Nitrification process in recirculating aquaculture system

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UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE

NITRIFICATION PROCESS IN RECIRCULATING AQUACULTURE SYSTEMS

MASTER THESIS

Mashkhura Babadjanova

Zagreb, June, 2017

UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE
Environment, agriculture and resource management
(INTER-EnAgro)

NITRIFICATION PROCESS IN RECIRCULATING AQUACULTURE SYSTEMS

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Mentor: Assist.Prof. Daniel Matulić, PhD

Zagreb, June, 2017

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STUDENT'S STATEMENT

ON ACADEMIC RECTITUDE

I, Mashkhura Babadjanova, JMBAG 0178108211, born on 06.08.1987 in Beruniy dstr Karakalpakstan republic, Uzbekistan, declare that I have independently written the thesis under the title of:

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REPORT

ON EVALUATION AND MASTER'S THESIS DEFENSE

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1. Assist.Prof. Daniel Matulić, PhD mentor _____
2. Assoc.Prof. Željka Zgorelec, PhD member _____
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Mashkhura Babadjanova

Zagreb, 2017

Summary

Of the master's thesis - student Mashkhura Babadjanova, entitled:

Nitrification process in recirculating aquaculture systems

Aquaculture is the most probable and feasible solution to providing the aquatic products for an ever-increasing market demand. Sustainable use and management of water used for aquaculture of aquatic organisms is long lasting economical and ecological issue. Recirculating aquaculture systems (RAS) are the key technology that will allow the world aquaculture community to supply the world per capita needs for aquatic species over the coming decades and will do so in an environmentally friendly manner. A major problem in these systems is the maintenance of a constant and optimal water quality. Elimination of waste is generally managed through mechanical filtration to remove solids, and biofiltration for the conversion of toxic nitrogen metabolites into less toxic forms. This is accomplished in the nitrifying bioreactor using a specific nitrifying bacterial consortium. Nitrifying bacteria process dissolve nitrogenous waste products excreted by the aquatic organisms being cultured. The process of bacterial driven ammonia removal is called nitrification, and consists of the successive oxidation of ammonia to nitrite and finally to nitrate. A better understanding of nitrification process can help appropriate performance through operational modifications of RAS.

Key words: RAS, nitrification, biofiltration.

Sažetak

Diplomskog rada studenta/ice Mashkhura Babadjanova, naslova:

Proces nitrifikacije u recirkulirajućem akvakulturnom sustavu

Obzirom na sve veću potražnju za slatkovodnim i morskim proizvodima na tržištu, akvakultura se smatra ostvarivim rješenjem njihove globalne opskrbe. Održiva uporaba i upravljanje vodom koja se koristi u akvakulturi vodenih organizama, dugotrajno je ekonomsko i ekološko pitanje. Recirkulacijski sustavi u akvakulturi (RAS) su ključna tehnologija koja će zajednici svjetske akvakulture, na ekološki prihvatljiv način, omogućiti opskrbu svjetskih potreba za akvakulturnim vrstama tijekom narednih desetljeća. Glavni problem navedenih sustava je održavanje konstantne i optimalne kakvoće vode. Uklanjanjem otpada iz RAS-a se upravlja mehaničkim filtriranjem vode, čime se uklanjaju krute tvari, te biofiltracijom tj. pretvorbom toksičnih metabolita dušika u manje toksične oblike. Biofiltracija se postiže u nitrificirajućem bioreaktoru uz pomoć specifičnog konzorcija nitrificirajućih bakterija. Proces uklanjanja amonijaka bakterijskim putem naziva se nitrifikacija, a sastoji se od uzastopne oksidacije amonijaka do nitrita te konačno do nitrata. Općenito, procesom nitrifikacije razlažu se otpadni proizvodi dušičnog podrijetla koje izlučuju organizmi u akvakulturi. Bolje razumijevanje procesa nitrifikacije može pomoći pri operativnim modifikacijama sustava a time i postizanju odgovarajućeg učinka RAS-a.

Ključne riječi: RAS, nitrifikacija, biofiltracija

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1. INTRODUCTION

Aquaculture is globally the fastest growing sector of agriculture that needs to be sustainable and must also meet bioeconomic demands. In order to fulfill global needs for valuable animal protein, aquaculture is by far the fastest-growing food sector of agriculture (Kloas et al., 2015). Aquaculture has a long history with its origins dating back to at least 475 BC in China (Timmons and Ebeling, 2010). Sustainable use and management of water used for aquaculture of aquatic organisms is long lasting economical and ecological issue. The aquaculture sector have made significant developmental progress during the past two decades in order to improve fish feed composition and reduce environmental impact by various management and technical solutions (Pedersen, 2009).

The earliest scientific research on RAS conducted in Japan in the 1950's focussing on biofilter design for carp production was driven by the need to use locally-limited water resources more productively. Independently of these efforts, European and American scientists attempted to adapt technology first developed for domestic waste-water treatment (e.g. the sewage treatment activated sludge process, submerged and down-flow biofilters, trickling and several mechanical filtration systems) (Murray et al., 2014).

Recirculating aquaculture systems (RAS) are the key technology that will allow the world aquaculture community to supply the world per capita needs for aquatic species over the coming decades and will do so in an environmentally friendly manner. A major problem in these systems is the maintenance of a constant and optimal water quality. Elimination of waste is generally managed through mechanical filtration to remove solids, and biofiltration for the conversion of toxic nitrogen metabolites into less toxic forms (Maartje et al., 2010). The removal of ammonia and nitrite from aquaculture tanks is a key component in development of a RAS. This is accomplished in the nitrifying bioreactor using a specific nitrifying bacterial consortium. Nitrifying bacteria process dissolved nitrogenous waste products excreted by the aquatic organisms being cultured. The process of bacterial driven ammonia removal is called nitrification, and consists of the successive oxidation of ammonia to nitrite and finally to nitrate.

The aim of the research is to define biological filtration, with the emphasis on the process of nitrification, in respect to its use in recirculation aquaculture. The impact of biological water treatment in RAS on the environment will be highlighted. The advantages and disadvantages of the RAS system will be determined. Microbial ecology with biochemistry of nitrification process will be also explained.

Research will include a review of professional and scientific literature with the use of relevant data on biological filtration and the nitrification process in the field of recirculating aquaculture.

2. RECIRCULATION AQUACULTURE SYSTEMS

In the early 1970s, RAS were designed to rear fish in land-based tanks with continuous recycling of water to optimize water use (Kloas et al., 2015). Recirculation aquaculture is essentially a technology for farming fish or other aquatic organisms by reusing the water in the production. The technology is based on the use of mechanical and biological filters, and the method can in principle be used for any species grown in aquaculture such as fish, shrimps, clams, etc. (Bregnballe, 2015).

Aquaculture systems can be extensive, semi-intensive, or intensive, depending upon the number of organisms grown per volume of water and the water source and supply. Pond culture is extensive, cage culture is semi-intensive but intensive within the cage, and RAS are intensive systems. Pond and cage systems are open-air, and therefore there is always a risk of air or water-borne contaminants. Because water quality control is more difficult in pond and cage systems, the number of organisms that can be grown effectively is limited. The principles of RAS for the water environment can be employed in the open air, but you lose total control of the environment (Timmons and Ebeling, 2010).

Typically recirculating (closed) aquatic production systems have higher capital and operating costs than many of the extensive systems such as cage culture in natural waters and raceway and/or pond culture systems. However, when the control provided by recirculating systems and the benefits this environmental control provides in terms of marketing, waste control, product quality, product availability, and other factors are considered then recirculating systems become much more attractive (Timmons and Ebeling, 2010). Recirculation enables the fish farmer to completely control all the parameters in the production, and the skills of the farmer to operate the recirculation system itself becomes just as important as his ability to take care of the fish (Bregnballe, 2015). Indoor RAS offers the advantage of raising fish in a controlled environment, permitting controlled product growth rates and predictable harvesting schedules. RAS conserve heat and water through water reuse after reconditioning by biological filtration using biofilters. RAS allow effective economies of scale, which results in the highest production per unit area and per unit worker of any aquaculture system. RAS are environmentally sustainable; they use 90-99 % less water than conventional aquaculture systems; less than 1 % of the land area; and provide for environmentally safe waste management treatment. The RAS assumes a tilapia culture system with a density of 100 kg/m^3, a 1 % feeding rate, and a feed conversion of 1 to 1 and a system volume discharge rate of 5 % per day. Some current commercial RAS are using less water (2 or 3 % system discharge per day and of course some use much more), higher densities and similar feed conversions. RAS allow year-round production of consistent volumes of product, and complete climate control of the environment (Barbu et al., 2008). Because RAS

can be set up to produce the same volume of fish every week, week in and week out, they have a competitive advantage over outdoor tank and pond systems, which are seasonal and sporadic in harvest (Timmons and Ebeling, 2010). The RAS allows the fish farmer to observe directly the biological material behavior and to adjust the growth technology as necessary. In a recirculation system it is necessary to treat the water continuously to remove the waste products excreted by the fish, and to add oxygen to keep the fish alive and well (Bregnballe, 2015). A recirculation system is in fact quite simple (Figure 1).

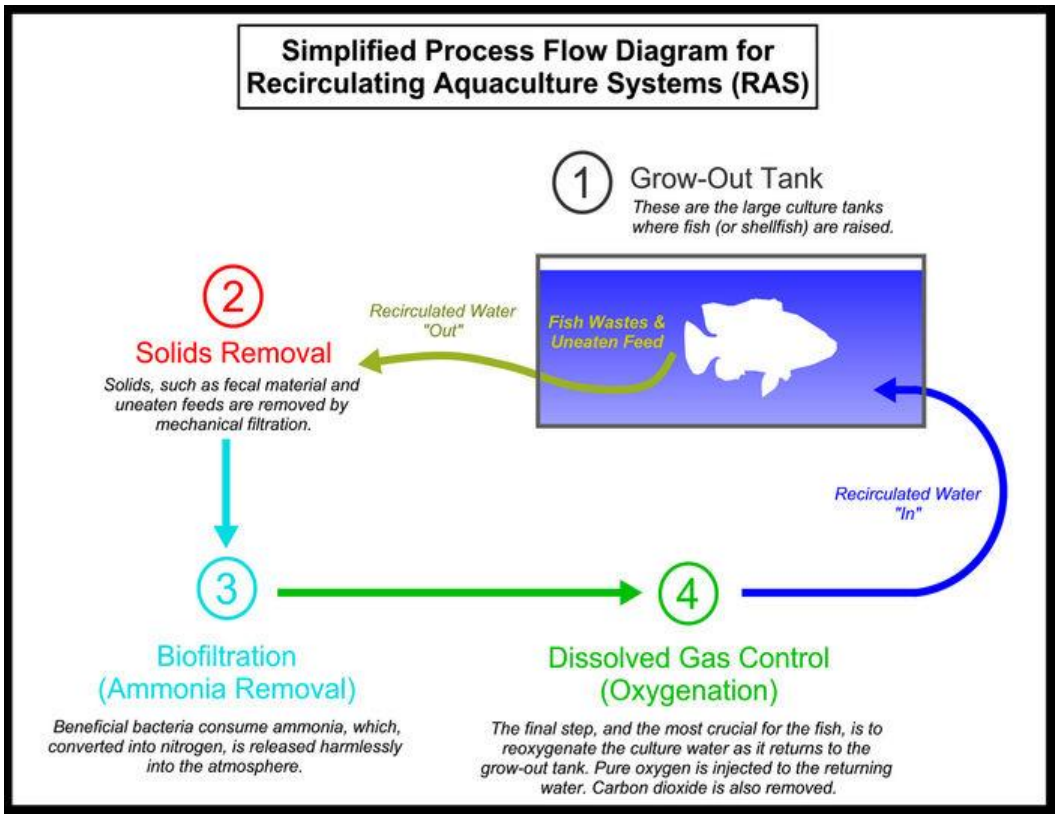


Figure 1. Structure of RAS (Source: Blue ridge aquaculture, 2017).

RAS are essentially small water treatment facilities that support the growth of species in culture tanks. A typical RAS may have water treatment and culture elements arranged as in Figure 1 although many variations are available dependent on the specifications required, and the interpretation of the designer.

3. COMPONENTS OF A RAS

3.1. Culture tanks, plumbing and pumps

Generally RAS utilize tanks for the culture of fish or other species, but may, on occasion, be associated with pond grow-out systems. Tanks are usually constructed of non-toxic, inert, non-corrosive materials including fibre-glass (fibre reinforced plastic, FRP), moulded plastic polyethylene, welded high density polyethylene plastic sheet, concrete or lined sheet metal. The interior finish of tanks needs to be smooth and as hard and durable as possible to prevent damage to fish and assist with cleaning. Tank bases are generally flat to slightly sloping (<15 degrees) to allow workers to work within tanks with safety. Tanks shapes include round, square with rounded corners and rectangular with D-ends and a central divider. Tank colour should be dull or dark to minimize stress, although white or lighter coloured bases assist with fish husbandry and management. Small windows installed in the sides of tanks can be useful for observing stock.

Different operational requirements for weaning, nursery culture and purging may require RAS to include a number of tanks of different sizes/volumes, which add further flexibility within the facility. Tanks should be designed to provide as much "self-cleaning" as possible. Water flow characteristics of the tank design can be used to promote the concentration of wastes towards outlet points. Circular tanks are preferred, mainly because it is easier to manage water flow patterns required to concentrate and remove settle able solids, whilst providing a relatively uniform culture environment. The major disadvantage with circular tanks is the space required to house them when compared to the more space efficient tank layouts achievable with square, octagonal or rectangular tanks. Inlet pipe design should allow water inflow at several depths and also allow current direction to be changed to manage water flow and circulation. Small grating covered sumps beneath the floor of tanks can be fitted with a double-drain system that allows separation of settle able solid wastes from suspended solids.

There is an obvious need to match pipe sizes for the delivery and discharge of water, if the water flow specified in the system design is to be achieved. The plumbing system should separate water supply and effluent discharge. Commercial RAS generally use PVC or ABS piping and fittings, both relatively non-toxic and inert compounds.

Distribution lines should ideally be constructed in a manner that allows access to them for cleaning purposes and to allow complete drainage. This can be achieved using capped inspection points, barrel unions and flange connections strategically located within the plumbing layout.

Most RAS are pump driven to provide the water movement required to deliver oxygen to fish and remove wastes from culture tanks. Centrifugal pumps are the most common type used in RAS although mixed flow, axial flow and air-lift pumps are also used in some systems. Pump

installations include submersible pumps, shaft-driven submerged pumps, flooded suction and suction lift pumps.

Pump selection is based upon performance specifications required within the RAS design. Pump performance is described by capacity (e.g. L/min), head, power, pump efficiency, suction head, and specific speed (rpm). Pump efficiency at the desired performance criteria is particularly important to reduce the operating cost of pumping. Pump selection criteria should include:

• Operational duty rating - pumps used in RAS need to operate continuously so should be industrial or 100 % duty pumps. Although more expensive, they are critical to RAS operation and are preferred to cheap pumps (e.g. swimming pool pumps) that are not designed for continuous operation.

• Pump construction materials - in corrosive saltwater, use all ferrous (i.e. cast iron housings and impellers with 316 or 316 L stainless steel shafts), fibreglass or plastic pumps, or pumps internally lined with resistant rubber or epoxy coatings.

Three phase power (415 v) - essential for efficient pump operation and longevity. The cost of pumping is directly proportional to the height (head) to which water is pumped. The system configuration should account for this during design to reduce operating costs. Where possible, the use of high-volume, low-head pumps should be incorporated into the design of RAS.

The minimum number of pumps should be used to provide the performance required, as a single larger capacity pump is more cost effective than multiple small capacity pumps. Bearing this in mind, the RAS design should also provide operational flexibility through the use of at least two pumps for each application, thereby allowing one to be used as a backup during maintenance or to provide extra capacity if required. Pump switching control can be installed to operate all pumps routinely to promote even wear. Foam Fractionation RAS will accumulate dissolved organic material and fine suspended solids between 5 – 10 μm that are not easily removed by conventional methods of mechanical filtration and sedimentation. Foam fractionation is a simple, relatively low cost method for removal of fine suspended solids and dissolved organic material (DOM), originating from proteins that accumulate within RAS, and are ultimately responsible for water turning yellow or brown over time. This material comes from sources such as decomposing feed and faces, urine and mucous. Foam fractionation works very well in saltwater in which foam production is easier, although it has been reported to work in freshwater with high concentrations of dissolved organic material.

Foam fractionation used in RAS is a process in which air is mixed with water to form bubbles that concentrate fine suspended solids (generally < 30 μm) and dissolved organics (surfactants) at the bubble surface. The bubbles and concentrated surfactants rise to the water surface and form foam that can be easily removed from the culture system.

Many types of foam fractionators are available. All are typically columns into which culture water enters and fine air bubbles are introduced using diffusers or ventures driven by pumps. A collector at the top of the device (above the operating water level) receives and allows breakdown of foam to produce a concentrated solution that is discharged to the effluent system (Hutchinson et al., 2004).

According to Hutchinson et al (2004) additional benefits of foam fractionation include:

Reduction in ammonia due to removal of organics.

Removal of bacteria.

Increased pH through removal of organic acids

3.2. Mechanical filtration

High stocking densities in RAS require high inputs of feed and with this comes high levels of waste production (i.e. faeces and uneaten feed). Mechanical filtration is used to remove the coarse particulate matter from the system. Turbidity, excessive backwashing and removal of finer solids are common problems that must also be addressed using mechanical filtration.

For RAS design it is essential to know the expected nature and load of solids produced during culture operations and the impact within the RAS if these are not removed efficiently. Typical fish feces contain digested and undigested material bound within a mucous coating. Efficient mechanical filtration will greatly reduce oxygen demand within the RAS as breakdown of these organic solids consumes significant amounts of oxygen within the culture system. Biological degradation (biological oxygen demand - BOD) of fish waste and unconsumed feed is attributed to micro-organisms that occur on all surfaces (i.e. tanks, pipes, filters, and solids particles) and throughout the water column. In the absence of adequate mechanical filtration, the presence of high levels of solids within the RAS encourages population growth of these micro-organisms, due to “feed availability,” to the extent that they can become a major user of dissolved oxygen (DO) within the system. In addition, ammonia is produced during decomposition of fish wastes that is typically high in nitrogen, placing further demands on the biological filter. There is no universally accepted design layout of RAS components apart from general acceptance that efficient mechanical filtration is critical and should precede biological filtration and other water treatment components. Commercial RAS design should incorporate filtration methods that achieve rapid separation and removal of solids from the system before this material (mostly feces and uneaten feed) begins to breakdown. If this occurs within the system it will greatly increase the oxygen demand on the biological filter and reduce the amount of oxygen available to fish. This will in turn significantly increase oxygen supply needed within the system to sustain high levels of fish growth and biological filter efficiency. Coarse settle able solids (>100 μm) are

generally removed from RAS using some form of settlement device (e.g. swirl separators, settlement chambers, inclined plate separator). Removal of settle able solids can be enhanced by the use of components located within culture tanks such as modified sumps or separate plumbing configurations to provide double drainage points from each tank. These configurations aim to intercept coarse solids from the recycled water at the tank exit point and direct this stream to a separation device (e.g. swirl separator, sludge collector, clarifier) for concentration and sludge removal. Suspended solids (<100 μm) can be removed using an efficient mechanical filter that may operate using depth filtration (e.g. pressure sand filters, cartridge filters, filter matting) or screen filtration (e.g. inclined screens, rotating drum filters, conveyor filters). Micro screens, typically installed as rotating drum filters service high flow rates at the filtration level required (20 – 100 μm), low head loss during operation and efficient (low water use typically <10 % system volume per day) automated cleaning options. Although other filtration methods are available, the organic solids loading within RAS presents operational problems for the use of pressure sand filters, cartridge filters and bag filters. If possible, mechanical filter selection should achieve treatment of the entire recirculated water flow to a minimum of 100 μm and if possible down to 20 μm (Hutchinson et al., 2004).

Biological filtration is widely covered in the next section.

3.3. Management of dissolved gases: Oxygen, carbon dioxide and ozone

RAS design will need to provide the dissolved oxygen (DO) requirements of all components of the system and allow removal of carbon dioxide produced through fish respiration. This is often overlooked in RAS design and is of particular importance at the high stocking densities required for economic operation of these systems. Management of dissolved nitrogen also requires consideration within RAS design due to the potential for mortality associated with relatively low levels of super-saturation (>102 %) when using pressurized components. (Hutchinson et al 2004).

Effective management of DO is a key factor in the operation of commercial RAS. Generally, intensive RAS attempt to maintain system DO at 100 % saturation to optimize growth and system performance (i.e. biofilter operation). At higher levels of saturation, loss of DO to the atmosphere can be significant.

There is a direct relationship between oxygen consumption of fish, feeding and growth rate. If oxygen is not at near saturation levels, growth rates will be reduced, extending grow-out time and thus reducing potential profit. Another advantage of using pure oxygen is the reduction in pumping costs (i.e. operating, pipe and plumbing size) by delivering water at levels of

saturation greater than 100 %. The overall size (i.e. buildings, tanks) of the RAS may also be reduced using oxygen, providing further savings during construction.

RAS tend to be divided into two levels of intensification based upon methods of oxygen supply:

• Low-density systems (<30 – 40 kg/m^3) provide oxygen requirements through aeration (i.e. oxygen from air), supplied by air blowers and re-aeration components (i.e. diffusers, air lifts, re/degassers etc).

• High-density systems (>60 – 100 +kg/m^3) receive oxygen as pure oxygen from either liquid oxygen stored on-site or an oxygen generation system.

Oxygen requirements of fish will vary depending on metabolic rate (related in part to feed consumption), fish size and holding conditions. Other information required includes total biomass at full stocking, water flow rate, desired DO content of water flowing into tanks, minimum desired DO content of out flowing water (typically 80 – 100 % saturation) and the efficiency of the oxygen transfer devices.

A rule of thumb is that for each kilogram of feed added, approximately 0.50 - 0.56 kg of oxygen will be consumed by fish and bacteria.

Use of oxygen in RAS should be as efficient as possible. Oxygen transfer devices will be either of an open un-pressurised type such as low head oxygenators, fine diffusers in culture tanks and packed columns; or closed pressurised types such as U-tubes and oxygenation cones. The design of a RAS should include consideration of the type of contactor best suited to the application, absorption efficiency (O_2 absorbed per unit O_2 applied) and transfer efficiency (power required per unit O_2 transferred).

Oxygen produced by generators will contain approximately 10 % nitrogen. This limits the selection of transfer devices used with oxygen generators to un-pressurised types, due to the possibility of promoting nitrogen super saturation. (Hutchinson et al 2004).

Carbon dioxide (CO_2) is a by-product of fish and bacteria respiration within RAS and production is directly related to the amount of oxygen consumed as for every 1.0 g of O_2 consumed, 1.27 g of CO_2 is produced. Carbon dioxide reacts with water to form carbonic acid that reduces pH in RAS. High levels of circulating CO_2 lead to a reduction in blood pH of fish that impairs the oxygen carrying capacity of haemoglobin, even at high levels of dissolved oxygen. Carbon dioxide does not accumulate in low stocking density systems, as these tend to use high water exchange and aeration. Accumulation does occur in high intensity RAS that use oxygen injection and minimal agitation. In these types of systems carbon dioxide should be managed so that levels do not exceed 20 mg/L. This level can be exceeded in oxygenated systems at high stocking density, particularly when high efficiency contactors are used to

provide the DO saturation required to support increased feed consumption with corresponding increased levels of CO_2 production.

In these systems, a well-designed gas-stripping device needs to be installed to remove CO_2. Carbon dioxide is many times more soluble in water than oxygen. Consequently, it is harder to strip than dissolve oxygen. Thus gas-stripping devices must provide a very high airflow, about 3 – 10 times the air volume to water volume treated. This is achieved using a fan which forces air through towers packed with open plastic degassing media. These devices should be installed in well-ventilated areas or be ducted to the outside of buildings. (Hutchinson et al 2004).

Ozone (O_3) is a powerful oxidizing agent that is increasingly being used for disease control and water quality enhancement in RAS, particularly within saltwater systems where it is often used in combination with foam fractionation.

In seawater, ozone reacts with bromine to produce the residual oxidant hypo-bromous (bromic) acid that is reactive. Degradation is dependant on organic loading and harmful effects of hypobromous acid can be minimised through use of an Oxidation Reduction Potential (ORP) controller. Ozone is a highly unstable gas that must be generated and used within RAS prior to reaching the culture species, as it is toxic.

Apart from bacterial and viral inactivation, ozone use provides a number of beneficial interactions with organics resulting in lower turbidities, including:

• Oxidation of organic compounds.

• Coagulation of particles into larger ones that are more easily removed by mechanical filtration.

• Break down of large organic molecules into more biodegradable ones.

Corona discharge type ozone generators are used in RAS. These units generate ozone as oxygen passes through a high voltage produced across two electrodes. These ozone units are often supplied with pure oxygen or an oxygen concentrator, which is fed by an air drier to improve the efficiency and capacity of ozone production.

Ozone is generally applied within a contact chamber, which is designed to allow the desired treatment time and allow ozone to revert to oxygen. In aquaculture systems containing high organic carbon loads, the half-life of ozone may be less than a few minutes. Typical dosage levels for ozone disinfection within RAS are between 0.01 - 0.10 mg/L water flow with retention time for treatment between 0.5 and 20 minutes. Residual ozone can be removed as water is passed through activated carbon or a packed column degasser vented back to the contact system or to an ozone destructor and released to the outside of the building (ozone gas is highly dangerous to human health).

In aquaculture systems, ozone dosage is monitored by redox potential through an ORP (Oxidation Reduction Potential) meter working in conjunction with a controller. Generally,

ozone is dosed automatically to maintain a redox potential between 300 –350 mv. Ozone is very corrosive and all connections, distribution plumbing and contacting elements should be constructed of resistant materials. In contrast to UV, ozone is generally added before the mechanical and biological filter elements as it decomposes dissolved and solid organic material and thus improves the performance of mechanical filtration and reduces the load on biological filters (Hutchinson et al., 2004).

3.4. UV disinfection

Ultraviolet irradiation (UV) is widely used to control pathogens in aquaculture. The UV used is typically produced by mercury vapour bulbs/lamps that emit radiation at wavelengths from 100 – 400 nm, that is between the blue - violet range of the visible spectrum and the shorter wavelength X-rays (Hutchinson, 2004). UV disinfection works by applying light in wavelengths that destroy DNA in biological organisms. The treatment has been used for medical purposes for decades and does not impact the fish as UV treatment of the water is applied outside the fish production area. It is important to understand that bacteria grow so rapidly in organic matter that controlling bacterial numbers in traditional fish farms has limited effect. The best control is achieved when effective mechanical filtration is combined with a thorough biofiltration to effectively remove organic matter from the process water, thus making the UV radiation work efficiently (Bregnballe, 2015).

3.5. Environmental control: Water temperature, salinity, lighting and water supply

Water temperature is one of the primary environmental factors that influence the growth rate of fish. RAS must be able to control water temperature so that optimum growth performance and economic return can be achieved to justify the cost of operation.

Direct heating of recirculated water through the use of immersion heaters, gas fired boilers and heat exchangers, or electric heat (and chill) pumps are the most efficient methods.

Space heating can also be considered but is not as efficient as other water heating methods. The use of waste heat generated from pumps and aerators can be used to effectively remove the necessity for dedicated heating components within RAS in well insulated buildings, thereby saving operating costs. Thermostatic control systems are available for all heating or chilling equipment.

There are also a number of other natural sources of energy that can be utilized to control water temperature in RAS such as solar heating, saline solar ponds and geothermal water/heat. (Brown, 2013).

Also, the effect of temperature on the nitrification performance of the fixed film the ammonia removal cycles were 67 days, 63 days, 52 days, and 38 days at 14 °C, 18 °C, 22 °C and 26 °C that an increase in temperature results in greater ammonia removal and growth of nitrifying bacteria. (Kir, 2009).

Toxic concentrations of ammonium and nitrite depend on the species being cultured and salinity levels. For Pacific white shrimp, ammonium toxicity levels range from 2.44 to 3.95 mg/L of total ammonium-nitrogen, while nitrite toxicity levels range from 6.1 to 25.7 mg/L of nitrite-nitrogen. Decreases in salinity, in the range of 35 to 15 g/L, result in increased sensitivity to ammonium and nitrite (Brown, 2013).

Fish are visual feeders so RAS can incorporate day-length control of feeding duration to optimise growth. Superimposed upon the artificial day-length provided are the natural circadian feeding peaks that generally occur at dawn and dusk for most fish species. Of more importance may be light intensity as some species such as Murray Cod are less stressed and feed better in dim lighting conditions.

Incandescent or fluorescent bulbs mounted in appropriate water resistant housings with diffusers can provide lighting in RAS. Natural lighting can be used to supplement artificial lighting but this may encourage algal growth if directed on to the water in culture tanks. It is beneficial to incorporate a separate light circuit that can be dimmed to avoid sudden exposure to light and dark that will stress fish and may cause them to jump from tanks or injure themselves by running into tank walls.

Before construction, it is recommended that the site selected be able to provide at least 20 % of system volume for exchange daily. Additional water usage needs that are often not accounted for include requirements for purging, cleaning, flushing during emergencies and loss of well capacity. Prospective operators should expect bore recharge capacity to drop approximately 50 % over the first year from initial recharge levels. A complete water analysis should be completed when designing a RAS facility, as the results could influence system and species suitability for the chosen water source.

Components such as salinity, iron, manganese, ammonia, hydrogen sulphide and excessive carbonate hardness (>300−400 mg/L) are common problems in bore water sources. Some of these problems can be overcome relatively inexpensively, but others will limit or negate the usefulness of the water source for RAS. Recirculated water can be used, however is typically expensive and may require treatment before use (to remove chlorine, amines, etc.) (Hutchinson et al., 2004).

Also RAS can be supported by shed, backup power supply, microscopes and water quality monitoring equipment, miscellaneous equipment, storage and workshop, processing, administration and staff amenities.

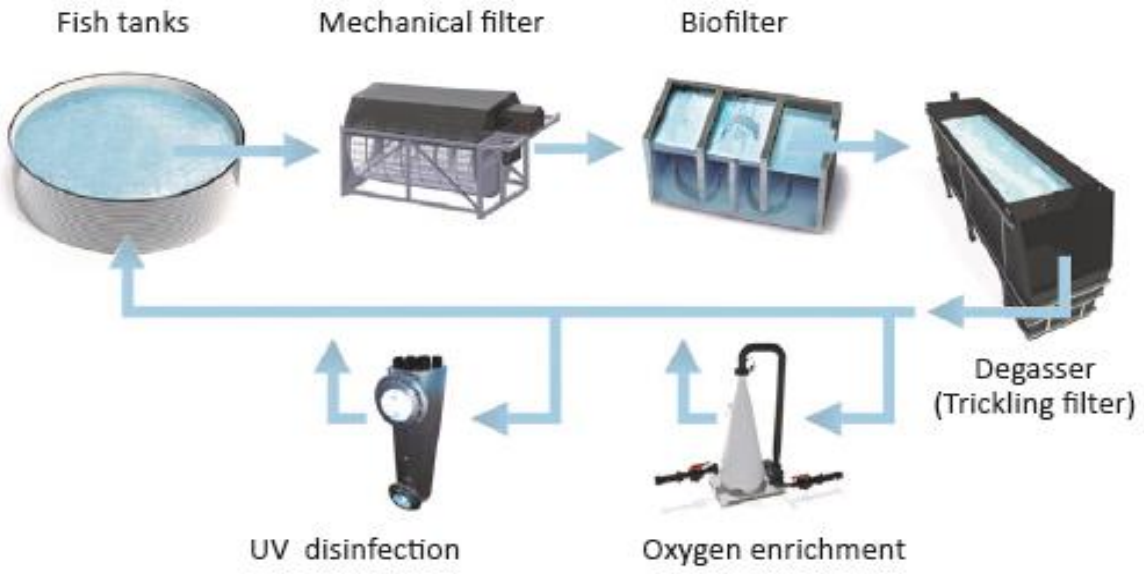


Figure 2. Principle drawing of a recirculation system (from Bregnballe, 2015).

4. BIOLOGICAL FILTERS AND MEDIA

4.1. BIOFILTERS

Nitrogen is an essential nutrient for all living organisms and is found in proteins, nucleic acids, adenosine phosphates, pyridine nucleotides, and pigments. In the aquaculture environment, there are four primary sources of nitrogenous wastes: (1) urea, uric acid and amino acid excreted by the fish; (2) organic debris from dead and dying organisms; (3) uneaten feed and feces; and (4) nitrogen gas from the atmosphere. The decomposition of these nitrogenous compounds is particularly important in intensive recirculating aquaculture systems because of the toxicity of ammonia, nitrite, and to some limited extent, nitrate. The process of bacterial driven ammonia removal in a biological filter is called nitrification, and consists of the successive oxidation of ammonia to nitrite and finally to nitrate. The reverse process is called denitrification and is an anaerobic process where nitrate is converted to nitrogen gas (Ebeling and Timmons, 2012).

There is considerable debate (and significant competition) as to the most appropriate biological filter technology for intensive aquaculture applications. The task is further complicated by the wide variety of water quality requirements and environmental conditions

displayed by recirculating aquaculture systems. An ideal biofilter would maximize media specific surface area and remove 100 % of the inlet ammonia concentration, generate very little nitrite, maximize oxygen transfer, require a relatively small footprint, use inexpensive media, have minimal head loss, require very little maintenance to operate, and would not capture solids. Unfortunately, there is no one biofilter type that meets all of these ideals, each biofilter has its own strength and weaknesses and areas of best application. Currently large scale commercial recirculating systems have been moving towards using granular filters (expanded beds, fluidized beds and floating bead beds) (Timmons and Ebeling, 2010).

Biological treatment processes employ bacteria that grow either attached to a surface (fixed films) or that grow suspended in the water column. Almost all recirculating systems use fixed-film bioreactors, where the nitrifying bacteria grow on either a wetted or submerged media surface. The ammonia removal capacity of biological filters is largely dependent upon the total surface area available for growth of the nitrifying bacteria. For maximum efficiency, the media used must balance a high specific surface area, i.e., surface per unit volume, with appreciable voids ratio (pore space) for adequate hydraulic performance of the system. The media used in the biofilters must be inert, non-compressible, and not biologically degradable. Typical media used in aquaculture biofilters are sand, crushed rock or river gravel, or some form of plastic or ceramic material shaped as small beads, or large spheres, rings, or saddles. Biofilters must be carefully designed to avoid oxygen limitation or excessive loading of solids, biochemical oxygen demand, or ammonia (Timmons and Ebeling, 2010).

The ideal material has a high surface area per volume, low in cost, durable, does not clog easily and promotes a uniform spread of water to be treated. Plastic materials fulfill most of these criteria and are increasingly being used. Various configurations are commercially available; the specific surface area usually varies from 150 to 350 m^2 m^{-3}, with maximum ammonia removal rates (where NH_4-N stands for total ammonia-N) between 0.28 and 0.55 g NH_4-N m^{-2} day^{-1} (van Rijn, 1996).

Biofilter bacteria include members of Actinobacteria, Bacterioidetes/Chlorobi, Firmicutes, Nitrospirae, Planctomycetales, and Proteobacteria. Chloroflexi and Synergistetes have also been found in a few cases. Recent studies have also suggested involvement of Archaea. Significant differences in microbial communities have been found in RAS biofilters, culture water, and communities representing unique and complex environments. Because every fish species introduces its own unique microbial flora, filter diversity varies from RAS to RAS. Differences in communities also reflect utilization of UV disinfection and ozonation treatments, which play significant roles in perturbing these communities but appear to have little effect in deep layers of filter biofilms (Schreier et al., 2010).

4.2. MICROBIAL DISTRIBUTION IN RAS

Microorganisms play main role in the biofilters to keep water quality in the tank. For that reason, clear understanding of microbially mediated nitrogen transformation processes in RAS can help improve design through proper operational modifications. Each compartment in RAS provides a specific biotope with a specific microbial community. Besides the culture animals, the biofilter is the largest reservoir of microorganisms in RAS. Two types of biofilters can be distinguished: fixed film (attached growth) biofilters in which media provide substrate for microorganisms to attach and grow, and single sludge biofilters in which sludge and microorganisms are maintained in suspension. Outside the different filter compartments, suspended free-floating microorganisms are often not homogeneously distributed through the system. A mixture of fungi, bacteria and algae proliferating on organic wastes might create fouling on walls of tanks and pipes and inside air diffusers, causing clogging and changes in flows of water and gasses. The biofilter shelters the highest amount of microorganisms in comparison with other compartments of the RAS system. (Rurangwa and Verdegem, 2015).

Bacteria and archaea in RAS biofilters (Table 1). Besides the nitrite-oxidizing bacteria (NOB), nitrospirae and ammonia-oxidizing archaea (AOA) were abundant nitrifiers in a shrimp RAS. AOA are adapted to life under nutrient limiting conditions which ammonia oxidizing bacteria (AOB) cannot survive (Rurangwa and Verdegem, 2015).

Table 1. Microorganisms associated with recirculation aquaculture systems biofiltration (from Rurangwa and Verdegem, 2015).

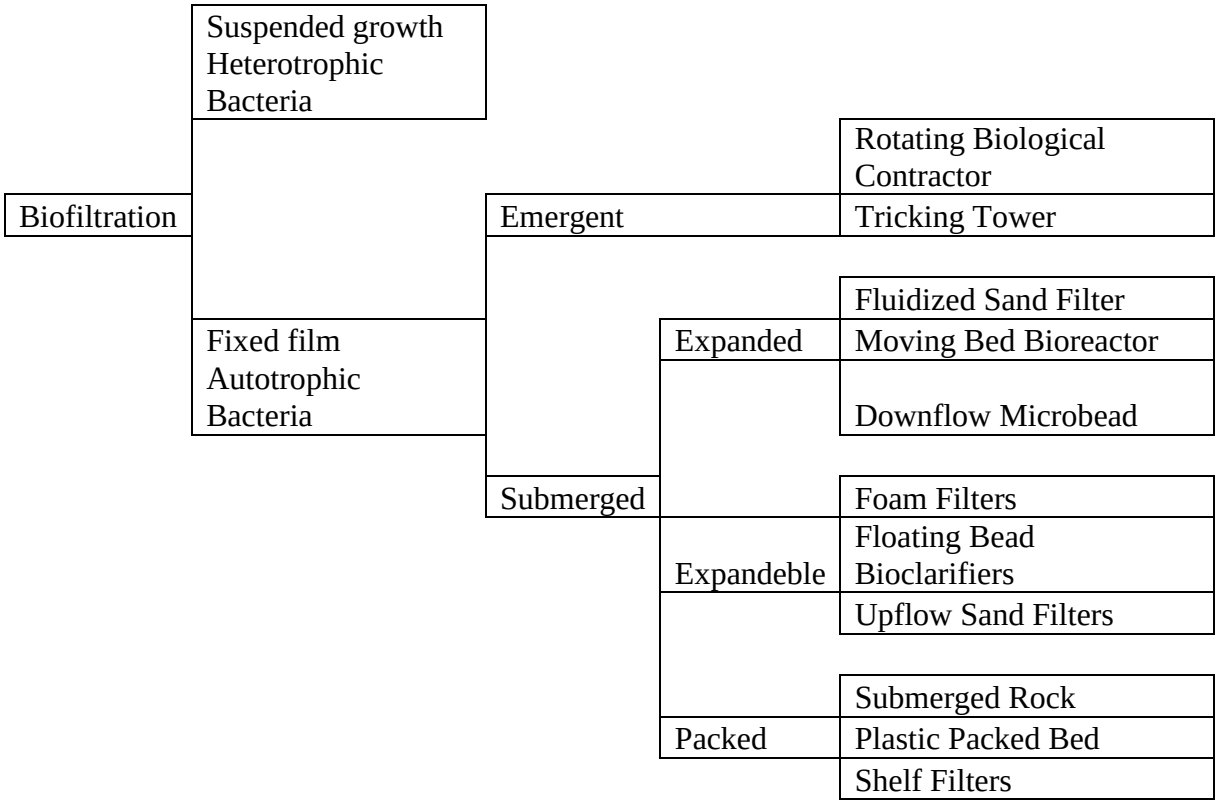
Process	**Phylum and Genus**
Nitrification	
Ammonium-oxidizing bacteria	*Nitrosomonas sp., Nitrosococcus sp*., Nitrosospira
Ammonium-oxidizing archaea	Nitrosopumilus
Nitrite-oxidizing bacteria	*Nitrospira sp., Nitrobacter sp.*
Denitrification	
Autotrophic	*Thiomicrospira sp., Thiothrix sp., Rhodobacter sp., Hydrogenophaga sp.*
Heterotrophic	*Pseudomonas sp., Paracoccus sp., Comamonas sp.*
Dissimilatory nitrate reduction to ammonia	Various Proteobacteria and Firmicutes
Anaerobic ammonium	*Planctomycetes sp., Brocadia sp.*

oxidation (Anammox)	
Sulphate reduction	*Desulfovibrio sp., Dethiosulfovibrio sp., Fusibacter sp., Bacteroides sp.*
Sulphide oxidation Methanogenesis	*Thiomicrospira sp.* Methanogenic Archaea

These AOB depend on the availability of ammonium and use ammonia oxidation as their sole source of energy. Specifically, nitrosopumilus-type AOA were more abundant than nitrosomonas marina-type AOB in the biofilter of a shrimp RAS. Similarly, the AOA Candidatus Nitrosopumilus maritimus and the AOB Nitrosospira were dominant in biofilters in three RAS each with a different marine fish species. Ammonia- and nitrite-oxidizing species are different in marine and freshwater biofilters. Fungi have been associated with assimilatory nitrate reduction in RAS. Aspergillus niger NBG5 removed simultaneously ammonium, nitrite and protein at low temperature but shifted to metabolize carbon at high temperature. Both autotrophic and heterotrophic bacteria are present in RAS. Autotrophic bacteria derive carbon from CO_2 and energy from oxidation of inorganic nitrogen, sulphur or iron compounds. Heterotrophic bacteria obtain carbon and energy from organic matter including carbohydrates, amino acids, peptides and lipids. Heterotrophic bacteria mineralize organic matter from uneaten feeds, dead fish and faeces in RAS. Other microbial processes involved in nitrogen cycling and removal in RAS include anaerobic ammonium oxidation and denitrification (Rurangwa and Verdegem., 2015).

Biofiltration, in this context the microbial degradation of organic matter, TAN, and nitrite, is facilitated by biofilter units connected to the rearing facilities. Various types of nitrifying biofilters have been developed for RAS (Table 2) all to control and degrade ammonia and nitrite. Aquaculture biofilters ideally maximize available surface area in a confined space while still ensuring oxygen and substrate transfer to support optimal conditions for the beneficial nitrifying microorganisms. Fixed film biofilter are far the most applied type in salmonid RAS, though suspended growth (biofloc technology) recently have gained new focus to non-salmonid species (Pedersen, 2009).

Table 2. Schematic representation of various types of nitrifying biofilters (from Pedersen, 2009).



4.3. SUSPENDED GROWTH AND FIXED FILM

The first juncture separates the two fundamental approaches to bacterial culture, suspended growth, or fixed film. Suspended growth systems were rarely found in production aquaculture until recently with the increased utilization of microbial floc systems for the production of very hardy species such as tilapia and marine shrimp. In these systems, heterotrophic bacterial growth is stimulated through the addition of organic carbonaceous substrate, for example molasses, sugar, wheat, cassava, etc. At high organic carbon to nitrogen feed ratios (greater than -14 C/N ratios), heterotrophic bacteria assimilate ammonia-nitrogen directly from the water replacing the need for an external fixed film biofilter (Timmons and Ebeling, 2010).

In the traditional intensive recirculating aquaculture production systems, large fixed-film bioreactors are used that rely on the nitrification of ammonia-nitrogen to nitrate-nitrogen by Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB). In intensive recirculating systems, the growth of heterotrophic bacteria and the accumulation of organic carbon are minimized intentionally through the rapid removal of solids from the system and through water exchange (Figure 3).

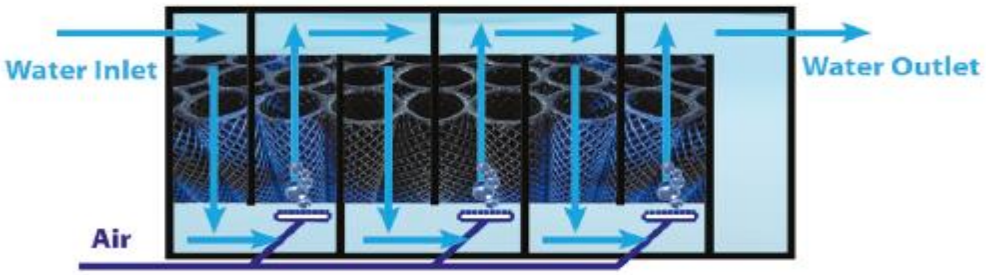


Figure 3. Fixed bed film biofilters (from Bregnballe, 2015).

In general, fixed film bioreactors are more stable than suspended growth systems. In a fixed film biofilter, a thin bacterial biomass coats the filter media and the dissolved nutrients and oxygen are transported by diffusion into the biofilm. Numerous types of media have been employed to support this biofilm, including rock, shells, sand, plastic, and others too numerous to list. Just about anything that will support a biofilm and has a reasonable specific surface area (and many that do not) have been used over the years. The major drawback to these types of filters is that they can be quickly 'smothered' by heterotrophic bacteria, resulting in significant performance degradation. Fixed film biofilters subdivided into four fundamental blocks distinguished by the strategy used to provide oxygen and the techniques used to handle excess biofilm growth (Timmons and Ebeling, 2010).

4.4. EMERGENT BIOFILTERS

The second juncture separates fixed film biofilters based on the two fundamental methods of oxygen transfer. The 'emergent' filters use a cascading mixture of water and air over the media to insure a high level of dissolved oxygen at the surface of the biofilm. ln the trickling filter, water is cascaded over the media in a tower opened to the ambient air. Whereas the rotating biological contactors create a similar effect by slowly rotating the media in and out of a tank of water, always keeping the media wet. Excessive biofilm is managed through the process of film sloughing or shedding, which demands a relatively high porosity to prevent clogging of the filter media with the sloughed film material. These filters provide a secondary benefit in the form of aeration and carbon dioxide stripping. (Timmons and Ebeling, 2010).

A substrate that has a high specific surface area (large surface area per unit volume) provides an attachment site for the bacteria. Some common substrates include sand or gravel, plastic beads, plastic rings, or plates.

4.4.1. Trickling towers

The trickling tower is a classical biofilter, combining both biofiltration, aeration, and degassing into one unit process. Water cascades over some media on which bacteria grow, oxygen diffuses into the water, and nitrogen and carbon dioxide diffuse out. They can be constructed to any diameter required. Effective distribution of the influent water over all the media both horizontally and vertically is a continual challenge (Ebeling and Timmons, 2012) (Figure 4).

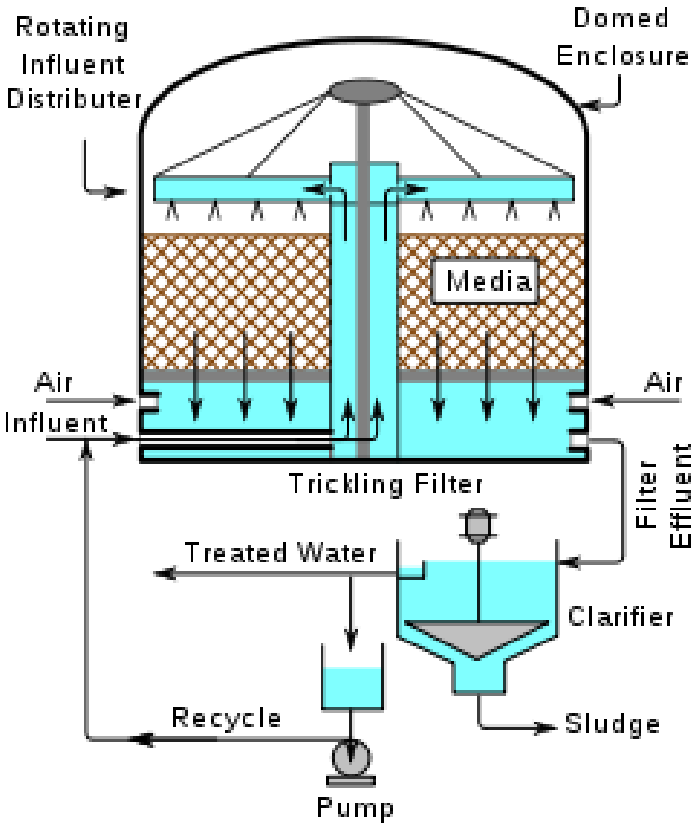


Figure 4. A schematic of a complete trickling filter system (Source: https://en.wikipedia.org/wiki/Biofilter).

4.4.2. Rotating Biological Contactors (RBC)

A rotating biological contactor or biodisc filter (Figure 5) is a fixed film bioreactor composed of circular plates aligned on a central axle, first developed for the treatment of treating domestic wastewater. The filler is usually staged in series within a flooded compartment through which recirculated water flows, with approximately half of the disc surfaces submerged, and half exposed to the air. The discs are rotated slowly (1.5 to 2.0 rpm) to alternately expose the biologically active media to the nutrient recirculated water and to the air, which provides oxygen to the biofilm. Early RBC designs were fabricated from discs of corrugated fiberglass roofing material.

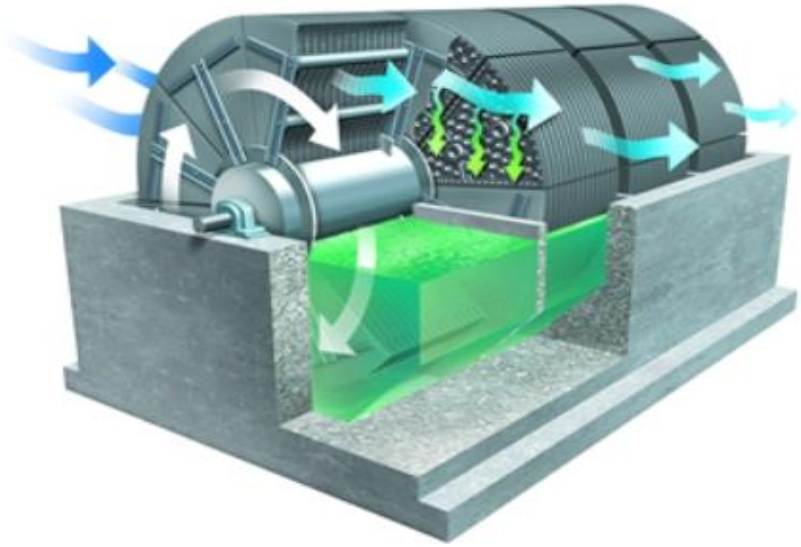


Figure 5. Fixed film: Rotating Biological Contactors (RBC) (Source:http://www.walker-process.com/prod_bio_RBC.htm).

Currently, media with a higher specific surface area (258 m^2/m^3) are utilized in construction of RBC, which reduce the physical size and increase the ammonia and nitrite removal capacity. Hochheimer and Wheaton recommended a maximum hydraulic loading design limit for RBCs of 300 m^3/m^2 day. Brazil determined an average total ammonia nitrogen area removal rate of 0.43 ± 0.16 g/m^2 day for an industrial-scale, air-driven RBC used to rear tilapia at 28 °C. Van Gorder and Jug-Dujakovic reported higher rates for multiple commercial scale systems of 1.2 g/m^2 day. In addition, carbon dioxide concentrations were reduced approximately 39 % as the water flowed through the RBCs and 65% of the estimated carbon dioxide generated was off-gassed by the RBCs.

Rotating biological contactors have inherent advantages for aquaculture, because they are self-aerating, require little hydraulic head, have low operating costs, provide gas stripping, and can maintain a consistently aerobic treatment environment. In addition, they tend to be self-cleaning due to the shearing of loose biofilm caused by the rotation of the media through the water. The main disadvantages of these systems are a) the mechanical nature of its operation, b) the substantial weight gain due to biomass loading of the media and the resultant load on the shaft and bearings, and c) the relatively high capital cost per unit of nitrification obtained (several fold higher than fluidized sand beds or microbead filters). Early efforts using RBC's often employed underdesigned shafts and mechanical components, which resulted in mechanical failure, but a properly designed RBC is very functional and reliable. Figure 5 shows an RBC (manufactured by Fresh-Culture Systems, Inc.) categorized as "floating/air-driven/rotating biologicalcontactor", which rotates using pumped air and/or water. Its weight is supported by the water column

resulting in very little resistance to the rotation of the biofilter. This also eliminates the mechanical difficulties often reported with the gear motors, pillow blocks, chain drives and shafts used with many commercially available RBC's (Timmons and Ebeling, 2010).

4.5. SUBMERGED BIOFILTERS

The second major category of fixed film biofilters, submerged filters presume that sufficient oxygen can be transported to the biofilm in the water circulated through the filter. This is accomplished by the use of high recirculation rates, internal recycling, or through oxygen enrichment of the influent water. In addition, the assumption is made that ammonia diffusion into the biofilm is the rate limiting parameter and not dissolved oxygen. Thus, the goal of submerged filters is to first maximize the specific surface area in order to enhance nitrification. The three general types of submerged biofilters are categorized by the strategy used to manage biofilm accumulation (Table 2). The first major category of submerged biofilters employ a fixed, static packed bed of media that has no active management of either the biofilm or solids accumulation. Examples of fixed, static packed beds are submerged rock biofilters, plastic packed beds and shell filters.

Submerged packed beds relay entirely upon endogenous respiration to control biofilm accumulation. The water can flow either from the bottom up (upflow) or from the top down (downflow). Thus, the hydraulic retention time can be controlled by adjusting the water flow rate. Solids from the culture tank can accumulate within the submerged filter, along with cell mass from nitrifying and heterotrophic bacteria. This process can eventually block the void spaces, requiring some mechanism to flush solids from the filter for successful long term operation. To provide large void spaces to prevent clogging of the filters, the media used for submerged biofilters has been traditionally of large size, such as uniform crushed rock over 5 cm in diameter or plastic media over 2.5 cm in diameter. However, 5 cm diameter crushed rock would only have a specific surface area of 75 m^2/m^3 and avoid fraction of only 40 to 50 %. Random packed plastic media would also have a relatively low specific surface area of 100-200 m^2 /m^3, but a much higher void fraction, greater than 95 %. Drawbacks of this type of filter include problems of low dissolved oxygen and solids accumulation, resulting from heavy loading of organic matter (feed) and the difficulty of backflushing. Although this type of filter was promoted and used in aquaculture in the past, it has since been replaced in aquaculture due to the inherent high construction costs, biofouling problems, and operational expense. Packed submerged biofilters are still used in lightly loaded systems such as display aqua1ia and seafood-holding/display systems, where oyster shells are often used to help maintain calcium carbonate

concentrations and other important trace minerals. The second category of submerged biofilters utilizes a static bed that is intermittently "expandable" using air, water, or mechanical mixers. Excessive biofilm growth is removed by the process of abrasion as the media is agitated, then allowed to settle out before reintroducing the flow stream. Expandable biofilters are able to operate as mechanical filters for solids removal, biofilters for ammonia removal and as bioclarifiers accomplishing both solids capture and nitrification depending up design and backflushing frequency. Examples of expandable biofilters include upflow sand filters, floating bead bioclarifiers and foam filters. (Timmons and Ebeling, 2010).

4.5.1. Pressurized up flow sand filters

Still part of the second category of submerged biofilters, up flow pressurized sand filters or often a typical swimming pool filter are principally used as mechanical filters, although they may contribute some nitrification. They usually make for poor biofilters due to the high rate of backwashing and slow biofilm growth rates. Sand filters have been widely used for display aquaria. Up flow gravel filters have seen some utilization in large public aquariums, but are rarely used today because of the high water loss during backflushing. Very high flow rates are required through these biofilters to initiate their expansion. (Timmons and Ebeling, 2010).

4.5.2. Floating bead filters

Floating bead filters use beads that are slightly buoyant. The beads provide surface area for bacteria and also trap solids, thus doing two jobs for the price of one filter. Water is introduced below a bed of packed bead media and travels upward through the filtration chamber where mechanical and biological filtration takes place. Backwashing of the filter is accomplished either mechanically with a motor/propeller or with air bubbles (Figure 6).



Figure 6. Propeller washed bead filter for biofiltration and solids capture (from Ebeling and Timmons, 2012).

At some predetermined rate, a desired backwash or mixing cycle (after mixing and breaking up the static floating bed, the bed is allowed to settle for a minute or so, and then the settled sludge is discharged by opening a valve at the bottom of the filter) will be imposed to clean the beads and remove the resulting sludge. Newer designs for bead filters have the capacity to minimize water loss during the cleaning cycle. This is particularly advantageous in marine systems where the loss of saltwater is minimized and thus operating costs are decreased (Ebeling and Timmons, 2012).

4.5.3. Fluidized sand beds

Usually used in large-scale cool or cold-water applications, fluidized sand beds provide large surface area for bacteria in a small footprint. These filters get their name because as the water flows up through the sand bed, the sand becomes suspended in the flow or fluidized. Numerous designs have been investigated and found to perform effectively, particularly for cool water applications that use fine sands. Fluidized sand beds can be relatively more expensive to operate than other filters due to the high pump rates and pressures required to fluidize the sand bed (Ebeling and Timmons, 2012).

4.5.4. Downflow micro-bead biofilter

Downflow micro-bead biofilters have been used for several years due to their simplicity and low cost for media. The filters use small plastic beads (1 to 3 mm) that float in the biofilter as the water flows down through them. The high specific surface area, low head loss, and small

footprint makes them a strong competitor to other biofilter designs. The Styrofoam micro-beads are also a fraction of the cost of other bead media (Ebeling and Timmons, 2012).

According to Timmons et al (2006) floating bead filters work in pressured vessels and use a media that is only slightly buoyant, thus the media is a relatively expensive component of the overall filter design. In contrast, microbead filters use a polystyrene bead (microbead) that is 1–3 mm in diameter with a bulk density of 16 kg/m^3 and a specific surface area of 3936 m^2/m^3 (for the 1 mm beads). This material can be obtained commercially in bulk for roughly 4 kg / 1 $ of material. Microbead filters are considered a low-cost design alternative similar to fluidized sand filters because of their ability to be scaled to large production systems.a key advantage of microbead filters is that their cost of operation will be approximately 50 % of a fluidized sand bed due to the ability to use low head high volume pumps for their operation. For design purposes, microbead filters can be assumed to nitrify approximately 1.2 kg of TAN/(m^3 day) of media for warm water systems with influent ammonia–nitrogen levels from 2 to 3 mg/L. For cool water applications, rates should be assumed to be 50 % of warm water rates. These rates are similar for design purposes with fluidized sand beds. Microbead filters will have low nitrification efficiencies of 10–20 % primarily because of the low hydraulic retention time within the bead bed volume. Bead filters are limited in depth to approximately 50 cm; this will increase the required footprint of the bio-filter as compared to a fluidized sand bed that can be made several meters tall (Figure 7).

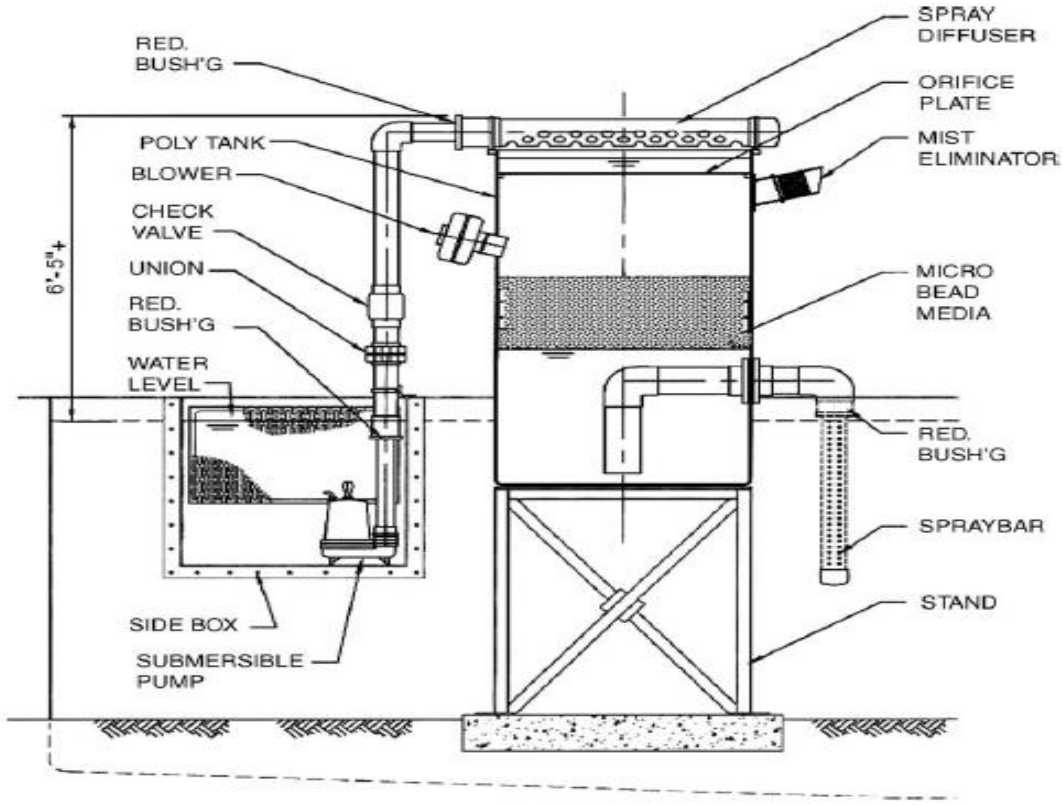


Figure 7. Generic microbead filter design (from Timmons et al., 2006).

4.5.5. Moving bed bioreactors

Moving bed bioreactors (MBBR) have been introduced over the last several years and appear to be one of the most competitive of all the biofilter types (Figure 8). The media remain in suspension as the water flows through the biofilter, which is actively aerated. The high turbulence and aeration provide good mixing and contract with the media.

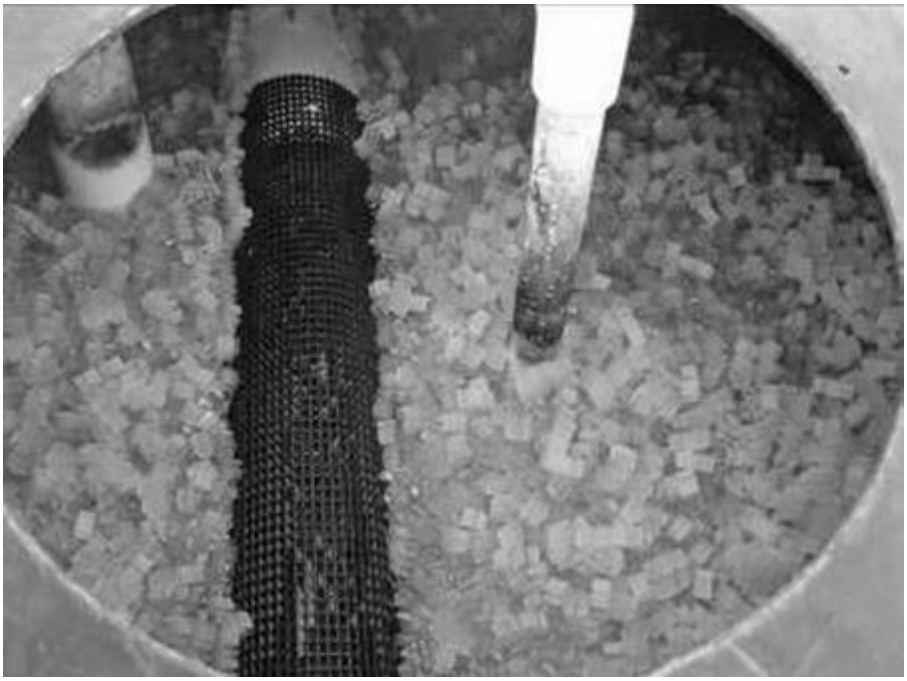


Figure 8. Media and discharge manifold in a MBBR (from Ebeling and Timmons, 2012).

Each biofilter described it's advantages and disadvantages that need to be taken in consideration during the early design phase. One of the chief advantages of both the trickling biofilter and the MBBR is that they both add oxygen to the water flow during normal operation. In addition, they can provide some carbon dioxide stripping. In contrast, the submerged biofilters, floating bead filters, micro-bead filters, and fluidized-bed biofilters are all net oxygen consumers and must rely solely on the oxygen in the influent flow to maintain aerobic conditions for the biofilm. If, for whatever reason, the influent flow is low in dissolved oxygen or the incoming flow to the biofilter is too low, interrupted anaerobic conditions will be generated within the biofilter. The application of low specific surface area media is a distinct disadvantage for both the trickling biofilters and the MBBR. Since the capital cost is proportional to the total surface area of the filters, the result is physically large and requires more costly filters. In contrast, floating bead filters, and especially fluidized-bed filters and downflow micro-bead filters, use media with high specific surface area resulting in reduced cost and space requirements for the equivalent surface area (Ebeling and Timmons, 2012).

5. NITRIFICATION IN RAS

5.1. CHEMICAL PROCESS AND MICROBIAL ECOLOGY

Profitability of recirculating systems depends in part on the ability to manage nutrient wastes. Nitrogenous wastes in these systems can be eliminated through nitrifying and denitrifying biofilters. (van Rijn, 2006).

There are nitrogen (N) exists in a number of oxidation various states in the nature, including nitrogen gas (N_2), positive two in nitric oxide (NO_2), positive one in nitrous oxide (N_2O).

The sum of the two ($NH_4^+ + NH_3$) is called total ammonia or simply ammonia. It is common in chemistry to express inorganic nitrogen compounds in terms of the nitrogen they contain, i.e., NH_4^+ (ionized ammonia nitrogen), NH_3 (un-ionized ammonia nitrogen), NO_2 (nitrite nitrogen), and NO_3 (nitrate nitrogen). This allows for easier computation of total ammonia-nitrogen (TAN = $NH_4^+ + NH_3$) and easy conversion between the various stages of nitrification (Timmons and Ebeling, 2010).

Ammonia is produced as the major end-product of protein catabolism and is excreted by fish primarily as unionized ammonia across their gills (Timmons and Ebeling, 2010).

The concentrations of various nitrogen species are important in aquaculture because several nitrogen compounds are toxic to aquatic organisms at various concentrations. Un-ionized ammonia is toxic at concentrations of ranging from 0.8 to 2 mg/L ammonia-nitrogen; consequently farmers are advised to maintain total ammonia (sum of un-ionized and ionized ammonia) below 1 mg/L nitrogen. Nitrite above 5 mg/L nitrite-nitrogen changes blood hemoglobin to methemoglobin, which does not carry oxygen and can cause death. Researchers have shown that chronic exposure to nitrate concentrations above 200 mg/L nitrate-nitrogen has a negative impact on growth (Brown, 2013).

Ammonia, nitrite, and nitrate are all highly soluble in water. An increase in pH or temperature increases the proportion of the un-ionized form of ammonia nitrogen. For example, at 20 ^{0}C and a pH of 7.0, the mole fraction of un-ionized ammonia is only 0.004, but at the same temperature the mole fraction increases to 0.80 at a pH of 10.0. Un-ionized ammonia is toxic to fish at low concentrations. Nitrification is a two-step process, where ammonia is first oxidized to nitrite and then nitrite is oxidized to nitrate. 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 are usually controlled by ammonia oxidation, and, as a result, there is usually no appreciable amount of nitrite accumulation. Equations 1, 2, and 3 show the basic chemical

conversions occurring during oxidation by Nitrosomonas and Nitrobacter and the overall oxidation reaction

Ammonia oxidation- Nitrosomonas: $NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H+ + H_2O$ (1)

Nitrite oxidation - Nitrobacter: $NO_2^- + 0.5O_2 \rightarrow NO_3^-$ (2)

Overall: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H+ + H_2O$ (3)

Nitrification is a two-step process in which ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA) and nitrite is then oxidized to nitrate by nitrite oxidizing bacteria (NOB) (Figure 9). The sensitivity of AOB and NOB to a wide variety of environmental factors is well known, so much so that nitrification has been regarded as the "Achilles heel" of wastewater treatment. In recirculating aquaculture settings, the challenges associated with accumulation of ammonia and nitrite are similar to those in the wastewater treatment field and include problems with low dissolved oxygen levels, pH outside the optimal range for nitrifying microbes (7.5 – 8.6), and accumulation of trace amounts of toxic sulfides due to the activity of sulfate reducing microbes when dissolved oxygen concentrations are low. Less is known about the sensitivity of AOA because they have not been under study for as long as the AOB. However, research has shown that AOA have adapted to survive under ammonia limited conditions where AOB cannot grow, AOA utilize a different ammonia oxidation pathway than AOB, and AOA use a different carbon fixation pathway.

AOB mediate the first step in nitrification. They use ammonia as their energy source and carbon dioxide as their carbon source, although some AOB can also use organic carbon as their carbon source. AOB and were first isolated in the 19th century by Winogradsky from soil. Since then, researchers have continued to investigate the diversity of AOB in various terrestrial and aquatic environments. All known AOB share a common ammonia oxidation biochemical pathway, in which ammonia is oxidized to hydroxylamine by an ammonia monoxygenase (AMO) complex and hydroxylamine is oxidized to nitrite by a hydroxylamine oxidoreductase (HAO) complex. These microbes can only respire under aerobic conditions, although some groups may be tolerant of low oxygen or anoxic environments. Studies have shown that AOB can reduce nitrite under anoxic conditions to nitrogen gas. The known AOB belong to two lineages of the *Proteobacteria*, the beta-subclass and the gamma-subclass. The genera *Nitrosomonas, Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio* fall within the *Betaproteobacteria*, and the genus *Nitrosococcus* is affiliated with the *Gammaproteobacteria*. *Betaproteobacteria*-AOB can vary from no salt requirement to obligate halophilic, while *Gammaproteobacteria*-AOB are obligate halophilic. AOB are one of two microbial populations known to oxidize ammonium to nitrite (Brown, 2013).

The second step of nitrification is mediated by NOB. There are five phylogenetically distinct groups of aerobic NOB, *Nitrobacter*, *Nitrospina*, *Nitrococcus*, *Nitrospira*, and *Nitrotoga*. The genus *Nitrobacter* is part of the *Alphaproteobacteria* and the genera *Nitrospina* and *Nitrococcus* belong to the *Gammaproteobacteria*. The genus *Nitrospira* falls within the separate phylum *Nitrospirae*, which is closely related to the *Deltaproteobacteria*. A cold-adapted betaproteobacterial NOB, Candidatus *Nitrotoga arctica,* was recently cultivated from the Siberian Arctic. Researchers have also identified a nitrite oxidizing bacterium in the phylum *Chloroflexi*. Nitrite oxidizers use the enzyme nitrite oxidoreductase to oxidize nitrite to nitrate. For many years, the general consensus was that *Nitrobacter* species were the dominant NOB in most environments because these were isolated most frequently. However, since molecular methods have been applied to environmental samples, researchers have found that the dominant nitrite oxidizers in most environments are *Nitrospira* species. Studies have shown that *Nitrospira*-like bacteria can exploit low amounts of nitrite and oxygen more efficiently than *Nitrobacter* (Brown, 2013).

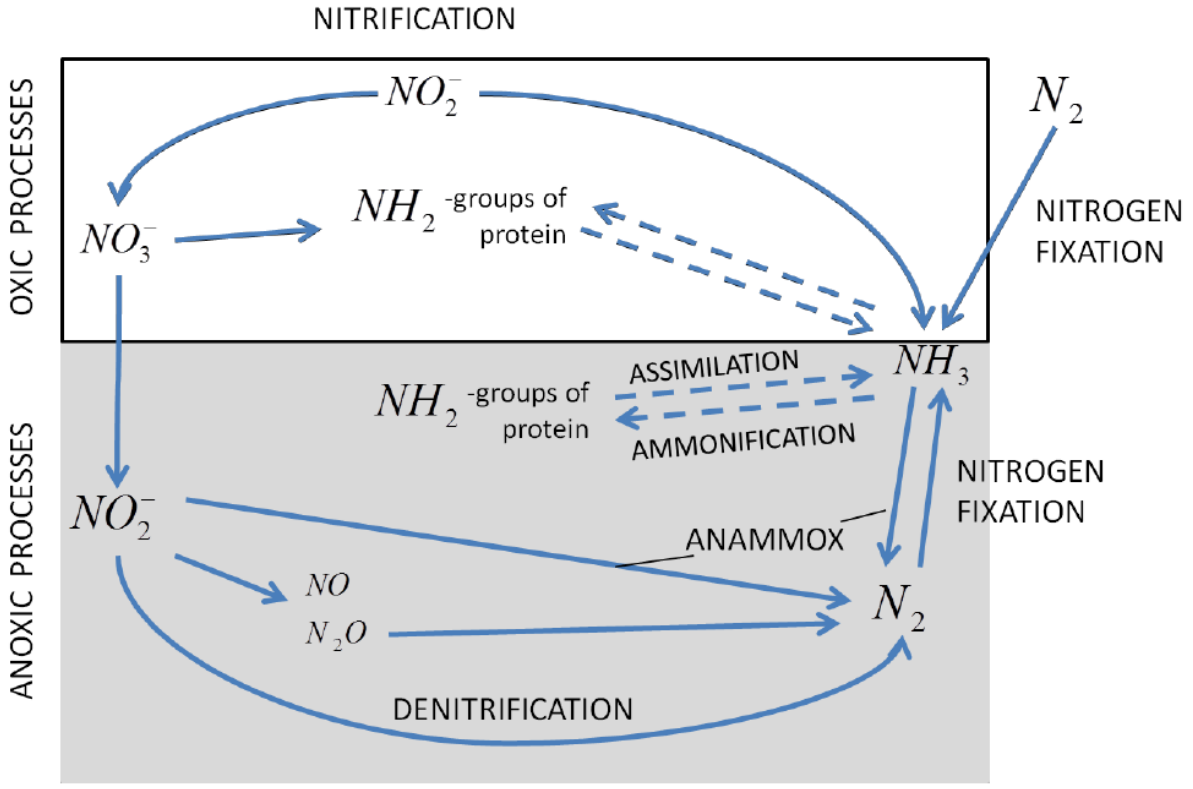


Figure 9. Diagram of the nirogen cycle. The solid arrows denote oxidation or reduction reactions (from Brown, 2013).

The growth rate of these chemolithotrophic, autotrophic microorganisms is very slow (doubling time in days) due to the low energy yield compared to growth of heterotrophic bacteria with doubling times in few hours (Pedersen, 2009).

Abundant AOB and NOB microorganisms in aquaculture are typically *Nitrosomonas oligotropha* and *Nitrospira sp*, respectively. *N. oligotropha* is the dominant AOB in systems with low TAN due to a high ammonium affinity which generally favour their growth in freshwater aquaculture systems.

Only recently, with the implementation of culture independent molecular methods, the abundance and significance of *Nitrospira sp*. instead of genus *Nitrobacter* as a major dominant NOB has been documented. *Nitrospira spp*. has, compared to *Nitrobacter* a competitive advantage at low nitrite levels under oligotrophic aquaculture conditions due to a high nitrite affinity. AOB and NOB have been suggested to coexist in a beneficial, though fragile mutualism, where NOB strongly depends on AOB for its preferred electron donor and AOB depends on NOB to remove toxic nitrite. NOB is usually distributed/localized in a deeper layer behind AOB, and hence more prone to oxygen limitations due to the additional diffusion path. With sufficient oxygen present NO_2^- oxidation occurs at a faster rate than NH_4^+ oxidation, though with a lower energy yield per molecule. Besides AOB, the activity of ammonia oxidizing Archaea (AOA) and heterotrophic ammonium assimilation is also likely to contribute to the removal of TAN (Pedersen, 2009).

Based on these relationships, 4.57 g of O_2 and approximately 7.14 g of alkalinity as $CaCO_3$ are needed for the complete oxidation of 1 g of ammonia-nitrogen. Alkalinity (all alkalinity is measured or defined in terms of calcium carbonate, $CaCO_3$) is generally added by using sodium bicarbonate/baking soda ($NaHCO_3$) or calcium carbonate, slaked lime (CaO), or hydrated lime, $Ca(OH)_2$. Sodium carbonate has the advantage of being rapidly dissolved and being very safe to handle. Conversely, the various lime compounds are not easy to dissolve (mixing tanks are required) and they are dangerous to handle. The lime products though are often much less expensive per unit of alkalinity than baking soda. The ammonia removal capacity of biological filters is largely dependent upon the total surface area available for biological growth of the nitrifying bacteria (Ebeling and Timmons, 2012).

5.2. AMMONIA

There is considerable confusion about design target values for ammonia concentration. Definitive values for the toxic levels of ammonia and the differentiation between the toxic NH_3 form and the supposed nontoxic NH_4^+ have not been precisely determined. The apparent toxicity

of ammonia is extremely variable and depends on more than the mean or maximum concentration of ammonia (Ebeling and Timmons, 2012).

According to The European Inland Fishery Advisory Commission (EIFAC) of FAO has set 0.025 mg/L as the maximum allowable concentration for un-ionized ammonia (NH_3 or ANH_3-N). A good rule of thumb is values of 1 mg/L for total ammonia nitrogen (TAN) for cool water and 2 or 3 mg/L for warmwater fish. You should always check your TAN target value selection by assuming some pH and temperature that you intend to maintain and see if your NH_3 concentrations will exceed the 0.025 mg/L value using readily available ammonia-ammonium pH temperature tables from several texts.

The rate of ammonia generation is considered to be a "soft" number. For simplicity, one could simply assume 10 % of the protein in the feed becomes the ammonia-N generation rate. For a more precise estimate and one that is affected by protein content, the following equation for the production rate of ammonia (PTAN) can be used:

$$PTAN = F \times PC \times 0.092 \qquad (4)$$

Here, F is the daily feeding level and PC is the protein content of the feed being fed. In this equation, the time period used is one day. In RAS, feed can be fed uniformly over a twenty-four-hour period, thus distributing the ammonia load uniformly over the entire day as well. If a uniform twenty-four-hour feeding is not used, then the above equation should be adjusted and the time period should be the time between feedings, or if a single feeding per day is used, then use four hours as the time period as an estimate of the time for the ammonia to be excreted from a feeding event. Note that in the above equation, the feed is assumed to be fed uniformly over a twenty-four-hour period. If the feeding period is concentrated to some fraction of the day, then the production equation for TAN must be accordingly adjusted upward. For example, if you feed over a twelve-hour period, then the PTAN term will be twice as large for design purposes, requiring your biofilters to be twice as large. This is why in RAS, you generally feed the fish over a twenty-four-hour period when possible (Ebeling and Timmons, 2012).

5.3. NITRATE NITROGEN

Typically, nitrate nitrogen is not considered in the mass balance equations to determine maximum required flow rate. In saltwater systems, though, nitrate should be considered. Nitrate nitrogen (NO_3-N) is the end product of the nitrification process. In general, concentrations of nitrate are not extremely adverse to RAS water quality. Nitrogen is essentially conserved throughout the nitrification process. Thus, if 1 kg per day of TAN is being produced, then 1 kg of nitrate-N is being produced. The equilibrium concentration of nitrate will therefore be directly dependent upon the overall water exchange rate throughout the system. Nitrate-nitrogen is

relatively nontoxic to freshwater fish and as such will not influence the controlling flow rates in the system. One can choose some value such as 200 mg/L, if you want a number to work with. More recent unpublished information seems to indicate that for various salmonids, the nitrate-N levels should not exceed 40 to 50 mg/L. Design information is very limited relative to this water quality parameter, especially for saltwater systems (Ebeling and Timmons, 2012).

6. FACTORS AFFECTING NITRIFICATION PROCESS IN RAS.

Parameters important to bioreactor performance such as pH, temperature, alkalinity, and concentrations of dissolved oxygen, turbulence and organic matter.

6.1. pH

The effect of pH on the nitrification rate for biofilters has been researched for more than sixty years, yet there is a wide range in reported pH optima. This suggests that the history and condition under which the bacteria are cultured may affect their response to pH. The literature suggests that the optimum range of pH for nitrification can range from 7.0 to 9.0. The optimum pH for Nitrosomonas ranges from 7.2 to 7.8 and from 7.2 to 8.2 for Nitrobacter. Nitrifying biofilters have been operated over a much broader range from 6 to 9, due to the adaptation of the bacteria in a filter to actual operating conditions. It is probably a good idea to maintain pH near the lower end of the optimum pH for the nitrifying bacteria to minimize ammonia stress on the cultivated fish species. In addition, rapid changes in pH of more than 0.5 to l.0 units over a short time span will stress the filter and require time for adaptation to the new environmental conditions (Timmons and Ebeling, 2010).

6.2. TEMPERATURE

Temperature plays a significant role in the nitrification reaction rate in suspended growth systems as it does in all chemical and biological kinetic reactions, although limited research is available to quantify the effects of temperature on fixed film nitrification rates. The impact of temperature on the nitrification rate for fixed film nitrification was less than predicted by the van't Hoff-Arrhenius equation. More specifically, in the case of no oxygen limitation, temperatures from 14 to 27 °C had no significant impact on the nitrification rate of a fixed film bioreactor. Although originally assumed to be an important factor in biofilter design, temperature is increasingly being viewed as a minor factor in controlling biofiller carrying capacities. Other researchers have determined a small but significant impact of temperature on nitrification rates. For example, the nitrification rates at l7 °C would only be 77 % of the rates obtained at 27 °C, or a 23 % reduction in rate. This is less severe than the 50 % reduction that would be predicted

based upon Q-l 0 (Arrhenius relationship) effect for a 10 °C drop in water temperature. There is a wide range of optimum temperatures reported for nitrification, suggesting that nitrifying bacteria are able to adapt to a wide range of environmental temperature, if acclimated slowly. In practical application. however, the temperature at which the biofilter operates is normally determined by the requirements of the species being cultured, not by the needs of the biofilter bacteria (Timmons and Ebeling, 2010).

6.3. ALKALLIITY

Alkalinity is a measure of the buffering capacity of an aquatic system. From the relationships above, it was determined that for every gram of ammonium-nitrogen reduced to nitrate-nitrogen, 7.05 grams of alkalinity is consumed. This loss of alkalinity is easily made up by the addition of sodium bicarbonate, referred to commonly as baking soda ($NaHCO_3$), or other bicarbonate supplement. A rule-of-thumb is 113 g (0.113 kg) of baking soda per pound of feed, 0.25 kg per 1 kg. Nitrification is an acid-forming process. and if the biofilter system's water is poorly buffered, the system pH will decline and affect the biofilter performance.

Timmons and Ebeling (2010) show that dramatically the impact of low alkalinity on nitrification. Ammonia in the form of ammonia chloride was added daily and in addition baking soda until Day 55 to maintain a constant alkalinity. The impact of not adding baking soda was a steady decline in alkalinity and a significant increase in ammonia-nitrogen (Day 62) when the alkalinity fell below 100 mg/L as $CaCO_3$. When the alkalinity was increased above 150 mg/L, nitrification resumed and ammonia-nitrogen quickly dropped to very low levels. Note that the alkalinity consumption value of 7.05 g alkalinity (or 7.14 g in absence of heterotrophic growth) per g of ammonium-N nitrified is not accounting for the alkalinity being added when fish secrete ammonia (NH_3) across their gills (paper on this subject under construction by authors) into the same water that has the nitrification system. Ammonia (NH_3) acts as a base when added to the water (can take on an H^+ ion). So, starting from the fish excreting NH_3 which then is protonated with a hydrogen ion in the water column (absorbing acid), the net alkalinity consumption is only half that when you start with ammonium (NH_4^+) or 3.57 g of alkalinity (one equivalent) per g of ammonia-N nitrified.

6.4. DISSOLVED OXYGEN

Oxygen can become the rate-limiting factor in certain biofilters because of the low levels in the influent and the competing demands of the heterotrophic bacteria. For every gram of ammonia-nitrogen oxidized to nitrate-nitrogen, 4.57 g of oxygen is required. Nitrification with a mixed culture reactor and reported that DO affected the growth rate of Nitrosomonas very little at DO levels above 2.0 mg/L, but Nitrobacter exhibited a reduced growth rate at DO levels of

less than 4 mg/L. Biofilter effluent levels of at least 2 mg/L of oxygen are probably adequate to maintain maximum nitrification rates (Timmons and Ebeling, 2010).

6.5. TURBULENCE

Turbulence affects the thickness of the stagnant water film covering the bacteria and thus the transfer rate of the nutrients from the bulk liquid into the biofilm. The ammonia removal rate is seen to increase as the flow rate through the bubble-wash bead filter increases. Currently there is limited information on the impact of turbulence and the design role it plays in improving the nitrification rate in biofilters. Excessive shear (high water velocity) or abrasion (sand particles) would be assumed to have a negative impact on biofilm growth and film thickness. (Timmons and Ebeling, 2010).

6.6. ORGANIC MATTER

Recirculating aquaculture systems by their very nature have significant quantities of both dissolved and particulate organic matter. This organic matter provides substrate for heterotrophic bacteria, which compete with the nitrifying bacteria for growing space. Heterotrophic bacteria have a maximum growth rate of five times and yields of two to three times that of autotrophic nitrifying bacteria. An exponential decrease in nitrification rate with the increase in the COD/N ratio in a laboratory study using a chemically fed reactor series system with a floating bead filter, a fluidized sand filter, and a submerged biocube filter (Timmons and Ebeling, 2010). The nitrification process was strongly inhibited by the heterotrophic processes when organic carbon was present. The addition of sucrose carbon with a carbon/nitrogen ratio of C/N=1.0 or 2.0 reduced TAN removal rate nearly 70% as compared with a pure nitrification process (C/N=0). The potential of heterotrophic inhibitory impact on nitrifiers decreased with the increase in organic carbon concentration when C/N above 1.0 (Zhu and Chen, 2001). The message of these studies is that organics, i.e. solids, need to be removed immediately from the recirculating aquaculture system (Timmons and Ebeling, 2010).

7. DENITRIFICATION

Biological denitrification is the microbial reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to nitrogen gas (N_2) by heterotrophic and autotrophic facultative aerobic bacteria and some fungi widely found in the environment. A relative broad range of bacteria can use either nitrate or oxygen to oxidize organic material, easily shifting between oxygen respiration and nitrogen respiration, depending upon the oxygen concentration. Denitrifiers are common among the heterotrophic, Gramnegative Proteobacteria, such as Pseudomonas, Alcaligenes, Paracoccus, and Thiobacillus. Some Gram-positive bacteria, including Bacillus, can denitrify. In addition to

heterotrophic microorganisms, several autotrophic microorganisms are also capable of denitrification. These organisms, which use reduced inorganic sulfur and iron compounds or hydrogen as their electron donor and derive carbon from an inorganic source, are often dominant in organic-poor environments where such donors are present (Timmons and Ebeling, 2010).

Denitrification proceeds in a stepwise manner in which nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and finally N_2 gas (Timmons and Ebeling, 2010).

Heterotrophic denitrifiers derive electrons and protons required for nitrate reduction to elemental nitrogen from organic carbon compounds. Such compounds include carbohydrates, organic alcohols, amino acids and fatty acids. For example, utilization of acetate as a carbon source for denitrification proceeds as follows:

$$5CH_3COO^- + 8NO_3^- + 3H^+ \rightarrow 10HCO_3^- + 4N_2(g) + 4H_2O \qquad (5)$$

The C/N ratio required for complete nitrate reduction to nitrogen gas by denitrifying bacteria depends on the nature of the carbon source and the bacterial species. For most readily available organic carbon sources, a $COD/NO_3^- - N$ (w/w) ratio from 3.0 to 6.0 enables complete nitrate reduction to elemental nitrogen, where COD stands for chemical oxidation demand and is expressed as MgO_2/L (van Rijn et al., 2006).

8. CONCUSIONS

The RAS is agreeable in the point of view of managing waste, water conservation, reduced land use, and limited impact on receiving water quality when compared to conventional pond culture. Effective control of diseases, solid waste and ammonia removal are environmentally acceptable as well.

The biological filteration (biofilter) is necessary to purify the water and remove toxic (ammonia) harmful waste products and uneaten feed. The procedure occurs on the biofilter through the process of nitrification.

Nitrification process and its management is of key importance to maintain water quality in recirculation aquaculture. The management of ammonia removal as a nitrification process can lead to reuse limited resource in many regions. There are various nitrogen species toxic to aquatic organisms at various concentrations. Nitrogen biofilter microbes carried out the processes. These microbial communities in RAS provides a healthy, stable life of farmed cultures.

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APPENDIX: GLOSSARY AND ABBREVIATIONS

AERATE	To expose to the air, or add air to a liquid.
AERATION	Introduction of air into water.
AEROBIC	Free oxygen is present in the environment.
AIR	What makes up our atmosphere. A mixture of nitrogen, oxygen and other gases. A mixture of gases around the earth: about 78% nitrogen, 21% oxygen, 0.9% argon, 0.03% carbon dioxide and traces of helium, krypton, neon and xenon, plus water vapour.
AMMONIA	A form of nitrogen found in water and may be toxic to fish and other stock under certain conditions.
ANAEROBIC	Free oxygen is absent from the environment.
AOA	Ammonia-oxidizing archaea
AOB	Ammonia oxidizing bacteria
AUTOTROPHIC	Organisms not requiring organic carbon in their diet. Able to grow on inorganic salts only. Usually refers to plants that utilize sunlight and carbon dioxide through the process of photosynthesis to produce organic nutrients.
BACTERIA	Single-celled micro-organisms that lack chlorophyll. Bacteria are important agents of decay and some species are responsible for human, animal and plant disease.
BIOLOGICAL FILTER	Part of a closed recirculating water system where dissolved metabolic by-products are converted to less toxic forms by microbial action. The most important function is the oxidation of ammonia to nitrite and nitrite to nitrate.
BOD	Biological oxygen demand
CARBON DIOXIDE	CO2, an atmospheric gas. It is used by plants to produce organic matter during photosynthesis and is released during combustion, respiration, or organic decomposition.
COD	Chemical oxygen demand
DISEASE	Unhealthy condition. A derivation from the normal state of an organism which may be inherited or caused by parasites, bacteria and other organisms, dietary deficiencies, or by

	physical and chemical factors in the environment.
DISSOLVED OXYGEN	DO- The amount of elemental oxygen (O_2) present in a solution. Sometimes represented as parts per million (ppm) and sometimes as percent of saturation level (%). Measured with electronic probes or through titration methods.
DRAIN	Partial – only part of the water is taken out of the culture container. Whole – culture container is completely drained.
EFFLUENT	Water or other liquids discharged from ponds, tanks, etc.
ENVIRONMENT	The total of all internal and external conditions that may affect an organism.
FILTERING	Removal of particles from the water column.
FLOW RATE	The volumetric movement of water past a given point in a unit of time.
FRESHWATER	Water with salinity below 3 ppt, generally able to be consumed by livestock.
FUNGUS OR FUNGI	Member of the class of primitive vegetable organisms including mushrooms, yeasts, rusts, moulds and smuts.
GILL	The respiratory and excretory organ which allows absorption of oxygen, water, certain mineral nutrients and other substances into the fish, mollusc or crustacean body and nitrogenous wastes, carbonaceous wastes, excess water, excess minerals and other excretory products to be released from the body. In bivalves it is also used to filter food from the water.
HARDNESS	Concentration of divalent ions (primarily calcium and magnesium) present in water.
HETEROTROPHIC	Incapable of manufacturing organic compounds from inorganic raw materials, therefore requiring organic nutrients from the environment.
H_2O	Water.
INTENSITY	1) Stocking density. 2) Level of inputs (extensive to intensive).
m^3	Measurement unit: cubic meter = 1000 L
MECHANICAL FILTRATION	Any filtering process which functions by separating out

	physical particles, as distinguished from chemical or biological filtration.
METABOLIC WASTE	By-products of metabolism excreted from the body of an organism.
MICROORGANISM	A microscopic plant or animal, especially bacteria, protozoans, and viruses.
NH_3	Ammonia – a gas, toxic to many aquatic animals when dissolved in water.
NH_3-N	unionized ammonia nitrogen (ammonia)
NH_4^+ - N	ionized ammonia nitrogen (ammonium)
NO_2^-- N	nitrite nitrogen
NO_3^-- N	nitrate nitrogen
NITROGENOUS PRODUCTS	Primarily consist of waste product ammonia, but may include ammonia breakdown products nitrite and nitrate, and other nitrogen based waste products such as urea and uric acid NO_3 Nitrate - a nutrient, can be toxic if in high concentrations. Results from bacterial breakdown of nitrite during nitrification. NO_2 Nitrite - can be highly toxic. Results from bacterial breakdown of ammonia during nitrification.
NOB	Nitrite-oxidizing bacteria
O_2	Oxygen
O_3	Ozone – highly unstable oxidiser used as a disinfecting agent to treat water for disease organisms and breakdown of organic compounds.
ORGANIC	Containing carbon and hydrogen, to do with living organisms.
OXIDATION	To combine with oxygen.
OXYGEN	O_2
OXYGENATION	In aquaculture: the input of pure oxygen into the culture medium to enhance or supplement its oxygen content; this promotes lower water exchange rates in the system.
ORP	Oxidation Reduction Potential
ppm	Parts per million, same as mg/L ppt parts per thousand.
pH	Expression of the acid-base relationship; defined as the

	negative logarithm of the reciprocal of the hydrogen-ion activity (<7 = acid, 7 = neutral, >7 = alkaline).
PRODUCTIVITY	1) Amount of food in area = number of stock that can be produced in an area. Similar to Carrying Capacity. 2) Amount of product grown in a specific area over a specific time.
RACEWAY	A long narrow pond where the water inlet and outlet are at opposite ends, giving a unidirectional flow.
RAS	Recirculating aquaculture system
RECIRCULATING	Being re-used. Usually refers to water moving through a system and after some form of treatment and then returned to the system.
RESPIRATION	1) The release of energy by oxidation of fuel molecules. 2) The taking in of O_2 and release of CO_2; breathing.
RISK	The probability of injury, disease or death for persons or groups of persons undertaking certain activities or exposed to hazardous substances. Risk is sometimes expressed in numeric terms (fractions) or qualitative terms (low, moderate or high).
SPECIES	Smallest unit of classification used, i.e. the group whose members have the greatest mutual resemblance and can produce viable offspring.
STOCKING DENSITY	The quantity (kg) of fish kept in a particular volume (m^3) of water.
STRESS	1) To subject an organism to physically disruptive forces that are harmful to that organism's growth and survival. 2) Any condition inimical to the health or growth of an organism.
SUBSTRATE	Material on which shellfish or other organisms can settle. Can be plants, shells, rocks, gravel, wood, metal or plastic. Sometimes used to represent the sea floor.
TAN	NH_4^+-N + NH_3-N
TURBIDITY	A cloudy condition of water, usually caused by particles such as clay, phytoplankton, bacteria or impurities, which limits the penetration of light. May result from wave action stirring up bottom sediments. Degree to which the penetration of light

	into the water is limited by the presence of suspended or dissolved material.
VIRUS	An infectious agent, containing either DNA or RNA as its genetic material, which requires a host for its replication.
WASTES	Byproducts produced as a result of a process. In aquaculture wastes consist of urine and faeces.
WATER COLUMN	The part of the water that is neither the surface nor the benthos but the area in between. Can refer to any body of water.
WATER FLOW	Movement of water in a particular direction, as a result of pumping or gravity.