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WP 2 – Enhancement of secondary outputs

Task 2.3 – Valorisation of shellfish industry by-products

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GLOSSARY OF ACRONYMS

Acronym	Definition
ABP	Animal by-product
AOB	Ammonia-oxidizing bacteria
RAS	Recirculation Aquaculture Systems

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Executive summary

The Task 2.3 of the GAIN project deals with the valorisation of bivalve shells, an important residue from the bivalve canning industry: e.g. discarded mussel shells account for 147,000 t y^{-1} in the EU. One of the pillars of the project is the eco-intensification of European aquaculture through the implementation of principles of circular economy. This document provides a review of the state of the art regarding different applications for the valorisation of bivalve shells, as a basis for the development and the demonstration of the suitability of the valorization processes which will be investigated in GAIN. Three candidate processes were identified at the proposal preparation stage:

1) the use of discarded shells as packaging material in biofilters and as phosphorus sorbent in recirculating aquaculture systems (RAS),
2) the use of shells in the production of seaweed seedlings,
3) the potential use of shells as filler for the cement industry.

During the first year of GAIN, activities were focused on the first process: preliminary results presented in this deliverables led to the design of the biofilter, which will be tested in the second year.

The second and third processes are currently being investigated. Results will be presented in GAIN Deliverable D2.8, due by Month 30.

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1. Introduction

GAIN is a collaborative project funded by the European Union (EU) designed to support the ecological intensification of aquaculture in the EU and the European Economic Area (EEA), with the dual objectives of increasing production and competitiveness of the industry, while ensuring sustainability and compliance with EU regulations on food safety and environment. One of the main objectives of this project is to add value to the cultivation of finfish and shellfish by means of innovation in both by-products and side-streams, ensuring improved re-use of secondary materials, increase in profit, and minimization of environmental footprint.

Within WP2, Task 2.3, the use of bivalve shell residues in several processes will be evaluated in four (or three?) innovative processes:

1) as packaging material in RAS biofilters, using coarsely crushed mussel shell as a possible alternative substrate for bacterial growth and nitrification-denitrification processes, substituting the usual plastic rings,
2) by developing a filtration column containing crushed and calcined mussel shell for phosphorus removal from RAS effluents,
3) the use of shells in the production of seaweed seedlings,
4) the potential use of shells as filler for the cement industry.

In this deliverable, after reviewing the state of art of shellfish production and by product valorisation, the first two GAIN innovative processes are described in detail and the design of a biofilter packed with mussel shells as a medium is presented.

2. Shellfish production

World aquaculture production has been increasing steadily during the last thirty years and currently is the fastest growing food sector. Fish are currently the main product with a 49.1% of the whole production, followed by seaweeds and molluscs (27.3% and 15.6%, respectively). The global mollusc production exceeded 17 Mt in 2016, mainly consisting of cupped oysters, Japanese carpet shell, scallops, marine molluscs and sea mussels (FAO, 2018).

In the EU, aquaculture production reached 1,292,597 tonnes in 2016. Mollusc production represented 46.6% of this amount, with an economic value of 502 M€. Mussels (Mediterranean and blue mussel) are the main species produced in the EU, with 387,884 tonnes followed by cupped oysters (88,205 t) and Japanese carpet shell (35,436 t) (Eurostat,

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2018). The main shellfish producing countries are Spain, based on mussel farming, followed by France (oysters) and Italy (clams). These three countries accounted for 73.8% of the total European aquaculture mollusc production in 2016 (Eurostat, 2018). The Spanish mussel sector produces an average of 267 Mt per year, 45% of EU mussel production, with an estimated first sale value of 124.9 M€. 97% of this production is located in the autonomous community of Galicia where the sector stands out as an engine of social and economic development with 3,337 production units distributed in 47 cultivation areas. Rafts are the mussel farming system commonly used in Galicia. They are floating wood structures anchored to the bottom. Mussels are grown in hanging ropes, maximum 500 per raft. Each raft yields between 100 and 150 t per year, depending on the production cycle and commercial objective. In Spain, the canning industry alone generates shell mussel discard equivalent to more than 40 % of fresh live weight as shells and 30 % as cooking effluents, an average of 35,000 and 26,000 t of each by-product respectively per annum. In France, more than 56,000 t of oyster shells are generated which represent 75% of the biomass of harvested oysters. In Korea, about 300,000 t of oyster shells are generated annually (Hamester et al., 2012) and in Taiwan more than 160,000 t per year, which are either dumped into coastal water or landfilled.

3. Shell by-product valorization: state of the art

Humans have valued marine mollusc shells since prehistoric times. Mollusc shells are one of the hardest animal tissues. This fact, together with their beauty and diversity of sizes, shapes and colours gave mollusc shells different uses over the history such as trumpets, money, tools, medicine, pieces of games, as well as ornaments (Vitezovic et al., 2017).

Shells were used as a form of currency in many cultures. In fact, the oldest form of currency was a shell called "cauri", original from China but soon spread throughout the world as a form of payment. Other use widely spread through cultures and ages is jewellery; mollusc shells and jewellery have been intricately linked through history. At least 100,000 years ago, people from current North Africa and Israel made beads from shells. Religions like hinduism, buddhism and christianity have given shells symbolic significances and functions in religious rituals. A scallop shell is frequently used to dip water in the baptism, and in many churches and cathedrals holy water is stored in *Tridacna gigas* giant shells. Shells also have been attributed a great variety of health benefits and healing properties, and hence shell powder has been used for medicinal purposes. Finally, shells have also been used as building material in several coastal settlements around the world.

The development and expansion of mollusc aquaculture worldwide led to the production of a large quantity of shell waste, causing environmental concerns and the need to develop management solutions. Shells are an abundant residue in regions where shellfish aquaculture and canning industries are well developed. In fact, from the total amount of shellfish

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produced, around 40 to 90% of biomass consists on shells treated as a residue or as a by-product discarded in the transformation process.

Classification of shell waste depends on the presence of flesh of soft tissue remnants. If this is the case, shells are considered animal by-products (ABPs) according to Regulation (EC) no. 1069/2009, whereas clean shells are considered a residue subjected to Directive 2008/98/EC on waste. EU policies and strategies on waste (European Commission, 2005; 2011) encourage the prevention and the recycling of residues. Likewise, Regulation no. 1069/2009 foresees that shells pose only a limited risk to public or animal health and hence, competent authorities may authorise their application as fertilisers after adequate treatment, on the basis of Category 2 and Category 3 materials. Nevertheless, and despite the opportunities contemplated in regulations, in view of the scarcity of information about shell valorisation in the EU (e.g. EUMOFA, 2018; Morris et al., 2018), it is reasonable to think that virtually all shell waste produced is disposed of, as foreseen in Directive 2008/98/EC. In fact, the use of mussel shell as a liming agent for agriculture soils in Galicia seems to be the most remarkable exception to the general absence of further uses for this resource.

Shells are a good source of carbonate, since they are composed of 95% to 99% of calcium carbonate, being the rest (1-5%) organic matter and other compounds. This high level of calcium carbonate has raised the interest on this residue: several articles related to shellfish valorisation have been published recently, and reviewed by Morris et al. (2018). These studies suggest the use of shells as construction material, feed supplement, fertiliser or agricultural agent. Other recent uses include the mitigation of contamination and as de-icer substance, as summarized in the following paragraphs.

3.1. Shells in the building sector

Shells were used as a simple material for construction or incorporated into aggregate and mortar mixes due to their characteristics that might make them suitable for certain construction aggregates. Whole oyster shells are used for simple wall structures in coastal villages in China, and crushed scallop shells have been used as a simple path aggregate on the Mull Island in Scotland. In Spain, Galician mussel shells have been tested for their suitability in aggregate mixes. Biovalvo project (https://proyectobiovalvo.wordpress.com/,), carried out by the University of Coruña has demonstrated that mussel shells thermally processed at 135 °C for 30 minutes can be used as aggregates for mass concrete. Percentages of substitution of up to 25% of natural aggregates by mussel shell aggregate (sand or gravel) are suitable for structural concrete.

The potential use of shells in the cement industry was also investigated. A study assessing the incorporation of mussel shell waste in Spain into mortars found that differences in particle microstructure between quarried limestone (rounded particles) and mussel waste $CaCO_3$ (elongated prismatic particles) resulted in mussel waste-derived mortars showing improved setting times and final strength (Ballester et al. 2007). The authors concluded that ground

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mussel shell waste could be incorporated into cement mixes, reducing the cement mix cost as well as the providing environmental benefits of reduced quarried limestone reliance. In France, a study investigated the incorporation of crushed *Crepidula* sp. (slipper limpet) shells into pervious concrete mixes and concluded that shell incorporation did not have an adverse effect on the concretes mechanical strength and increased porosity allowed for better water permeability, an important characteristic of pervious concretes (Nguyen et al. 2013). Further studies have found similar viability of shell incorporation in various aggregate mixes (Lertwattanaruk et al. 2012; Kuo et al. 2013; Othman et al. 2013).

Another potential use in the building industry is the incorporation of shells into green roofing structures, since they can help with the neutralisation of acid rain, and the reduction of heavy metal contamination in drainage water (Chiou et al. 2014).

3.2. Water treatment

Du et al., 2011 indicated the use of shells for heavy metal removal in wastewater treatment facilities, since the calcium carbonate rich powder is good as lead (Pb) sorbent, whilst the use of mixed shells rich in both calcite and aragonite should be optimised for heavy metal removal. The shell preparation should be optimised via simple technological treatments to avoid expensive end-products. For instance, the same authors suggested a process based on washing, airdrying and pulverisation. Another study from Hossain and Aditya (2013) indicated that the shell dust coming from invasive *Physa acuta* could act as an effective cadmium (Cd) sorbent.

Besides heavy metals, calcareous shells have been previously tested as nitrate, sulphate and/or phosphate sorbents (Morris et al., 2018). For instance, the use of shells for carbon sequestration has been studied by several authors, highlighting that a previous pre-treatment via calcination or pyrolisation to convert the shells in calcium oxide (CaO) is required. (Ma & Teng 2010; Castilho et al. 2013). This "ad hoc" product displays a good performance; however, pyrolisation of shells may require heating over 400 ºC, which makes this solution not sustainable at large scale in terms of costs and environmental impact. Few studies, e.g. Paradelo et al., (2016), Monneron-Gyurits et al. (2018) have worked with uncalcined or unpyrolysed shells that could represent a low-cost solution.

3.3. Shell in agriculture and livestock production

Lime or calcium hydroxide is a common chemical agent used to increase pH to optimum values for the growth of many crops in agriculture. Liming technique can create better environmental conditions for the growth of acid-intolerant microorganisms, thus increasing microbial biomass and soil respiration in acid soils (Lee and Jose, 2003). Other chemicals that can be used for increasing pH include calcium and magnesium carbonates and oxides and magnesium hydroxide. Therefore, bivalve shells have a high potential to be used as a liming material in

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agriculture, as recently postulated and investigated by several researchers (Ahn et al., 2010, Mohamed et al., 2012). On a large scale, Galicia is the major region in Europe currently utilising shell waste as a liming agent. The main reasons are related to the proximity of acid agricultural soils to the shellfish aquaculture sites, and also due to the presence of large shell processing facilities providing mussels to cannery industry. Indeed, the Spanish legislation about fertilizer products (RD 506/2013) contemplates limestone products as one of the subcategories of fertilizers.

The use of calcium supplementation in livestock feed is well established. Since the 1940s, several experiments were developed about the addition of mussel and oyster shell as ingredients to animal feed to improve the health of laying birds. Trials show that an increase of calcium ingests of only 5% with regard to control diets improved the quality and strength of eggshells (Heuser & Norris, 1946; Guinotte and Nys, 1991; Pizzolante et al., 2009). In Galicia, at least one company produces calcium supplements for laying hens using 100% crushed mollusc shell base, containing 12 natural minerals. Its composition includes high calcium content (36%) and active elements that enhance bird appetite, due to their organic origin. Furthermore, a recent Galician project called MEXICAL is also evaluating the use of mussel shells as bird fodder. This project is led by the company Galaytec, and other three companies, INDUTEC Ingenieros, TRESIMA and Vitalmar are participating in it.

3.4. De-icer grit

Chlorine-based compounds, such as rock salt (NaCl), are commonly used as de-icing chemicals. However, it is well known that chlorine-based road grits could damage the urban environment (and also the natural one) due to their corrosive effects. In fact, they are forbidden in airports, as they can damage aircrafts. Calcium magnesium acetate or any calcium acetate could be environmental-friendly potential alternatives as road grit (Clarke et al., 2002). Therefore, the use of shells as calcium donor in the formation of calcium acetates could be also an alternative use for shells valorisation. The use of potassium carbonate as de-icer and the combination of scallop shells mixed with apple pomace waste from Aomori Prefecture in Northern Japan was reported (Hartl et al., 2002).

3.5. Water treatment in aquaculture applications

Besides the neutralisation of acid rain or the elimination of heavy metals from water described above, physico-chemical properties of bivalve shells can also be exploited for the removal of dissolved nitrogen and phosphorus, important pollutants discharged by aquaculture facilities. Studies have demonstrated that oyster shells are a suitable material for the uptake of ammonia nitrogen and phosphorus from municipal wastewater (Liu et al., 2010), and are also appropriate to remove nutrients from wastewaters discharged to natural environments such as wetlands (Luo et al., 2013). Nevertheless, reports about the use of shells to treat water in aquaculture systems are scarce. Yen and Chou (2016) studied the use of oyster shells as

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biofilter medium in RAS, demonstrating an efficient water treatment and nitrification in an aquaponics setup combining oyster shells as biofilter followed by a cultivation of water spinach. Oyster shells support the growth of bacteria involved in nitrification, i.e. the oxidation of ammonia nitrogen to nitrate (Buzin et al., 2015; Yen and Chou, 2016). The nitrification and denitrification processes are described in detail in Section 4 of this document.

As for phosphorus uptake, the performance of adsorbent materials is apparently related to their chemical composition and structure. Fe-rich materials such as fly ash or bauxite are particularly efficient (Kwon et al., 2004), but treatment with lime or other cheap carbonate sources is a low-cost method to remove phosphorus from wastewater, based on the precipitation of hydroxyapatite produced by the reaction of phosphate and dissolved calcium. Since bivalve shells are rich in calcium carbonate, they could also be potentially used to remove phosphorus from wastewater and particularly aquaculture effluents. Paradelo et al. (2016) performed experiments with calcined and non-calcined mussel shells to uptake dissolved phosphorus from water samples. Lab-scale experiments showed a higher uptake capacity in calcined samples, of around 600-800 mmol of P/kg. This is due to calcination of shells at 500 ºC or higher, which transforms the crystalline structure of calcium carbonate from aragonite to calcite; moreover, part of the calcium carbonate is transformed in the much more soluble calcium oxide, thus enhancing the reaction with phosphate and the precipitation of hydroxyapatite. In non-calcined shells, adsorption is the predominant mechanism of phosphorus removal, and therefore the process is mostly limited to the shell-water interface.

4. GAIN innovative processes

The aim to valorise bivalve shell waste within the concept of circular economy through applications in aquaculture arises from the amount of this raw material that is generated yearly in the EU, mainly in Galicia as a residue from the mussel aquaculture and processing industry and also in France, from oyster aquaculture. Roughly, it can be estimated that 35,000 t of mussel shell and 56,000 t of oyster shell were generated in 2017 in Galicia and France respectively.

Since the main objective of GAIN is increasing productivity and competitiveness of the EU aquaculture industry through ecological intensification, the innovative processes proposed within Task 2.3 deal with the integration of shell residues into aquaculture practices, instead of searching for applications in other fields, e.g. as described in Section 3 of this deliverable. Taking this requirement into account, two cost-effective options to valorise shell waste are investigated in this deliverable:

1) packaging material for biofilters in RAS. Shells will be the substrate for the growth of nitrifying and denitrifying bacteria

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2) sorbent material to uptake phosphorus released from fish excretions and uneaten feed, also in RAS

Current practice in RAS focuses in decreasing ammonia levels from recirculating water, whereas nitrate and phosphorus are partially removed through water exchange. GAIN proposes innovative applications of shells to decrease the levels of both substances, thus reducing the nutrient load of RAS effluents and the need for water renewal. In contrast with previous studies which combine bacterial nitrification and nitrogen uptake by vegetables in aquaponic systems (Yen and Chou, 2016) or filter feeders (Buzin et al., 2015), in GAIN Task 2.3 shells will be used as the only element enabling nitrogen and phosphorus removal.

ANFACO is the responsible partner for the development of these valorisation processes, and hence mussel shells have been selected, due to the local abundance of this material. Based on the results of the pilot study being carried out in GAIN, other types of shells could be tested for these applications, perhaps requiring different pretreatments, e.g. cleaning or crushing.

4.1. Use of shells in RAS biofilters for nitrogen removal

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 under natural water resources, but also the volume of waste generated (Lahav et al., 2009). One of the key points in a RAS design (Figure 1) is associated to the biological filtration systems, also known as biofilter.

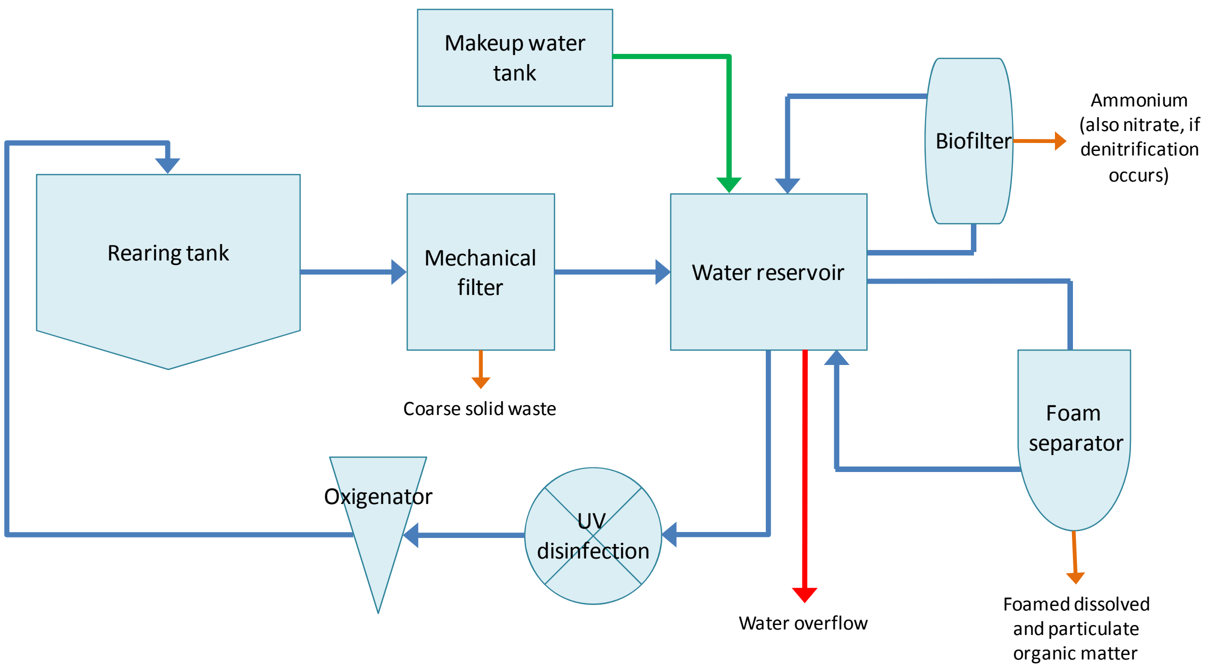

Figure 1. Simplified schematic design of a typical RAS, showing the most important elements of water fluxes and treatment. Blue arrows: recirculated water; green arrow: clean makeup water inlet; red arrows: used recirculated

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water outlet; orange arrows: waste separation processes and type of removed waste. Other RAS components such as degassing columns or heat exchangers are omitted for simplicity.

Ammonia is released from fish excretes 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 mg/l. 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 cylindrical bioreactor filled with different substrates, also known as biofilter medium. The function of this medium is maximizing the contact area, in order to promote the growth of the bacterial consortium, which cover the surface of the substrate particles as a biofilm (Avnimelech, 2006). Plastic rings or beads are the most usual materials to fill biofilters.

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 (AOB) which oxidize ammonia to nitrite, according to the mass balance Eq. 1 and nitrite oxidizing bacteria, which oxidize nitrite to nitrate, according to Eq. 2. AOB can be found among the β-proteobacteria and Gamma proteobacteria classes, but they belong mainly to *Nitrosomonas* genus. The second step (is carried out mainly by bacteria of the genus *Nitrobacter* and *Nitrospira*.

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2\,H^+ \quad (1)$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \quad (2)$$

As a consequence of ammonia oxidation, nitrate tends to accumulate in the recirculated water. Although aquaculture organisms can resist high nitrate concentrations, this is 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. On the other side, water replacement involves 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 (N_2) by facultative anaerobic bacteria, generally using organic matter as electron donor. The formula to reduce the nitrate using methanol as carbon source appears in equation (3):

$$6\,NO_3^- + 5\,CH_3OH \rightarrow 3\,N_2 + 5\,CO_2 + 7\,H_2O + 6\,OH^- \quad (3)$$

This denitrification step is carried out by heterotrophic bacteria including *Pseudomonas*, *Paraccocus*, *Alcaligenes*, *Thiobacillus*, *Bacillus*, among others (Schreier et al., 2010). Most of

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these organisms are facultative anaerobes and use nitrate as a final electron acceptor in the absence of oxygen.

Whereas in wastewater treatment plants the use of anaerobic denitrification to remove nitrate is a common process, in commercial RAS this is not yet widely applied due to its level of efficiency and complexity (Pungrasmi et al., 2016). 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 (van Rijn et al., 2006). High oxygen concentrations may inhibit denitrification and lead to an excessive, aerobic consumption of the organic matter provided (Singer et al., 2008). Thus, two separate reactors are needed for nitrification and denitrification.

Nevertheless, the advantages of integrating a denitrification step within the biofiltration step in a RAS should be carefully considered, as removal from effluents and the subsequent reduction in water renewal and nitrate may lead to decrease the overall operational costs; moreover, beneficial effects of appropriate water quality on fish health and welfare may enhance productivity.

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 (Figure 2).

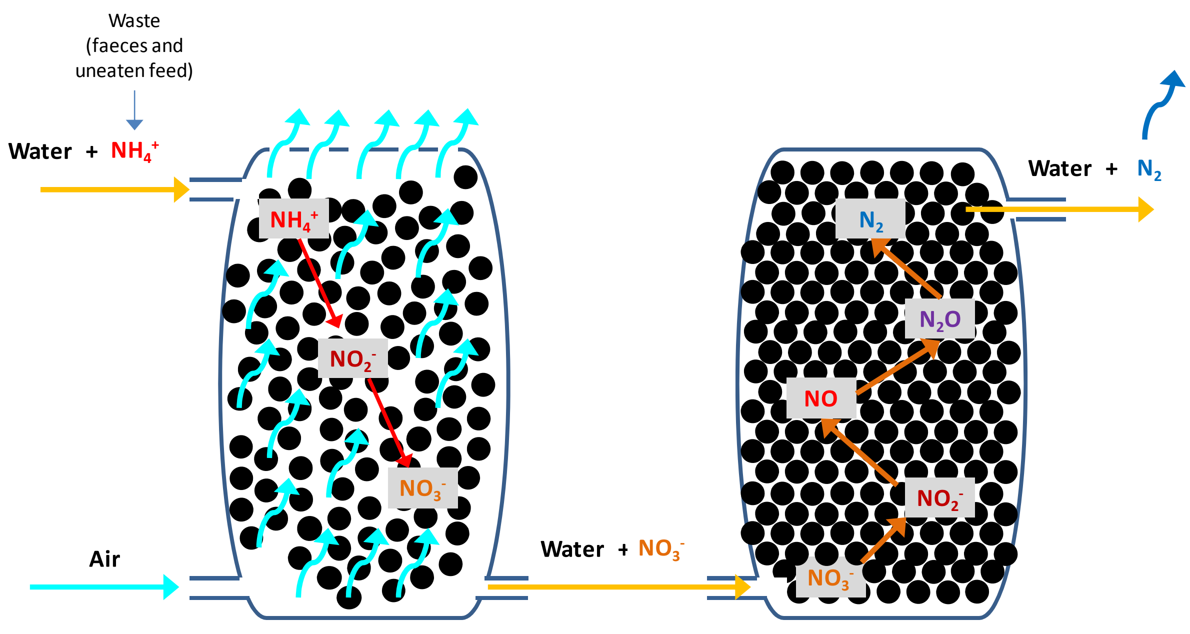

Figure 2. Schematic diagram of nitrifying and denitrifying biofilters, showing water and air fluxes and the

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intermediate and final products of nitrification and denitrification. Left: in nitrifying biofilters, air is injected to supply oxygen, thus keeping particles in suspension (fluidized bed reactor). Bacteria oxidize ammonium to nitrate, which tends to accumulate. Right, the denitrifying biofilter is a packed bed reactor where the absence of aeration and the low water exchange help to maintain an anoxic environment that enables the reduction of nitrate to elemental nitrogen, which is finally released to the atmosphere.

Within GAIN Task 2.3, the use of raw mussel shells as biofilter packaging material in both aerobic and non-aerated phases will be tested, in order to assess its performance as growth substrate for nitrifying and denitrifying bacteria. The expected main advantages related to the use of shells as packaging material are:

1) pH and alkalinity control in the nitrification process. As the oxidation of ammonia progresses, pH and alkalinity levels tend to decrease. Shells, in this case mussel shells, would gradually dissolve, being a source of alkalinity and thus increasing pH. Nevertheless, this fact also involves that the biofilter would require a periodical replacement of the packaging material.
2) Surface area can be controlled through grinding or crushing to guarantee a high settlement rate for microorganisms.
3) Disposed mussel shells can be managed as organic waste and thus their impact is much lower than that of plastic materials and residues.

4.1.1 Biofilter design

The structure of the biofilter, designed by ANFACO, is shown in
Figure ***3***. The methacrylate tank, 150 mm diameter, 3 mm thick and 800 mm high, allow one to look inside the reactor. The 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 packaging where bacteria grow. Wastewater from fish tanks is fed into the bottom compartment (150 mm height) through a 20-mm (outside, 17-mm inside) inlet pipe connected to the feeding pump; aeration can also be provided from this section to the whole biofilter. The intermediate part (500 mm height) contains the filtering media i.e. crushed or whole mussel shells, and the top compartment (150 mm height) allows to collect the water by overflow from the outlet, located at the top and with the same as feeding 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 and is expected to work well until overfeeding and solids accumulation in the packed bed shells provokes excess of bacterial growth, inhibiting the water penetration. This situation will be solved by blowing air to detach excess bacterial biofilm or draining to wash out the accumulated solids.

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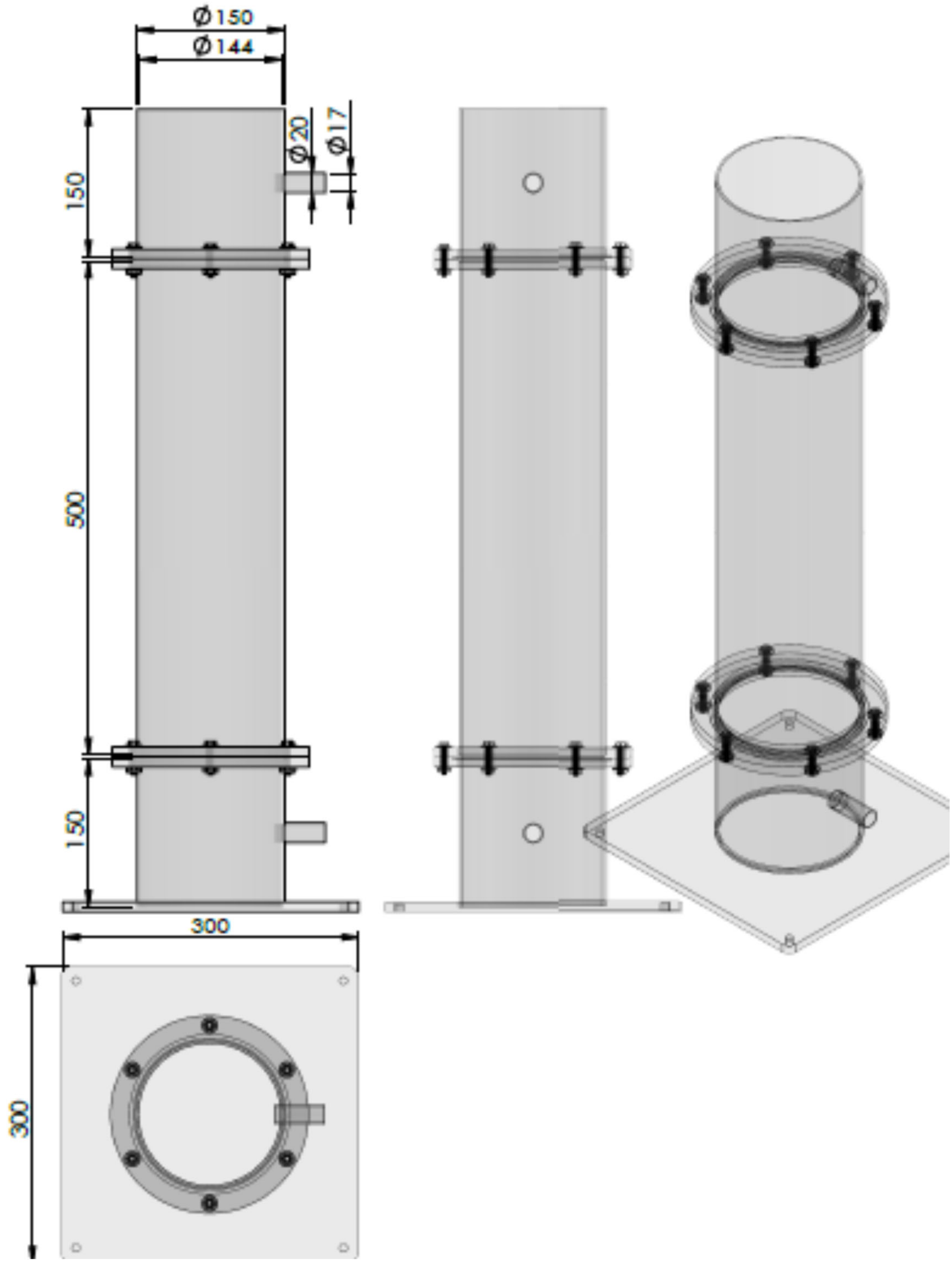

Figure 3. ANFACO biofilter design. Water is pumped from a water reservoir through the inlet in the bottom part of the column. The central section contains the packaging material and the nitrifying or denitrifying bacteria. As water is pumped in, excess volume overflows from the top of the column through an outlet pipe and returns to the water reservoir.

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Figure 4. Built biofilter prototypes. Aeration is provided through an inlet at the bottom connected to an airstone.

Three units of the biofilter have been built and deployed at ANFACO laboratories (Figure 4).Hydraulic tests to verify robustness, flow speed, volume, etc. are being carried out. In a following step, experiments to assess the nitrification and denitrification capacity of mussel shell-packed prototypes, compared to prototypes packed with plastic beads or other standard packaging material will be performed. Temperature, pH and dissolved oxygen will be monitored on a daily basis. Ammonia, nitrite and nitrate will be measured every second day.

Sodium acetate will be used as cheap external carbon source at a COD:N ratio of 5:1 and the denitrification reactor will be gassed with nitrogen, trying to guarantee anoxic environment with minimum gas exchange. The carbon source supplement should not have negative effects on fish.

4.2. Use of shells in RAS as phosphorus sorbent

Likewise ammonia, phosphorus is released from fish faeces and uneaten feed, and it is discharged to the environment through the effluent, contributing to eutrophication in fresh

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and marine waters. Currently, RAS do not include specific processes to remove phosphorus from the recirculating water; therefore, the development of solutions to decrease phosphorus discharge from RAS effluents constitutes an innovative approach to reduce the contribution to eutrophication from aquaculture constitutes an innovative approach to reduce the contribution to eutrophication from aquaculture activities.

Research in GAIN project will build on the capacity of bivalve shells to adsorbe/precipitate dissolved phosphorus, developing an innovative process to remove this nutrient from aquaculture wastewater using mussel shells as a sorbent. The main objective will be to assess the conditions for maximum phosphorus absorption by mussel shells. Since the development of a low-cost, low-energy footprint process is a prerequisite, shells will not be subjected to a calcination step, even when this would enhance the efficiency of phosphorus removal, as explained in section 3.5. Instead, shells will be crushed to increase the available surface for phosphorus adsorption. Determining the saturation point (g of removed phosphorus per kg of mussel shell and time) will be crucial to evaluate the system efficiency from a technical and economical point of view the efficiency of this system. The biofilter prototypes designed and built for nitrification and denitrification assays (see Section 4.1.1.) will be used in further tests to assess the phosphorus removal dynamics. In a second phase, phosphorus and ammonium or phosphorus and nitrate removal may be jointly assessed to test the capacity of the biofilter to the simultaneous uptake of the different types of nutrients.

4.3. Advantages of the use of shells as packaging and P sorbent materials in RAS

The benefits of replacing plastic beads, usually called bio balls, as packaging material in biofilters, or as sorbent material to remove dissolved phosphate, must be considered from an integrative perspective, taking diverse aspects into account.

- 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 to a correct 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.

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

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 benefit 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 proposed in this document.

The developed uses of shells in nutrient removal are susceptible of being transferred to other treatment processes dealing with water rich in ammonium, nitrate and phosphate, such as effluents from household or food manufacturing. This potential of diversification would largely increase the chances and the market for bivalve shells for water treatment applications. Technical or economic advantages over current technologies, and moreover, the existence of strict regulations regarding effluent discharges would be crucial for the development of these applications.

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5. Concluding remarks

Mussel aquaculture in Spain, namely in Galicia, generates a large amount of shells as a by-product that is necessary to treat to avoid environmental problems; in this context, the development of valorisation solutions is of high interest.

The different options to use bivalve shells for different applications found in the revised scientific literature, as well as the objectives of GAIN project, led to identify two promising avenues for shell valorisation, i.e. the removal of nutrients –nitrogen and phosphorus- in RAS. In this aquaculture systems nitrate and phosphorus tend to accumulate to high levels, thus posing issues from the point of view of effluent treatment or discharge to the environment.

The capacity of shells to remove important quantities of nitrogen and phosphorus from aquaculture wastewater, as well as to provide alkalinity, will be explored in purpose-made equipment –i.e. biofilters- that will be continuously operated. The data of nitrogen and also phosphorus removal will be used to control the efficiency and to estimate further quantities to be applied in high technological readiness level equipment.

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