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Innovative circular processes for bivalve shells valorisation

Martiña Ferreira, Diego Méndez & Leticia Regueiro
ANFACO-CECOPESCA

June 2021

Hello and welcome again to this unit coordinated by Anfaco, and produced by Martiña Ferreira, Diego Méndez and Leticia Regueiro.

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Part 5: Innovative processes studied in the GAIN Project (B) The use of shells in recirculated aquaculture system biofilters

Innovative circular processes for bivalve shells valorisation

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In this part we introduce the second of the two innovative uses for bivalve shells that were investigated as part of the GAIN project, which is their use as a substrate in aquaculture biofilters.

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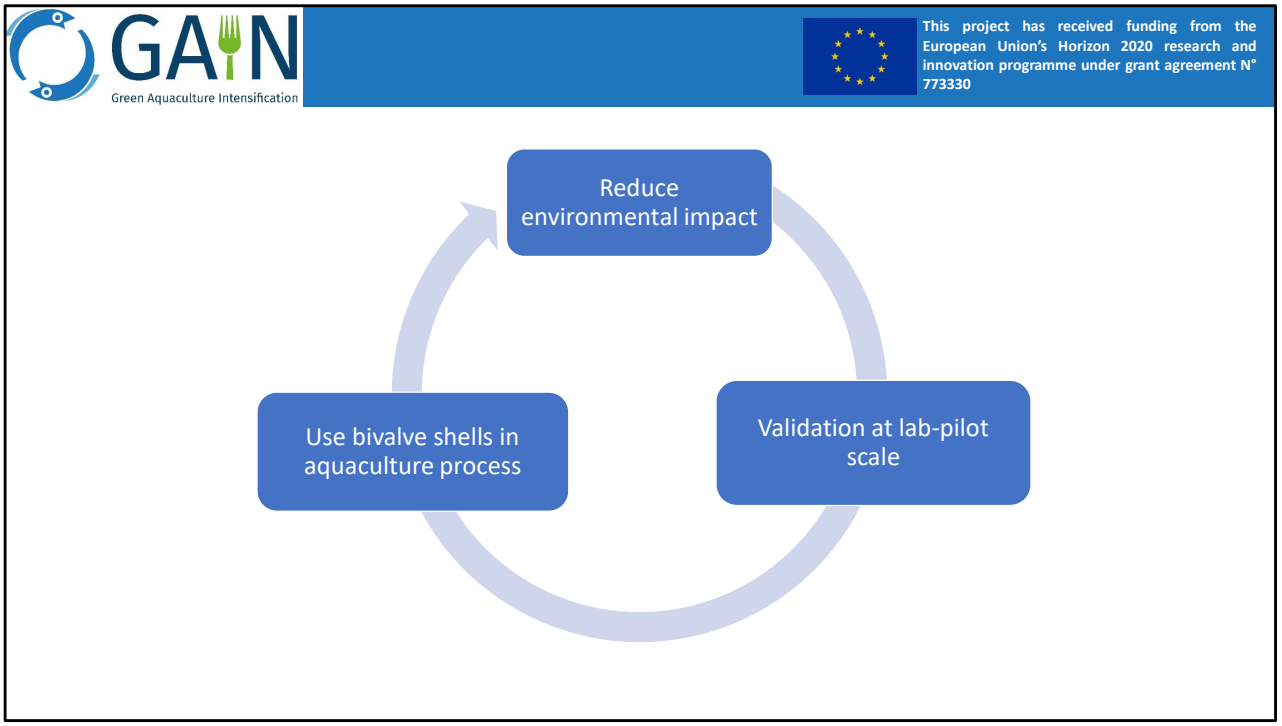

The use of bivalve shells in recirculating aquaculture biofilters has two potential advantages. Firstly as a cost-effective means of reducing the environmental impact caused by plastic biofilter material, and secondly providing enhanced functionality to the biofilter to also remove phosphorus.
Laboratory and pilot scale trials were carried out under the Gain project to assess the performance of bivalve shells for ammonia and nitrite removal, and also as a sorbent material to take up phosphorus released from fish excretions and uneaten feed.

Current practice in recirculation systems (RAS) focuses on using a biofilter to decrease ammonia and nitrite concentrations in recirculating water, whilst nitrate and phosphorus are partially removed through water exchange. The GAIN project investigated the use of bivalve shells to decrease the concentrations of both nitrogen and phosphorus, thus reducing the nutrient load of RAS effluents and the need for water renewal. In contrast with previous studies which combine bacterial nitrification and nitrate uptake by vegetables in aquaponic systems, in these trials the shells were used as the only element enabling nitrogen and phosphorus removal.

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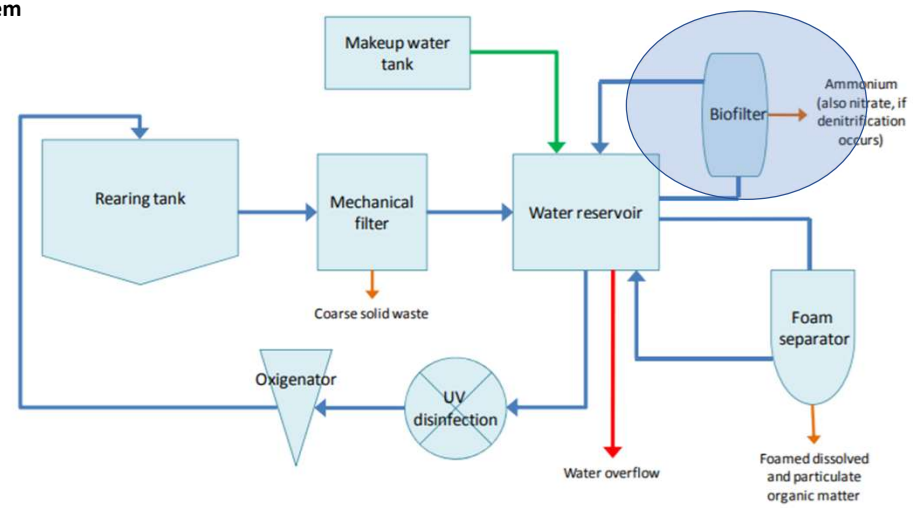
RAS system



Recirculated aquaculture systems (RAS) allow the intensive rearing of marine and freshwater fish with a minimum exchange of water, since wastewater passes through treatment processes which remove solid and dissolved residues and restore water quality. This solution not only reduces the pressure on natural water resources, but also the volume of waste generated. One of the key points in a RAS design, shown in this figure, is associated with the biological filtration system, also known as biofilter.

Ammonia accumulates from the excretion of the fish and the decay of uneaten feed. It is extremely toxic to most aquatic animals, having deleterious effects for fish at concentrations as low as 1 milligram per liter. Biofilters or biological filters are the specific part of the RAS where ammonia is removed from water due to the physiological activity of a consortium of different types of bacteria. A biofilter usually consists of a bioreactor container (e.g. tank) filled with one of a variety of different substrates, also known as biofilter medium. The function of this medium is to provide as great a surface area as possible exposed to the water upon which the bacterial consortium can grow. This results in the surface of the substrate being coated with a biofilm. Plastic rings or beads are the most usual materials to fill biofilters, but plastic has a relatively high environmental impact.

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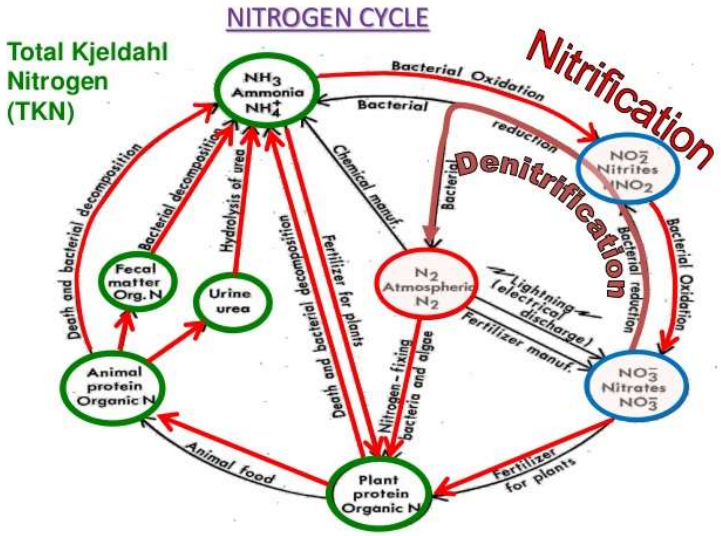
Nitrogen cycle



This diagram shows the nitrogen cycle and particularly the processes of nitrification and denitrification which are particularly relevant for aquaculture biofilters.

The steps in the nitrogen cycle, which are not altogether sequential, fall into the following classifications: nitrogen fixation by plants, bacteria and algae; nitrogen assimilation as organic nitrogen in animal protein; ammonification by death and bacterial decomposition or through feces or urine; nitrification from ammonia to nitrite and nitrate; and finally, denitrification to reach again the atmospheric nitrogen.

Two of them are important in biofilters: Nitrification that involves the conversion of reduced nitrogen compounds into oxidized forms, and Denitrification that involves the conversion of oxidized nitrogen compounds into reduced form.

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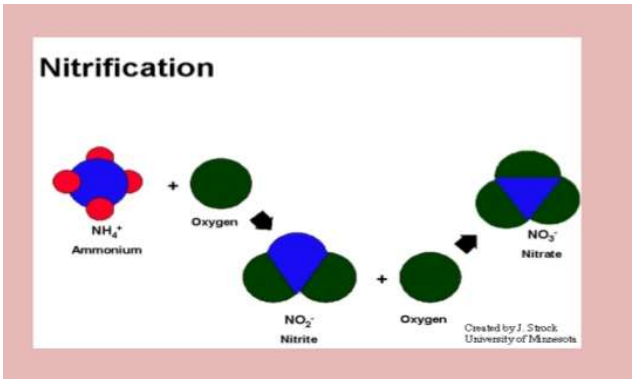
Nitrification process

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2\,H^+$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^-$$



Nitrification is the main process for ammonia removal in biofilters. Nitrification is an aerobic process which involves the simultaneous action of two bacteria communities: ammonia oxidizing bacteria, called AOB, which oxidize ammonia to nitrite, according to the mass balance of equation 1, and nitrite oxidizing bacteria ,called NOB, which oxidize nitrite to nitrate, according to Equation 2.

As you can see in both equations, oxygen is required in ammonium and nitrite oxidation; since ammonia-oxidizing and nitrite-oxidizing bacteria are aerobes.

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Two steps of nitrification process

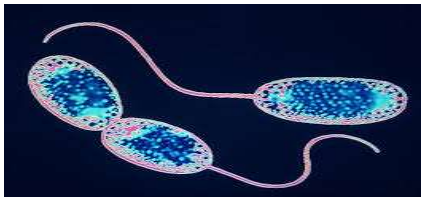
Nitrification of Ammonia Occurs in Two Steps

NH_3-N Ammonia N → NO_2-N Nitrite N

Nitrosomonas

NO_2-N Nitrite N → NO_3-N Nitrate N

Nitrobacter



Therefore, according to the previous slide, the nitrification process is a two-step process going from ammonium to nitrate.

The ammonium oxidizing bacteria can be found among the β-proteobacteria and Gamma proteobacteria classes, but they belong mainly to *Nitrosomonas* genus. The second step is carried out by the nitrite oxidizing bacteria, represented mainly by bacteria of the genus *Nitrobacter* as shown on the slide, but also from *Nitrospira*.

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First step of nitrification: AOB

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2\,H^+$$

-AOB were classified by cell morphology into the five different genera: *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio* and *Nitrosolobus*

-Recently, on the basis of 16S rRNA sequence homology, *Nitrosospira, Nitrosovibrio* and *Nitrosolobus* were proposed to be combined into one common genus *Nitrosospira*

-With the exception of *Nitrosococcus*, all genera represent closely related organisms of the β subclass of Proteobacteria

The transformation of ammonia to nitrite is usually the rate limiting step of nitrification. Biochemically, ammonium oxidation occurs by the stepwise oxidation of ammonium to hydroxylamine by the enzyme ammonium monooxygenase in the cytoplasm, followed by the oxidation of hydroxylamine to nitrite by the enzyme hydroxylamine oxidoreductase in the periplasm. Electron and proton cycling are very complex, but as a net result only one proton is translocated across the membrane per molecule of ammonium oxidized.

Nitrosomonas is the most frequently identified genus associated to this step, although other genera including *Nitrosococcus* or *Nitrospira* can do this task. In fact, traditionally, AOB were classified by cell morphology into the five different genera: *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio* and *Nitrosolobus*. Recently, on the basis of 16S rRNA sequence homology, *Nitrosospira, Nitrosovibrio* and *Nitrosolobus* were proposed to be combined into one common genus *Nitrosospira*. With the exception of *Nitrosococcus*, all genera represent closely related organisms of the beta subclass of Proteobacteria.

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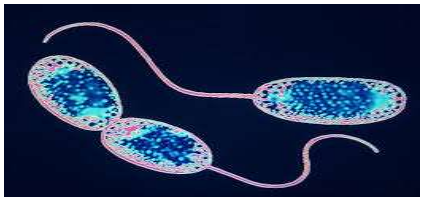
Second step of nitrification: NOB

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$$

Nitrite-oxidizers mainly comprise six bacterial genera: *Nitrobacter, Nitrospira, Nitrotoga, Nitrococcus, Nitrospina*, and *Nitrolancetus*, which are affiliated with *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*, and *Deltaproteobacteria*, as well as the phyla *Chloroflexi* and *Nitrospirae.*

However, only *Nitrobacter*- and *Nitrospira*-like NOB are believed to play important functional roles in terrestrial ecosystems.

Nitrobacter-like NOB are *r*-strategists, which prefer high substrate concentrations and have lower substrate affinity, while *Nitrospira*-like NOB are *K*-strategists with affinity for lower nitrite and oxygen concentration



Nitrite-Oxidizing Bacteria Community Composition and Diversity Are Influenced by Fertilizer Regimes, but Are Independent of the Soil Aggregate in Acidic Subtropical Red Soil

Shun Han[1], Xiang Li[1], Xuesong Luo[1,2], Shilin Wen[1], Wenli Chen[1,*] and Qiaoyun Huang[1,2*]

Nitrite reduction is much simpler, with nitrite being oxidized by the enzyme nitrite oxidoreductase coupled to proton translocation by a very short electron transport chain, again leading to very low growth rates for these organisms. Oxygen is required in ammonium and nitrite oxidation, meaning that both nitrosifying and nitrite-oxidizing bacteria are aerobes. As in sulfur and iron oxidation, NADH for carbon dioxide fixation using the Calvin cycle is generated by reverse electron flow, thereby placing a further metabolic burden on an already energy-poor process.

Nitrite-oxidizers mainly comprise six bacterial genera: *Nitrobacter, Nitrospira, Nitrotoga, Nitrococcus, Nitrospina*, and *Nitrolancetus*, which are affiliated with *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*, and *Deltaproteobacteria*, as well as the phyla *Chloroflexi* and *Nitrospirae*. However, only *Nitrobacter*- and *Nitrospira*-like NOB are believed to play important functional roles in terrestrial ecosystems. *Nitrobacter*-like NOB are *r*-strategists, which prefer high substrate concentrations and have lower substrate affinity, while *Nitrospira*-like NOB are *K*-strategists with affinity for lower nitrite and oxygen concentration.

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Nitrification

-Nitrification is extremely energetically poor leading to very slow growth rates for both types of organisms.

- Nitrifying bacteria are slower growing than the heterotrophic bacteria.

- Therefore, it is necessary to provide suitable environmental conditions for the nitrification process

Nitrification is extremely energetically poor, leading to very slow growth rates for both types of organisms.
One of the main requirements for nitrification to occur, therefore, is that the process should be controlled so that the net rate of accumulation of biomass is less than the growth rate of the nitrifying bacteria, since the latter ones are slower growing that the heterotrophic bacteria.

Therefore, it is necessary to have suitable environmental conditions for the nitrification process.

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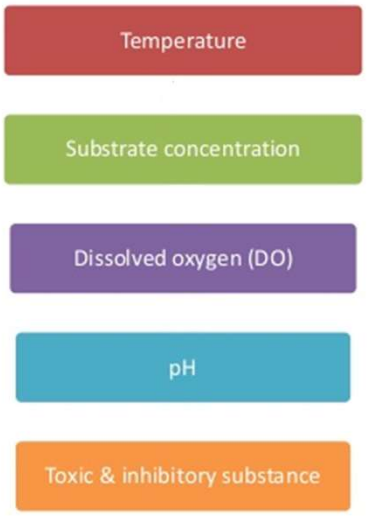
Nitrification

Main factors affecting the nitrification process



On this slide it is possible to see the main factors affecting the nitrification process: temperature, substrate concentration, dissolved oxygen, pH and the presence of toxic and inhibitory substances for the nitrification bacteria.
Other factors such as alkalinity, hydraulic retention time in the reactor, and nutrients (related to substrate concentration) are important.

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Nitrification

Temperature	Optimum temperature is between 20 to 30ºC but at least 8ºC is required
Substrate concentration	The ratio BOD/NTK is a primary determinant of the degree of nitrification that can be expected.
Dissolved oxygen (DO)	Nitrification requires 4.33 mg/l of oxygen per mg/l of NH_4^+-N.
pH	Nitrifiers prefer a tighter pH range, typically 6.8 – 8.2.
Toxic & inhibitory substance	Thiourea, cyanide, phenol, anilines, and heavy metals (silver, copper, nickel, chromium, mercury, and zin

Regarding temperature, the nitrifying bacteria are mesophilic, with optimum temperature being around 30 ºC. Below this, the rate of nitrification rapidly decreases until it stops completely below 8 ºC. Although nitrification can be achieved at elevated temperatures as high as 43ºC, the rate of ammonia removal is inhibited.
Regarding substrate concentration, without sufficient residual ammonia, nitrification cannot be supported. It is somewhat counter-intuitive, but some systems lose nitrification when the influent ammonia drops below a given amount. Since the first path of ammonia removal from the wastewater is via nutrient uptake by heterotrophic bacteria, the ratio of carbon-based material (BOD) to Total Kjeldahl Nitrogen (TKN) is a primary determinant of the degree of nitrification that can be expected. Also, as the BOD/TKN ratio increases, the fraction of nitrifying organisms decreases.
Going to dissolved oxygen , is necessary to consider that Nitrification is an oxidative process and both Nitrobacter and Nitrosomonas are strict aerobes. Nitrification requires 4.33 milligram per liter of oxygen per milligram liter of ammoimum. Dissolved oxygen residuals in the aeration tank of a nitrifying system must be maintained at residual DO levels of 1.0 – 4.0 mg/l to ensure adequate oxygen availability.

Nitrification is a very pH dependent process. Whereas carbonaceous bacteria function quite well throughout the range of 6.0 – 9.0, nitrifiers prefer a much tighter pH range, typically 6.8 – 8.2.

Regarding toxic substances, the nitrifying bacteria are much more susceptible to toxicity and inhibition than heterotrophic bacteria. Both *Nitrosomonas* and *Nitrobacter* are inhibited by unionized ammonia, which is present at elevated pH values. Since *Nitrosomonas* are more sensitive than *Nitrobacter*, the result may be a high level of nitrite in the final effluent. There are many other compounds that can exert inhibition on nitrifiers, such as thiourea, cyanide, phenol, anilines, and heavy metals (silver, copper, nickel, chromium, mercury, and zinc.

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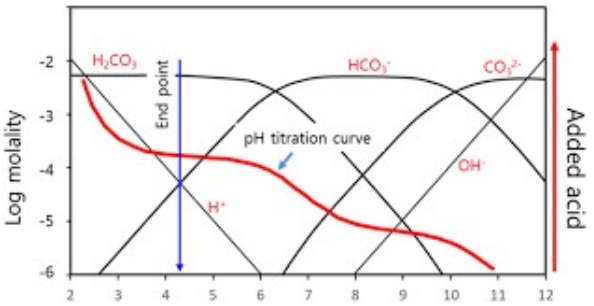
Nitrification

Hydraulic retention time: The time required for nitrification is directly proportional to the amount of nitrifiers present. Because the rate of oxidation of ammonia is essentially linear, short-circuiting must be prevented. For wastewater treatment, the minimum aeration basin retention time is around 4 hours at 22 ° to 24 °C, although in practice, values of between 5 to 30 hours are used.

Alkalinity: In addition to the pH requirement, nitrification requires that attention is paid to the alkalinity available. Each mg/l of ammonia that is oxidized (converted to nitrate) requires 7.15 mg/l alkalinity. Typically, systems are controlled to a residual alkalinity of 50 – 100 mg/l alkalinity, as CaCO3.

$$\text{Hydraulic Retention Time (HRT)} = \frac{\text{Volume of Aeration Tank (V)}}{\text{Influent flowrate (Q)}}$$



Another factor that is really important in the nitrification process is the hydraulic retention time; that it is the average retention time of the wastewater in the reactor. The volume of the tank divided by the influent flowrate is the hydraulic retention time.

The time required for nitrification is directly proportional to the amount of nitrifiers present. Because the rate of oxidation of ammonia is essentially linear, short-circuiting must be prevented. For wastewater treatment, the minimum aeration basin retention time is around 4 hours at 22 ° to 24 °C, although in practice, values of between 5 to 30 hours are used.

Finally, another important factor in nitrification is the alkalinity. Nitrification requires that attention is paid to the alkalinity available. Each milligram per liter of ammonia that is oxidized (that is, converted to nitrate) requires 7.15 milligram per liter of alkalinity. Typically, systems are controlled to a residual alkalinity of 50 – 100 milligram per liter alkalinity, as calcium carbonate, and this is an important point since as we said in previous slides, the shells are around 95% calcium carbonate.

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Denitrification process

Denitrification is the **process** that converts nitrate to nitrogen gas, thus removing bioavailable nitrogen and returning it to the atmosphere. Dinitrogen gas is the ultimate end product of **denitrification**, but other intermediate gaseous forms of nitrogen exist

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$

Denitrifying bacteria are a diverse group of bacteria that encompass many different phyla.

Denitrifying bacteria have been identified in over 50 genera with over 125 different species and are estimated to represent 10-15% of bacteria population in water, soil and sediment. Denitrifiers include for example several species of *Pseudomonas, Alkaligenes , Bacillus* and others.

Denitrification is not considered in normal RAS systems since nitrate is not toxic for fish until high concentrations.

As a consequence of ammonia oxidation after the nitrification step in a RAS system, nitrate tends to accumulate in the recirculated water. Although aquaculture organisms can resist high nitrate concentrations, excessive concentrations are controlled by daily water replacement up to 40% of the total RAS treated volume. Nevertheless this solution is not sustainable from the point of view of water consumption and provides only limited nitrate mitigation capability. Furthermore, water replacement means that nitrate is eventually disposed of into the environment. In typical wastewater treatment systems, nitrification is usually followed by a denitrification step, in which nitrate is reduced to gaseous nitrogen.
Denitrifying bacteria are a diverse group of bacteria that encompass many different phyla.
Denitrifying bacteria have been identified in over 50 genera with over 125 different species and are estimated to represent 10-15% of bacteria population in water, soil and sediment. Denitrifiers include for example, several species of *Pseudomonas, Alkaligenes , Bacillus* and others.

In wastewater treatment plants the use of anaerobic denitrification to remove nitrate is a common process. However, in commercial RAS this is not yet widely applied due to its complexity and low efficiency. Since denitrification is mostly heterotrophic and

occurs in the absence of oxygen, the aquaculture setup must provide a compartment with an anoxic environment, plus a source of organic matter, which may be external or endogenous, where denitrifiers may thrive and transform nitrate into elemental nitrogen. High oxygen concentrations may inhibit denitrification and lead to an excessive, aerobic consumption of the organic matter provided. Thus, two separate reactors are needed for nitrification and denitrification. Nevertheless, the advantages of integrating a denitrification step within the biofiltration process of a RAS should be carefully considered, as removal of nitrate from effluents and the subsequent reduction in water renewal requirements may lead to a decrease in the overall operational costs. Moreover, there can be beneficial effects of improved water quality on fish health and welfare which may enhance productivity.

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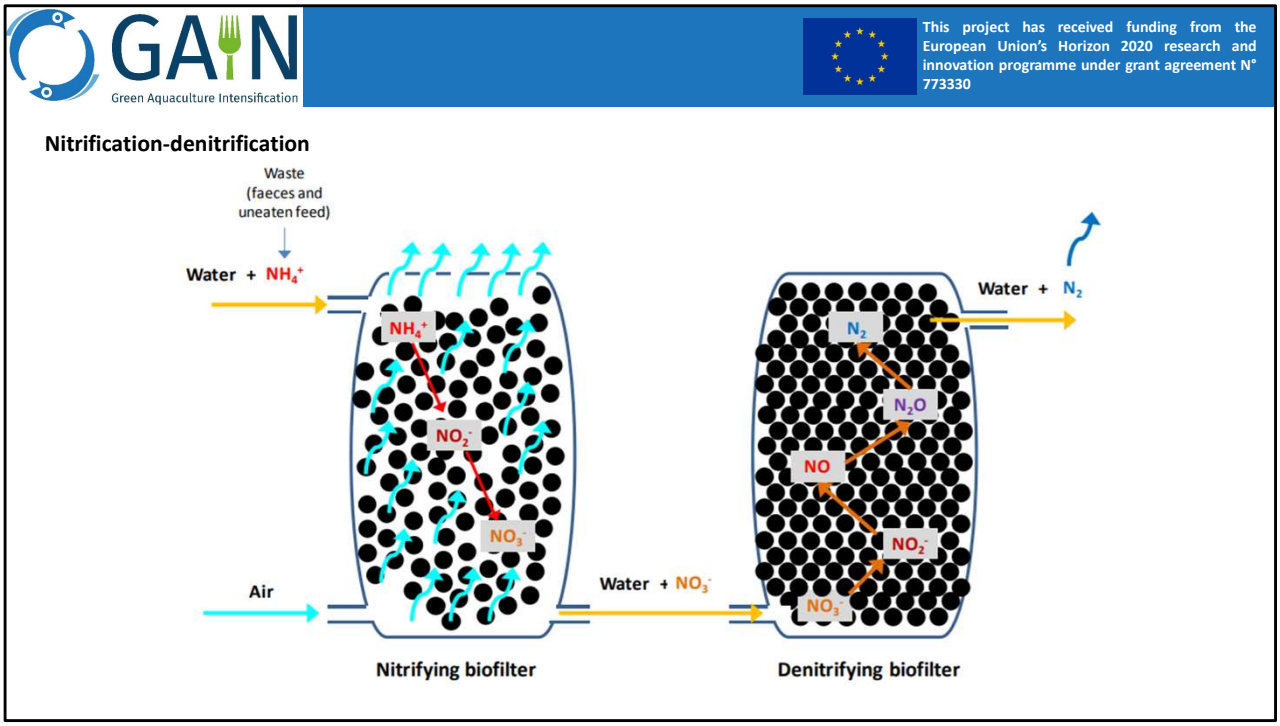

Having reviewed the processes that take place in nitrifying and denitrifying biofilters, we can now consider the design and arrangements of the reactors themselves.

Here you can see a schematic configuration of a nitrification-denitrification system.

Due to their different oxygen requirements, nitrification and denitrification reactors have different configurations. Common nitrifying biofilters are configured as fluidized bed reactors, where the particles that serve as substrate for bacterial growth are kept suspended in the water column by air injection, which also supplies oxygen. In contrast, denitrification biofilters are configured as non-aerated, packed bed reactors, in order to provide the required anoxic conditions

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Biofilter with plastic rings

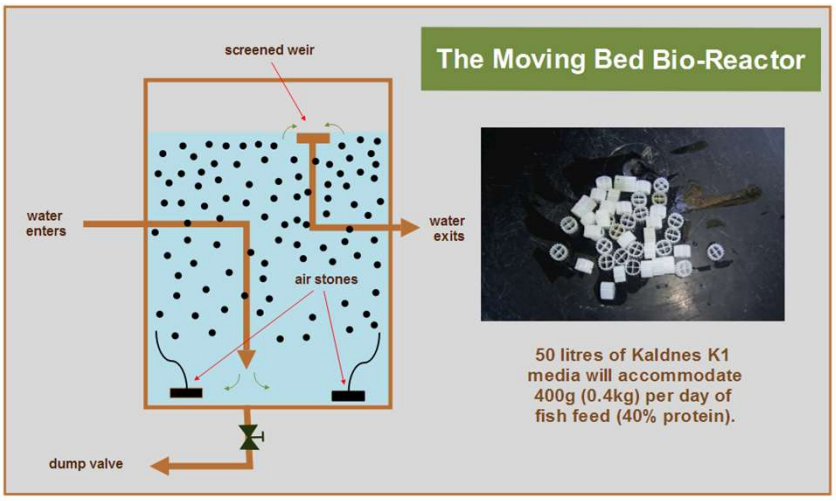

Image from: https://garydonaldson.net/2017/11/understanding-filtration/

Normally the reactors such as fixed bed or moving bed reactors contain Kaldnes plastic media or similar, to support the bacterial growth.

AOB and NOB attach to the bed material to form biofilms.

AOB occupied the outside layers of the biofilm whereas NOB occupied the inside layer of the biofilm.

A high cell concentration is possible if the biomass is imobilized due to higher retention of solids.

To size a biofilter for use in an RAS, the primary concern for the designer is to provide enough biofilter capacity to control the total ammonia-nitrogen concentration in the culture tanks to a preset upper limit. Knowing this concentration is very important, as the removal rate of a biofilter is related to the concentration of ammonia-nitrogen available to the bacteria in the filter. The lower the limit of the TAN concentration selected by the designer, the lower the removal rate will be for a biofilter. The result will be the requirement of a large biofilter for a given application.
Also critical to the process of sizing a biofilter is specifying the maximum feed rate for

the system. The ammonia-nitrogen production rate can be estimated based on the rate of feed addition and the protein content of the feed used within the system. Therefore, previous assays with the water that are going to use are necessary to define the size of the biofilter.

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Possible benefits coming from use of shells

- Bivalve shells provide a natural, biodegradable alternative to a plastic material, thus avoiding the negative impacts associated with its manufacture and disposal. Shell valorisation transforms a residue into a resource; moreover, little processing would be required for the use of shells as biofilter packaging: manufacturing costs and impact are thus low.
- In RAS, the use of shells as substrate for the growth of nitrifying bacteria may help to control pH and alkalinity, which tend to decrease due to the respiration both of reared fish and biofilter bacteria. Calcium carbonate of shells would gradually dissolve, contributing to restore pH and alkalinity levels, and hence maintaining stable water quality, which is beneficial to the health and growth of fish.
- The implementation of denitrification biofilters and phosphate sorbent filters in RAS would produce a net removal of N and P from the water, which would be released to the atmosphere as N_2 and sequestered in the mineral matrix of the shells respectively.
- Lower nutrient contents in the outlet water may help to decrease the taxes linked to effluent discharges that are charged to aquaculture companies, as well as reducing the risk of eutrophication.

The possible benefits coming from the use of mussels as biofilter filling material are:
Firstly, the bivalve shells provide a natural, biodegradable alternative to a plastic material, thus avoiding the negative impacts associated with its manufacture and disposal. Shell valorisation transforms a residue into a resource; moreover, little processing would be required for the use of shells as biofilter packaging: manufacturing costs and impact are thus low.
Secondly, In RAS systems, the use of shells as substrate for the growth of nitrifying bacteria may help to control pH and alkalinity, which tend to decrease due to the respiration both of reared fish and biofilter bacteria. Calcium carbonate within the shells would gradually dissolve, contributing to restore pH and alkalinity levels, and hence to maintain stable water quality, which is beneficial to the health and growth of fish.
Moreover, the implementation of denitrification biofilters and phosphate sorbent filters in RAS would produce a net removal of Nitrogen and Phosphorous from the water, which would be released to the atmosphere as nitrogen and sequestered in the mineral matrix of the shells respectively.

Finally, the lower nutrient contents in the outlet water may help to decrease the taxes linked to effluent discharges that are paid by aquaculture companies, as well as reducing the risk of eutrophication.

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Possible economic benefits coming from use of shells

- In economic terms, bivalve shells are a material that in most cases could be obtained at no cost, transportation likely being the only expenditure. Prices of plastic bio balls for high-scale applications such as recirculating aquaculture are within the range 150-450 $/m^3, which makes shells a competitive product.
- The potential market within the EU, i.e. RAS facilities, is still small, but it is expected to grow in forthcoming years. In Spain and Portugal, only two companies have recirculating nursery and ongrowing facilities, for the rearing of Senegalese sole.
- In contrast, Denmark is the EU country with the highest implementation of recirculation; in 2014, 30 % of the Danish trout production was reared in RAS farms, and this value is continuing to grow (agri benchmark, 2017).
- RAS benefits in this country from the tight environmental laws regarding effluent discharges, which force farmers to intensify their processes. This regulatory framework is highly favourable to the implementation of measures that reduce the nutrient load in discharged water, such as the introduction of bivalve shell in biofilters and phosphate sorbent units.

Having established the possible benefits coming from the use of mussels as biofilter material we also paid attention to economic points.

Firstly, in economic terms, bivalve shells are a material that in most cases could be obtained at no cost; transportation likely being the only expenditure. Prices of plastic bio balls for high-scale applications such as recirculating aquaculture are within the range 150-450 dollars per cubic meter, which makes shells a competitive product.

Secondly, the potential market within the European Union, for instance in RAS facilities, is still small, but it is expected to grow in forthcoming years. In Spain and Portugal, only two companies have recirculating nursery and ongrowing facilities, for the rearing of Senegalese sole. But, in contrast, Denmark is the European country with the highest implementation of recirculation; since 2014, 30 % of Danish trout production was reared in RAS farms, and this proportion is continuing to grow.

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End of Part 5
Thank you for you attention

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This is the end of part 5. We hope you have found it interesting. When you are ready, you can proceed to Part 6.