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GLOSSARY OF ACRONYMS

Acronym	Definition
HRT	Hydraulic Retention Time
NOB	Nitrite oxidizing bacteria
RAS	Recirculation Aquaculture Systems
TAN	Total ammonia nitrogen
TRL	Technology Readiness Level
XRF	X-ray fluorescence

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TABLE OF CONTENTS

The project has received funding from the European Union's Horizon 2020 Framework Research and Innovation Programme under GA n. 773330

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Executive summary

The Task 2.3 of the GAIN project deals with the valorisation of bivalve shells, as was stated in previous deliverable D2.4.Activities were focused on testing the different applications selected in GAIN project for shells valorisation, i.e.: the use of shells as substrate for conchocelis growth as part of the cultivation cycle of the red seaweeds *Porphyra* spp. and *Pyropia* spp., as filler for the cement industry, and their application as filling material for biofilters in recirculating aquaculture systems (RAS).

Preliminary data showed that mussel and cockle shells, which are by-products of EU bivalve aquaculture and canning industry, promote good conchocelis growth, are robust and allow a good handling of. These findings are mainly based on visual analysis and image analysis and further analysis with control techniques (image analysis, statistical data, etc.) should be used to see if the use of shells in this field can be interesting and economically relevant. Thus, both mussel and cockle shells are suitable alternatives to oyster shells traditionally used as substrate in the cultivation of *Pyropia* and *Porphyra*, which provides a new valorisation option.

The assessment of bivalve –mussel- shells as a partial substitute for limestone in cement production was another objective of GAIN and a matter of high interest for the Norwegian cement industry. Nevertheless, after the first analyses of this material as is described in this report, the collaborator cement manufacturer showed reticence to use it due to their high alkalinity. Thus, the use of mussel shell in cement production was unfortunately not investigated beyond preliminary characterization and company assessment.

Finally, the preliminary results at TRL 4-5 regarding shells as filling material for biofilters were promising, showing a possible alternative for this waste that could be scale-up in the future. The inoculum selection seems to be important to promote the nitrogen elimination, regardless the filling material applied. The shells also showed good results in terms of phosphorous adsorption. Given the potential advantages of shells over plastic rings as filling material for aquaculture biofilters, this valorisation route was deeply investigated, from the technical point of view in GAIN Task 2.3, and its environmental and economic sustainability was assessed, respectively in Task 4.1 and 4.3. The results of the Life Cycle Assessment, presented in detail in GAIN deliverable D4.4, suggests that environmental impact of shell as filler media is slightly lower, compared with that of plastic rings. This result, however, may be affected by the TRL of the system analysed. The economic analysis, presented in detail in deliverable 6.9, showed that mussel shells could be more cost-effective than plastic material for a period of 3 years in biofilter systems.

The results presented in this document also demonstrate the technical and economic viability of two valorisation routes for bivalve shells: *Porphyra*/*Pyropia* aquaculture and use in RAS as filling material for biofilters, being the latter more interesting a priori due to the potential demand of large quantities of shells.

The third valorisation option assessed, the use of shells in cement production, did not lead to the expected results according to Norwegian requirements for cement and concrete products. Nevertheless, shell aggregates could meet quality criteria in other countries and be used in this type of products; therefore, this research line could be continued.

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1.Introduction

At present, around 550 Mt per year of mussels are produced in the EU, of which around 45% comes from Galicia (Eurostat, 2018). The canning industry is very relevant in this region and annually produces around 35,000 tonnes of shells as by-products which, at present, are treated as waste and disposed via incineration or landfilling.

Regarding the innovative valorisation techniques proposed in D2.4, this deliverable is focused only on those possibilities that do not require high-energy processing, and that should be simple, sustainable and potentially economically viable, according to circular economy concepts. The processes studied are summarized below.

- **1) The use of discarded shells as packaging material in biofilters and as phosphorus sorbent in recirculating aquaculture systems (RAS).**
  As it was stated in deliverable 2.4, RAS systems have been developed to reduce nutrient contamination problems in aquaculture and, at the same time, to reduce water necessities of conventional terrestrial aquaculture facilities (Martins et al., 2010). Nitrifying biofilters are one of the components of most RAS systems and, since their performance is based in biological processes, this is one of the most critical components for an operational success (Badiola et al., 2012). Biofilters are applied to remove nitrogenous waste by-products, mainly coming from fish feed and fish faeces. Biofilters support the nitrification steps where the oxidation of ammonium and nitrite ions occurs in the bacterial film of the biofilter. This is crucial in RAS systems since ammonia and nitrite are toxic for the fishes, depending on pH and the aquatic organism studied (Randall and Tsui, 2002). Literature suggests to keep ammonium and nitrite levels below 1 ppm of ammonium and not exceeding 2 ppm of total nitrogen (Timmons and Ebeling, 2010). Also, the removal of phosphate from aquaculture wastewaters is important in the context of eutrophication in the receiving waters and for this reason the possible adsorption in the shells has been studied.

- **2) The use of shells in the production of seaweed seedlings.**
  Bivalve shells play an essential role in the life cycle of the rhodophyta belonging to the geni*Porphyra* and *Pyropia*, both of commercial importance. Several *Pyropia* species constitute raw material for the manufacturing of nori, and some *Porphyra* species are also used for human consumption. Their life cycle comprises two phases, a laminar gametophyte and a filamentous sporophyte that develops from spores that attach to bivalve shells to develop a filamentous thallus which penetrates the shell. Cultivation of *Pyropia* and *Porphyra* spp. requires the replication of its life cycle and thus the use of bivalve shells as a substrate for development of the conchocelis phase.

- **3) The potential use of shells as filler for the cement industry as was stated in**

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Deliverable 2.4 is one of the current possibilities for shells that could be evaluated.

The objective of this deliverable is to show the results concerning the abovementioned valorisation options. The following activities were carried out.

- The use of whole and crushed shells as filler material in nitrification biofilters was validated at lab-scale and the results were compared with those obtained with the same biofilter operating with plastic rings. Also, it was compared the use of shells as material for phosphorous sorption in biofilters with the use of a calcinated material, in this case mainly calcite coming from a mine (>95% of calcite in the limestone).

- Secondly, a complete characterization of the mussel shells for their integration in the cement industry was done. In this sense, SHP is collaborating with a Norwegian cement producer with an annual production capacity of 1.2 million tonnes, which is looking at ways for achieving a CO_2-neutral cement production. As a first stage, this company is screening suitable candidate fillers from the shellfish industry and evaluating their properties under a variety of processing conditions.

- Finally, shells from commercial production have been tested to evaluate their suitability as a growth substrate for conchocelis, the microscopic phase of *Pyropia*.

The following sections of this deliverable describe:

- Methodology applied in experimental test
- Results obtained in all valorisation possibilities
- Main conclusions and next steps

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2.Methodology and set-up

2.1 Biofilters for nitrification at lab-scale

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 sense ANFACO proposed the use of mussel shells (whole and crushed) to be tested as filler material in lab-scale aerated biofilters, as a potential alternative to plastic balls.

The biofilter prototypes used in this task were shown in deliverable 2.4 (Soula et al., D2.4 GAIN).

Figure 1. Lab-scale biofilters

Three filters of 10 L volume were set up, to reach the expected TRL of 4 that was established for the GAIN project, each of them containing different filler material (plastic rings. 0.7 kg, crushed mussel shells, 1.5 kg and whole mussel shells, around 1 kg). We used both crushed and whole shells, since the use of crushed shells would allow a greater specific surface for the adherence of nitrifying bacteria, i.e., theoretically it would be better and more similar to plastic filler considering that the grinding would increase the 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.

Biofilters were fed with seawater and ammonium acetate was added as a nitrogen source, until TAN concentrations typical of aquaculture wastewater were reached (around 5 ppm of $N\text{-}NH_4^+$). Other studies (Sharrer et al., 2007 and Tossavainen et al., 2019) found TAN concentrations around 2 to 6 ppm in RAS. Elimination of ammonia and nitrite was followed in the three reactors.

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The nitrification process was investigated in two stages.

1st stage (50 days): Biofilters operation started with an N-NH_4^+ concentration of 5 ppm, and with an approximate ratio C/N of 2. A vial of Biodigest Standard (Prodibio) was selected as inoculum composed by *Nitrosomonas* and *Nitrobacter* bacteria in order to promote the growth of nitrifying bacteria. Samples were taken twice per week from the outlet of each filter to measure ammonia, nitrate and nitrate using Nanocolor (Macherey-Nagel, Düren, Germany) 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. The temperature was controlled and kept at 17°C all the experiment since it is the lab constant temperature.

2nd stage (175 days): Biofilters operation was re-started as a problem with the pumping systems caused by the accumulation of salts in the pumps led to stop the first stage. The COVID-19 situation delayed the arrival of new pumps. In this second period different inocula were studied, in order to investigate the effect of the inoculum source (synthetic, sludge) in ammonia and nitrite elimination and trying to speed up the nitrification rates compared to the first period. 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 decide to study the influence of the inoculum source in this second period.

To do it, firstly, the biofilters were inoculated with Biodigest Standard ProdiBio vials, added on days 40 and 58. After day 58 onwards the biofilters were fed every 2 days (Monday, Wednesday and Friday) with seawater with ammonium acetate to maintain an initial nitrogen concentration as ammonium in 5 ppm in the feeding and a hydraulic retention time (HRT) of 1 day since it started the ammonium elimination. Finally, on day 106 the biofilters were inoculated with a mixture of *Nitrobacter* and *Nitrospira* (Bio S, Aquaforest), to introduce another nitrite oxidizing bacteria (NOB) since *Nitrobacter* alone seemed that did not work properly for nitrite elimination until this point. We decide to use a mixture of nitrite oxidizing bacteria (*Nitrospira* and *Nitrobacter*) since in this case one of the two NOB bacteria could be more resistant (one could be gradually adapted if both are competing for the same substrate) to this operation conditions (i.e. salinity conditions) and it can promote the nitrite elimination. According to Whang et al. (2017) and Zhang et al. (2018), *Nitrospira* species have an optimum pH close to 8-8.1, similar to the pH found in the three reactors (close to 8, as shows Figure 8 for the three bioreactors). Also, Wang et coworkers showed that an increase from 5 to 15 g/L in sodium chloride concentration provoked an increase from 2 to 10% in the *Nitrospira* percentage in a CANON system, and in our case the salinity of the system is close to 30 g/L, since we are working with seawater.

During this period also samples for ammonia and nitrite were taken twice or three times per week for analysis and pH were measured the same days in this second period.

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2.2 Biofilter for nitrification-denitrification at pilot-scale and assessment of shells as phosphorus sorbent

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 of 300L of useful volume from another project (Pleamar-BIOSHELL). The system displayed in Figure 2 consisted of two tanks, one for denitrification and the other one for nitrification working in recirculation, the best option to promote a good nitrogen elimination according to the results coming from the BIOSHELL Spanish project (http://www.anfaco.es/blog_ct/index.php/2020/01/31/filtros-biologicos-basados-en-cocha-de-mejillon-un-sistema-innovador-para-depuracion-de-aguas-piscicolas/). ANFACO used the equipment 1 week for GAIN purposes 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. The trial lasted 1 week. 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 steady state, using the outlet freshwater from Tres Mares ponds instead seawater, adding ammonium acetate in the feeding to reach the 5 ppm $N\text{-}NH_4^+$ concentration, and with a hydraulic retention time of 1 day. In this case whole shells were used as filling material.

Figure 2. Pilot plant for denitrification/nitrification

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2.3 Shells as substrate for conchocelis growth

This activity was carried out in cooperation between ANFACO and SaltenHavbrukspark (SHP). Cockle, oyster and blue mussel shells were inoculated with fertile *Porphyraumbilicalis* gametophyte, i.e. the conchocelis phase (Figure 3).

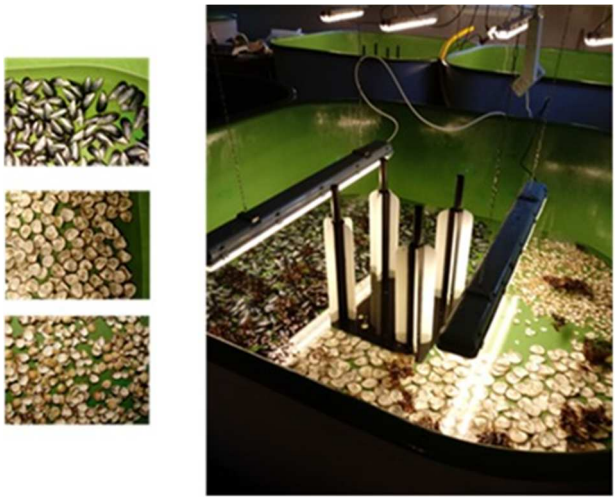

Figure 3. Shells used for *Porphyra umbilicalis* gametophyte inoculation

2.4 Shells analysis for their use in cement industry

Samples of mussel *Mytilus galloprovincialis* and cockle *Cerastodermaedule* were collected from a canning industry company located in Ribeira (Galicia) after cooking process (Annex 1), without any further processing. Shells from mussel and cockle were stored for two months at –18°C at ANFACO before sending to NORCEM HEIDELBERGCEMENT GROUP for chemical composition analysis. Chemical analysis using X-ray fluorescence (XRF) has been conducted to estimate the mineral composition in mussel and cockle shell.

2.5 Biofilters for phosphorus adsorption at lab-scale

Two of the three filters of 10 L volume described in deliverable 2.4 were used to evaluate the phosphorus adsorption by 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 as PO_4, using for that NaH_2PO_4 in the feeding. After that, on day 30 we added 1 g/L of phosphate to evaluate the elimination at high concentrations during 24 hours. The initial idea was to compare calcined and non-calcined shells, but due to the difficulties to find a company to “calcinate” low amount of materials, we decide to compare the shells with calcite from a Galician mine, since the only thing that shells experimented due to 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.

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3.Results and discussion

3.1 Shells as biofilter filler at lab-scale

3.1.1 First operation period

During this period the biofilters presented similar behaviour, regardless of the filling material, since the trends were similar in terms of ammonium and nitrite concentrations (Figure 4).

The chart on top of Figure 4 shows that the start-up period needed almost 15 days, it means in all reactors this was the necessary to period to reach almost cero values in ammonium concentration.

After the second feeding the biofilter filled with plastic material needed around 15 days more than mussel-shell biofilters to eliminate the ammonium. After this second feeding the mussel shells showed a sharp decrease in the ammonia concentration to nitrite (day 15 on), up to three times faster than in a biofilter packed with plastic balls (needed from day 15 to 29 to eliminate all the ammonium fed). However, the nitrate and nitrite accumulation were lower in this plastic biofilter, maybe due some denitrification was happening in this biofilter.

After the third ammonium acetate addition, the biofilter packed with whole mussel shells performed apparently better than other reactors in the second nitrification step (nitrite to nitrate conversion). However, this should be corroborated since the biofilters were stopped and no more data were collected. pH values were ranging between 7.2 to 7.9. These values are in the range that guarantee proper bacterial performance in nitrification step in the three biofilters. The same happened with dissolved oxygen that was all the time over 2 mg/L.

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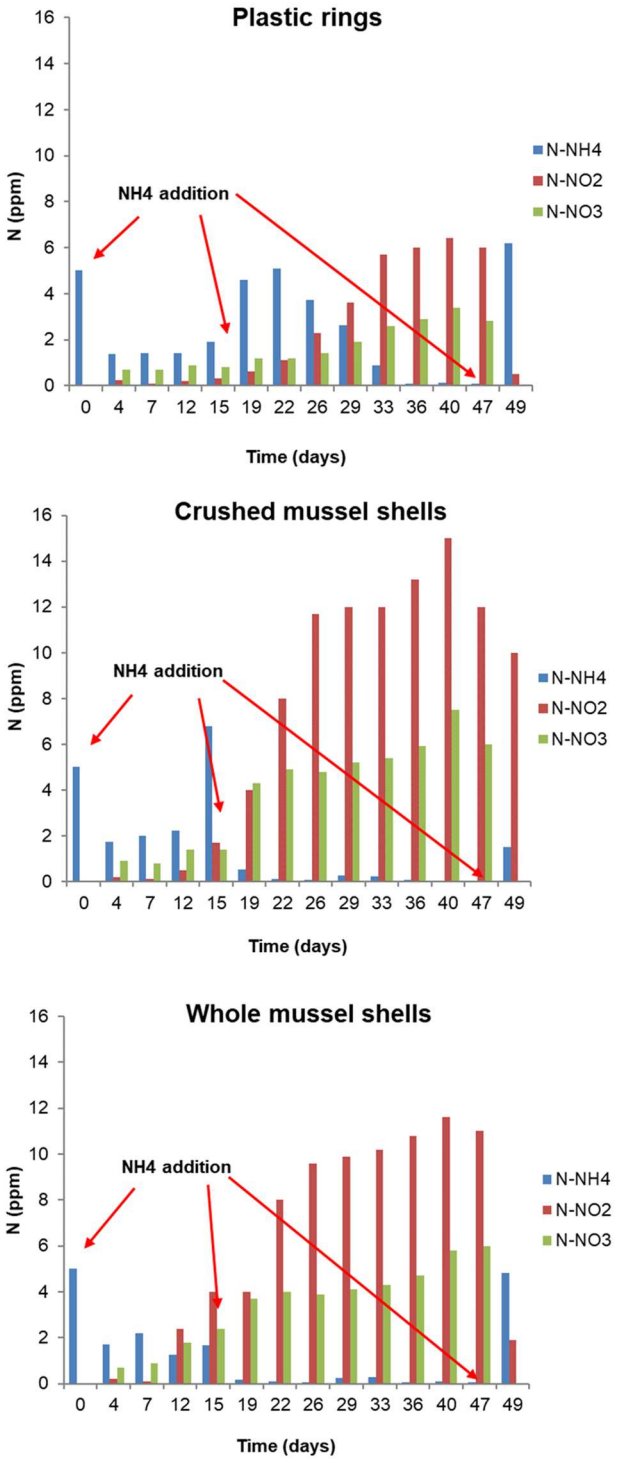

Figure 4. Changes in $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ over time in biofilters packaged with plastic balls, crushed mussel shell and whole mussel shell (first operation period).

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3.1.2 Second operation period

During this period, ammonium and nitrite were monitored, focusing on nitrite removal. The following figures (Figures 5, 6 and 7) show the evolution of ammonium and nitrite in the three biofilters, filled with plastic rings, crushed shells and whole shells. ProdiBio vials (1 per day and per reactor) were added to all biofilters again. This caused a decrease in ammonium concentration (Figures 5, 6 and 7, day 50 on) which reached zero values in the three bioreactors at day 60.

Data show that a clear decrease in the ammonium concentration in the three biofilters after day 58 (Figures 5, 6 and 7). It seems that *Nitrosomonas* were well established in all biofilters at that point, since they presented similar behaviour. Therefore, the first step of nitrification process (ammonia to nitrite) was completed (100% elimination), regardless of the filling material. However, the nitrite values remained high. To solve this problem, a partial water renovation (20%) was applied in the three biofilters from day 78 on, since until this moment no renovation to the water had been applied to ensure a proper biomass attachment to the fillers (plastic or shells). Apparently, this renovation caused an initial drop of nitrite concentration around day 80-85 in the three biofilters (Figures 5, 6 and 7), but nitrite concentrations recovered to previous values in the following 10days.

In the first experimental period a decrease in nitrite in the last point for two reactors (plastic rings and whole shells) was observed at day 49, as is shown in Figure 4. For this reason, it was decided not to use another inoculum promoting more NOB (nitrite oxidizing bacteria) species since apparently the Prodibio inoculum could work. However, in this second period, we did not observe the nitrite elimination after more than 20 days and this was the reason for trying a new inoculation with two NOB species. The reactor was reinoculated on day 106with a mixture of *Nitrobacter* and *Nitrospira*.

After this inoculation, and after stopping the water renovation during 12 days (until day 118 to avoid NOB washing out) the concentration of nitrite decreased almost to zero in whole shells and plastic rings biofilters. The crushed shells biofilter showed a slight decrease in the nitrite concentration but it did not reach “zero” values in this first days after reinoculation but also this bioreactor started from high nitrite concentrations in day 106 (Figure 6).

On day 118 the concentrations of nitrogen as nitrite and ammonia in plastic fill and whole shells, Figures 5 and 7, were zero, which means 100% elimination.

The biofilters continued to operate until day 175 and the elimination of ammonium and nitrite was clearly maintained in both mussels’ reactors. However, the plastic fill biofilter experienced important oscillations in the nitrogen concentrations (i.e ammonium and nitrite), mainly from day 145 on, due to the high pH oscillations. Only with the addition of buffer on day 155, 156 and 157, it was possible to control the pH values, but at the end of the experiment the pH trend was equally decreasing. Apparently, the pH control is a crucial point to guarantee the nitrogen eliminations in plastic biofilters

Taking into account these results, it could be said that both complete and crushed shells could

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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 respect to the commercial plastic filters.

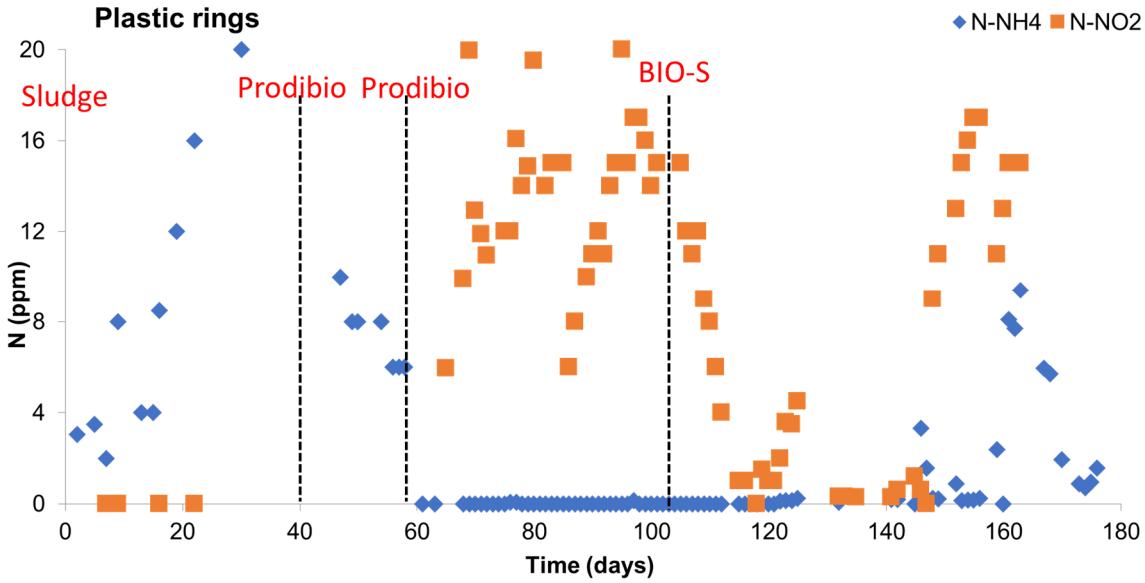

Figure 5. Changes in N-NH_4^+ and N-NO_2^- time in biofilters packaged with plastic balls (second operation period) (in red words the type of inoculum highlighting the inoculation moment).

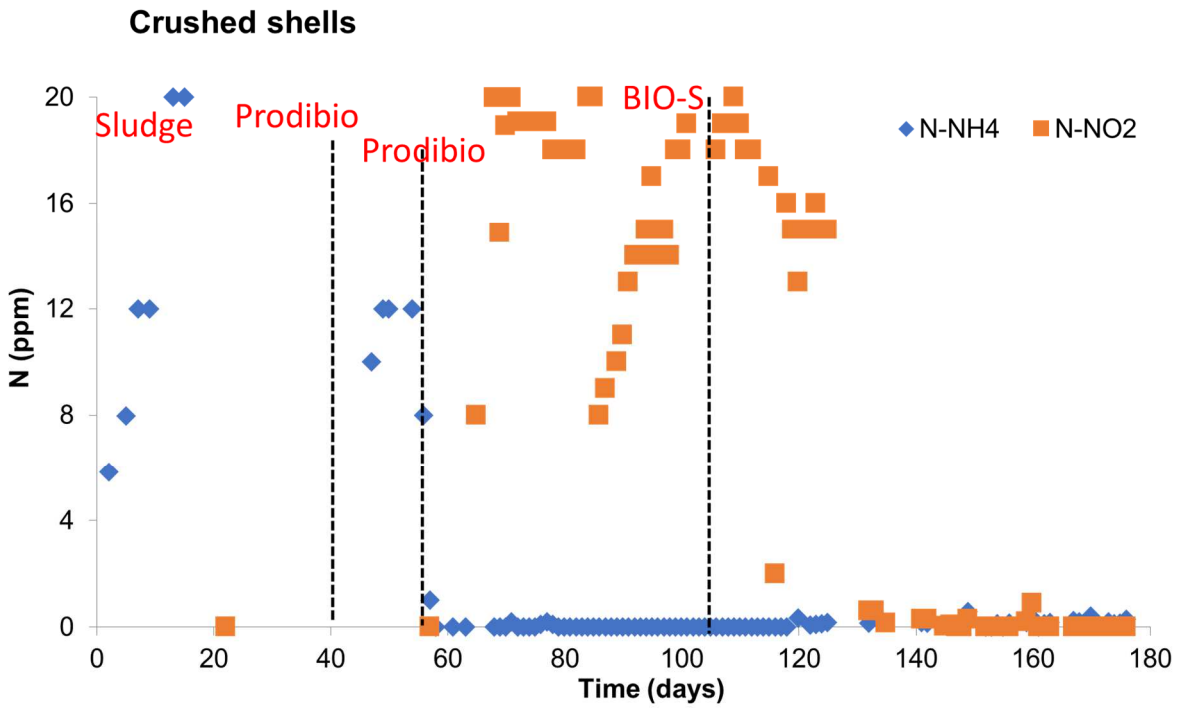

Figure 6. Changes in N-NH_4^+ and N-NO_2^- time in biofilters biofilter packaged with crushed mussel shell (second operation period). (In red words the type of inoculum highlighting the inoculation time).

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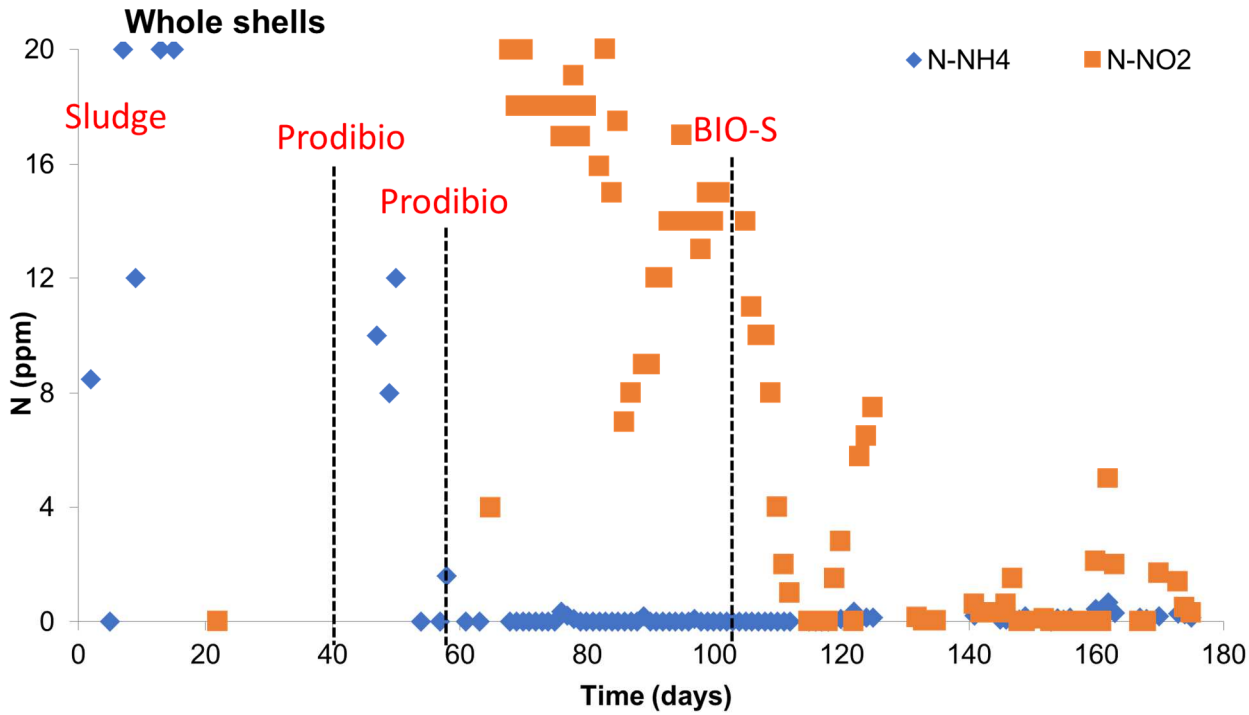

Figure 7. Changes in $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$ time in biofilter packaged with whole mussel shell (second operation period) (in red words the type of inoculum highlighting the inoculation moment).

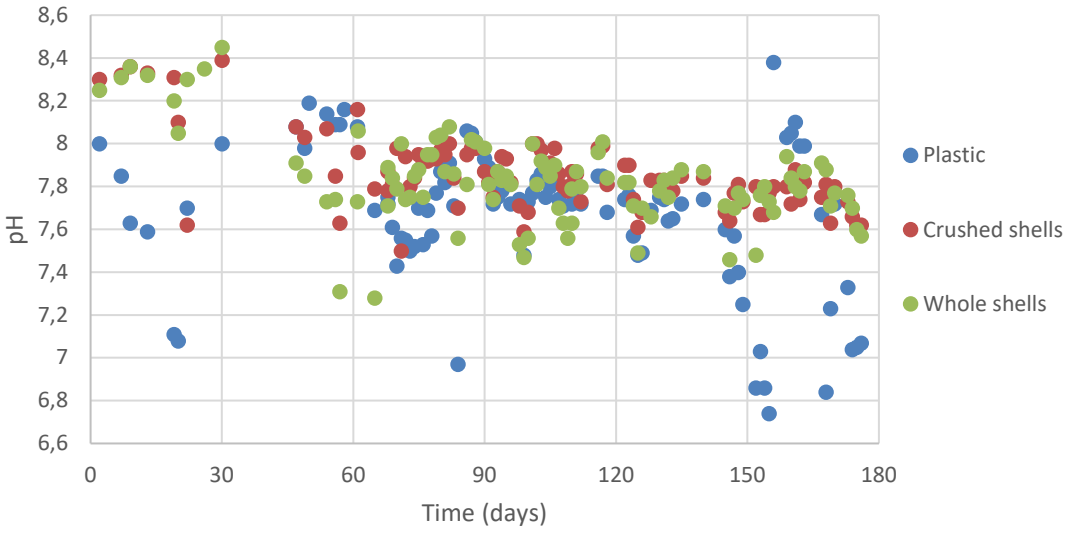

Figure 8. pH values during the second operation period in the three biofilters.

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3.2 Shells as biofilter filler at pilot-scale

Data coming from pilot-scale operation showed high yields of nitrite, nitrate and ammonia removals (Table 1). The pH value was maintained between 7.3 and 7.6 during this week, that it is the typical pH of the farming trout water but also the ideal pH for AOB and NOB Bacteria, and the temperature was around 12-15°C as maximum (reaching even low values during the night) that cannot be ideal but the system cannot be heated. At the same time during this period also the phosphate concentration was measured. Starting from an initial phosphate concentration of around 0.12 ppm the values were around 0.06 ppm after 7days period, meaning around 50% of phosphate adsorption in the shells.

Table 1. Ammonia, nitrite, nitrate and phosphate concentrations in the influent and effluent of the two-phase pilot scale reactor and removal efficiency obtained using whole shells as filling material

	$N\text{-}NH_4^+$	$N\text{-}NO_2^-$	$N\text{-}NO_3^-$	PO_4
Influent (ppm)	5	0	0	0,12
Effluent (ppm)	0	0	1,8	0,06
Removal Efficiency (%)	100	100	66	50

3.3 Shells as substrate for conchocelis

*Porphyra*conchocelis grew well in the tested shells. In the pictures below (Figures 9, 10 and 11) it can be recognized as red to purple colour. However, for practical handling, especially for the physical removal of contaminants (e.g. by a brush), blue shells are too fragile and cockles too small. Oyster shells show good conchocelis growth and exhibit a robustness and size that support handling in manual processes, as e.g. sorting or cleaning by a brush.

Figure 9. Oyster shells with conchocelis, visible as red to purple patches.

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Figure 10. Blue shells with conchocelis, visible as red to purple patches

Figure 11. Cockle shells with conchocelis, visible as red to purple patches

3.4 Shells for their use in cement industry

Chemical analysis using XRF has been conducted to estimate the mineral composition in mussel and cockle shell (Table 2).

Results observed in both species are in concordance with observed in other studies with high level of calcium upper than 98% of total dry weight (Awang-Hazmi et al. 2007, Ballester et al. 2007, Mohamed et al., 2012). In general, results of diffractograms of mollusk shells demonstrate that in all of them the minerals present are calcite and aragonite, two polymorphic varieties of calcium carbonate.

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Table 2. Chemical characterisation of mussel and cockle shells.

Chemical Parameters	***Mussel***	***Cockel***
Water dried-105°C	5,31%	5,13%
LossOnIgnition (LOI)	46,11%	45,05%
Water soluble Chloride (Cl)	0,40%	0,21%
SulfurTrioxide-IR (SO3)	0,15%	0,55%
XRF Analysis		
Silica Oxide (SiO2)	0,00%	0,34%
Aluminum Oxide (Al2O3)	0,00%	0,00%
Ferric Oxide (Fe2O3)	0,00%	0,01%
Calsium Oxide (CaO)	99,30%	98,45%
Potassium Oxide (K2O)	0,00%	0,01%
Sodium Oxide (Na2O)	1,67%	2,40%
Magnesium Oxide (MgO)	0,38%	0,18%
TitaniumDioxide (TiO2)	0,00%	0,00%
PhosphorousPentoxide (P2O5)	0,06%	0,04%
Manganic Oxide (Mn2O3)	0,00%	0,00%
Chloride (Cl)	0,53%	0,70%
Chromium Oxide (Cr2O3)	0,00%	0,00%
Zinc Oxide (ZnO)	0,00%	0,00%
Strontium Oxide	0,00%	0,00%
Sodium Oxide Equivalent (Na2O)	1,67%	2,41%

Although, the composition is quite similar between the two species and published studies, we observe a high ratio of chloride in shell composition. This ratio could be related to the presence of sodium oxide which represents the third compound more representative in the sample composition. These results coincide with those published by several authors for mussels, oysters and cockles (Adegoke and Adewuyi, 2008, Kelly 2009, Martinez Garcia, 2016).

It is necessary to highlight that the aggregates used in mortars and concretes have to comply with certain physical criteria and specific chemicals. In Spain, mussel shell aggregates meet the criteria of Spanish regulations on the quality of aggregates for concrete with the exception of chloride (should be lower than 15,000 mg/kg of dry matter). A higher value of chloride could produce the rapid corrosion of steel. Therefore, this reduce or severely limits the use of mussel aggregate in reinforced or pre-stressed concrete.

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Martinez-Garcia, 2016 suggest to resolve this issue by a thermal treatment of the shells to guarantee a safe handling since it eliminates all the bacteria and implies a low consumption of energy. With this treatment, the leaching results lead to the classification of the whole shell as an inert material, and all fractions crushed as non-hazardous material.

Analysing the result detailed, NORCEM HEIDELBERGCEMENT GROUP has shown many doubts to try to use this shell in their process, specially respecting to the high alkalinity that is the showstopper in Norwegian cement. This was validating also from other contacted companies.

Taking into account these conclusions from Norwegian cement industry we should focus on alternative paths for valorisation of the shells, such as biofilters in this task.

3.5 Biofilters for phosphorous adsorption operation at lab-scale

The results of the phosphate concentrations in the biofilters were similar in both biofilters regardless the packaging material. From day 15 on 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%. These removal rates are high according literature reports. However, a 10-ppm concentration, which is typical of aquaculture systems, is quite low to verify the potential adsorbing capacity of the filter.

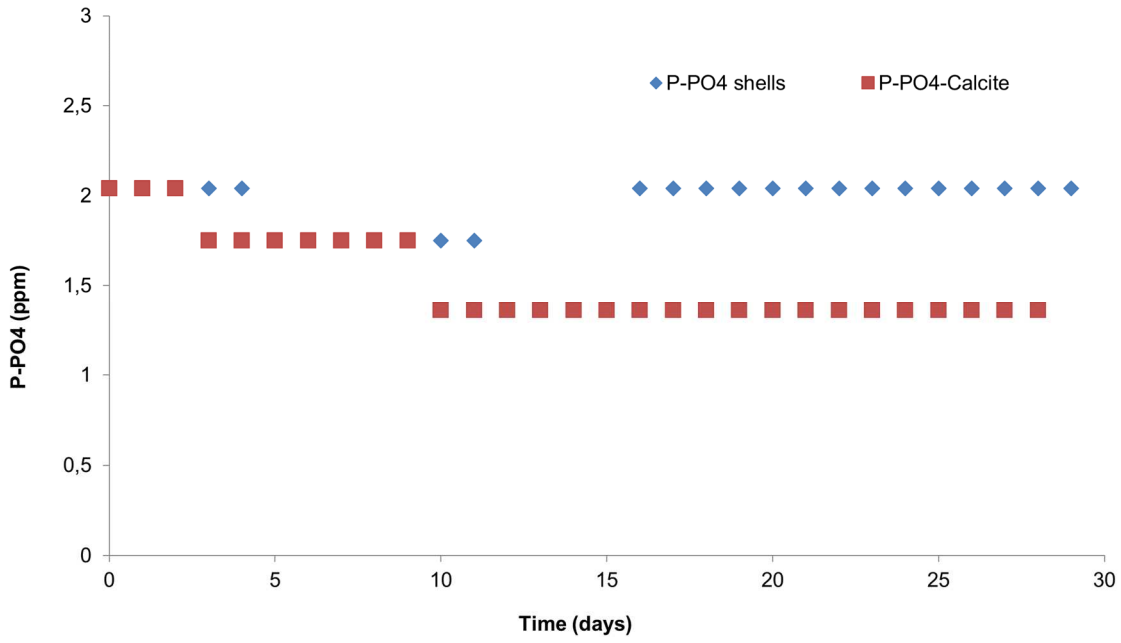

Figure 12. *Concentrations of P-PO4^{-} in the effluent comparing shells and calcite packaged biofilters*

The results of the phosphate concentrations after the g 1g/L spike (after 0 hours measurement) where the following:

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Table 3 Phosphate concentrations in ppm during time

	Time 0 h - $P\text{-}PO_4^-$	Time 1h - $P\text{-}PO_4^-$	Time 24 - $P\text{-}PO_4^-$
Mussel shells	2	843	677
Calcite	2	843	550

Results show that after 1 hour after the feeding the phosphate concentration was around 0.85 g/L, it 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 from Xion et al (2011) that demonstrated that calcination of mussel shell increased the phosphate removal capacity, in these case from 25% to 55%, as well from other authors such as Currie et al. (2007) and Kwon et al. (2004), whose studies obtained increases in phosphorous removal from 40 to 90%, and from 10% to 60%, when using calcined shell instead the raw material.

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4.Conclusions and next steps

The results of this task are promising to promote new uses of the shells. They allow us to think about the applications of them as substrates for Conchocelis in future aquaculture systems but also to reduce the impact of plastic material in current RAS systems using the shells as filling material. Both solutions are clearly in line with the principles of the circular economy pursed in the GAIN project. However, the use of shells for Norwegian cement industry has not the expected success, since they could not use it as a material in their formulation, but this would need to be investigated with other companies, since recent published articles showed that this is an interesting line for shell by-products.

The main conclusions for the potential use of shellfish in aquaculture as filling material in RAS systems are:

- **Similar efficiency of shell filled systems** (whole and crushed shells**) compared to bioplastic fillers** in ammonia and nitrite removal,reaching almost 100% working at lab-scale treating simulated seawater.
- High removal efficiencies in nitrification (100%) and almost 70% in 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, that is the ammonia and nitrite removals.

Regarding the use of the shells as filler media in biofilters, it would be necessary to consider the following points for bringing this innovation to higher TRLs :

- No replacement of shells was necessary after 175 days operating the biofilters, since no biofilm formation was observed. Therefore, the shell waste consumption for this use will be minimal in an aquaculture farm. 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.
- The economic analysis, presented in detail in GAIN deliverable D6.9 , shows that mussel shells can be more cost-effective than plastic material when applying to a bio filter system that runs for a period of 3 years, provided that mussel shells are free of costs, except for transport, and that their disposal costs is much lower, compared to plastic material.
- Also,promoting stricter regulations regarding effluent discharges (i.e., zero ammoium and nitrite values) and water consumption in current aquaculture systemswould be crucial for the development of these applications in a commercial way.
- The GAIN data showed that the use of plastic fillers in biofilters is not necessary for the correct elimination of nitrogen, clearly confirming the mussel shells as

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an alternative as filling material. The future now involves evaluating the process over longer periods of time and on an industrial scale, to assess its economic viability.

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5. References

- Adegoke, A.P. & Adewuyi T. (2008). Exploratory study of periwinkles shells as coarse aggregates in concrete works. Hitachi, Japan & Ogbomoso, Nigeria: Asian Research Publishing Ntework, ARPN Journal of Engineering and Applied Sciences, 2008. pág. 6. Vol. 3
- Awang-Hazmi, A.J.; Zuki, A.B.Z.; Nordin, M.M.; Jalila, A.; and Norimah, Y. (2007). Mineral composition of the cockle (Anadara Granosa) shells of west coast of peninsular Malaysia and its potential as biomaterial for use in bone repair. Journal of Animal and Veterinary Advances, 6(5), 591-594.
- Badiola, M., Mendiola, D., and Bostock, J. (2012). Recirculating Aquaculture Systems (RAS) analysis: main issues on management and future challenges. Aquac. Eng. 51, 26–35. doi: 10.1016/j.aquaeng.2012.07.004
- Ballester, P., Mármol, I., Morales, J., Sánchez, L. (2007). Use of limestone obtained from waste of the mussel cannery industry for the production of mortars. Cement and Concrete Research 37, 559–564
- Bioshell project: https://fundacion-biodiversidad.es/es/biodiversidad-marina-y-litoral/proyectos-convocatoria-ayudas/conchas-de-mejillon-como-biorrelleno
- Blackburne, R., Vadivelu, V.M., Yuan, Z. & Keller J. 2007. Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. WaterResearch., 4, 3033-3042
- Currie, J. A., Harrison, N. R., Wang, L., Jones, M. I., & Brooks, M. S. (2007). A preliminary study of processing seafood shells for eutrophication control. Asia-Pacific Journal of Chemical Engineering, 2(5), 460-467.
- Eurostat (2018). https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Fishery_statistics#Aquaculture_statistics.%20Accessed%20on%20March%2014th%202019
- Kelley, Kristy Noel (2009). Use of recycled oyster shells as aggregate for pervious concrete. Florida, EEUU: University of Florida, 2009. Master Thesis.
- Kwon, H. B., Lee, C. W., Jun, B. S., Weon, S. Y., & Koopman, B. (2004). Recycling waste oyster shells for eutrophication control. Resources, Conservation and Recycling, 41(1), 75-82.
- Martinez Garcia, C. (2016). Estudio del comportamiento concha de mejillón como árido para la fabricación de hormigones en masa. Proyecto de fin de grado. Universidad de Coruña. P 46- 48.

The project has received funding from the European Union's Horizon 2020 Framework Research and Innovation Programme under GA n. 773330

- Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O., Blancheton, J. P., et al. (2010). New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. Aquac. Eng. 43, 83–93. doi: 10.1016/j.aquaeng.2010.09.002
- Randall, D., and Tsui, T. K. (2002). Ammonia toxicity in fish. Mar. Pollut. Bull. 45, 17–23. doi: 10.1016/S0025-326X(02)00227-8
- Sharrer, M. J., Tal, Y., Ferrier, D., Hankins, J. A., & Summerfelt, S. T. (2007). Membrane biological reactor treatment of a saline backwash flow from a recirculating aquaculture system. Aquacultural engineering, 36(2), 159-176.
- Soula, M., Regueiro, L., Mendez, D., Ferreira, M., & Johansen, J. Innovative processes for valorising shellfish by-products. Deliverable 2.4. GAIN - Green Aquaculture INtensification in Europe. EU Horizon 2020 project grant nº. 773330.
- Timmons, M.B., Holder, J. L., Ebeling, J. M., 2006. Application of microbead biological filters. Aquacultural Engineering, 34, 332-343
- Timmons, M. B., & Ebeling, J. M. (2010). Recirculating aquaculture. Cayuga Aqua Ventures.
- Tossavainen, M., Lahti, K., Edelmann, M., Eskola, R., Lampi, A. M., Piironen, V., ... &Romantschuk, M. (2019). Integrated utilization of microalgae cultured in aquaculture wastewater: wastewater treatment and production of valuable fatty acids and tocopherols. Journal of Applied Phycology, 31(3), 1753-1763.
- Wang, Y., Chen, J., Zhou, S., Wang, X., Chen, Y., Lin, X., ... & Han, H. (2017). 16S rRNA gene high-throughput sequencing reveals shift in nitrogen conversion related microorganisms in a CANON system in response to salt stress. Chemical Engineering Journal, 317, 512-521.
- Xiong, J., Qin, Y., Islam, E., Yue, M., & Wang, W. (2011). Phosphate removal from solution using powdered freshwater mussel shells. Desalination, 276(1-3), 317-321.
- Zhang, X., Zhou, Y., Ma, Y., Zhang, N., Zhao, S., Zhang, R., ... & Zhang, H. (2018). Effect of inorganic carbon concentration on the stability and nitrite-oxidizing bacteria community structure of the CANON process in a membrane bioreactor. Environmental technology, 39(4), 457-463.

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