogenetic affiliation of the cloned sequence with the help of fadedicated computer program. The advantages of this method include: a) can be used for very precise taxonomic studies; b) can cover most microorganisms; c) can indentify microorganisms that have not

been cultured or identified. It also has a lot of disadvantages, such as time consuming and laborious, which make it unpracticle for large amount of samples, many clones have to be sequenced to ensure most of individual species in the samples are covered and it can not be used for quantative determinations (Sanz and Köchling, 2006).

2.8.2 Denaturant gradient gel electrophoresis (DGGE)

In DGGE, denatured DNA fragments of the same length, but with different sequence can be separated. The separation is based on the idea that DNA mixture can be seperated by denaturant gradient electrophoresis on an acrylamide gel with a decreasing urea/formamide gradient. When the double-stranded DNA migrate to the positive pole, it degenerates when it reach the correponding denaturant concentration, which is decided by the DNA sequence. Beacause the electrophoretic mobility of double-stranded DNA fragment is significantly reduced by their partial denaturation (Peters and Robinson, 1991), the molecular DNA with the same sequence will hal at different points on the gel, which results in different bands. Every band that corresponds to a different microorganism can be cut from the gel and then the DNA can be extracted and sequenced.

The general procedure of the DGGE can be ascribed as follows (Chan *et al.,* 2001): a) DNA extraction; b) PCR amplification for 16S rRNA with universal primers to give the mixture DNA with same length; c) DGGE the PCR-amplified 16S rRNA; d) cut the DGGE bands from the gel and then do phylogenetic analysis. The advantages of DGGE include simple easy and fast to obtain an overview of the dominant species of an ecosystem and adequate for analysis of a large number of samples. The disadvantages include (Sanz and Köchling, 2006): not always possible to separate DNA fragments which have a certain amount of sequence variation; the sequences of the bands obtained from a gel just correspond to a short DNA fragment, which limit the amount of sequence information for phylogenetic inferences as well as for probe degsin.

2.8.3 Fluorescent in situ hybridization with DNA (FISH)

Fluorescent in situ hybridization with rRNA-targeted nucleic acid probe can be used to identify, localize and quantify microorganisms in a few hours (Wagner *et al.,* 2003). The general theory is that rRNA sequence labbed on the probe is hybridized with the microorganisms in the sample. The probes are generally 15-25 nucleotides in length and are labeled covalently at the 5'-end with a fluorescent dye (Wagner *et al.,* 2003). The process of FISH technology is as follows (O'Connor, 2008): a) make either a fluorescent of the probe sequence or a modified copy of the probe sequence that can be rendered fluorescent later in the procedure; b) denature the target and the probe sequence with chemical or heat, which is necessary for new hydrogen bonds to form between the target and the probe during the subsequent hybridization step; c) mix the probe and target sequence and then the probe can be hybridized to its complementary sequence on the chromosome; d) using the fluorescence microscope to detect the hybrids formed between the probe and their chromosomal. The advantages of this method include: it can generally quantify the bacteria; it is easy to process and has no requirement for specialized personnel. There are also some disadvantages: not all bacterial and archaeal cells can be permeabilised by oligonucleotide probe using standard fixation protocols (Wagner *et al.,* 2003); the accuracy of this quantification method is relatively low in densely colonized biofilms.

3. Materials and methods

This case study was carried out in the Fish laboratory at the Norwegian University of Life Sciences (UMB) in Ås, Norway. The Fish laboratory has three separated aquaculture recirculating systems (RAS). Two of them are used for cold water species, mainly Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), while the third one is used for the warm water species Nile tilapia (*Oreochromis niloticus*).

This study focuses on the RAS for tilapia, in particular the biofilter which include the moving bed biofilm reactor (MBBR).

The tilapia RAS consists of two fish rearing rooms and one separated water-treatment room. The layout is shown in Figure 3.1 and the simplified flow chart is shown in Figure 3.2.

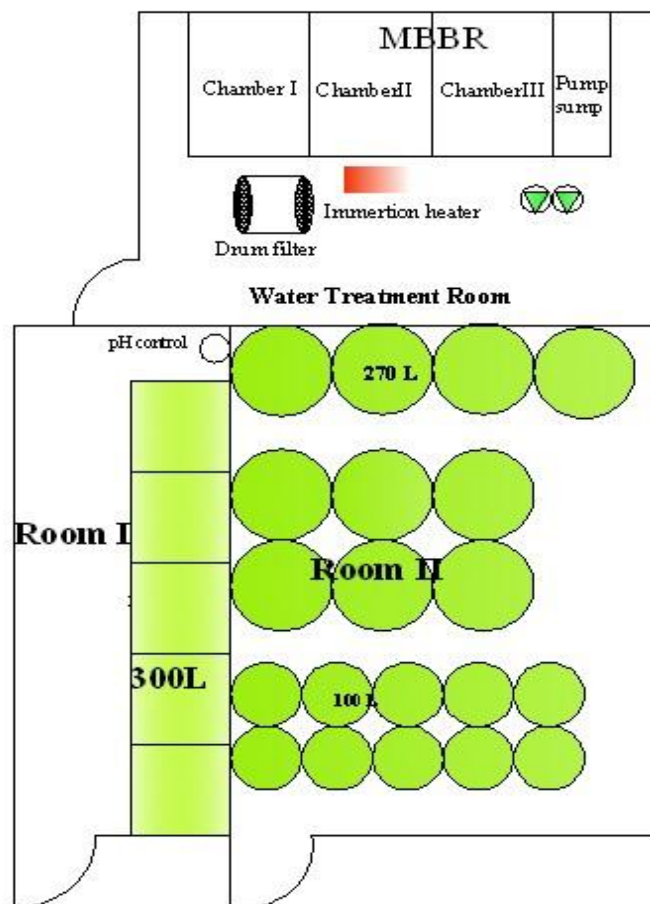


Figure 3.1 Simplified sketch of the tilapia RAS, Fish laboratory, UMB.

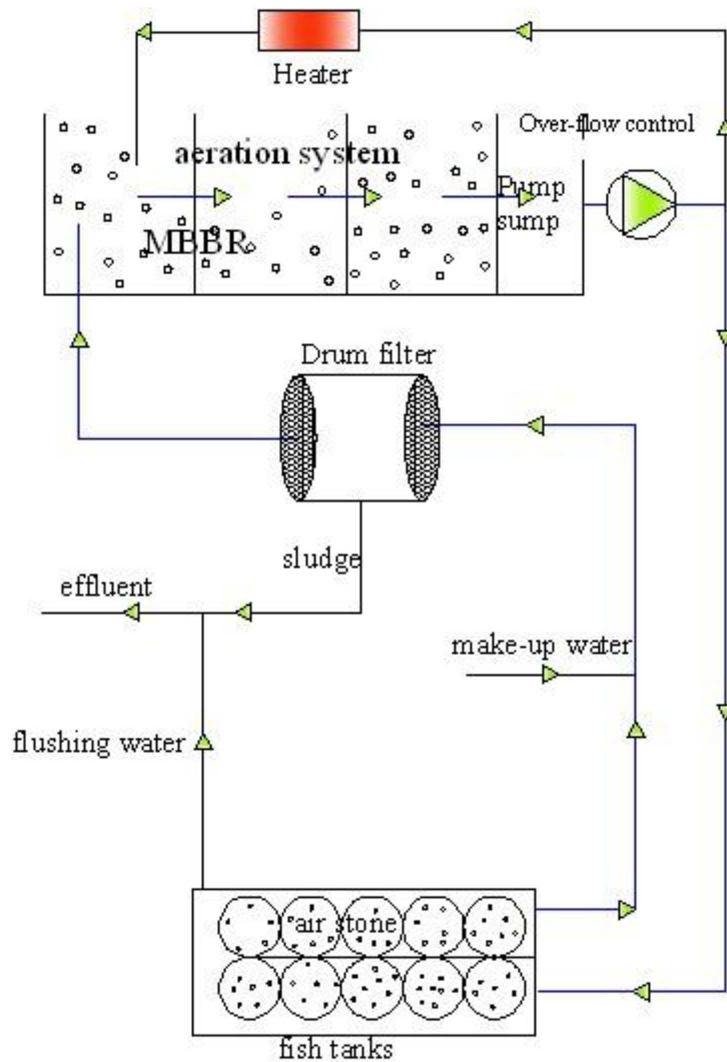


Figure 3.2 Flow chart of the tilapia RAS, Fish laboratory, UMB.

3.1 Nile tilapia in RAS

Nile tilapia (*Oreochromis niloticus)* in the Fish laboratory was imported from Singapore in 2006 (about 1000 fry). This was progeny from the 16th generation of selected Nile tilapia (from the GenoMar GIFT program).

When this study started, it was 213 kg brood fish (average weight 1 kg) and 30 kg smaller fish (average weight 50 g) in the system. Due to the start of a new feeding experiment, the biomass was reduced remarkably. However, to some degree, the growth of the smaller fish compensated the biomass reduction afterwards. Detailed information about the biomass is shown in Table 3.5.

3.2 Fish tanks

Room I consists of 5 square tanks, used mainly for brood stock tilapia. Room II has 10 small round tanks and 10 big round tanks used for start feeding, feeding studies and technical experiments. Details about these tanks are included in Table 3.1. A pictorial view of the two rearing rooms is shown in Figure 3.3.

Pipelines for inlet and outlet are made of PVC material. All tanks have valves to regulate inlet. The outlet system makes it possible to flush sludge water directly into the municipal waste-water system. All the fish tanks are aerated separately by aquarium air stones.

Table 3.1 Fish tanks in the system.

Room	Tank style/number	Volume(l)
Room I	Square tanks/5	300 x 5
Room II	Small circular/10	100 x 10
	Big circular/10	270 x 10

Figure 3.3 Inside the two rearing rooms (Room I at left).

3.3 Components of the water treatment system

From the fish tanks water flows by gravity trough a 125 mm PVC pipeline to the water treatment room. First step is filtration (drum filter) to remove waste solids which originate from waste feed and feces. Second step is the MBBR, in which ammonia and nitrite are oxidized to nitrate. An aeration system is installed at the bottom of the

MBBR. The last step is pumping water back to the fish tanks. Details are listed below. The pictorial view of the water treatment room is shown in Figure 3.4.

Figure 3.4 Water treatment room.

Drum filter

From the fish tanks water flows by gravity into the drum filter (Hydrotech HDF501-1H, Hydrotech AB, Vellinge, Sweden). The screen has a mesh size of 40 µm and adopts the back-flushing theory. Sludge water is discharged to the municipal waste water system. The backwash process causes the major water loss in the tilapia RAS, on average 300 l/day.

Biofilter and aeration system

Water flows by gravity from the drum filter into the MBBR. The basin is made of PE (polyethylene) material (Muliplast AS, Ski, Norway). The basin is separated into 4 chambers by transverse partitions. These have a cutout, covered by a perforated plate with 8 mm round holes to keep media separated (shown in Figure 3.5). The across area is 0.35 m^2 and area available for water to flow through is 0.15 m^2.

Prior to this study the first chamber was without media, while the second and third one were filled with a mixture of Kaldnes K1 (KaldnesMiljøteknologi AS, Tønsberg, Norway) and 1” plastic Pall Ring (Vereinigte Füllkörper-Fabrikenj GmbH & Co, D-56235 Ransbach-Baumbach). The small chamber at the end of the basin is a pump

sump. Here is installed an overflow drain. Detailed information about the MBBR is included in Table 3.2

Aeration system for the MBBR is installed at the bottom of each chamber. Adding air this way combines three important processes: adding oxygen, stripping off CO_2 and maintaining the media in motion. The air blower (SAH 55, Gardner Denver, USA) takes air directly from the room, which is well ventilated. The air is distributed by a grid of PVC pipes with several 2 mm round holes. Air flow in each chamber is adjusted by valves in such a way that media move properly. When water enters the pump sump, the oxygen saturation is normally above 90% and CO_2 less than 2 mg/l. The air distribution grid is shown in Figure 3.6.

Table 3.2 Parameters from the MBBR setup.

Chamber	Length x width x depth (cm)	Water depth (cm)	Volume of water (l)	Volume of media(l)	% of media
C1	85 x 90 x 135	120	830	0	0
C2	85 x 90 x 135	120	830	274	33
C3	85 x 90 x 135	120	830	266	32
Pump sump	35 x 90 x 135	120	310	0	0

Figure 3.5 The perforated partitions in the MBBR.

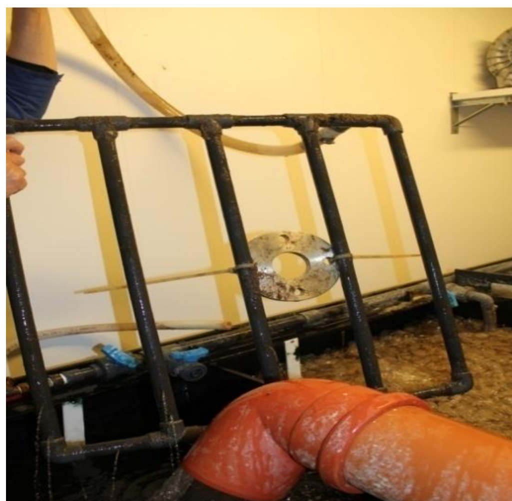


Figure 3.6 Aeration pipes in MBBR.

Circulation pumps

The pump sump is the last chamber in the MBBR basin, located directly after the C3. Two centrifugal pumps (ITT HydroAir AV 150, USA) are installed to lift water back to the rearing rooms through a 90 mm PVC pipeline.

Heaters

Two immersion heaters (Elecro Engineering Ltd, Hertfordshire, UK) each of 3 kW, are installed to keep the temperature at appropriate level (26-27 °C). During this study, a bypass water flow (37.5 l/min) was pumped back from the outlet of the MBBR through the heaters and into C1 again.

Monitoring system

Continuous online monitoring system Oxyguard Commander (OxyGuard International A/S, Birkerød, Denmark) is installed in the pump sump, which transfer date directly to the PC. Here is also installed a float switch (level alarm) connected to the alarm system in the Fish laboratory.

3.4 Make-up water

Make-up water is added to compensate for water loss and regulate alkalinity. It is a mixture of ground water (>75%), tap water and water from the cold water RAS in the Fish laboratory. On average 1.5-2 l/min is added. The make-up water is added into the outlet of one fish tank so it will be well mixed before entering the MBBR. The quality of the make-up water is very stable, pH 7.3-7.5, alkalinity 2.4-2.6 mmol/l. The relatively high alkalinity helps to keep the system alkalinity above 1 mmol/l (which is recommended for the nitrification process). The amount of make-up water added to the system is presented in Table 3.3.

Table 3.3 Physical parameters of the tilapia RAS.

P[a]	Make-up water (l/min)	Total water flow (l/min)	Recirc[b] (%)	Bypass through heaters (l/min)	Hydraulic retention[c] time (min)
1	2.7	155	98.3	37.5	16.1
2	2.0	173	98.8	37.5	14.4
3	1.8	150	98.8	37.5	16.6
4	1.8	150	98.8	37.5	16.6
5	2.8	195	98.6	37.5	12.8
6	1.8	188	99.0	37.5	13.2
7	1.6	184	99.1	37.5	13.5
8	2.1	180	98.8	37.5	13.8
9	1.4	171	99.2	37.5	14.6
10	1.3	173	99.2	37.5	14.4

a: Experimental periods, see section 3.7.3.

b: Definition of recirculation is expressed as the ratio between amount of make up water (A) and the total waterflow (T); Degree of recirculation (%)= (1-A/T) × 100

c: Hydraulic retention time (min) = Volume of MBBR/water flow

3.5 Flushing routines

Flushing of the fish tanks is an important routine to prevent organic matter to settle and block the outlet. Besides backwash of the drum filter, this process makes up the main water loss in the RAS.

Fish tanks in room I are flushed twice a week, while tanks in room II are flushed every day. The normal procedure is to flush out 8-10 l of water from each tank. Flushed water from the fish tanks is discharged to the municipal waste-water system. The flushing process causes a water loss in the RAS of about 200 l/day (40% of the total water loss). Each tank in room II is installed with a strainer at the outlet to collect uneaten feed and feces. The strainers have a mesh size of 1 mm. They are normally emptied once a day.

3.6 Feed and feeding routine

The amount of feed offered to the tilapia in the room I was according to the experience of engineers working in the Fish lab mainly because these are brood stock

(average feeding rate is about 0.7 % of bodyweight/day). Automatic disc-feeders offered feed 6 times a day (24 h non-stop). Commercial feed (Aller Aqua, Christiamsfeld, Denmark) was used. The feed ingredients and amount are presented in Table 3.4 and Table 3.5.

Table 3.4 Protein ratioes of different type feed used.

Feed Type	APC Feed 1	APC Feed 2	APC Feed 3	APC Feed 4	APC Feed 5	Commercial Feed
Protein ratio (%)	0.25	0.29	0.32	0.36	0.39	0.32

The feeding experiment by APC (Aquaculture Protein Center) was held from October 28th to November 27th 2011 (during the exchange process) in room II. Information about feed amount and feed ingredients is provided by the researcher from APC. It is shown in Table 3.4 and 3.5. Automatic belt feeders were used during the exchange process. Feeding periods were 09:00-09:30, 13:00-13:30, 17:00-17:30 and 21:00-21:30. The uneaten feed and feces were collected after the feeders were run out.

Table 3.5 Biomass, feed and feeding routines.

	Room I		Room II		Total	
P[a]	Biomass (kg)	Feed type/ Amount(kg)/day	Biomass (kg)	Feed type/ Amount(kg)/day	Biomass (kg)	Feed/day (kg)
1	213	commercial/1.5	30	commercial/0.3	243	1.8
2	188	commercial/1.2	33	APC/1.2	221	2.4
3	188	commercial/1.2	37	APC/1.3	225	2.5
4	188	commercial/1.2	42	APC/1.6	230	2.8
5	175	commercial/1.2	47	APC/1.6	222	2.8
6	145	commercial/0.8	51	APC/1.6	196	2.4
7	145	commercial/0.8	56	APC/1.7	201	2.5
8	145	commercial/0.8	62	APC/1.9	207	2.7
9	145	commercial/0.8	56	APC/0.9	201	1.7
10	145	commercial/0.8	0	APC/0.0	145	0.8

a: Experimental periods, see section 3.7.3.

The amount of make-up water per kilogram feed fed is calculated. The results are shown in Table 3.6. According to the information given by Hydrotech (Hydrotech, Veolia Water), the normal value for RAS ranges from 0.02 to 0.05 m^3/kg feed. Martins *et al.* (2010) defined feed loading rate > 50 m^3/kg feed as flow through, 1-50 m^3/kg as reuse; 0.1-1 m^3/kg as conventional recirculation and <0.1 m^3/kg as innovative RAS.

Table 3.6 The amount of make-up water used per kilogram of feed.

Period[a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Feed loading rate (m^3/kg)	2.1	1.2	1.0	0.9	1.4	1.1	0.9	1.1	1.2	2.3

Feed loading rate = make up water (m^3/day) / feed (kg/day).

a: Experimental periods, see section 3.7.3.

3.7 Exchange process set up

3.7.1 General description of the exchange process

The purpose of this case study was to replace the "old" media in chamber 2 and 3 with new Anox K5 and BiofilmChip M. In order to afford bacterial source for the "new" media, "old" media in chamber 2 were moved to the empty chamber 1 firstly. New Anox K5 were then placed in chamber 2. "Old" media in chamber 3 were then gradually taken out, on average 50 l at once. After chamber 3 was empty, new BiofilmChip M were filled in chamber 3. Emptying of chamber 3 took 25 days in total. Until this process, the chamber 1 was filled with "old" media, chamber 2 was filled with Anox K5 and chamber 3 was filled with BiofilmChip M. Afterwards, "old" media in chamber 1 were taken out gradually. Because of very low TAN or NO_2 levels, the pace of taking out media in chamber 1 was faster than that of chamber 3. The detailed schedule is shown in Table 3.7 and Figure 3.7.

3.7.2 The amount of "new"media filled in

As one of the initial purposes of this study was to compare the MBBR's efficiency before and after the exchange process, we decided to put the same volume of "new" media as that of "old" media. However, because of the size difference, most of the Kaldnes K1 were sucked into the Pall Ring. The volume of "new" media we filled in is the total volum of Kaldnes K1 and Pall Ring when we measured them separately. So in chamber 2 we filled in 270 l of Anox K5 instead of 220 l. However, as no nitrification was observed in chamber 3, we decided to put the same amount of BiofilmChip M as that of "old" media, which was 263 l.

3.7.3 Division of the exchange period

The exchange process was divided into 10 periods according to the amount and the type of media used in each chamber. Which day to take out the "old" media (Mixture of the Kaldnes K1 and Pall Ring) and the amount of media to take out was decided on the basis of the system water quality. Several samples were taken during each period on different days. The detailed information about the sampling and measuring schedule is shown in Appendix I. The average value was taken as the result of one period. Number of samples in each period and other detailed information is shown in Table 3.7 and Figure 3.7.

Table 3.7 Schedule of the exchange process.

P	Date	Duration (days)	No of samples	C1 (V/type)	C2 (V/type)	C3 (V/type)
1	21/10-26/10	6	5	0	220 /M	263/M
2	27/10-30/10	4	3	220/M	270 /K5	263/M
3	31/10-04/11	5	4	220/M	270/K5	213/M
4	05/11-09/11	5	5	220/M	270/K5	163/M
5	10/11-14/11	5	5	220/M	270/K5	113/M
6	15/11-16/11	2	2	220/M	270/K5	33/M
7	17/11-21/11	5	5	220/M	270/K5	263/BC
8	22/11-25/11	4	4	170/M	270/K5	263/BC
9	26/11-28-11	3	3	90/M	270/K5	263/BC
10	29/11-02/12	4	4	0	270/K5	263/BC

M = Mixture of Kaldnes K1 and Pall Ring; K 5 = Anox K5; BC = BiofilmChip M; V = Volume of the media (l).

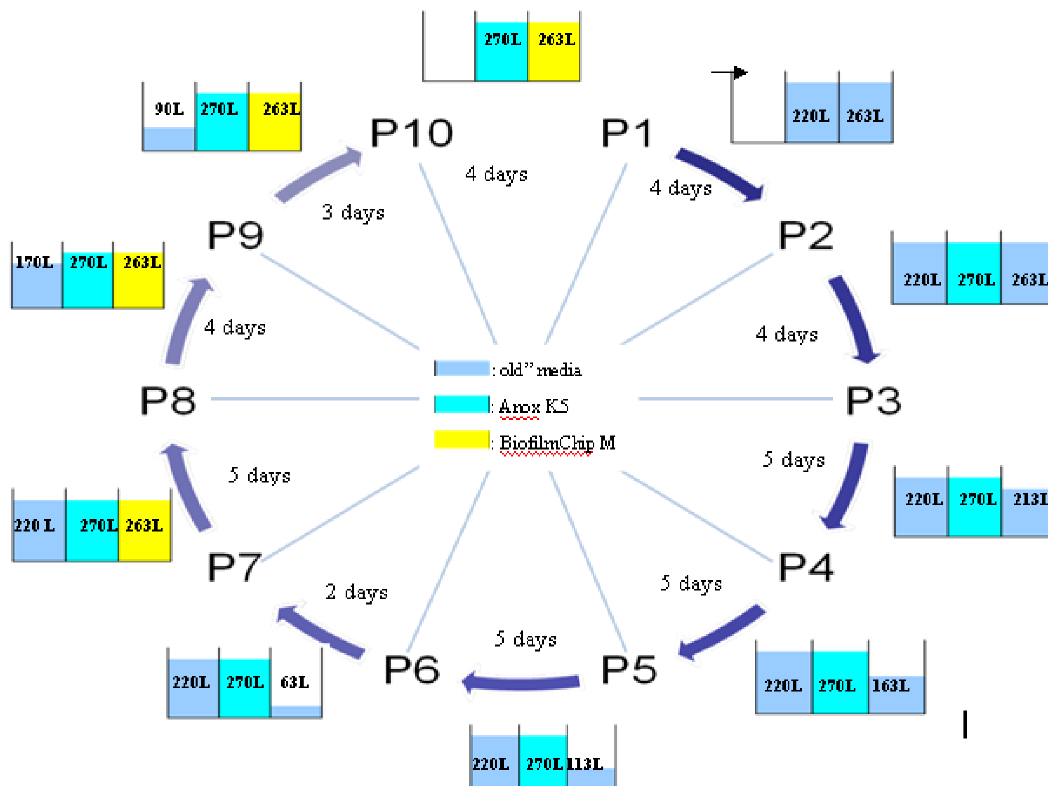


Figure 3.7 Schedule of the exchange process.

The transfer of media was done in a very gentle way using a fine-mesh landing net and a 25 l bucket to avoid destruction of the biofilm. The media were never out of water for more than 30 seconds. The transfer of media was done between 14:00 and 15:00 while water sampling was done between 10:00 and 11:00. In case of dramatical reduction of nitrification efficiency, the taken out media were stored in a well aerated bucket for some days.

3.8 Sampling routines and measurement methods

3.8.1 Sample preparation

Water samples were taken at 4 points. The location of sampling points is shown in Figure 3.8.

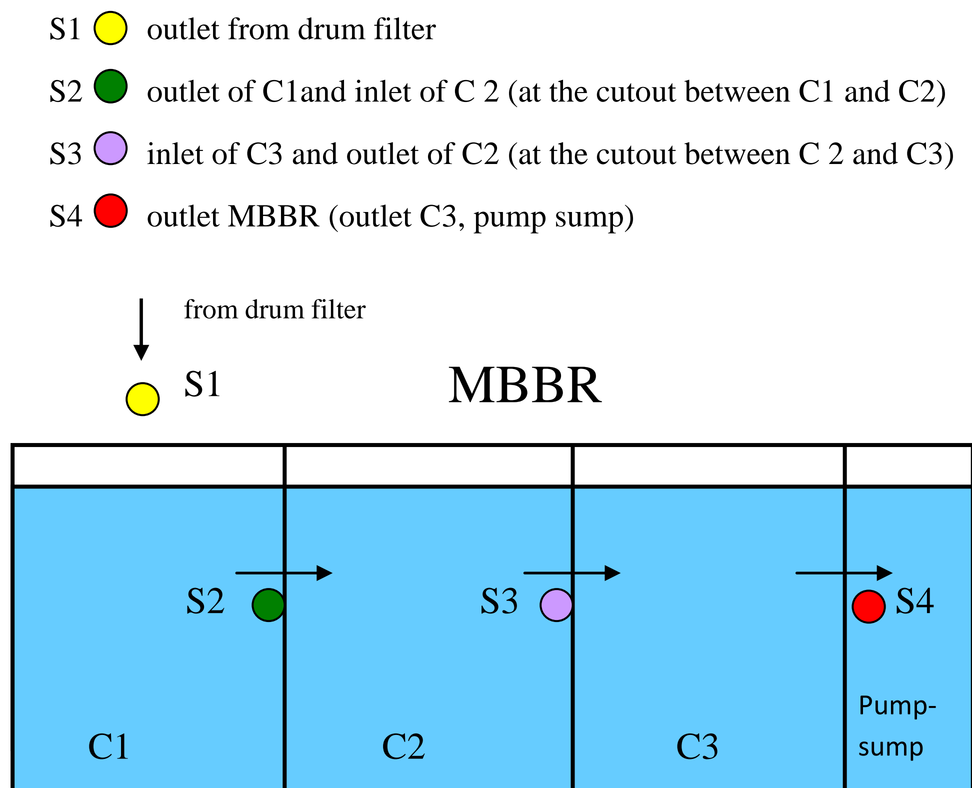


Figure 3.8 The location of sampling points.

Samples from the MBBR were taken at 0.5 m depth and stored in bottles (Polyethylene, 500 ml), which were washed by sample water three times before they were finally filled up. Samples were immediately analyzed for pH, alkalinity, NH_4-N and NO_2-N.

3.8.2 Water flow

Total water flow was measured at the outlet of the drum filter by using stop watch and a 25 l bucket. The average of three samples was used. The same method was used to measure the amount of make-up water.

3.8.3 Fish biomass

Fish biomass in room I was measured once before starting the experiment. These tilapia was mainly brood stock with an SGR (specific growth rate) close to zero. A lot fish from this room were taken out during the exchange period. The data of biomass in room II was provided by the researcher from APC. Results are shown in Table 3.5.

3.8.4 Oxygen, temperature, pH and alkalinity

Oxygen and temperature were measured directly at sampling points S1 and S4. Samples for pH and alkalinity were also taken at S1 and S4.

Oxygen and temperature were measured by OxyGuard Handy oxygen meter (OxyGuard International A/S, Birkerød, Denmark). Temperature was also read separately from a spirit thermometer (27-1000-10, Stener Fish Tech AS, Langhus, Norway).

pH was measured immediately after the samples were taken to the laborotary by using Oxyguard Handy pH meter (OxyGuard International A/S, Birkerød, Denmark), which was calibrated every day with standard buffer solutions pH 4 and pH 7.

Alkalinity was measured by titration with 0.1 M HCl to pH 4.5. The method is described in Britian Standard-Water quality determination of alkalinity (BS EN ISO 9963-2: 1996).

Temperature, pH and dissolved oxygen (DO) kept stable during the exchange process. Temperatures were within the range of 26-27 °C. DO levels in the inlet and outlet were around 6.8 mg/l and 7.2 mg/l respectively. DO levels in the outlet were always higher than that in inlet. pH appeared stable both in inlet and outlet of the MBBR for the whole exchange process, 7.3-7.5 in inlet and 7.4-7.7 in outlet. pH was always higher in the outlet than in the inlet. No consistent trend was observed for alkalinity in the inlet and outlet of the MBBR. More detailed information is shown in Table 3.8.

Table 3.8 Water quality parameters for the whole system during the exchange process: temperature (ºC), DO (mg/l), pH, alkalinity (mmol/l).

P	Temperature (ºC)	DO inlet	DO outlet	pH inlet	pH outlet	Alkalinity inlet	Alkalinit y outlet
1	26	6.5	7.4	7.4	7.5	1.0	1.0
2	26	6.9	7.4	7.4	7.5	1.1	1.1
3	27	6.9	7.4	7.4	7.6	1.3	1.3
4	27	6.8	7.3	7.4	7.5	1.3	1.3
5	27	6.7	7.3	7.4	7.5	1.2	1.2
6	27	6.8	7.4	7.5	7.6	1.3	1.3
7	27	6.5	7.2	7.4	7.5	1.4	1.4
8	27	6.6	7.1	7.3	7.4	1.3	1.3
9	27	6.5	7.1	7.4	7.4	1.4	1.3
10	26	7.1	7.4	7.5	7.7	1.5	1.5

3.8.5 NH_4-N concentration

The concentration of NH_4-N was determined by using Spectroquant® Ammonium test (1.14752.0001, Merck KGaA, Darmstadt, Germany), which is shown in Figure 3.9. The method is analogous to United Stated Environmental Protection Agency (EPA 350.1), American Public Health Association (APHA 4500-NH3 D), International standard organization (7150/1) and German Institute for Standardization (DIN 38406 E5). More information about the reagents is shown in Appendix II. Table 3.9 shows the characteristic quality data of the method. Figure 3.11 shows typical colors of prepared samples.

Table 3.9 Characteristic quality data of the method (1.14752.0001), 10-mm cell.

Parameters	Value
Standard deviation of the method (mg/l NH_4-N)	±0.023
Co-efficiency of variation of the method (%)	±1.6
Co-efficiency interval(mg/l (mg/l NH_4-N))	±0.06
Number of lots	35
Measuring range (mg/l NH_4-N)	0.05-3.00
Accuracy of the measurement value (mg/l NH_4-N)	max.±0.08

The second measurement was taken if strange results were obtained.

3.8.6 NO_2-N concentration

The concentration of NO_2-N was determined by using Spectroquant Nitrite Test (1.14776.0001, Merck KGaA, Darmstadt, Germany), which is shown in Figure 3.9. The method is analogous to EPA 354.1, APHA 4500-NO_2-B, and DIN EN 26 777 D10. Detailed information about the reagents used is shown in Appendix III. Table 3.10 shows the characteristic quality data of the method. Figure 3.12 shows typical colors of prepared samples

Table 3.10 Characteristic quality data of the method (1.14776.0001), 10-mm cell.

Parameters	Value
Standard deviation of the method (mg/l NO_2-N)	±0.008
Co-efficiency of variation of the method (%)	±1.5
Co-efficiency interval (mg/l NO_2-N)	±0.02
Number of lots	37
Measuring range (mg/l NO_2-N)	0.02-1.00
Accuracy of the measured value (mg/l NO_2-N)	max.±0.03

The second measurement was taken if strange results were obtained.

Figure 3.9 Test kits for NH_4-N and NO_2-N determination.

Figure 3.10 Spectroquant® NOVA 60A photometer.

Figure 3.11 NH_4-N determination: typical colours of prepared samples (highest consentration to the right).

Figure 3.12 NO_2-N determination: typical colours of prepared samples (highest consentration to the left).

The reagents and sample water were mixed in 15 ml glass bottles, which were washed three times with sample water before using. All the procedures were processed according to the instruction provided by the test kits' producer. The amount of samples and reagents were measured and transferred by using appropriate pipettes (No.4642090 and No.4642100, Finnpippette, Finland). Prepared samples were placed in 10 mm

cuvette and the results were read from a Spectroquant® NOVA 60A photometer (Merck KGaA, Darmstadt, Germany), which is shown in Figure 3.10. The photometer was calibrated by using standard solutions and the predefined bar code for the current methode.

3.9 Characteristics of the biofilm media

Because of the hydraulic mode of MBBR operation the active biofilm is primarily formed on the inner, hollow surface areas of media (Suhr and Pedersen, 2010). In this study, only the protected surface area (specific biofilm area) is used (Table 3.11), which is significantly smaller than the total surface area (Rusten *et al.,* 2006). Information about the biofilm media is shown in Table 3.11. Pictorial view of different biofilm media is shown in Figure 3.13.

Table 3.11 Data for some biofilm media.

	Type of media			
	Pall Ring[a]	**Kaldnes K1**[b]	**Anox K5**[c]	**Kaldnes BiofilmChip™ M**[c]
Nominal diameter (mm)	25	9.1	25	48
Nominal thickness (mm)	/	7.2	3.5-4	2.2
Bulk density (kg/m^3)	/	150	118	225
Protected surface area (in bulk) (m^2/m^3)	220	500	800	1200

a: Lekang and Kleppe, 2000; b: Rusten et al., 2006; c: Anox kaldnes, 2009.

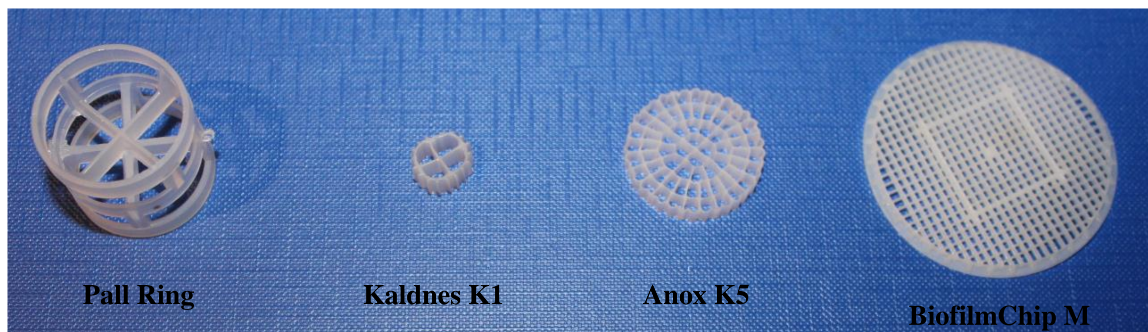


Figure 3.13 Photo of different biofilm media.

3.10 Calculation methods

3.10.1 Calculation of total protected surface area

The protected surface area was calculated as the mixture of Kaldnes K1 and Pall Ring. Because of the size difference and turbulence, a lot of K1 had fastened in the holes of the Pall Ring as shown in Figure 3.14. On average (3 samples), 2 l of the mixture contained 2 l of Pall Ring and 0.425 l of Kaldnes K1. The volume of K1 was 21.25% of the total volume. The calculation of total protected surface area of the mixture ("old" media) can be expressed as Equation 3.1.

Figure 3.14 The mixture of the Kaldnes K1 and Pall Ring.

$$\mathbf{A_M = (220\ m^2/m^3 \times V_M + 500\ m^2/m^3 \times 21.25\%V_M} \quad \textbf{......Equation 3.1}$$

Where: A_M is the total protected surface area of the mixture; V_m is the volume of mixture (Kaldnes K1 and Pall Ring).

The protected surface area of Anox K5 can be calculated with Equation 3.2**.**

$$\mathbf{A_A = 800\ m^2/m^3 \times V_A} \quad \textbf{......Equation 3.2}$$

Where: A_A is the total protected surface area of Anox K5; V_A is the volume of Anox K5.

The protected surface area of BiofilmChip M can be calculated with Equation 3.3.

$$\mathbf{A_B = 1200\ m^2/m^3 \times V_B} \quad \textbf{......Equation 3.3}$$

Where: A_B is the total protected surface area of BiofilmChip M; V_B is the volume of BiofilmChip M.

3.10.2 TAN and NH_3-N calculation from the measured NH_4-N consentration

As discussed in the literature review part, the ratio of NH_4-N and NH_3-N in TAN is determined by the temperature, pH and salinity (salinity = 0 in this case because fresh water was used). The percentage of NH_3-N in TAN is shown in Table 2.1. The calculation of NH_4-N is shown in Equation 3.4; NH_3-N is shown in Equation 3.5.

$$\mathbf{C_{TAN} = C_{NH4\text{-}N} / (1 - P_{NH3\text{-}N})} \quad \textbf{Equation 3.4}$$

$$\mathbf{C_{NH3\text{-}N} = P_{NH3\text{-}N} \times C_{TAN}} \quad \textbf{Equation 3.5}$$

Where C_{TAN} is the concentration of TAN (mg/l); $C_{NH4\text{-}N}$ is the concentration of NH_4-N (mg/l), which is measured by the Spectroquant® NOVA 60 A photometer (MERCK, Merck company, Darmstadt, Germany); $P_{NH3\text{-}N}$ is the percentage of NH_3-N in TAN under different pH and temperatures, which can be read from Table 2.1; $C_{NH3\text{-}N}$ is the concentration of NH_3-N (mg/l).

3.10.3 Calculation of MBBR's inlet water quality

The inlet of MBBR is a combination of water from the drum filter and bypass water through the heater, which is pumped from the pump sump. The calculation method is shown in Equation 3.6 (taking the TAN level as example).

$$\mathbf{TAN_I = P \times TAN_d + TAN_O\,(1-P)} \quad \textbf{Equation 3.6}$$

Where TAN_I is the TAN concentration in inlet of MBBR (mg/l); P is the percentage of water from drum filter in total water flow; TAN_d is the TAN concentration after drum filter (mg/l); TAN_O is the TAN level in pump sump (mg/l).

3.10.4 Calculation of areal TAN removal rate

Areal TAN removal rate can be calculated by using Equation 3.7 (Malone and Beecher, 2000):

$$\mathbf{ATR = K_C\ (TAN_I - TAN_O)\ Q/A} \text{..........} \textbf{Equation 3.7}$$

Where ATR is the g TAN removed per m^2 of biofilm media per day (g TAN m^{-2} d^{-1}); Q is the flow rate through the biofilter (l/min); K_c is the unit conversion factor of 1.44 (24 h×60 min/1000); TAN_I is the inlet TAN concentration (mg/l); TAN_O is the outlet TAN concentration (mg/l); A is the biofilm media's protected surface area (m^2).

3.10.5 Calculation of areal nitrite removal rate

Since NO_2-N is produced when TAN is converted, the level of NO_2-N removal rate should be connected with the areal TAN removal rate (Malone and Beecher, 2000). The areal NO_2-N removal rate can be expressed as Equation 3.8:

$$\mathbf{ANR = ATR + K_C\ (NO_2\text{-}N_I - NO_2\text{-}N_O)Q/A} \text{..........} \textbf{Equation 3.8}$$

Where ANR is the g NO_2-N removed per m^2 of biofilm media per day (g NO_2-N m^{-2} d^{-1}); ATR is the areal TAN removal rate (g TAN m^{-2} d^{-1}); Q is the flow rate through the biofilter (l/min); K_c is the unit conversion factor of 1.44 (24 h×60 min/1000); NO_2-N_I is the inlet NO_2-N concentration (mg/l); NO_2-N_O is the outlet NO_2-N concentration (mg/l); A is the biofilm media's protected surface area (m^2).

3.10.6 Calculation for the amount of alkalinity consumed per day

$$\mathbf{A_{Alkalinity} = K_C\ (ALK_I - ALK_O)\ Q} \text{..........} \textbf{Equation 3.9}$$

Where $A_{Alkalinity}$ is amount of alkalinity consumed per day (g $CaCO_3$/day); K_c is the unit conversion factor of 1.44 (24 h×60 min/1000); ALK_I is the alkalinity inlet of MBBR (g $CaCO_3$/l); ALK_O is the alkalinity outlet of MBBR (g $CaCO_3$/l); Q is the flow rate through the biofilter (l/min).

3.10.7 Calculation forf the amount of TAN oxidized per day

$$A_{Alkalinity} = K_C (TAN_I - TAN_O) Q \text{ .... Equation 3.10}$$

Where $A_{Alkalinity}$ is the amount of alkalinity consumed per day (g/day); K_c is the unit conversion factor of 1.44 (24 h×60 min/1000); TAN_I is the inlet TAN concentration (mg/l); TAN_O is the outlet TAN concentration (mg/l); Q is the flow rate through the biofilter (l/min).

3.11 Bacteria sampling method

In order to study the microbial community's development and composition at the "new" biofilm media, samples were taken before, during and after the exchange process. It included biofilm media and water samples. The time schedule of taking out the bacteria samples is shown in Appendix I. Samples of the "old" media were taken before the exchange process. During and after the exchange process, samples of the "new" Anox K5 and BiofilmChip M were taken from chamber 2 and 3 repectively. Samples of the make-up water for bacterial analysis were also taken before, during and after the exchange process, two for each stages.

Water samples for bacterial analysis were always taken at the same place as the samples for water quality analysis, which is shown in Figure 3.8. 100 ml of water was taken for each sample and was kept in a cooler (4 °C) until harvesting the micro biomass. 50 ml of this sample (well mixed) was filtered through a 0.22 μm membrane filter (MF-Millipore Membrane, Merck KGaA, Darmstadt, Germany) by using a vacuum filteration system (Figure 3.15). The filtration system was cleaned very carefully before each sample's filtration. After filtration, the filter was placed in a small plastic tube and stored at -80 °C until the extraction of genomic DNA. The tubes were well marked with information about the date, sampling point and amount of water used for filtration.

In order to avoid the bacterial interaction, gloves were used to take out the biofilm media from the MBBR. 5 pieces Anox K5 and 3 pieces BilfilmChip M were taken for every sampling. They were placed in a zip-lock bag. The bags were well marked (Figure 3.15) and stored at -80 °C until the extraction of genomic DNA.

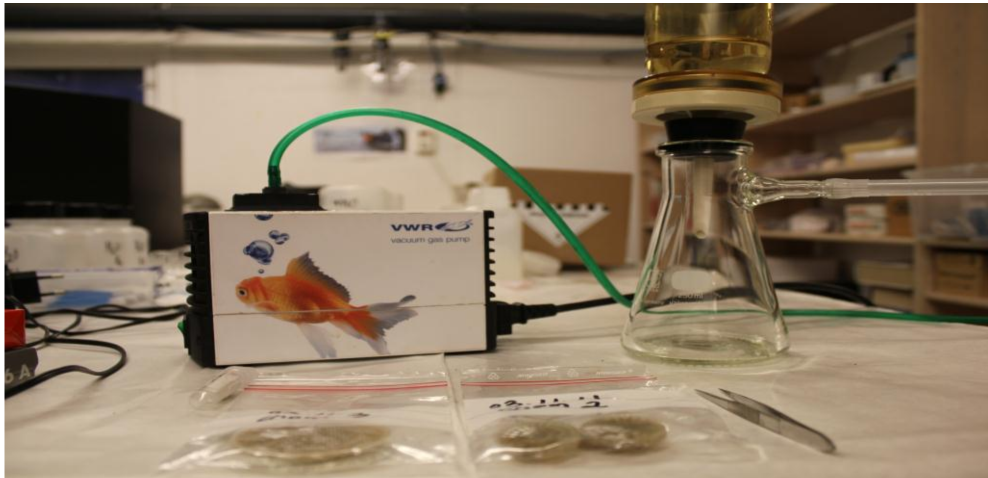


Figure 3.15 Filtration system and bacterial samples

4. Results

4.1 MBBR's situation before the exchange process

Samples were taken daily 5 days before exchanging media. In general MBBR's TAN loading level showed a reduction when closer to the start of the exchange process. NO_2-N variation showed the same trend as that of TAN level. Relatively high values of areal TAN removal rate and areal NO_2-N rate were observed. The highest values were 0.19 g TAN m^{-2} d^{-1} and 0.29 g NO_2-N m^{-2} d^{-1}. Detailed information about the MBBR's situation before the exchange process is shown in Table 4.1.

Table 4.1 MBBR's situation before the exchange process: TAN and NO_2-N (mg/l); ATR (areal TAN removal rate, g TAN m^{-2} d^{-1}); (areal NO_2-N removal rate, g NO_2-N m^{-2} d^{-1}).

Date	TAN inlet	TAN outlet	NO_2-N inlet	NO_2-N outlet	ATR	ANR
21.10.11	0.38	0.25	0.18	0.16	0.18	0.20
22.10.11	0.39	0.25	0.17	0.15	0.19	0.21
23.10.11	0.35	0.21	0.14	0.13	0.19	0.20
25.10.11	0.29	0.19	0.12	0.09	0.14	0.19
26.10.11	0.33	0.22	0.19	0.09	0.15	0.29

4.2 MBBR's situation during the exchange process

In general TAN levels both for inlet and outlet of the MBBR showed a reduction during the exchange process. At the same time, no TAN accumulation appeared in the MBBR. TAN levels in outlet were always lower than that of inlet. The difference between the inlet and outlet TAN level was largest at the beginning of the process and smallest at the end of the process. As for the poisonous part of TAN, NH_3 were at very low levels during the whole exchange process. The highest NH_3 level in the outlet of MBBR was 0.01 mg/l. More information about TAN level variations is shown in Table 4.2, Figure 4.1 and Figure 4.2.

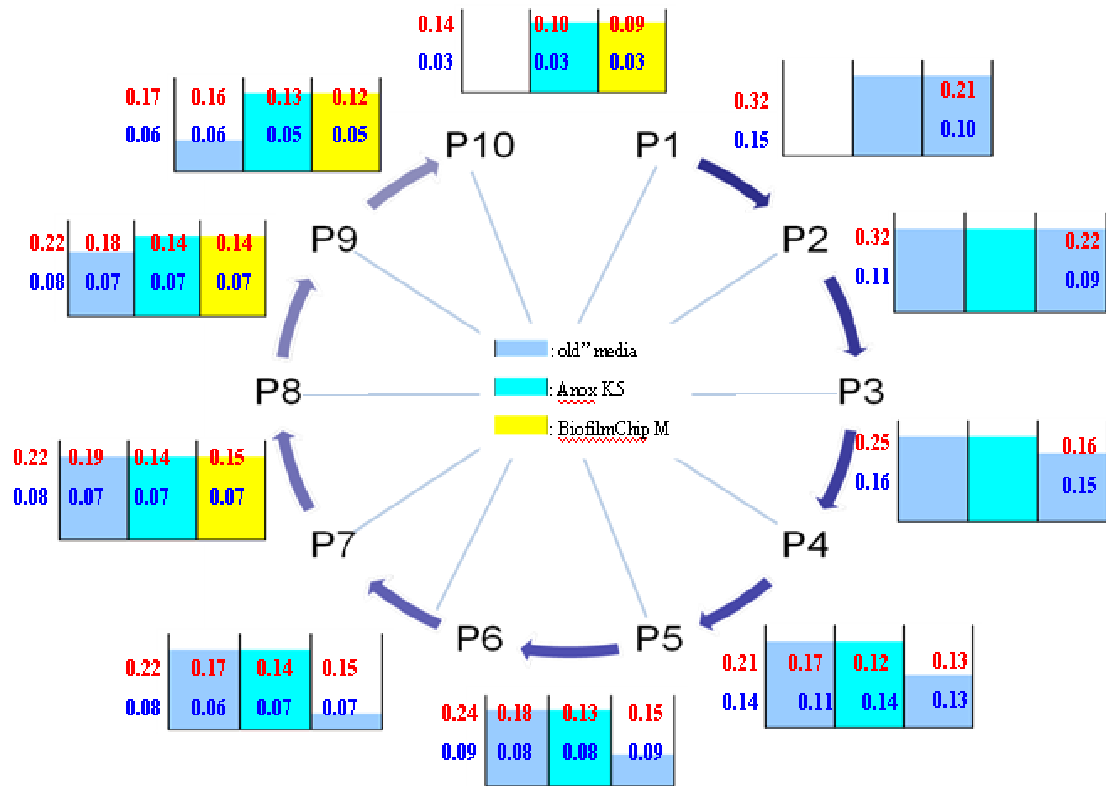


Figure 4.1 TAN and NO_2-N level variations in the MBBR throughout the exchange process. TAN values are in red color and NO_2-N values in blue. The inlet values are put outside of the three columns (chamber 1, 2 and 3). Values inside the columns stand for the TAN and NO_2-N levels in chamber 1, 2 and 3.

Table 4.2 MBBR's situation during the exchange process: TAN (mg/l), NH_3 (mg/l), NO_2-N (mg/l). The results are given as mean ± S.E (standard error, n in each period is the same value as that sample amount shown in Table 3.7).

P	TAN inlet	NH_3 inlet	TAN outlet	NH_3 outlet	NO_2-N inlet	NO_2-N outlet
1	0.35±0.02	0.01	0.23±0.01	0.01	0.16±0.01	0.12±0.01
2	0.32±0.01	0.01	0.22±0.00	0.01	0.11±0.01	0.09±0.01
3	0.25±0.03	0.01	0.16±0.02	0.00	0.16±0.02	0.15±0.02
4	0.21±0.02	0.01	0.13±0.01	0.00	0.14±0.01	0.13±0.01
5	0.24±0.02	0.01	0.15±0.01	0.00	0.09±0.01	0.09±0.01
6	0.22±0.01	0.01	0.15±0.02	0.00	0.08±0.01	0.07±0.01
7	0.22±0.01	0.01	0.15±0.00	0.00	0.08±0.00	0.07±0.00
8	0.22±0.01	0.00	0.14±0.01	0.00	0.08±0.01	0.07±0.01
9	0.17±0.01	0.00	0.12±0.01	0.00	0.06±0.01	0.05±0.01
10	0.15±0.00	0.00	0.11±0.00	0.00	0.04±0.00	0.04±0.00

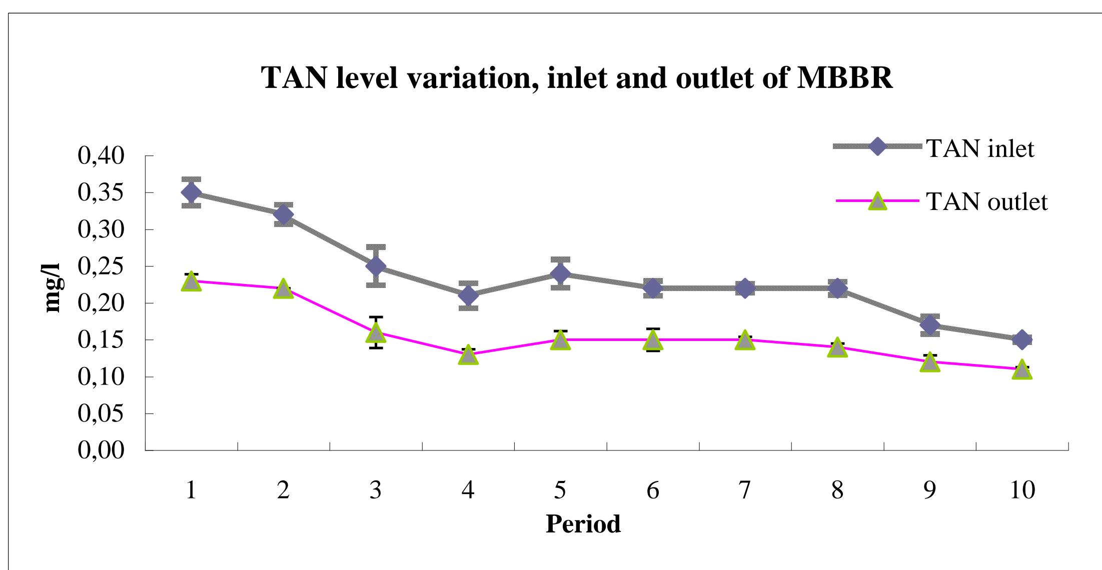


Figure 4.2 TAN level variation, inlet and outlet of MBBR

There were reduction in NO_2-N levels for both the inlet and outlet during the exchange process. Except for period 1 and 2, NO_2-N levels in inlet and outlet showed the same value (Figure 4.3, Table 4.2). Period 3 had the highest levels of NO_2-N in the outlet which was 0.15±0.02 mg/l.

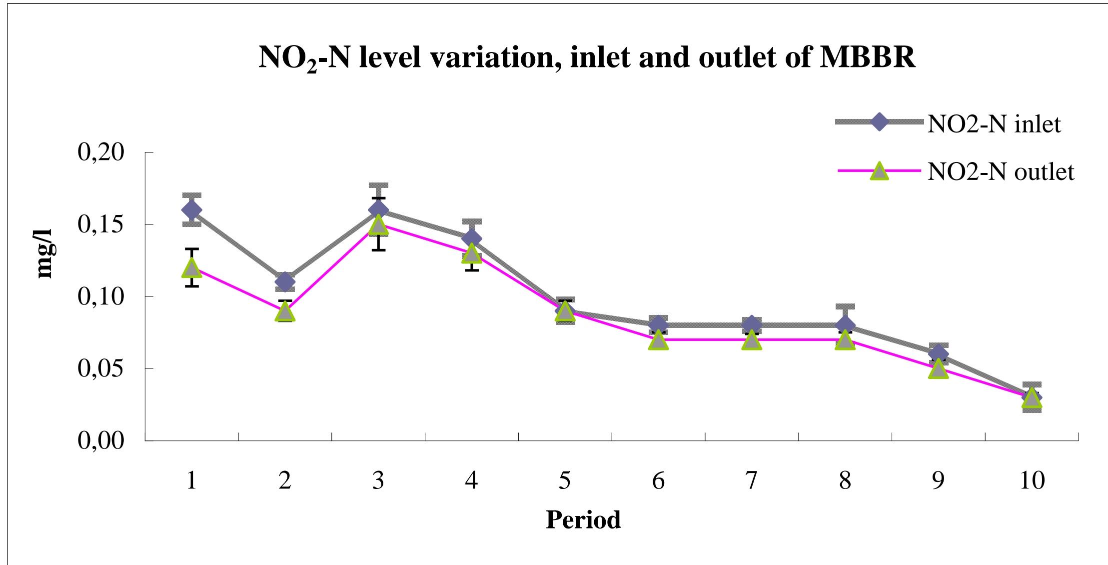


Figure 4.3 NO_2-N level variations, inlet and outlet of MBBR.

4.2.1 Chamber 1's situation during the exchange process

The development of C1 was divided into two phases. Phase 1 was the period before taking out "old" media (period 2 to 7) and phase 2 (period 8 and 9) was when the "old" media were gradually taken out.

The MBBR's inlet was considered as the inlet of chamber 1. The measurement for the outlet of chamber 1 started from period 4. There was always a reduction in TAN from inlet to outlet during the exchange process. No TAN accumulation was observed during phase 2 when the "old" media were gradually taken out. Unexpectedly, the difference between the inlet and outlet TAN level was bigger in period 8 (220 l "old" media) than that of period 7 (270 l "old" media). Details about the TAN level variation in chamber 1 are shown in Table 4.3 and Figure 4.4.

Table 4.3 Chamber 1's situation during the exchange process, phase I and II: TAN, NO_2-N (mg/l); ATR (areal TAN removal rate, g TAN m^{-2} d^{-1}); ANR (areal NO_2-N removal rate, g NO_2-N m^{-2} d^{-1}). The results are given as mean ± S.E (standard error, n in each period is the same value as that sample amount shown in Table 3.7).

Phase	P	TAN inlet	TAN outlet	NO_2-N inlet	NO_2-N outlet	ATR	ANR
I	2	0.32±0.01	/	0.11±0.01	/	/	/
	3	0.25±0.03	/	0.16±0.02	/	/	/
	4	0.21±0.02	0.17±0.01	0.14±0.01	0.11±0.01	0.13±0.03	0.12±0.08
	5	0.24±0.02	0.18±0.02	0.09±0.01	0.08±0.01	0.22±0.02	0.27±0.04
	6	0.22±0.01	0.17±0.02	0.08±0.01	0.06±0.01	0.20±0.01	0.24±0.03
	7	0.22±0.01	0.19±0.01	0.08±0.00	0.07±0.00	0.12±0.00	0.14±0.03
II	8	0.22±0.01	0.18±0.01	0.08±0.01	0.07±0.01	0.14±0.01	0.17±0.03
	9	0.17±0.01	0.16±0.01	0.06±0.01	0.06±0.01	0.05±0.01	0.01±0.08

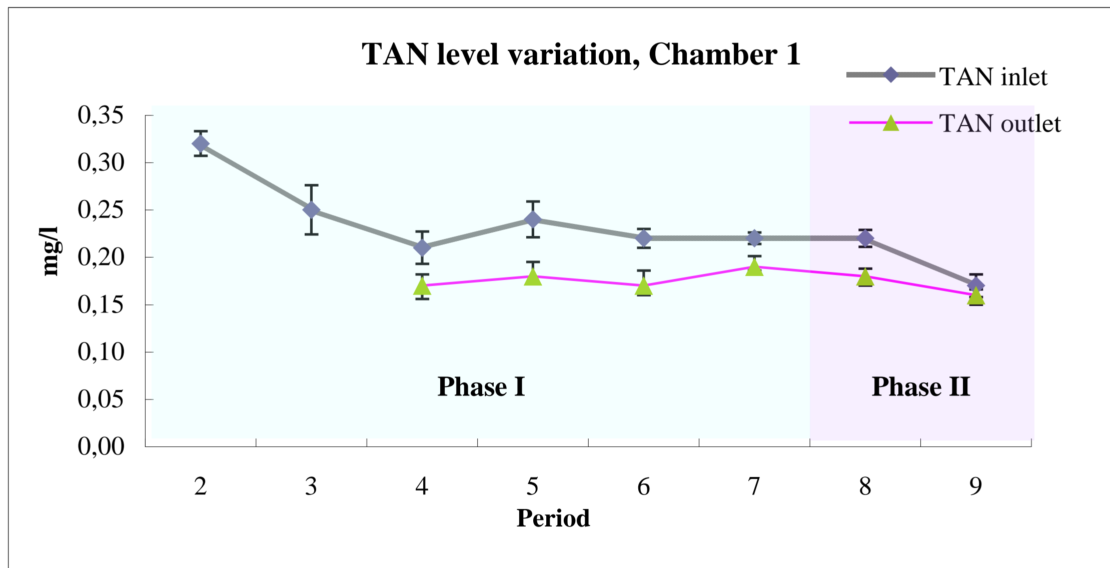


Figure 4.4 TAN level variation, Chamber 1.

In general, both the inlet and outlet NO_2-N decreased during the exchange process. Slight NO_2-N level reduction in outlet was observed in period 4 and 6. Detailed information about NO_2-N level variation is shown in Table 4.3 and Figure 4.5.

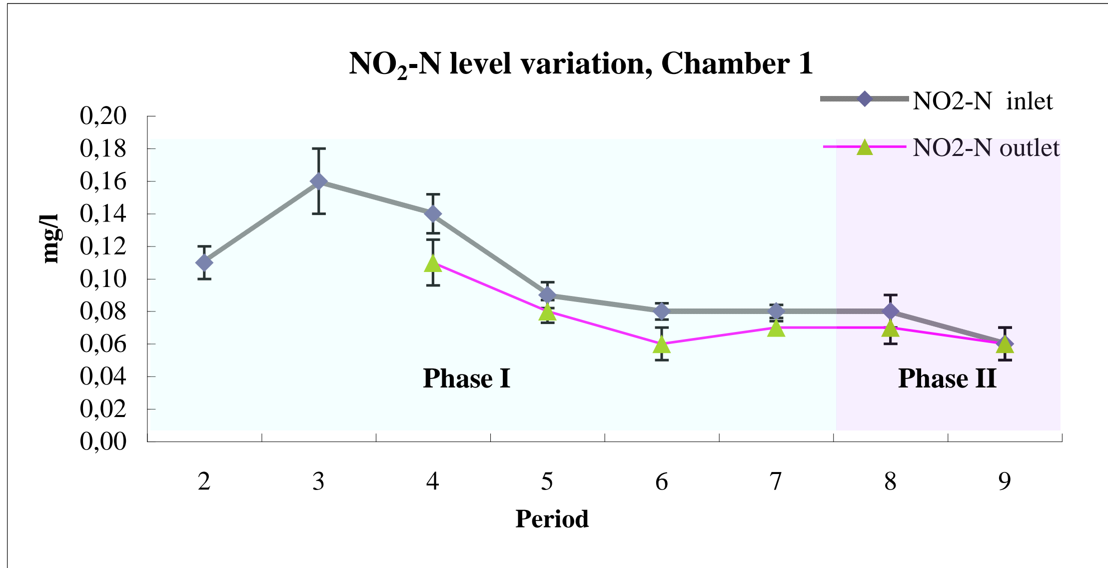


Figure 4.5 NO_2-N level variation, Chamber 1.

ATR and ANR showed the same variation pace. Compared with the values in period 4, significant increase of ATR and ANR was observed in period 5. However, both values kept on decreasing until the end of phase I (period 7). In phase II, ATR and ANR values increased slightly after taking out 50 l "old" media in period 8. However, ANR dropped to 0 when only 90 l of "old" media were left (period 9). Detailed information about ATR and ANR variation is shown in Table 4.3 and Figure 4.6.

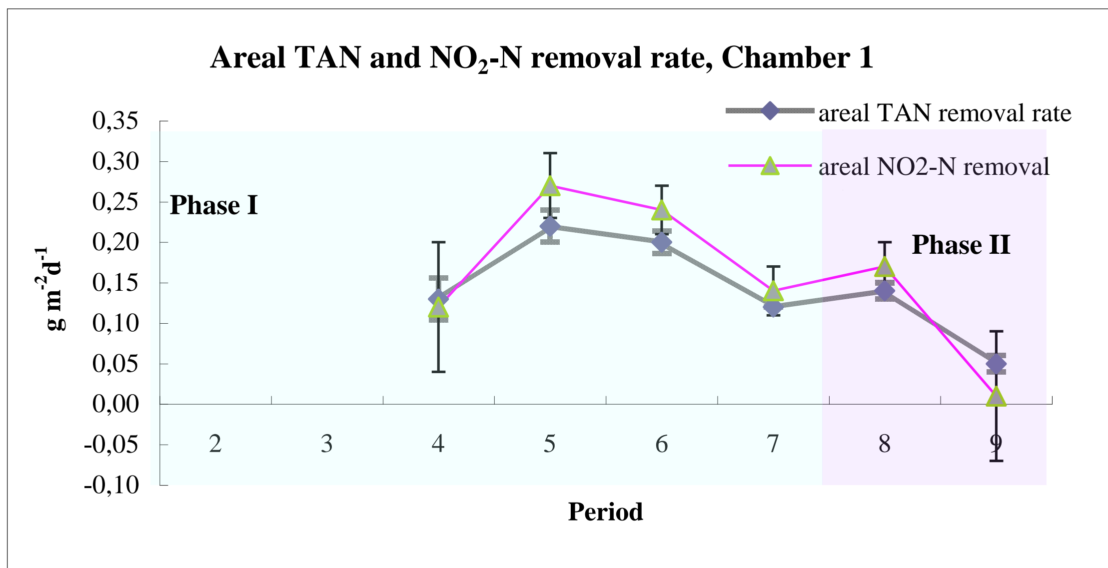


Figure 4.6 Areal TAN and NO_2-N removal rate variation, Chamber 1.

4.2.2 Chamber 2's situation during the exchange process

"New" Anox K5 in chamber 2 showed quite high suspension at the first 24 hours because of the low density of the material. It is better to fill in the new media gradually to insure the new biofilm media can rotate in short time. The new biofilm media could rotate well after two days in the MBBR. No clogging has been observed after running in the MBBR for nearly one year.

Measurement for C2 started from period 4 (05.11.12), 9 days after filling in Anox K5. TAN levels in chamber 2 appeared quite stable and were within the range of 0.14-0.19 mg/l (Figure 4.7; Table 4.4). Marked reduction in TAN levels was measured from period 4, 9 days afte filling in the "new" media. As can be seen from Figure 4.7, TAN levels in outlet were lower than the inlet levels during the whole exchange process, except for period 6. Furthermore, the difference between inlet and outlet TAN level got larger after period 10, even with lower TAN inlet level. Details about the TAN level variation are shown in Table 4.4 and Figure 4.7.

Table 4.4 Chamber 2's situation (period 2 to10) during the exchange process: TAN and NO_2-N (mg/l); ATR (areal TAN removal rate, g TAN m^{-2} d^{-1}); ANR (areal NO_2-N removal rate, g NO_2-N m^{-2} d^{-1}). The results are given as mean ± S.E (standard error, n in each period is the same value as that sample amount shown in Table 3.7).

P	No[a]	TAN inlet	TAN outlet	ATR	NO_2-N inlet	NO_2-N outlet	ANR
2	0	/	/	/	/	/	/
3	4	/	/	/	/	/	/
4	9	0.17±0.01	0.12±0.01	0.04±0.01	0.11±0/.01	0.14±0.02	0.01±0.01
5	14	0.18±0.02	0.13±0.01	0.06±0.01	0.08±0.01	0.08±0.01	0.06±0.01
6	19	0.17±0.02	0.14±0.03	0.04±0.02	0.06±0.01	0.07±0.02	0.03±0.02
7	21	0.19±0.01	0.14±0.01	0.06±0.01	0.07±0.00	0.07±0.00	0.07±0.02
8	26	0.18±0.01	0.14±0.00	0.05±0.00	0.07±0.01	0.07±0.01	0.06±0.01
9	30	0.16±0.01	0.13±0.01	0.03±0.00	0.06±0.01	0.05±0.01	0.05±0.01
10	33	0.17±0.01	0.11± 0.00	0.05±0.00	0.04±0.00	0.04±0.00	0.06±0.00

a: number of days after filling in Anox K5 in chamber 2

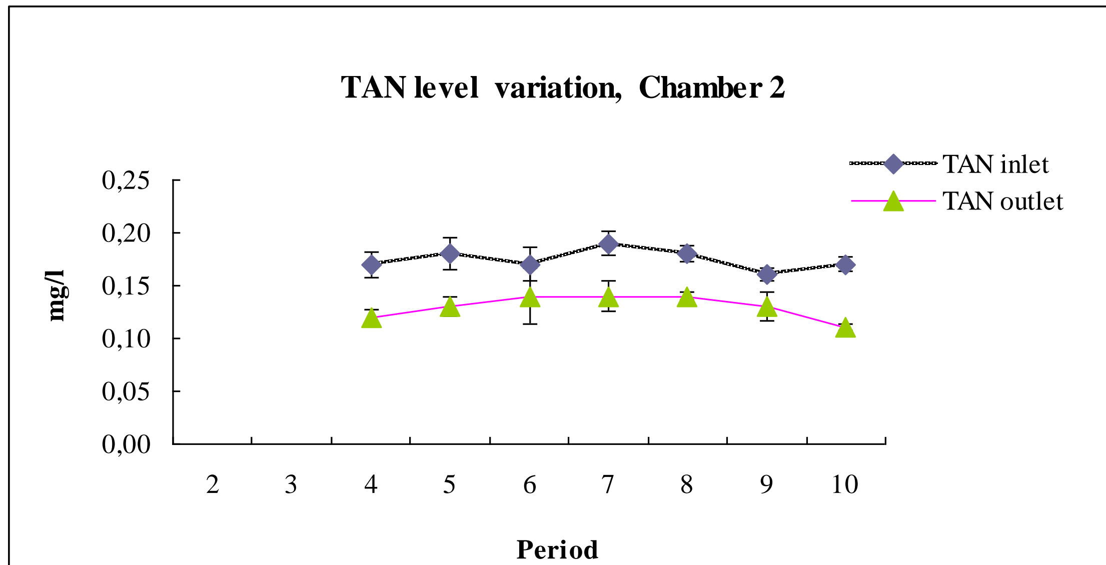


Figure 4.7 TAN level variation, Chamber 2.

NO_2-N levels both in the inlet and outlet showed a reduction during the whole exchange process with period 4 showing the highest level. From period 5 to 10, NO_2-N levels in the inlet and outlet were not significantly different, which means chamber 2 could manage to transfer the NO_2 produced to NO_3. Detailed imformation is shown in Table 4.4 and Figure 4.8.

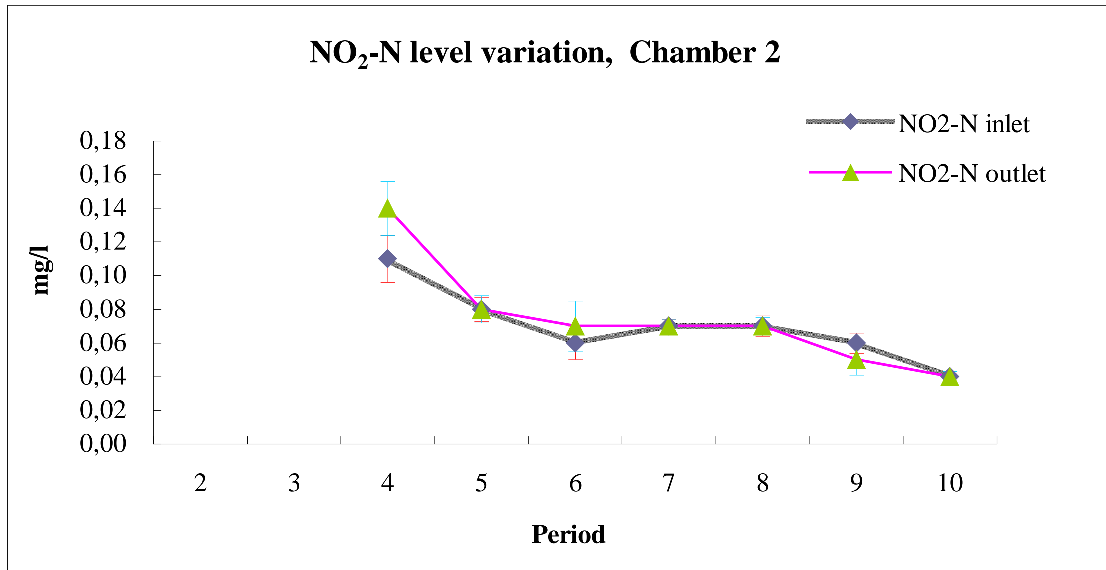


Figure 4.8 NO_2-N level variation, Chamber 2.

Chamber 2 had nitrification rate of 0.04 g TAN m^{-2} d^{-1} in period 4, which was 9 days after filling in the "new" Anox K5. The largest value appeared in period 5 and 7, which was 0.06 g TAN $m^{-2}d^{-1}$. All ATR values from period 4-10 were above 0.

ANR's variation showed the same trend as that of ATR. Obvious NO_2-N removal activity started from period 5 (14 days after filling Anox K5 in chamber 2) which had a value of 0.06±0.01 g NO_2-N m^{-2} d^{-1}. Detailed information about ATR and ANR is shown in Table 4.4 and Figure 4.9.

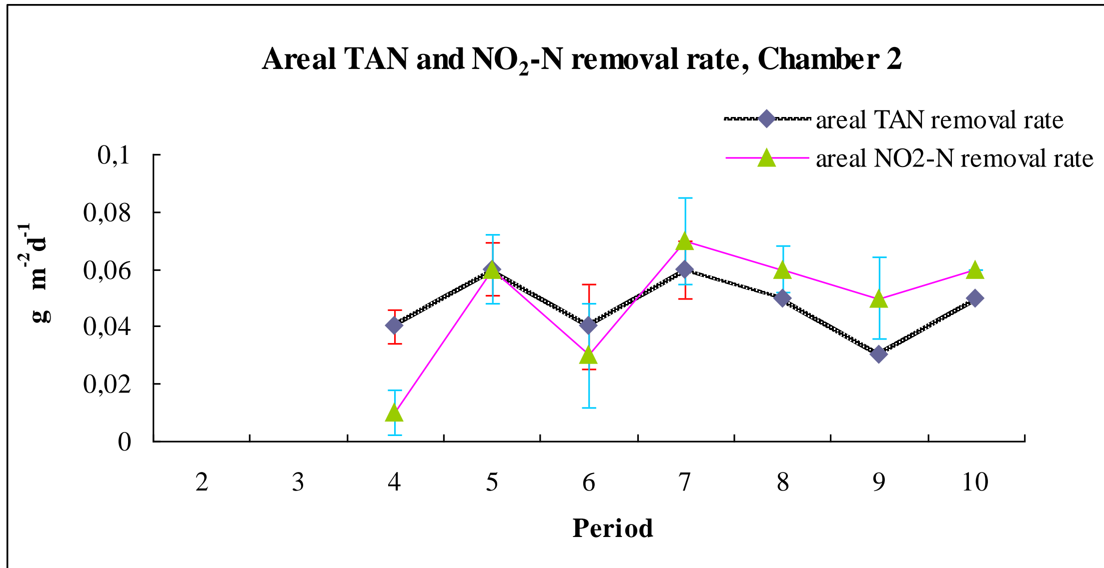


Figure 4.9 Areal TAN and NO_2-N removal rate variation, Chamber 2.

4.2.3 Chamber 3's situation during the exchange process

The development in C3 was divided into two phases. Phase I (period 2 to 6) was the period when gradually taking out "old" media and phase II (period 7 to 10) was after filling in "new" BiofilmChip M. BiofilmChip M has the largest protected surface area among all media used in this study. At the same time, it has the smallest mesh size (Figure 3.13), which could easily cause clogging. However, no clogging has been observed in this case after one year operation in MBBR.

C3 had the lowest TAN loading levels in the MBBR. They were within the range of 0.11-0.14 mg/l (Table 4.5; Figure 4.10). Generally, TAN levels in both inlet and outlet showed a reduction during the whole exchange process. Inlet and outlet TAN level kept the same during the periods of taking out "old" media, independent of the amount of media left. Moreover, no difference was observed between inlet and outlet TAN level after filling in BiofilmChip M. Detailed information is shown in Table 4.5 and Figure 4.10.

Table 4.5 Chamber 3's situation during the exchange process, phase I and II: TAN and (mg/l); ATR (areal TAN removal rate, g TAN m^{-2} d^{-1}); ANR (areal NO_2-N removal rate, g NO_2-N m^{-2} d^{-1}). The results are given as mean ± S.E (standard error, n in each period is the same value as that sample amount shown in Table 3.7).

	P	TAN inlet	TAN outlet	ATR	NO_2-N inlet	NO_2-N outlet	ANR
	2	/	0.22±0.00	/	/	0.09±0.01	/
	3	/	0.16±0.02	/	/	0.15±0.02	/
I	4	0.12±0.01	0.13±0.01	-0.03±0.01	0.14±0.02	0.13±0.01	-0.03±0.01
	5	0.13±0.01	0.15±0.01	-0.13±0.05	0.08±0.01	0.09±0.08	-0.14±0.06
	6	0.14±0.03	0.15±0.02	-0.27±0.27	0.07±0.02	0.07±0.02	-0.27±0.03
	7	0.14±0.01	0.15±0.00	-0.01±0.01	0.07±0.00	0.07±0.00	-0.01±0.01
II	8	0.14±0.00	0.14±0.01	0.00±0.00	0.07±0.01	0.07±0.01	0.00±0.01
	9	0.13±0.01	0.12±0.01	0.01±0.00	0.05±0.01	0.05±0.01	0.01±0.01
	10	0.11± 0.00	0.11±0.00	0.01±0.00	0.04±0.00	0.04±0.00	0.00±0.00

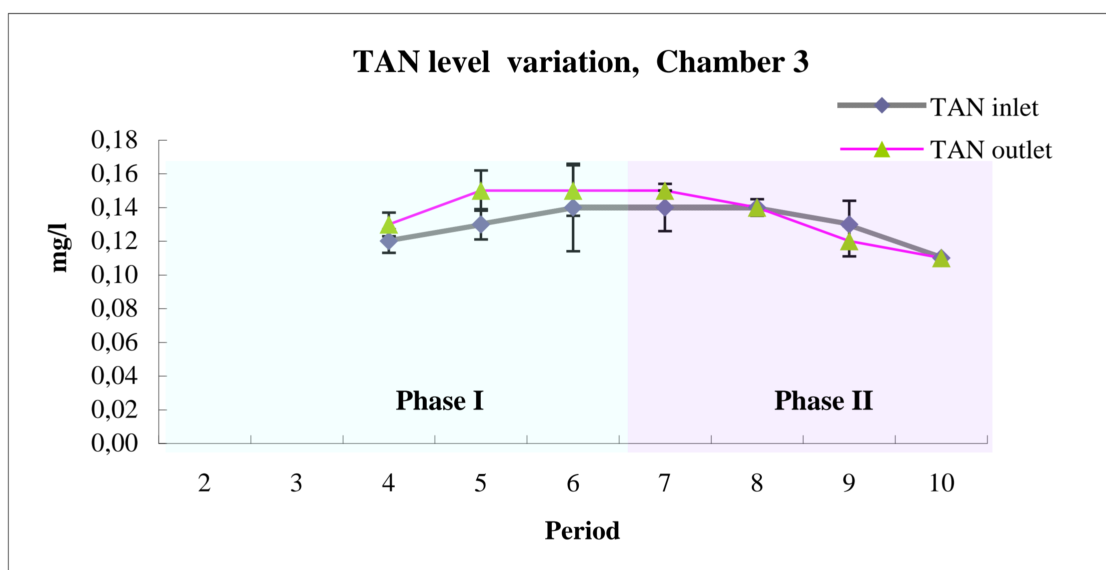


Figure 4.10 TAN level variation, Chamber 3.

NO_2-N level variation showed the same situation as that of TAN. No difference was observed between inlet and outlet NO_2-N, neither in the process of taking out "old" media nor after filling in BiofilmChip M. Detailed information is shown in Table 4.5 and Figure 4.11.

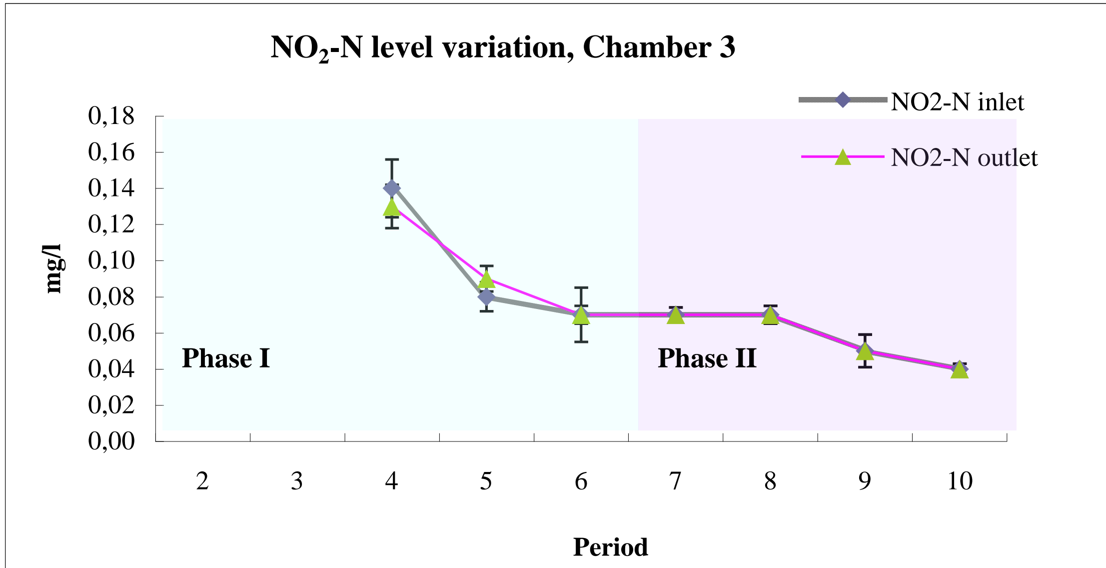


Figure 4.11 NO_2-N level variation, Chamber 3.

As shown in Figure 4.12, both ATR and ANR values were not significant different from zero, neither during phase I nor II. However, from period 8, the result could indicate an initial nitrification.

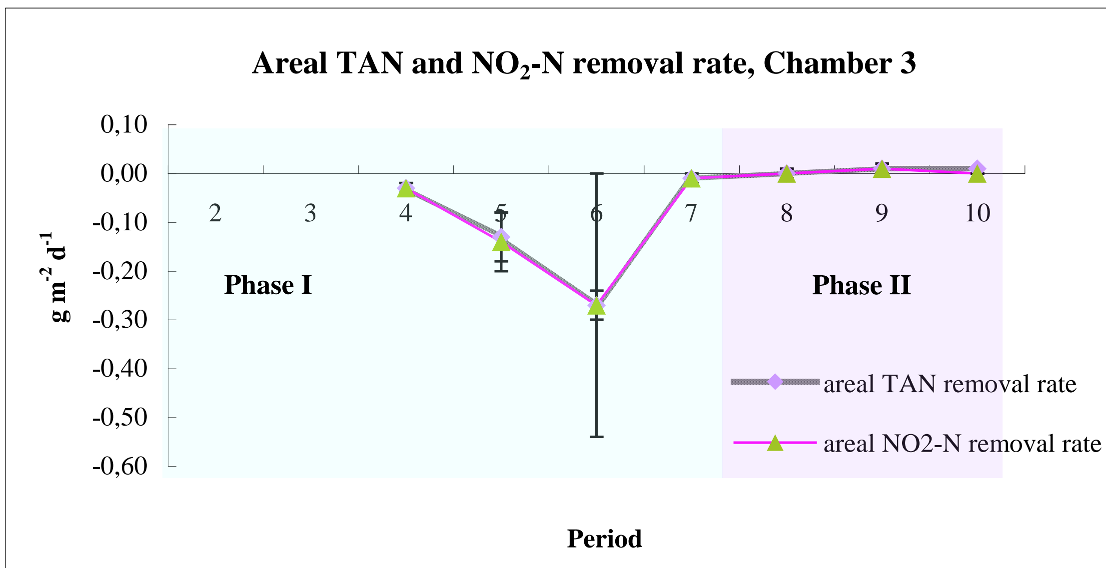


Figure 4.12 Areal TAN and NO_2-N removal rate, Chamber 3.

4.3 MBBR's stuation after finishing the exchange process.

Several samples were taken to describe the situation after the exchange process. The results on daily basis are shown in Table 4.6.

Table 4.6 MBBR's situation afte the exchange process: TAN and NO_2-N (mg/l); ATR (areal TAN removal rate, g TAN m^{-2} d^{-1}); ANR (areal NO_2-N removal rate, g NO_2-N m^{-2} d^{-1}).

	TAN			NO_2-N			ATR		ANR	
Date	**inlet**	**C2[a]**	**C3[b]**	**inlet**	**C2**	**C3**	**C2**	**C3**	**C2**	**C3**
03.12.11	0.14	0.11	0.10	0.04	0.03	0.03	0.03	0.01	0.04	0.01
04.12.11	0.14	0.11	0.11	0.04	0.03	0.03	0.04	0.00	0.06	0.00
05.12.11	0.14	0.10	0.09	0.03	0.03	0.03	0.04	0.01	0.04	0.01
07.12.11	0.14	0.11	0.10	0.03	0.03	0.03	0.03	0.01	0.01	0.01
12.12.11	0.13	0.09	0.09	0.04	0.03	0.03	0.05	0.00	0.01	0.00
14.12.11	0.13	0.10	0.08	0.03	0.03	0.03	0.04	0.02	0.00	0.02
19.12.11	0.14	0.10	0.09	0.03	0.02	0.02	0.05	0.01	0.01	0.01
22.12.11	0.14	0.10	0.09	0.03	0.02	0.02	0.05	0.01	0.01	0.01
29.12.11	0.12	0.09	0.08	0.01	0.01	0.01	0.03	0.01	0.00	0.01
02.01.12	0.13	0.08	0.08	0.01	0.01	0.01	0.05	0.00	0.00	0.00

a: sample from the outelet of chamber 2, sampling point S3 in Figure 3.8.

b: sample from the outlet of chamber 2, sampling S3 in Figrure 3.8.

TAN loading levels were quite stable after finishing the exchange process. They were in the range of 0.12-0.14 mg/l. TAN levels in chamber 3 (same as outlet MBBR) were mainly within 0.08-0.09 mg/l. Significant differences were observed between TAN level in inlet of MBBR and chamber 2. However, the difference between TAN level in chamber 2 and outlet of MBBR was not big. Detailed information about the TAN levels in inlet of MBBR, chamber 2 and chamber 3 after the exchange process is shown in Table 4.6 and Figure 4.13.

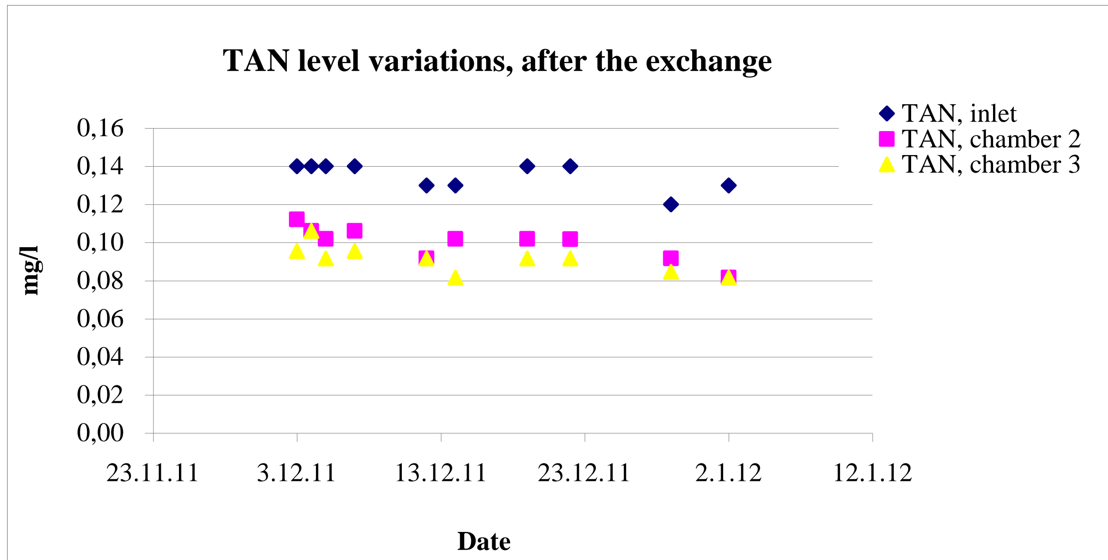


Figure 4.13 TAN level variation after the exchange process, inlet of MBBR, Chamber 2 and 3.

There were low NO_2-N loading levels to the MBBR after the exchange process. It was within the range of 0.01-0.04 mg/l. Slight difference excisted between the inlet of MBBR and chamber 2's NO_2-N level. However, no difference was observed between chamber 2 and 3's NO_2-N level. Detailed information is shown in Table 4.6 and Figure 4.14.

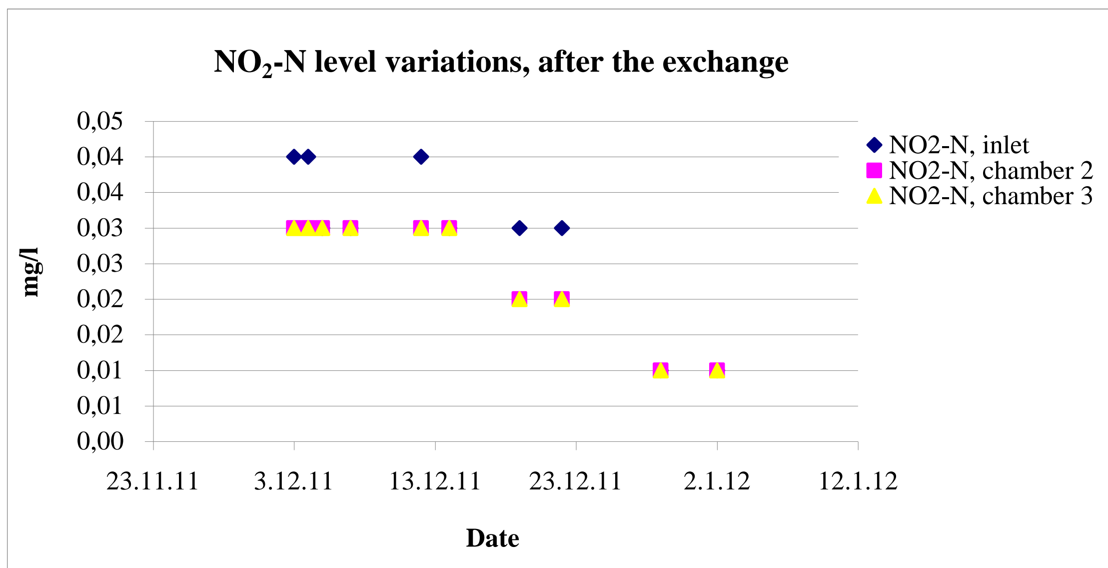


Figure 4.14 NO_2-N level variations in inlet of MBBR after the exchange process, Chamber 2 and 3.

Chamber 2 showed relative high ATR value. It was within the range of 0.03-0.05 g TAN m^{-2} d^{-1}. ATR values in chamber 3 were much lower. The highest was 0.02 g TAN m^{-2} d^{-1}. Detailed information about the ATR variation is shown in Table 4.6 and Figure 4.15.

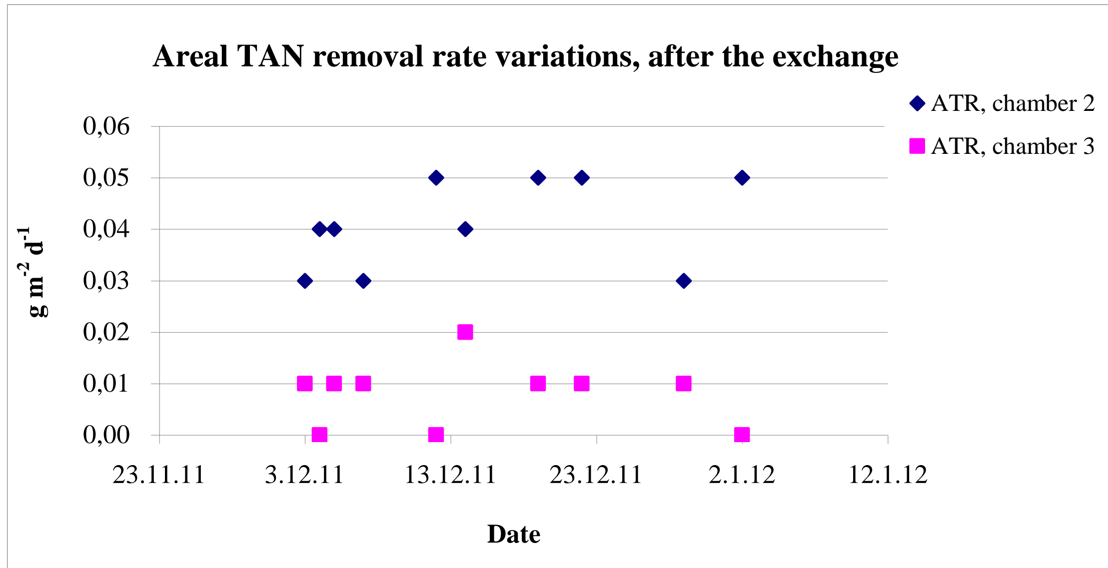


Figure 4.15 Areal TAN removal rate variations after the exchange process, Chamber 2 and 3.

As can be seen from Figure 4.16, ANR showed relatively high values on the date of 03.12, 04.12 and 05.12. They were within the range of 0.04-0.06 g NO_2-N m^{-2} d^{-1}. However the value decreased afterwards. ANR values in chamber 3 varied from 0.00 to 0.02 g NO_2-N m^{-2} d^{-1}. Detailed information is shown in Figure 4.16.

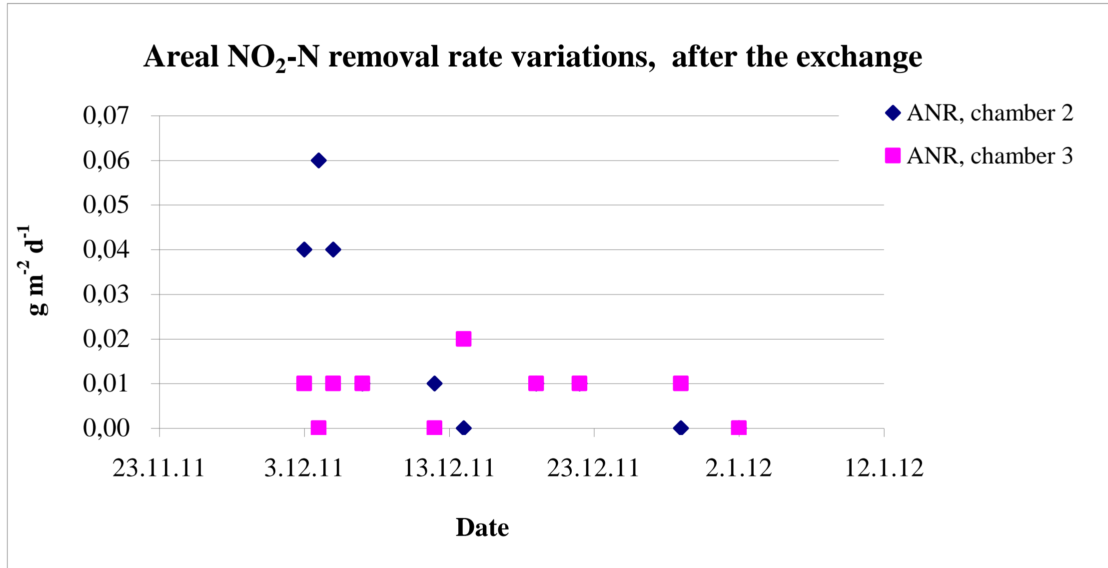


Figure 4.16 Areal NO_2-N removal rate variations after the exchange process, Chamber 2 and 3.

4.4 Daily variation of TAN

In order to check out the representativeness of the samples taken between 10:00 am and 10:30 am, samples were taken at different periods during one day (23.11.2011). Two parallel samples (duplicates) were taken at 11:00, 13:00, 15:00 and 17:00 after drum filter (sample point S1, see Figure 3.8). The results are shown in Table 4.7. There were no significant differences between TAN levels except at 17:00. As for the NO_2-N levels, no significant differences were observed..

Table 4.7 Daily variations of TAN and NO_2-N (mg/l) loading levels. The results are given as mean ± S.E (standard error, n = 2).

	11:00	**13:00**	**15:00**	**17:00**
TAN	0.26±0.02	0.28±0.01	0.28±0.01	0.34±0.01
NO_2-N	0.08±0.00	0.09±0.01	0.10±0.01	0.10±0.00

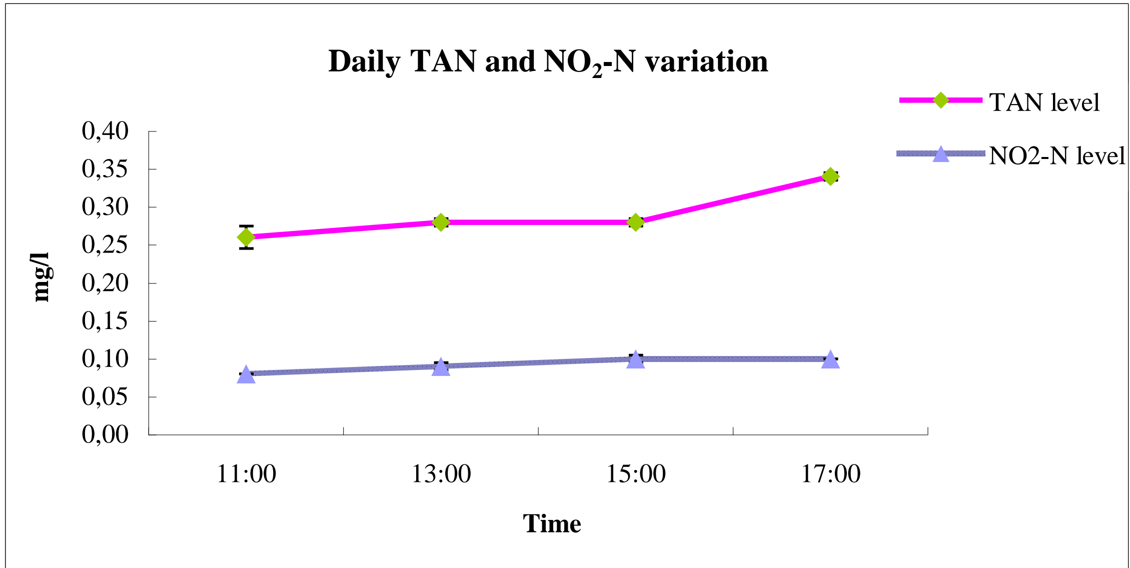


Figure 4.17 Daily variations of TAN and NO_2-N loading levels.

4.5 Alkalinity consumption variation with the amount of TAN oxidized

Alkalinity was measured both in outlet and inlet of the MBBR. The result for each period is shown in Table 4.8. The amount of alkalinity consumed per day was calculated using Equation 3.8. The amount of TAN oxidized per day was calculated by using Equation 3.9. Results are shown in Table 4.8. No obvious trend was shown in alkalinity levels at the inlet and outlet as values fluctuated at different periods. Moreover, the amount of alkalinity consumed shows large standard error.

Table 4.8 Alkalinity (mg $CaCO_3$/l) inlet and outlet; The amount of alkalinity consumed per day ($A_{Alkalinity}$, g $CaCO_3$/day); The amount of TAN oxidized per day (A_{TAN}, g TAN/day). The results are given as mean ± S.E (standard error).

P	alkalinity inlet	alkalinity outlet	$A_{Alkalinity}$	A_{TAN}	$A_{Alkalinity}/A_{TAN}$
1	51.9±1.4	52.6±1.5	-142.1±75.20	25.4±2.1	-5.60±3.3
2	52.9±1.4	55.1±0.3	-557.3±291.4	23.1±3.0	-24.1±10.2
3	63.8±2.8	62.5±2.8	285.8±418.3	18.5±2.4	15.5±29.2
4	64.2±2.0	63.1±2.0	244.9±171.3	17.6±2.5	13.9±11.2
5	61.1±0.9	62.4±1.2	365.8±238.1	24.5±2.2	-15.0±10.3
6	67.4±0.1	65.0±0.1	620.5±103.4	19.4±0.5	-32.1±7.10
7	68.0±1.2	70.4±2.7	-636.5±405.5	19.7±0.8	-32.3±19.5
8	63.9±1.2	65.9±1.1	620.5±103.4	19.7±0.8	26.3±4.5
9	66.3±0.7	67.0±0.0	-636.5±405.5	12.4±0.8	0.00±0.0
10	71.2±2.8	78.5±2.9	-517.1±84.40	10.8±0.4	-40.9±37.6

4.6 Relation between TAN levels and biomass variation

In general, TAN levels measured after drum filter (S1) decreased during the exchange process. This coincided with the variation observed for the biomass, which decreased from 232 kg at the beginning of the experiment to 145 kg at the end of the experiment. But this coincidence did not apply to every period. At period 2 and 3, biomass increased from 221±0.6 kg to 225±0.7 kg, while TAN level decreased from 0.32±0.02mg/l to 0.24±0.05 mg/l. However, it is opposite for period 5 and 6. Biomass decreased from 222±0.8 kg to 196±0.6 kg, but the TAN level stayed constant for these two periods. The relation between the TAN level variation and biomass is shown in Figure 4.18. TAN levels and biomass values are shown in Table 4.2 and Table 3.5 repectively.

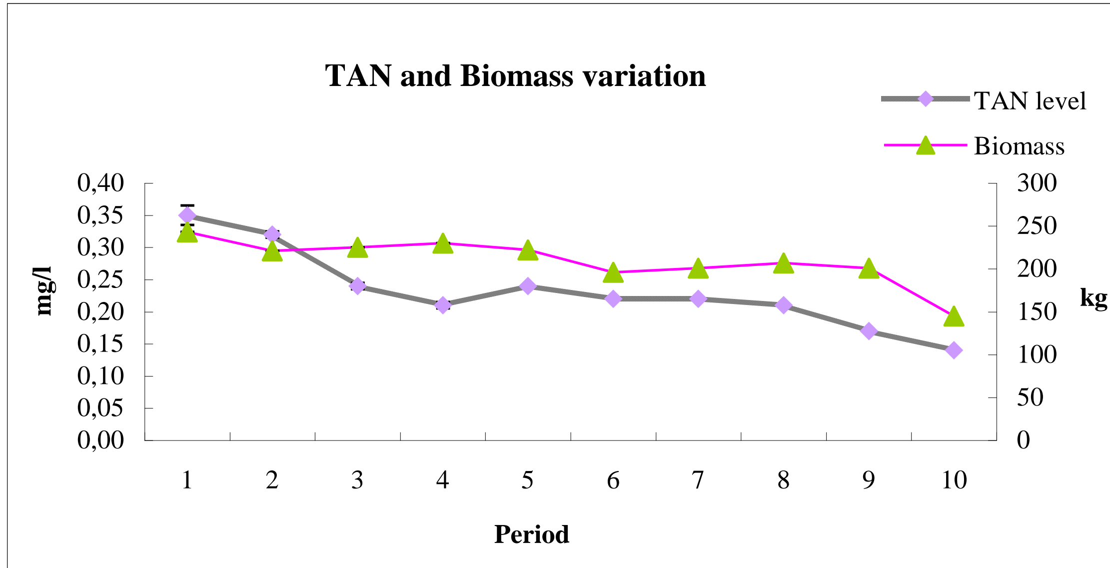


Figure 4.18 TAN levels after drumfilter versus biomass.

5. Discussion

5.1 Set- up of the exchange plan

The plan for the exchange of biofilm media was prepared in cooperation with Krüger Kaldnes and personnel in the Fish laboratory. In connection with the start up of a feeding experiment, biomass and thereby feeding was reduced prior to and during the exchange process.

In general, the exchange of media was successfully carried out. Water quality parameters like TAN, NO_2, pH, alkalinity, dissolved oxygen and temperature were all within the range for optimum growth of Nile tilapia (Table 3.8, Table 4.2). There were no symptoms of stress for the fish during the exchange process which might have been attributed to bad water quality. Especially because of the concurrent ongoing feeding experiment, one major challenge was to keep water quality as good and stable as possible during the exchange process.

The reduction of TAN was fairly stable and no NO_2 accumulation was observed throughout the exchange process (Figure 4.2 and 4.3). This can be due to the low system loading level, but also to the fast established nitrifying bacteria on the new media.

5.1.1 The advantage of the exchange plan

Instead of taking out all “old” biofilm media at once, we exchanged it step by step (Table 3.7) to ensure the stability of the system which was presumably dependent on the survival of the nitrifying bacteria. Putting the “old” biofilm media in the empty inlet chamber (chamber 1) of the MBBR afforded the new media (which were put in chamber 2 and chamber 3) a source of nitrifying bacteria. Furthermore, it also had a backup function in the exchange process. For RAS fish farms which plan to increase biofilm media volume or exchange media, it affords good realistic bases.

5.1.2 Drawback of the exchange plan

1. The reduction of biomass resulted in the complexity of explaining the reason of the security of the exchange plan, which could be caused by too low TAN production in the system or high bacterial establish speed. However, from a practical point of view, it could be risky to increase the biomass in the process of reducing the nitrifiying bacterial biomass.

However, this laboratory situation of reducing biomass can be reflected in the practical case. Assuming one smolt fish farm that adopts RAS, needs to set out a large amount of smolt in a short time. TAN loading level to the MBBR will be reduced with the reducing biomass. By consequence, there will be a nutrient shock for the nitrifying bacteria. Further study could be done to find out how fast nitrifying bacteria can recover after a nutrient shock both for warm water RAS (e.g. tilapia) and cold water RAS (e.g. salmon). So for the fish farms that adopt RAS, sustain production plan should not only be made for the fish, but maybe also for the nitrifying bacteria in the MBBR.

2. In this case, the "old" biofilm media could have been taken at once from chamber 3. As can be seen from the results shown in Table 4.5, there was no nitrification process going on in chamber 3 in the process of taking out the "old" media.

3. The pace of taking out "old" media should be done according to recommended TAN and NO_2 levels for the fish cultured in the system. If there is no evidence of stress for the fish after taking out "old" media, then it can be continuously taken out.

4. Replicated samples should be taken for each sampling instead of taking one sample every day. According to the plan before starting the exchange process, one sample should be taken every day to describe the daily development of the biofilter. However, in order to increase the statistic accuracy of the results, the average value of several days's results was presented. From the practical point, this may not represent the real development of the biofilter. Because nitrifying bacteria have higher growth rate in warm water systems compared with that in the cold water, small changes can be going

on in the biofilter every day. Better sampling plan should be made before starting up the experiment considering both the accuracy of the results and the cost of chemical reagents. For example, replicated samples could be taken and analyzed 24 hours after each exchange.

5. More water quality parameters should be included, especially NO_3 and COD. However, because of the high cost of the reagents for NO_3 and COD, just NH_4-N and NO_2-N were measured.

6. The accuracy of the method for measuring NH_4-N and NO_2-N is low due to the low values in this case. Some of the measured values are within the range of the method's accuracy, which increase the difficulty to explain the results. 50 mm cell should be used instead of 10 mm cell when measuring the NH_4-N and NO_2-N.

5.2 Development of the new biofilm media

Starting up a new biofilter is a time consuming process, especially for MBBR used in aquaculture, which has low TAN loading level. It poses even bigger challenge for cold water RAS. According to Boller and Gujer (1986), nitrification rate becomes stable after more than one year in operation in case of municipal water which has much higher TAN loading level than aquacultural waters. Furthermore, there are more challenges for fish farms because the system contains the living creature cultured. It is recommended that the new biofilm media which has never been exposed to the fish or feed should be started at low load and with a low fish density in the fish tanks (Rusten *et al*., 2006).

5.2.1 The development of Anox K5 in the tilapia RAS

TAN reduction in chamber 2 was observed within 9 days after filling Anox K5, which could be a sign of establishment of nitrifying bacteria on the new media. However, this could also have been caused by the existing biofilm on the chamber walls. To

emphasize the establishment of biofilm on media, the following discussion was hold: We hypothesis that the nitrification process was just held on the chamber 2 walls, which has total surface area of 5.5 m^2. According to the calculation method shown in Equation 3.6, the chamber walls had nitrification rate of 1.6 g TAN m^{-2} d^{-1}, which was more than five times as the maximum TAN removal rate reported in Rusten *et al*'s report (2006). Based on this we can conclude there was a nitrification process going on with the Anox K5 within 9 days (did not measure water quality within 9 days) after filled in chamber 2. Further studies can be done to find out the structure and importance of biofilm established on the wet parts of RAS (inside pipelines, fish tanks, MBBR' walls, particles in the water etc). Maybe the biofilm on these surfaces is enough to afford bacterial resource or even be enough to reduce the TAN produced. If so, it might be possible to exchange the biofilm media in one operation.

The TAN removal efficiency was low in this case. After 14 days, chamber 2 had removal rate of 0.04 g NH_4-N m^{-2} d^{-1} at the temperature 26 ºC. This was even much lower than the start-up speed of the MBBR run under the temperature of about 10 ºC, which had removal rate of 0.1 g NH_4-N m^{-2} d^{-1} after 14 days (Ulgenes, 1997). The main reason for this phenomenon was most probably because of the low TAN loading level during the exchange process.

TAN removal efficiency and TAN loading levels showed the same trend during the whole exchange process. In general, TAN removal efficiency improved with the time. This can be seen from the difference between the inlet and outlet TAN levels. This difference became bigger in period 10 compared with period 4 (Figure 4.3).

5.2.2 The development of BiofilmChip M in the tilapia RAS

No nitrification process was observed in chamber 3 in the process of taking out "old" media (Table 4.5), despite the amount of old media left in chamber 3. The reason for this could be the low TAN loading level. This corresponds with the results shown in Zhu and Chen's paper (1999), in which the authors used a series reactor system. The

results showed that there was no nitrification process going on in the last reactor due to the low TAN level.

5.3 Where does the NO_3 go in the tilapia RAS?

As the final product of nitrification process, NO_3 level can be reduced either by denitrification or water exchange (Hamlin *et al.*, 2008). Since no denitrification device is installed in the tilapia RAS, theoretically the NO_3 will continuously increase until it reaches the maximum level, which is mainly decided by the water exchange rate. After reaching a certain level, the amount of NO_3 flushed out (overflow, flushing of fish tanks etc.) will balance the amount produced. The maximum NO_3-N level in RAS can be calculated by Equation 5.1.

Maximum NO_3-N concentration = NO_3-$N_{produced}/V_{makeup\ water}$ ……….Equation 5.1

According to the mass balance theory, the ammonia nitrogen part will all go to the NO_3-N part. 1 gram NH_4-N will be oxidized to 1 gram NO_3-N. Take the average NH_4-N value and average amount of make-up water during the exchange process, the maximum NO_3 during the exchange was around 21.4 mg/l. This result corresponds quite well with results from measurements done after this study (with same biomass and amount of make-up water), which is shown in Table 5.1.

Table 5.1 NO_3-N levels in tilapia RAS.

Date	Feb-12	Jun-12
NO_3-N (mg/l)	25.7	22.6

There can be other reasons for this phenomenon that the NO_3 level keeps constant. Firstly, denitrification can be going on in the RAS without a dentrification reactor. The MBBR in the fish laboratory is under aerobic environment which is not suitable for the denitrification process. However, as described in the literature review, there is a lack of oxygen in deeper part of biofilm, which afford the possibility for the

denitrification process. Secondly, anammox process can be going on in the deep layer of biofilm. According to Strous *et al*. (1999), the ammonia oxidation could be held under the anaerobic condition (anammox) with the help of planctomycetales. This organism can turn the combination of ammonia and nitrite directly to nitrogenous gas (Strous *et al*., 1999) without adding oxygen and carbon source.

5.4 Relation between TAN level and feeding routine

As the final product of protein metabolism, TAN level shares liner relation with feed and feeding routine. As shown in Figure 4.17, TAN level showed an increase at 13:00 (3.5 h after first feeding) when compared with that at 11:00 (1.5 hours after feeding). However, TAN level of 15:00 (1.5 hours after second feeding) was the same as that of 13:00. While big increase showed at 17:00 (3.5 hours after the second feeding).
The most possible reason for this phenomenon was the feeding routine in Room II (4 times a day, from 08:00-20:00), since feeders in Room I were 24 h non-stop, which might keep TAN level stable after 6 hours after first feeding (Zakes and Karpinski, 1999). However, no information is available about Nile tilapia's ammonia excretion routine corresponding with the feeding routine.

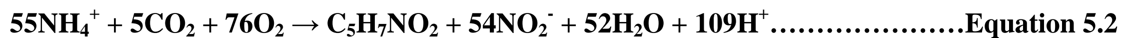
5.5 Amount of alkalinity consumed in the nitrification process

Nitrification process is an alkalinity consuming process. For every gram of TAN oxidiced, it needs approximately 7.07 g of alkalinity (as $CaCO_3$) (Chen *et al.,* 2006). However, the results gotten from this experiment differs (Table 4.8). The alkalinity in outlet of MBBR was equal or even higher than that of inlet during most periods. This phenomenon can be explained by the CO_2 stripping in the MBBR. According to the data afford by Fish labortary, CO_2 was around 5 in inlet of MBBR and around 3 in outlet of MBBR. In the process of CO_2 reduction, the pH increase. At the same time, nitrification is an acid producing process. However, during this study, pH values increased through the MBBR in general. This could indicate that the H^+ reduction

caused by CO_2 stripping compensated for the H^+ produced during the nitrification process. The amount of alkalinity can be expresse as Equation 5.1. As one of the alkalinity's use is to neutralize the H^+ produced, no use of alkalinity in this case. At the same time, nitrifying bacteria can use CO_2 instead of alkalinity for the cell mass formation, which is shown in Equation 5.2 and 5.3 (Timmons *et al.,* 2002).

$$\textbf{Alkalinity} = HCO_3^- + 2CO_3^{2-} + OH^- - H^+ \ldots\ldots \textbf{Equation 5.1}$$

Nitrosomonas:



$$55NH_4^+ + 5CO_2 + 76O_2 \rightarrow C_5H_7NO_2 + 54NO_2^- + 52H_2O + 109H^+ \ldots\ldots \textbf{Equation 5.2}$$

Nitrobacter

$$400NO_2^- + 5CO_2 + NH_4^+ + 195O_2 + 2H_2O \rightarrow C_5H_7NO_2 + 400\ NO_3^- + H^+ \ldots\ldots \textbf{Equation 5.3}$$

5.6 Effect of hydraulic retention time on the efficiency of MBBR.

As discussed in section 5.2, no nitrification was going on in chamber 3 because of the too low TAN level. This was mainly because of the pre-removal process held in chamber 1 and 2. If the board between chambers were removed, chamber 3 could act as the chamber 1 and chamber 2. The hydraulic retention time for each media would increases from 5 min to 15 min.

However, there is no information available about the influence of hydraulic retention time on the MBBR' nitrification effiency. Ulgenes (1997) reported an empty hydraulic retention time (HRT) in one MBBR as approximately 2.5 min., in which the MBBR was filled with 70% of Kaldnes K1. Moreover, he also reported the HRT as approximately 3.5 min for another MBBR which was filled with 67% of Kaldnes K1. However, little attention is paid to the HRT when deciding the volume of the MBBR. The volume is decided by the waste production, water quality limitations and the

filling percentage of the biofilm media. It is recommended the filling percentage of biofilm media should be less than 70% (Rusten *et al.*, 2006). While to treat waters with low TAN concentration, longer retention time is needed (Eding *et al.*, 2006). Further study can be done to find out the optimal retention time needed to achieve the maximum nitrification efficiency.

5.7 How to keep the water level in MBBR constant?

Water reuse percentage in tilapia RAS is normally between 98 and 99%. Make-up water is mainly used for compensating the water lost by flushing tanks, back flushing of drum filter and over-flow controlling.

Since there is no header tank in the tilapia RAS, water level in the MBBR drops a lot if too much water is flushed out from the system. To compensate happenings like this the MBBR is filled up with cold water which can cause a sudden change in temerature and water chemistry. This results in unstable environment for the nitrifying bacteria. An idea to solve this problem and thereby improve the tilapia RAS is described below. At normal operation excess water is drained out by the over-flow. This water could be directed to a storage tank of e.g. 500 l capacity. When water level in MBBR starts to drop a pump installed in the storage tank will start and fill up the MBBR basin and keep the water level constant. The pump is regulated by level sensors in the MBBR. The arrangement should not allow water stagnation in the storage tank.
The simplified layout is shown in Figure 5.1.

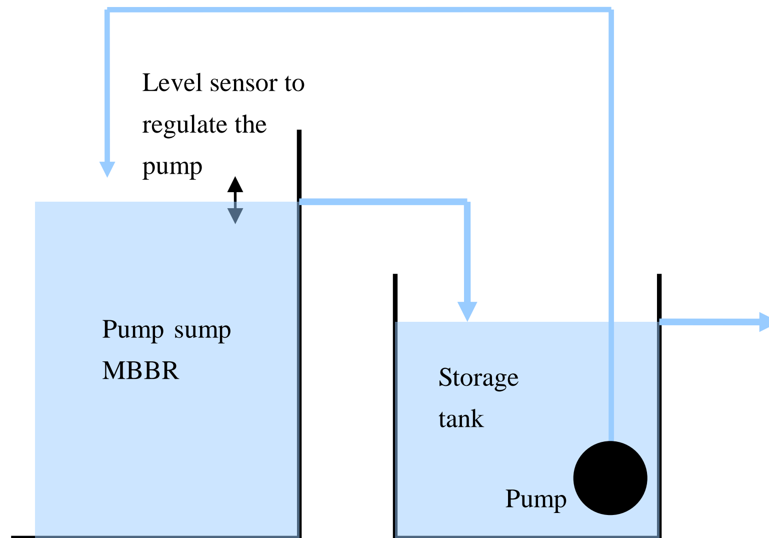


Figure 5.1 Layout of solution for increasing water reusing percentage and stabilize water quality in the tilapia RAS.

5.8 Further studys

1: Check out the feasibility of exchange “old” media with “new” media at once. Biofilm does not only establish on the biofilm media. It can be established on every wet part of the RAS, which can afford bacterial source for the “new” media;

2: Study the structure and efficiency of the biofilm established on the wall of pipes, tanks, MBBR and suspended particles in the RAS;

3: Study the Nile tilapia’ammonia expelling pattern based on different feeding routines;

4: Check the influence of sudden biomass reduction on the efficiency of MBBR;

5: Study the tract of NO_3 in the biofilter;

6: Check the influence of retention time on the nitrification rate.

6. Conclusion

The exchange process was a success. Water quality parameters, like pH, alkalinity, temperature, DO, TAN and NO_2-N were within the range for optimum growth of Nile tilapia (*Oreochromis niloticus*) and also for the nitrifying bacteria.

The new Anox K5 media in chamber 2 showed TAN reducing capacity within 9 days after it had been filled in. While the sign of reducing NO_2-N showed 14 days after. Almost no nitrification was shown going on in chamber 3 neither before nor during the exchange process. This was most probably caused by too low TAN levels.

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