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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 6: GAIN experiments on the use of shells in recirculated aquaculture system biofilters

Innovative circular processes for bivalve shells valorisation

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In this sixth and final part, we share the results of experiments carried out by the GAIN project on the use of shells in recirculated aquaculture system biofilters.

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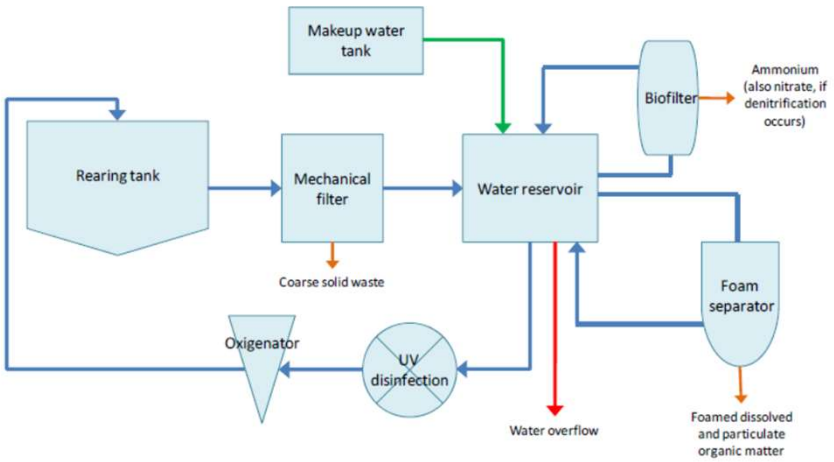
RAS



In a RAS, the biofilter is one of the key components of the recirculation process to treat the water of the fish tanks and to return clean water to them.

A series of treatment processes is utilized to maintain water quality in intensive fish farming operations. These steps are often done in order or sometimes in tandem. After leaving the vessel holding fish the water is first treated for solids in a mechanical filter before entering a biofilter to convert ammonia. Next, degassing, sterilization and oxygenation occur, often followed by heating/cooling. Each of these processes can be completed by using a variety of different methods and equipment, but regardless, all must take place to ensure a healthy environment that maximizes fish growth and health.

The biofilter is plays a key role in ammonium elimination, which is required due to its toxicity to fish.

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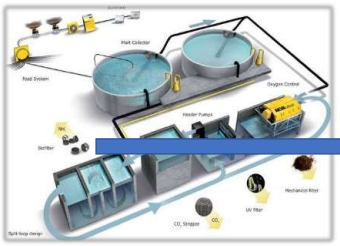
Reduce the use of plastics by using mussel shells as biofilter media in RAS systems

RECIRCULATORY AQUACULTURE SYSTEM



COMPARISON LAB SCALE

VS

USUAL

GAIN

Biological filters are used to eliminate ammonia and nitrite produced by the metabolism of fish, which in closed circuit conditions would accumulate until reaching concentrations harmful to the individuals. In this context, the GAIN project investigated the use of mussel shells (whole and crushed) as a filler material in lab-scale aerated biofilters, as a potential alternative to plastic balls.

The objective was two-fold; on the one hand to reduce the use of plastics in biofilters since the problem with plastic is known by everyone, and they should be eliminated as much as possible. On the other hand, to use a cheap material as filling media that achieves the objectives of the circular economy concept within the aquaculture sector, as is the case with mussel shells.

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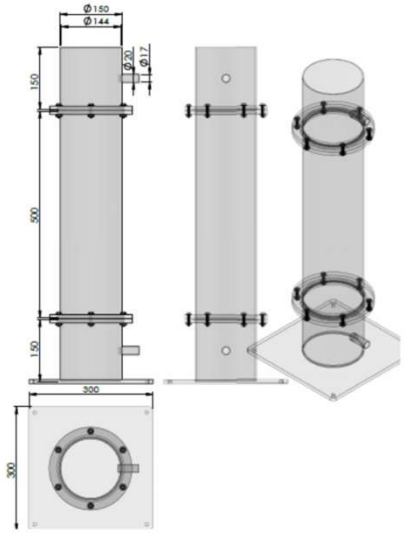
Biofilter design



In the GAIN project the first step was to design a biofilter to conduct assays at lab-scale with real aquaculture wastewater. As it was necessary to make a comparison between plastic and shells as filling material, at least two filters were required. But as in the Project we also wanted to evaluate the differences between crushed and entire shells, we decide to build 3 biofilters.

The structure of the biofilters, designed by ANFACO, is shown in this slide. Each methacrylate tank was 150 millimeters in diameter, 3 millimeters thick and 800 millimeters tall and transparent to allow one to look inside the reactor. Each biofilter has a bottom platform to guarantee the stability of the whole structure. The filter is divided into three parts, separated by a mesh which retains the packed media where bacteria grow. Wastewater from fish tanks is fed into the bottom compartment through an inlet pipe connected to the feeding pump; aeration can also be provided from this section to the whole biofilter. The intermediate part contains the filtering media (i.e. crushed or whole mussel shells or plastic rings) and the top compartment allows the filtered water to pass through an overflow outlet, located at the top of the biofilter of the same diameter as the inlet pipe. The top compartment can be easily removed and cleaned. The biofilter can operate in aerated or non-aerated conditions; therefore, it can be used for nitrification or denitrification, respectively. This prototype is inexpensive.

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Biofilter reactors

On this slide you can see two of the three biofilters that were built.
Following construction, hydraulic tests were conducted to verify robustness, flow speed, volume, etc.

Experiments were then carried out to assess the nitrification capacity of mussel shell-packed prototypes, compared to prototypes packed with plastic beads or other standard packaging material.

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Materials and methods

Three filters of 10 L volume were set up

- Different filler materials were used: plastic balls, crushed mussel shells, and whole mussel shells.
- Crushed shells would allow a greater specific surface area for the adherence of nitrifying bacteria
- Whole shells would be easier since they come directly from the industry and this would facilitate its handling if used in real plants.

In the Gain project, three filters of 10 L volume were set up, to conduct trials in accordance with the expected TRL (Technology Readiness Level) of 4, which is to have data at lab-scale. Each biofilter contained different filler material, plastic balls at an approximate amount of 0.7 kilograms, crushed mussel shells, at around1.5 kilogram and whole mussel shells, at around 1 kilogram of this material. The idea was to use either crushed or whole shells, since the use of crushed shells would allow a greater specific surface area for the adherence of nitrifying bacteria, i.e. theoretically it would be better and more similar to plastic filler considering this specific surface. However, the use of the whole shells would be easier, since they come directly from the industry and this would facilitate its handling if used in real plants.

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Materials and methods: lab-scale

Periods	N-NH_4	HRT	Inocula	Parameters
1 (50 days)	5 ppm	15-20 days	Prodibio	pH, Ammonium, nitrite, nitrate, disolved oxygen
2 (175 days)	5 ppm	1 day after day 120	Sludge +prodibio, Probiobio, Bio S Aquaforest	pH, Ammonium, nitrite, nitrate, disolved oxygen

At lab scale, two operational periods were studied to assess nitrification performance:

The first period lasted 50 days. Biofilter operation was started with an ammonium concentration of 5 ppm, and with an approximate Carbon to Nitrogen ratio of 2:1. In order to promote the growth of nitrifying bacteria, a vial of Biodigest Standard (Prodibio) was selected as inoculum composed of *Nitrosomonas* and *Nitrobacter* bacteria. Samples were taken twice per week from the outlet of each filter to measure ammonia, nitrite and nitrate using Nanocolor kits for photometric analysis, as well as API nitrite test and Marine test kits. Dissolved oxygen and pH were measured using digital meters (OxyGuard portable dissolved oxygen probe and XS instruments pH7+DHS; respectively) twice per month and twice per week respectively. Regarding temperature, the lab was maintained at a constant 17ºC.

The second period lasted for 175 days. In this period, the biofilters' operation was re-started after a after a period stopped due to a problem with the pumping systems caused by the accumulation of salts in the pumps. The COVID-19 situation delayed the arrival of new pumps. In this second period, different inocula were studied to see the effect on ammonia and nitrite elimination. The ammonia loading was also

increased to try to speed up the nitrification rates, since the amount fed in the first experiment was too low to be compared with a real scale plant. Also, the nitrite was not totally eliminated in the fist run.

Since oxidative reactions in nitrification are catalyzed by two groups of microorganisms called ammonium oxidants and nitrite oxidants, whose mechanisms are still not fully understood, we decided to study the influence of the inoculum in this second period, since it can be crucial to improve nitrogen elimination rates.

Firstly, the biofilters were inoculated with sewage sludge coming from the nitrification tank, as a low-cost bacterial source. At the same time, a Biodigest Standard (Prodibio) vial was added to increase the possibilities to establish a good biofilm sooner than in the first trial period. The proportions were 99:1 (Sludge:Prodibio). The biofilters were then fed with ammonium acetate, but a real start-up process was not observed. As the filters did not start eliminating ammonium, they were cleaned (the hypothesis being that a possible toxin, linked with the sewage sludge, was affecting the nitrification activity of the biofilm) and only the ProdiBio vials were added on days 40 and 58. After day 58, once ammonium elimination had started, the biofilters were fed every 2 days (Monday, Wednesday and Friday) with seawater with ammonium acetate to maintain an initial nitrogen concentration as ammonium of 5 ppm at the inflow, and a hydraulic retention time (HRT) of 1 day. Finally, on day 106 the biofilters were inoculated with a mixture of *Nitrobacter* and *Nitrospira* (Bio S, Aquaforest), since *Nitrobacter* alone (the nitrite oxidizing bacteria (NOB) in the Prodibio vials) did not work properly for nitrite elimination until this point. Samples for ammonia and nitrite were taken twice or three times per week for analysis and pH was measured on the same days in this second period.

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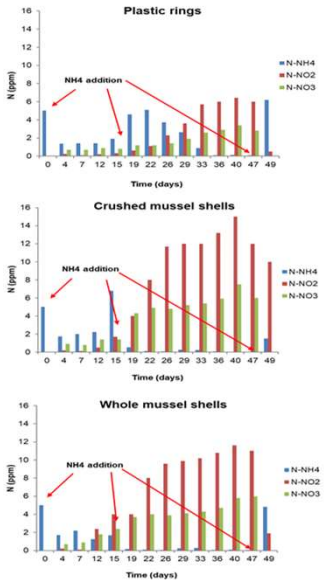
First period

During this period the biofilters presented similar behaviour regardless the filling material.

Start-up required almost 15 days.

After the second nutrient dosing, the biofilter filled with plastic material needed around 15 days more than mussel-shell biofilters to eliminate the ammonium. After this second dosing, the mussel shell filter showed a sharp decrease in the ammonia concentration and increase in nitrite (day 15 on), up to three times faster than in a biofilter packed with plastic balls.

After the third ammonium acetate addition, the biofilter packed with whole mussel shells performed apparently better than other reactors in the second nitrification step.



In this first study, our objective was to determine if shells can be used in similar equipment to plastic balls, and used as a future alternative filling material. During this period, the biofilters presented similar behaviour regardless the filling material, since the trends were similar with respect to ammonium and nitrite values as can be seen from the Figures on the right side.

The charts show that the start-up period required almost 15 days, which means that for all reactors, this was the period needed to show an ammonium decrease to close to zero.

After the second nutrient dosing, the biofilter filled with plastic material required around 15 days more than the mussel-shell biofilters to eliminate the ammonium. After this second dosing the mussel shells showed a sharp decrease in the ammonia concentration as it was converted to nitrite from day 15 onwards. This was up to three times faster than in a biofilter packed with plastic balls, which took from day 15 to day 29 to eliminate all the ammonium that was added. However, the nitrate and nitrite accumulation were lower in this plastic biofilter, maybe due some denitrification occurring in this biofilter.

After the third ammonium acetate addition, the biofilter packed with whole mussel shells apparently performed better than the other reactors in the second nitrification step, i.e. nitrite to nitrate conversion. However, this should be corroborated with further research since the biofilters were stopped and no more data were collected.

Throughout the trial, pH values ranged between 7.2 and 7.9. These values are in the range that guarantee proper bacterial performance in nitrification step in the three biofilters. Dissolved oxygen concentrations were also satisfactorily maintained at over 2 milligram per liter.

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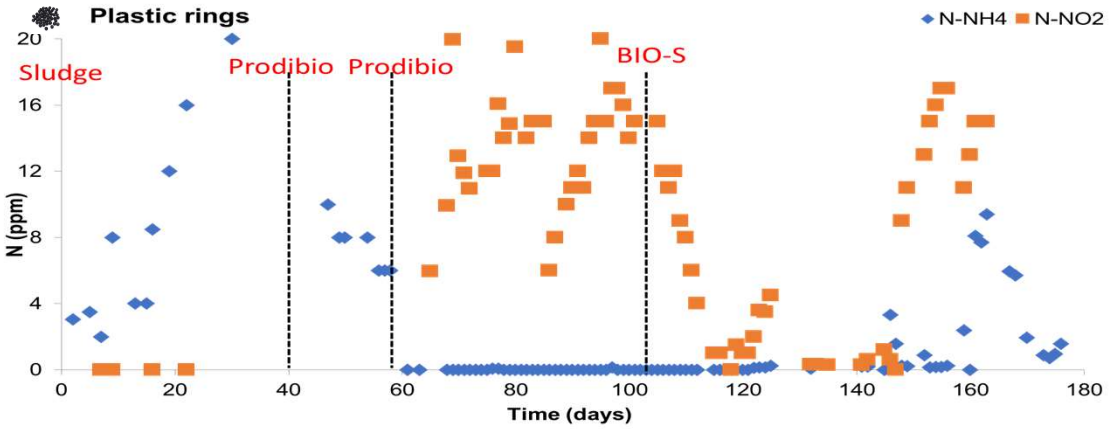
Second period



- Problems with inoculum for start-up (we use several inoculums: sludge, Prodibio, BIO-S)

This slide shows the data collected during the second operational period for the reactor filled with plastic rings.

In the second trial our objective was to validate previous data, with respect to the use of shells as filling material to reach similar nitrogen elimination rates compared to plastic filled system, but also to test different inoculums to reduce start-up period and develop a system that allows more continuous nutrient loading.

Following commencement of the trial we followed the ammonium and nitrite concentrations to determine which stages in nitrification were occurring. During the first phase of the trial no nitrite was detected. The biofilter reactors did not start-up properly with the inoculation of a mixture of sewage sludge coming from nitrification tank and Biodigest Standard (Prodibio) vial. Although this chart shows just the plastic ring filter, none of the filters showed elimination of ammonium. The sludge inoculum therefore seems inadequate for starting the filters, either because it was not very active at that moment or because bacteria did not thrive in salt water, although preliminary tests with freshwater did show a good start in another (BIOSHELL) project.

We decide to clean up the filters and add fresh seawater without ammonium acetate, then on days 40 and 58 adding 2 ProdiBio vials (1 per day and per reactor) to all biofilters again. After day 50 there was a decrease in ammonium concentrations, so we decided to resume addition of ammonium acetate every 2 days (Monday, Wednesday and Friday) so as to achive a nitrogen concentration as ammonium of 5 ppm at the inlet.

The results show a clear decrease in the ammonium concentration in all three biofilters after day 58 (here you see the results from the plastic ring filter) following the second Prodibio inoculation. It seems that *Nitrosomonas* were well established in all biofilters at that point, since the three biofilters presented similar behavior. Therefore, the first step of nitrification (ammonia to nitrite) was active, since we reached 100% elimination, regardless the filling material. However, the nitrite values remained high in all three biofilters. To solve this problem, a partial water replacement, up to 20%, was applied in the three biofilters from day 78 onwards, since until this moment no replacement of the water had been applied to help ensure a proper attachment of the biomass to the filter media. Apparently, this renovation caused an initial drop of nitrite concentration in the biofilters at around day 80-85, but nitrite concentrations recovered to previous values within 10 days.

As in the first experimental period, we observed a decrease in nitrite concentration on day 49 in the outlet of two reactors; that is plastic rings and whole shells. We decided not to use another inoculum to promote more nitrite oxidizing bacteria species since apparently the Prodibio inoculum could work. However, in this second period, we did not observe nitrite elimination after more than 20 days after water renovation.

We therefore decided to reinoculate the reactor on day 106 with a mixture of *Nitrobacter* and *Nitrospira* (using vials called Bio S, Aquaforest), since *Nitrobacter* alone, the nitrite oxidizing bacteria present in the Prodibio vials, cannot work properly with the simulated marine wastewater. We decided to use a mixture of nitrite oxidizing bacteria since in this case one of the two bacteria could be more resistant, or better able to adapt if both are competing for the same substrate under the trial operation conditions (salinity, temperature and nutrients etc). As we saw previously in the nitrification slides, both bacteria have different lifecycle strategies (k

or r) so ensuring both are present improves the likelihood that at least one will be successful.

Several studies showed that *Nitrospira* species have an optimum pH close to 8-8.1, similar to the pH found in the three reactors. These studies also showed that an increase from 5 to 15 grams per liter in sodium chloride concentration provoked an increase from 2 to 10% in the *Nitrospira* percentage. In our case the salinity of the system is close to 30 grams per liter, since we are working with seawater, therefore *Nitrospira* could work better.

After this inoculation, and after stopping the water renovation for 12 days until day 118, to avoid bacterial wash-out, the concentration of nitrite decreased almost to zero. This new inoculum seems to be much more effective in promoting nitrite elimination.

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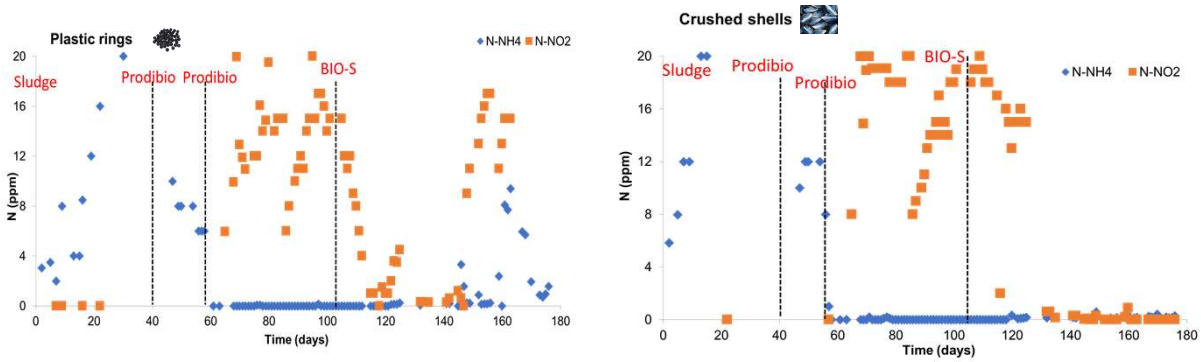
Second period



- After steady-state crushed shells had a better performance in terms of ammonium an nitrite elimination rates compared to plastic rings biofilter, mainly for pH shifts in the plastic reactor

Once the problems for nitrite elimination were solved, we wanted to compare the operation between plastic rings and shells.
On this slide you can see the comparison with crushed shells.

The biofilters continued to operate until day 175 and the elimination of ammonium and nitrite was clearly maintained in the crushed shell reactor. However, the plastic media biofilter experienced important oscillations in ammonium and nitrite concentrations, mainly from day 145 on, due to the high pH oscillations. Only with the addition of a buffer on day 155, 156 and 157, was it was possible to control the pH values, but at the end of the experiment the pH trend was still decreasing. Good pH control therefore appears a crucial point to guarantee nitrogen eliminations in plastic biofilters

These results show that both complete and crushed shells could be considered as a possible material to replace the traditional filling of plastic rings in bioreactors of RAS systems. In fact, the pH control without buffer requirements would be a clear advantage with respect to the commercial plastic filters.

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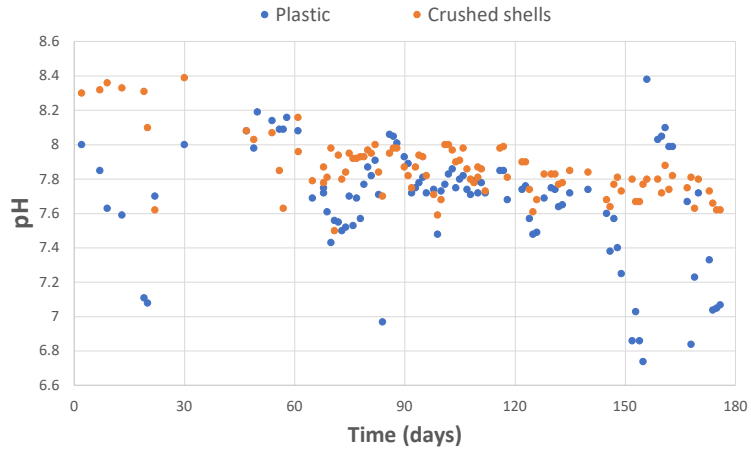
Second period



- Variation of pH was important (ranging from 6,7 to 8,4) in plastic rings biofilter. The addition of alkali was required across the operation period to control pH
- Mussels shell biofilters can maintain the pH without buffer requirements

On this slide, is posible to see the pH variation in both reactors.

The Variation of pH was important, ranging from 6,7 to 8,4, in the plastic rings biofilter. The addition of alkali was required across the operation period to control pH.

However, the mussels shell biofilter maintained the pH without buffer requirements, as was expected due to their calcium carbonate composition.

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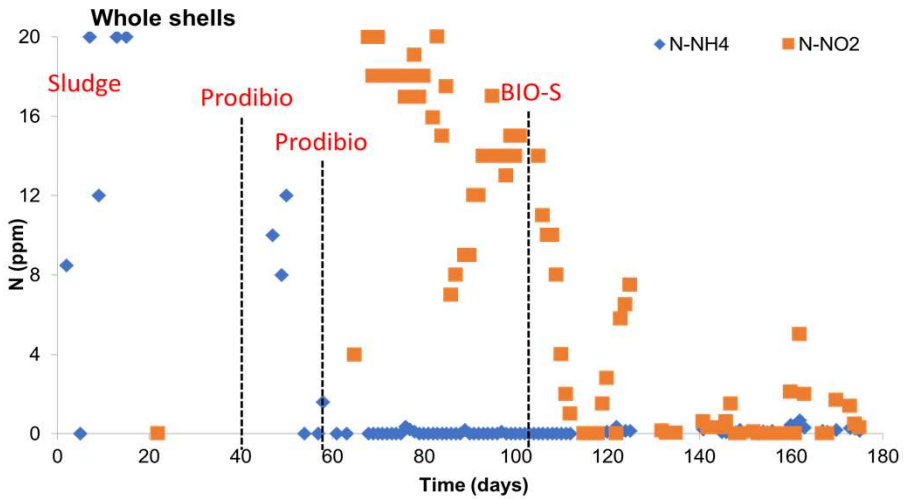

❑ Similar to crushed shells, but this reactor needed more time to reach zero nitrite after BIO-S reinoculation

The whole shell biofilter showed a similar performance profile to that of the crushed shell. However, the nitrite concentration did not reach "zero" values, probably due to a lower attached biomass than in the crushed shell filter, since it has lower specific surface area for attachment.

pH control appears a crucial point to guarantee the nitrogen eliminations in plastic biofilters whilst this whole mussel shell media did not need alkalinity additions.

Taking into account these results, it could be said that both complete and crushed shells could be considered as a possible material to replace the traditional filling of plastic rings in bioreactors of RAS systems. In fact, pH control without buffer requirements would be a clear advantage compared with commercial plastic filters.

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Materials and methods: pilot scale

Pilot-scale system installed in Grupo Tres Mares (Cee, Galicia)

5 ppm of ammonium concentration, and with a hydraulic retention time of 1 day

The initial idea was to study the possible phosphorous adsorption in the shells, but also some data coming from nitrogen elimination were obtained during this week.

ANFACO had installed in Grupo Tres Mares (Cee, Galicia) a company dedicated to the farming and processing of trout, a nitrification-denitrification pilot-scale system with a volume of 300 liters from another project (BIOSHELL). The system displayed in this slide consisted of two tanks, one for denitrification and the other one for nitrification working in recirculation - the best option to promote good nitrogen elimination, according to the results coming from the Spanish BIOSHELL project. ANFACO used the equipment for one week for the GAIN project in order to have some data at TRL 5 to provide data for techno-economic analysis and at the same time to have a preliminary study at pilot-scale. Unfortunately, we didn't have the possibility to operate the prototype for longer in the company.

The initial idea was to study the possible phosphorous adsorption in the shells, but also some data coming from nitrogen elimination were obtained during this week. This system was working in a steady state, using freshwater instead of seawater, and the system was using the water from the Tres Mares farm but with added ammonium acetate in the inlet to reach the desired 5 ppm of ammonium concentration, and with a hydraulic retention time of 1 day

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Materials and methods: pilot-scale

NITRIFICATION

DENITRIFICATION

	$N\text{-}NH_4^+$	$N\text{-}NO_2^-$	$N\text{-}NO_3^-$
Feed (ppm)	5	0	0
Effluent (ppm)	0	0	1,8
Removal Efficiency (%)	100	100	66

- High nitrification (100%) and denitrification yields (more than 60%)

Data from the pilot-scale operation showed high rates of nitrite, nitrate and ammonia removal as you can see in the Table. The pH value was maintained between 7.3 and 7.6 during this week, that it is the typical pH of the trout farm water, but is also the ideal pH for ammonium oxidizing bacteria and nitrite oxidizing bacteria. The temperature was around 12-15° C as maximum, reaching even lower values during the night. That was not ideal, but the system cannot be heated.

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Materials and methods: pilot-scale

NITRIFICATION

DENITRIFICATION

	PO_4
Feed (ppm)	0,12
Effluent (ppm)	0,06
Removal Efficiency (%)	50

- The shells also allow high yields of phosphorous adsorption close to 50%

During the trial period the phosphate concentration was also measured. Starting from an initial phosphate concentration of around 0.12 ppm the values were around 0.06 ppm after the 7-day period, meaning around 50% of phosphate was adsorbed in the shells.

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Materials and methods: phosphorous elimination

- Two of the three filters of 10 L volume were used to evaluate the phosphorus adsorption in the mussel shells.
- One of the filters was filled with crushed mussel shells, 1.0 kg, whereas the other one with the same quantity of calcite material.
- The filters were run for 30 days with concentrations of 10 ppm of phosphate. After that on day 30 we added 1 gram per litre of phosphate to evaluate the elimination at high concentrations over a 24 hour period.

Two of the three filters of 10 L volume used for the laboratory trials were repurposed to evaluate phosphorus adsorption in the mussel shells. One of the filters was filled with 1 kilogram of crushed mussel shells, and the other with the same quantity of calcite material. The initial idea was to compare calcined and non-calcined shells, but due to the difficulties to find a company to "calcinate" a low amount of material, we decided to compare the shells with calcite from a Galician mine, since the main feature of calcination is the promotion of the crystal structure of calcium carbonate; from the aragonite polymorph, with an orthorhombic crystal structure, to the calcite polymorph, with a trigonal-rhombohedral structure.

The filters were run for 30 days with concentrations of 10 ppm of phosphate, by dosing Sodium Phosphate in the inflow. After that on day 30, we made an addition of 1 gram per litre of phosphate to evaluate the elimination at high concentrations over a 24-hour period.

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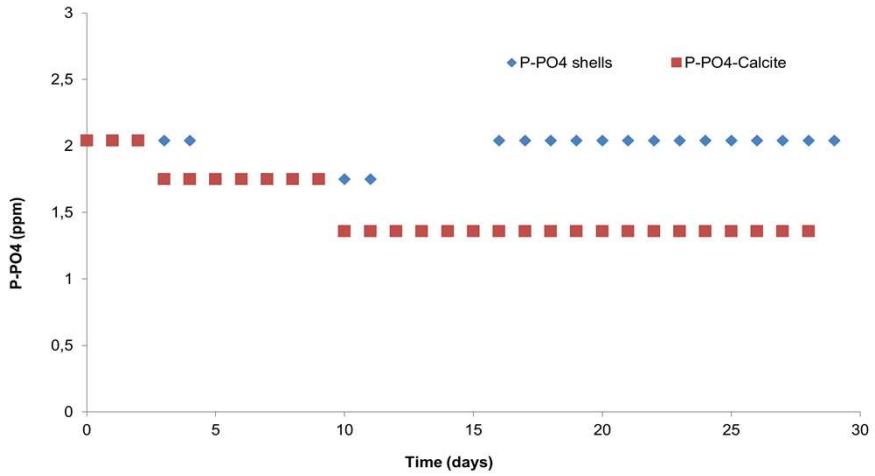
Results of phosphorous elimination



- Calcite material was better since it was able to adsorb around 86% of the phosphorus, whereas in the crushed shells this value was 79%.

The results of the phosphate concentrations in the biofilters were similar in both biofilters regardless the packing material. From day 15 onwards, the elimination rate was higher in the calcite material, since it was able to adsorb around 86% of the phosphorus, whereas in the crushed shells this value was 79%. This elimination rates are high according literature reports. However, the 10-ppm concentration, although aligned with concentrations expected in aquaculture systems, is quite low to evaluate the possibilities of the material as an adsorbent.

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Results of phosphorous elimination

	Time 0 h - $P\text{-}PO_4^-$	Time 1h - $P\text{-}PO_4^-$	Time 24 - $P\text{-}PO_4^-$
Mussel shells	1	843	677
Calcite	1	843	550

- Considering a 24 hours period the removal was around 32% in the crushed mussel shells and almost 45% in the calcite filled filter

For this reason, we did the assay with 1 gram per liter.

Results show that 1 hour after adding the sodium phosphate, the phosphate concentration was around 0.85 g/L, which means around a 15% of adsorption in the first hour. Moreover, after a 24 hours period, the removal was around 32% in the crushed mussel shells and almost 45% in the calcite filled filter. These data corroborate previous data that demonstrated that calcination of mussel shell increased the phosphate removal capacity, in one study from 25% to 55%, and in a second one from 40 to 90%, and in a third study from 10% to 60%, when using calcined shell instead the raw material.

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Conclusions of GAIN biofilter trials

The main conclusions are :

- Similar efficiency of shell filled systems (whole shells) compared to bioplastic fillers in ammonia and nitrite removal; reaching almost 100% working at lab-scale and treating simulated seawater.
- High removal efficiencies in nitrification (100%) and almost 70% in the denitrification step were reached using whole shells as biofilter packaging in pilot scale system.
- The selection of the inoculum seems to be important to promote both nitrification steps, i.e. both ammonia and nitrite removals.

The main conclusions of the trials are :

Firstly, it was shown that a similar efficiency of shell filled systems can be reached compared to bioplastic fillers in ammonia and nitrite removal; reaching almost 100% working at lab-scale and treating simulated seawater.

Secondly, high removal efficiencies in nitrification (100%) and almost 70% in the denitrification step were reached using whole shells as biofilter packing in a pilot scale system.

Thirdly, it is important to highlight that the selection of the inoculum seems to be important to promote both nitrification steps, i.e. both ammonia and nitrite removals.

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Considerations

Reagarding the possible use of the shells at high TRL, it would be neccessary to consider the following points for a future business plan:

- No replacement of shells was neccesary after 175 days operating the biofilters, since no excessive biofilm formation was observed. Therefore the shell waste for this use will be minimal in an aquaculture farm. For this reason the use of shells as a biofilter media can be extended to other wastewater treatment systems such as industrial or even municipal ones.
- Promotion of stricter regulations regarding effluent discharges (i.e zero ammoium and nitrite values) and water consumption for current aquaculture systems will be crucial for stimulating the development of these applications in a commercial way.
- The GAIN trials show that the use of plastic fillers in biofilters is not necessary for the correct elimination of nitrogen, clearly confirming the mussel shells as an alternative filling material. The future now involves evaluating the process over longer periods of time and on an industrial scale, to assess its economic viability and wider practical suitability.

Reagarding the possible use of the shells at a high Technology Readiness Level, it would be neccessary to consider the following points for a future business plan:

According to our experience, no replacement of shells was neccesary after 175 days operating the biofilters, since excessive biofilm formation was not observed. Therefore waste shell consumption for use in an aquaculture farm will be minimal, so will not impact greately on the total amount of shell waste generated (if you remember 35000 tonnes approximately). For this reason, the use of shells as biofilter media can be extended to other wastewater treatment systems such as industrial or even municipal ones.

Promoting stricter regulations regarding effluent discharges (i.e zero ammoium and nitrite values) and water consumption in current aquaculture systems could be crucial for stimulating the development of these applications in a commercial way. The promotion of circular economy policies would also help to promote their use or perhaps the banning of some plastic uses.

These trails have shown that the use of plastic fillers in biofilters is not necessary for the correct elimination of nitrogen, clearly confirming the mussel shells as a potential alternative filling material. The future now involves evaluating the process over longer periods of time and on an industrial scale, to assess its economic and practical viability.

GAIN
Green Aquaculture Intensification

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Thank you very much for you attention

If you would like more information or references on this subject, please contact us:
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Finally we reach the end of this Unit. We hope that you have found it interesting.
You can now attempt the quiz to check what you have learned.
Thank you very much for your attention