Crab Shell Chitin

Ireland Exploitation Report

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1 Purpose

The purpose of this report is to discuss the commercial feasibility and exploitation potential for the upscaling of the BlueShell extraction process for chitin from crab waste streams. Basic market analysis, life cycle assessment (LCA), mass balance analysis, price comparison and regulatory considerations are assessed to determine the best routes to exploitation of the results of the ERA-NET MBT BlueShell Project (2017-20).

2 Review of Current Commercial Aspects

There is a lack of scientific or commercial literature detailing industrial processing of shellfish waste streams for chitin. Chitin and chitosan from shrimp sources are available for purchase from several chemical suppliers, however most producers are small operations who keep their processes confidential. Up-scaled processing of crab waste streams is less commonly discussed in literature, mainly due to the under-utilisation of crab as a source of commercial chitin and chitosan[1]. As such, most literature details lab scale extractions and treatments as outlined in Section 3.1[2]. To define the best routes to exploitation, an understanding of the current state of the industrial chitin marketplace is essential. The following outlines the conditions in which any process must be able to operate to be commercially viable.

2.1 Raw Material Supply

Irish brown crab (*cancer pagurus*) landings totalled 5,500 tonnes in 2019, contributing a value of €16M to the Irish seafood sector[3]. Of this tonnage, only 30% is meat. Crab meat is the material of value while the other 70% is comprised of shell and connective tissue[4]. This material must be dealt with as "Category-3 Animal By-Product Waste" and as such has considerable waste management overheads associated with it. Therefore, a ready supply of raw material exists (~ 3,850 tonnes in 2019 for Ireland alone), of no value and a potential disposal cost.

2.2 Current Production

There is little literature on commercial extraction techniques for chitin, especially using crab shells. Most chitin producers use shrimp peels as a raw material, as shrimp constitutes a greater portion of the fisheries market globally (estimates for 2020 of about 5 million tonnes farmed

[1] L. Pighinelli, F. Arrouze, M. Essahli, *Nature*, 2008, **3**, 1–28.
[2] M. Hamdi, A. Hammami, S. Hajji, M. Jridi, M. Nasri and R. Nasri, *Int. J. Biol. Macromol.*, 2017, **101**, 455–463.
[3] Bord Iscaigh Mhara (BIM), 2019, 32.
[4] R. L. Olsen, J. Toppe and I. Karunasagar, *Trends Food Sci. Technol.*, 2014, **36**, 144–151.

shrimp[5], while worldwide production of crab will be about 3 million tonnes in 2024[6]), and is relatively easy to process for extraction of chitin[7]. There are few European companies currently producing chitin from either crab or shrimp. Merck is the largest supplier of pure crab and shrimp chitin but do not produce it in house. Merck do not provide the names of its suppliers and it is likely that they use several small-scale operations, more than likely from outside of Europe. In the European context there is a small list of independent producers including Advanced Biopolymers AS, NovaMatrix, Primex Ehf and Heppe Medical Chitosan GmbH. Looking specifically at the UK and Ireland there are two companies of interest - Bio-Marine Ingredients Ireland, based in Co. Monaghan and CuanTec based in Motherwell, Scotland.

Asia has the highest production volumes in the world, mainly in India and China. The largest producers are the Zhejiang Candrly Pharmaceutical Co., Ltd and the Zheijian Shinfuda Marine Biotechnology Corporation. Worldwide, the production of chitin was estimated at 28K tonnes in 2015[8].

2.3 Current Demand

At present most chitin produced worldwide is used as starting material for production of its soluble derivative, chitosan. Of the chitin that goes to market, the majority of this is utilised in low volume research settings. Chitin has low-level uses in bulking animal feedstock and as an excipient in recycled fibre materials such as cellulose and lichen products[9]. Due to primacy demand from research - both academic and commercial - high-quality is prized. The primary defining physiochemical property of chitin is the percentage degree of acetylation (%DA). A %DA of >50% means the product is insoluble chitin and a %DA of <50% means the product is the soluble chitosan. Depending on the intended use of the chitin, for example in drug encapsulation, electro-spinning, flocculation, metal recovery or bio-plastics manufacturing, the %DA of the starting material may vary. Discerning consumers demand high-quality chitin, with high purity, produced to a specific %DA. The chemical extraction of chitin often induces a degree of de-acetylation meaning it can be hard to maintain high %DA - especially when using shrimp or mushroom as starting material as these typically start at a lower %DA. Crab shell chitin, especially from brown crab, naturally occurs at ~ 98%DA[10]. This facilitates a wider scope of %DA products.

5 https://www.aquaculturealliance.org/advocate/goal-2019-global-shrimp-production-review/
6 https://www.prnewswire.com/news-releases/global-crab-market-to-2024-production-volume-is-expected-to-surpass-3-million-metric-tons-300768077.html
7 P. Charoenvuttitham, J. Shi and G. S. Mittal, *Sep. Sci. Technol.*, 2006, **41**, 1135–1153
8 http://sflyproteins.com/a-worldwide-market-with-a-strong-demand/
9 J. García-Romero, R. Ginés, M. S. Izquierdo, R. Haroun, R. Badilla and L. Robaina, *Aquaculture*, 2014, **422**–**423**, 239–248.
10 F. Ó Fearghail, M. Giltrap and P. Behan, 2017.

Similarly, the higher the %DA the more resistant the chitin is to degradation by chemical extraction, further enhancing the ability to produce a high-quality product.

Because of growing environmental awareness, crab meat producers are looking to divert waste streams to secondary products such as chitin. A crab processor can potentially reduce cost by redirecting the waste stream to a third-party chitin producer, thus avoiding waste disposal charges. Where demand is strong, a processor could charge for the crab waste stream because of its downstream value.

2.4 Environment, Energy and Economy (E^3)

In seeking to commercialise a chemical production process, the major aspects that must be considered are how environmentally, energetically, and economically sound a process is. These considerations are borne of the principles of "Green Chemistry". They are applied not only to reduce damaging environmental impact and energy waste but also to reduce the cost of production and create a commercially sustainable process[11].

The raw crab shell material is relatively cheap to source. However, at present, most crustacean chitin extractions are performed under harsh chemical conditions, usually at high temperatures, to remove the unwanted mineral and protein fractions at scale rapidly. This is especially the case for chitin which is intended for further processing to soluble chitosan. The quality of the chitin is not of major consideration as the de-acetylation process is particularly violent.

Thusly, the major E^3 consideration for an alternative commercial process is to reduce the use of high concentration reagents and to reduce the temperatures while still delivering a high purity product. The BlueShell extraction process was developed to do this using weaker reagents and lower temperatures to deliver high quality, high purity, and high %DA chitin. The application of mass balance analysis and LCA facilitates comparison between currently available commercial crab chitin and that produced by the BlueShell process.

3 Up-scaling for Commercialisation

Marine sourced chitin is predominantly produced from shrimp waste streams. Crab shells are massively under-utilised. The major output from the BlueShell research lies in demonstrating the commercial feasibility of the extraction of chitin, in an environmentally benign way, from crab shells.

[11] V. Ferraro, I. B. Cruz, R. F. Jorge, F. X. Malcata, M. E. Pintado and P. M. L. Castro, *Food Res. Int.*, 2010, **43**, 2221–2233.

Pilot upscaling was performed with project partners in Nofima AS, Tromsø, Norway. The up-scaled extraction was based on a lab scale chitin extraction procedure developed and optimised by TU Dublin.

3.1 Extraction Procedure

This section details the equipment, reagents and protocol used in the pilot upscaling of chitin production from the crab waste stream provided by Irish Fish Canners. The lessons learned from the pilot scheme are also discussed. The equipment necessary for up-scaling is listed in Table 1. The data from this pilot was used to undertake the LCA detailed in Section 4.3 and to guide the exploitation report in Section 4.4.

Table 1 - Equipment and Instrumentation used in up-scaled procedure

INSTRUMENT	MODEL/MAKE
REACTION VESSEL	ChemGlass 30L Jacketed Process Reactor
STIRRING ASSEMBLY	ChemStir ¼ HP Vertical Motor & Controller
HEATER	Julabo SL HighTech Heating Circulator
MASS BALANCE	Explorer Ohaus
CRUDE GRINDER	Kilia 20L Bowl Cutter Classical Series
FINE GRINDER	ColePalmer Waring 1L Blender
PARTICLE SIEVE	Retsch Vibratory Sieve Shaker AS 200 Basic
DRYING OVEN	ThermoFisher 396L HeraTherm

3.1.1 Pre-drying

Clean, whole crab carapaces were dried at 60°C for 12hrs overnight using a ThermoFisher 396L HeraTherm drying oven.

3.1.2 Grinding

Dried carapaces were crudely ground using a Kilia 20L Bowl Cutter Classical Series homogeniser. The crude ground material was then finely ground using a ColePalmer Waring 1L Blender.

3.1.3 Sieving

The ground material was separated into two distinct particle sizes using a Retsch AS 200 Basic Vibratory Sieve Shaker. Sieving was performed until there was 2kg of 0.3-1mm particles and 2kg of 1-2mm particles.

3.1.4 Demineralisation

Demineralisation was carried out the same way on both particle size samples separately. Each sample was placed in the reactor vessel. 0.5M HCl was added to each in the ratio of 5L per 1kg of dry sample. The mixtures were stirred at 240rpm at room temperature for 2hrs. After stirring, the samples were filtered and washed with process water[12] using 0.2mm mesh filter at the base of the reactor vessel. The steps were repeated twice more, to give a total of three demineralisation washes. Samples were washed with process water filtered to dryness as much as possible and stored in the reactor vessel for demineralisation.

3.1.5 Deproteinisation

Deproteinisation was carried out in the same way on both particle size samples. Each sample was placed in the reactor vessel and 0.5M NaOH was added to each in the ratio of 5L per 1kg of dry sample. The mixtures were stirred at 240rpm at >85˚C for 1hr. After 1hr each sample was cooled to room temperature using an ice bath and was filtered and washed with process water using 0.2mm mesh filter at the base of the reactor vessel. The steps above were repeated twice more, to give a total of three deproteinisation washes. Samples were washed with process water and filtered to dryness as much as possible and then transferred to a drying tray.

3.1.6 Drying

Extracted sample were dried at 60℃ for 12hrs overnight using a ThermoFisher 396L HeraTherm drying oven.

3.2 Commercial Feasibility Studies

To assess the real-world potential of scaling up and using this process to produce chitin in a commercially viable manner, two types of cost and impact analysis were carried out. The first was a simple mass balance analysis, looking at the lab scale production of chitin from crab. This incorporated material inputs, outputs and costs at a lab scale which allowed for a cost-per-gram comparison between the chitin produced in this work and the chitin already available on the market. The second analysis applied was Life Cycle Assessment using the data from the up-scaled production of crab chitin. This serves to act as the benchmark for defining the energy, material and environmental inputs, outputs, and costs of this process at scale. It is from this analysis that any future development toward exploitation of the process or products can start from.

[12] Process water is clean potable water for use in the cleaning of equipment, movement of bulk material and dilution of effluents in a manufacturing process

3.2.1 Mass Balance - Lab Scale Extraction

A mass balance simply defines the total inputs and total outputs of a given procedure[13]. Here, after defining the mass balance of the lab scale extraction optimised at TU Dublin, the costs of each starting material are assigned allowing for the determination of the cost-per-gram of chitin produced. Figure 1 details how the cost of each step was calculated resulting in the total capital cost of €3.11 per gram of dried chitin.

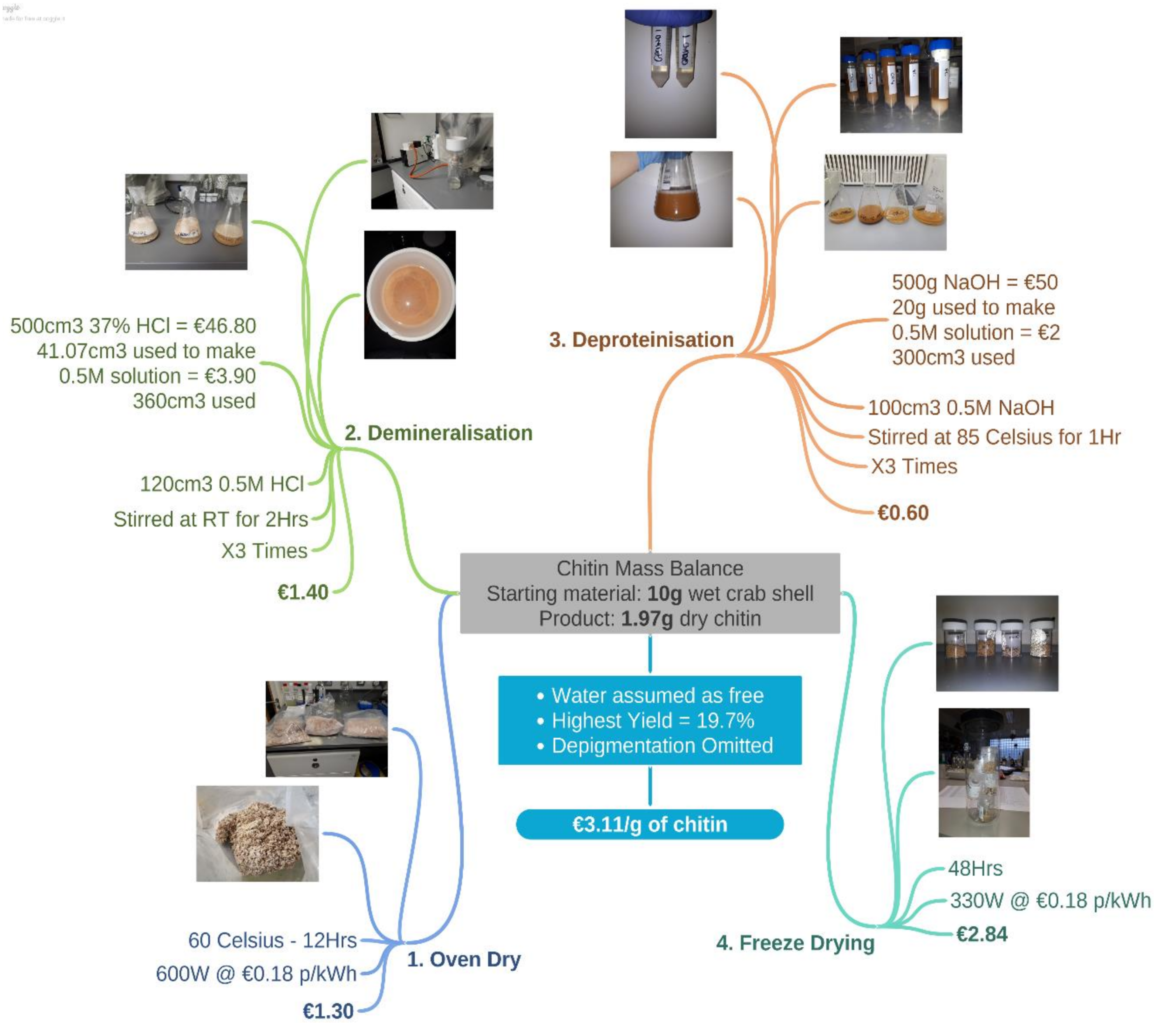

Figure 1 - Mass Balance analysis of lab scale extraction of crab chitin

3.2.2 Mass Balance – extrapolation

The mass balance exercise (section 3.2.1) was assessed in terms of performance for a scaled-up version, using the piloting process at Nofima in Tromso as the exemplar indicator. Each item in Figure 1 was critically assessed in terms of performance, cost and yield. From this, the partnership

[13] S. Okpighe, *Mass Energy Balanc.*, 2010, **5**, 84.

was able to deduce an improvement in performance in the range of 70 – 90%, leading to an estimated cost of €0.62 / gm of dried chitin.

Knowledge of the cost-per-gram allows for a quick comparison with chitin products currently on the market and helps to understand how close BlueShell is to commercially feasibility. The comparison indicates reasonable price competitiveness on a scaled volume basis with producers who are addressing the high-grade market (e.g. Merck and MP Biomedicals). Currently, there are no crab chitin products listed for sale by any producers in Europe and so Table 2 and Figure 2 list prices for shrimp chitin produced in Europe and America and crab chitin produced in Asia. Indeed, BlueShell could be the first European producer of crab chitin.

Producer	Source	Price per gram (Euro)
BlueShell Laboratory	Crab	3.11
BlueShell Scaled Volume	Crab	0.62
Merck High Grade	Shrimp	0.87
Merck Low Grade	Shrimp	0.21
MP Biomedicals High Grade	Shrimp	3.32
MP Biomedicals Low Grade	Shrimp	0.81
TCI America	Shrimp	0.68
Xi'an Huilin Bio-Tech Co., Ltd.	Crab	0.08
Xi'an Ceres Biotech Co., Ltd.	Crab	0.13
Beijing Be-Better Technology Co. Ltd	Crab	0.09

Table 2 - Price comparison of crustacean sourced chitin products currently available.

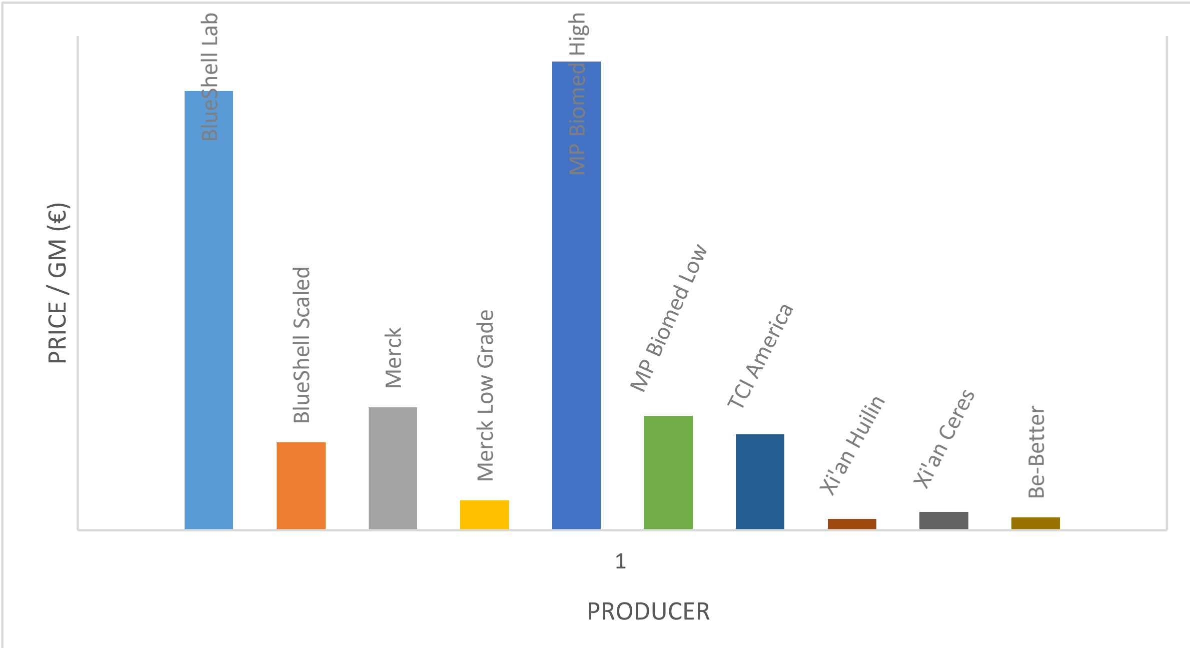

Figure 2 - Price comparison of crustacean sourced chitin products currently available

Note that major suppliers, Merck and MP Biomedicals, supply both a low grade and a high-grade product. This demonstrates well the price range accessible depending on the quality and purity of chitin produced. It is surprising that the chitin produced at lab scale by the BlueShell project is slightly cheaper than the high-grade MP Biomedicals chitin, which is encouraging. The estimated volume-scale pricing for high-grade BlueShell chitin, compares favourably with the market leaders, while, in addition, providing an environmental bonus (see LCA, next section). Of general concern are the three Asian producers, all of whom use crab as a starting material. Their price points are low because of the volumes produced. However, their market target is high-volume low-grade, which is not the positioning expected for BlueShell. In a way, BlueShell has the potential to be unique as a high-grade producer of chitin using crab waste, with exceptionally low environmental impact.

Further investigations are required to assess the actual quality and purity of competitive products. This could comprise part of a demonstration initiative.

3.2.3 Life Cycle Assessment - Up-scaled Extraction

To better understand the material and energy costs associated with production at scale, a Life Cycle Analysis (LCA) was undertaken using the data from the up-scaled extraction procedure detailed in Section 3.1 of this report. Ignoring the capital costs involved, the major goal of the LCA was in quantifying the environmental impact and the major energy sinks of the process. This analysis acts as a benchmark for the reliable chemical production of pure and high-quality chitin when assessing the feasibility of the experimental enzymatic or bacterial digestion techniques applied by other partners in the BlueShell project.

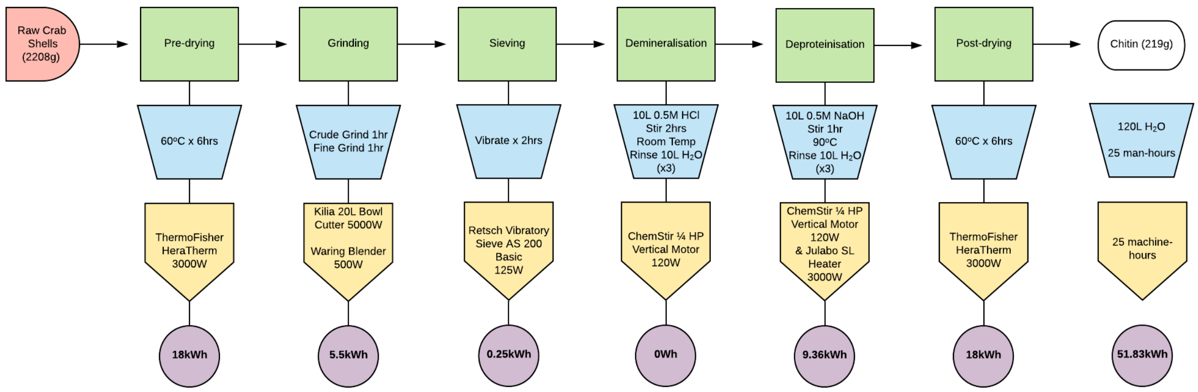

Figure 3 - Process Flow Diagram of up-scaled chitin production

The LCA was carried out using the SimaPro 7 software and database. Two commonly used, reliable environmental impact methods, Eco-indicator 99 and EPD 2008, were applied[14]. Aspects of the process considered included electricity consumption, raw materials, transportation, final products, and wastes. The environmental impacts associated with each of these aspects were derived from the SimaPro 7 database. The process flow of the scaled extraction, which includes all the material and energy inputs and outputs, is seen in Figure 3.

The process required 4 workdays to complete and produced 219g of chitin. The LCA model is based on monthly production capacity, taken as 20 workdays, and as such the total mass of product considered is 1.1kg. All LCA data is determined and presented with respect to this amount of chitin product.

The total inputs assigned with their environmental impact weighting can be seen in Figure 4. The input with the greatest impact is the electricity required to run the process (12.85Pt). It is also noted that the fact that the crab shells have been diverted from landfill reduces the extent of the environmental impact of the extraction process (-0.14Pt).

[14] D. V. Louzguine, A. Inoue, M. Saito and Y. Waseda, *Scr. Mater.*, 2000, **42**, 289–294.

In examining the nature of the environmental impacts, the Eco-indicator 99 methodology allows for comparison of the categories across which each aspect has an impact, as seen in Figure 5. This allows for a better understanding of exactly what the consequences are from utilising the different inputs of the process. In this process in particular, the high electricity consumption and

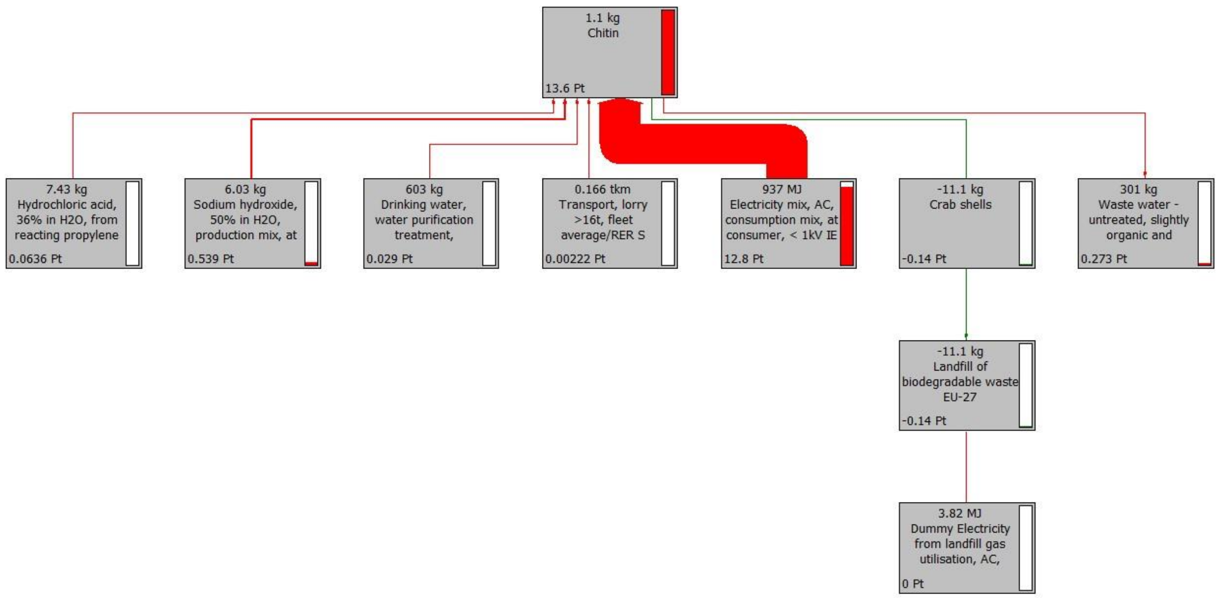

Figure 4 - Environmental impact assignations for total inputs

the use of low concentration corrosive reagents have the greatest degree and range of impacts.

The total environmental impact of the process is represented by a single value known as the Eco Point (Pt) which is calculated using the Eco-indicator 99. The production of 1.1kg of chitin from crab shells in by this process is assigned an Eco Point of 13.61Pt, which is considered “moderate” by the Eco-indicator 99 assignation.

The inputs with the greatest environmental impacts are electricity consumption and use of HCl and NaOH for the demineralisation and deproteinisation, respectively. The greatest

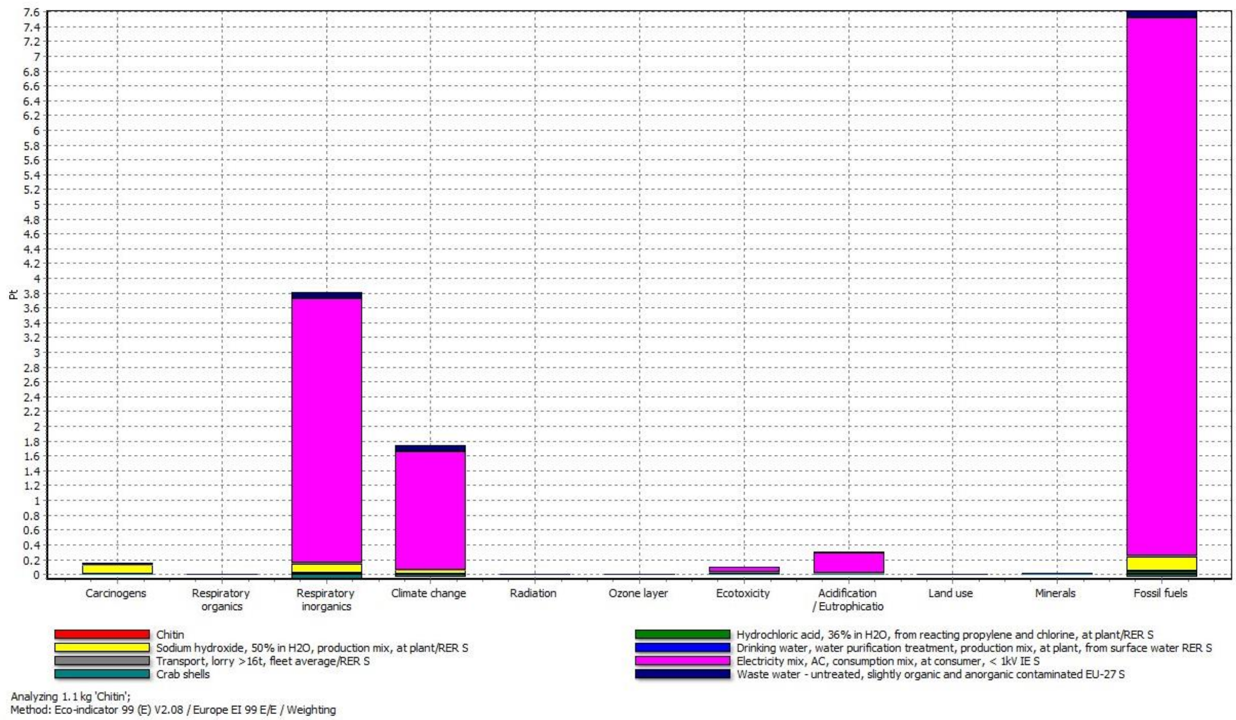

Figure 5 - Categories of environmental impact

environmental impact because of the above inputs is fossil fuel consumption to produce electricity and the production of industrial chemicals. Use of fossil fuels has associated impacts, specifically production of polluting emissions which are carcinogenic, reduce air quality, induce respiratory problems, and contribute massively to climate change. Similarly, use of HCl, NaOH and the process water in large quantities have secondary associated impacts, specifically contributing to acidification and eutrophication in the marine and inland aquatic environments.

Application of the EPD methodology also identifies that the highest impact is related to use of non-renewable fossil fuels in the production of electricity. The EPD methodology determined the specific amount of CO_2 emitted to the atmosphere because of the production of 1.1kg of chitin as 239.4kg CO_2-equivalent. To put this in perspective, a single tree can absorb CO_2 at a rate of approximately 22kg per year. This requires 11 trees absorbing for a whole year to offset the amount of CO_2 produced by the BlueShell process[15].

[15] H. K. Jo, H. M. Park and J. Y. Kim, *Sustain.*, , DOI:10.3390/su11133543.

3.3 Lessons Learned from Upscaling

As is often the case when scaling a process, certain challenges arise thanks to the change in physio-chemical environment at scale. The up-scaled process detailed in this report was scaled 400 times from the lab scale extraction. Discussed here are the major lessons learned from the scale up and recommendations for future work at production level.

Washing between each demineralisation step was found to be fundamental for the HCl to perform ideally. The reason for this is that each wash results in the formation of conjugate bases which reduce the efficacy of fresh HCl. Similarly, there is a high likelihood that when no washing occurs between HCl treatments the free minerals liberated in the previous HCl treatment remain adsorbed to the surface of the crab mass. This results in the fresh HCl in subsequent washes acting predominantly on already liberated minerals, thus resulting in little demineralisation of the remaining bound minerals. Application of higher temperature HCl washes may help as this method uses a HCl solution four times weaker than current methods discussed in the literature. Similarly, at scale, use of an elevated temperature of around 60°C makes it a lot easier to handle crab mass. Greater mixing occurs as the raw material is less likely to settle in the reactor and the reaction proceeds more continuously. However, use of higher temperature can results in higher energy costs and increased environmental impact.

During demineralisation, foaming is a recurring issue. To mitigate this, 5l of water was added first followed by the addition of 5l of 1M HCl to dilute to a final concentration of 0.5M. This gives greater control over the foaming, however there is a minimum threshold of acid concentration below which no reaction occurs. Once this threshold is reached, foaming occurs but in a more controlled manner than when directly applying the 0.5M HCl solution.

The application of alternating demineralisation/deproteinisation steps should be considered at scale. This may allow the HCl and NaOH to penetrate the shells deeper and more effectively unwind the protein-mineral-chitin nanofiber structure. Alternating washes may likely reduce the necessity for 3 washes of each treatment type therefore reducing overall HCl, NaOH and water consumption. However, to maintain efficacy of each treatment, thorough washing with process water between each step must occur.

Both large (1-2 mm) and smaller (0.3-1 mm) pieces of shell were used as starting material for extraction. It appears HCl is less effective in solubilising minerals in larger pieces. Therefore, mechanical grinding as a pre-treatment is advised to expose the greatest area of the protein-mineral-chitin structure to enhance the efficacy of demineralisation and deproteinisation.

4 Routes to Exploitation of BlueShell Results

4.1 Exploitable Results

The major exploitable outputs are:

1) Chemical extraction technique for crab shell chitin at TRL 5 which is more E^3 friendly than other chemical extraction processes used.

2) High quality, high purity, and high %DA crab shell chitin for use in a wide range of downstream products and processes.

3) Accurate and precise analytical suite of techniques allowing for characterisation of chitin produced from both chemical and biological extraction techniques.

4) National Funder reports, EU Interim reports, publications, and public outreach forming a base of knowledge and stakeholder awareness from which future work may progress.

4.2 Moving forward on these results

Due to the limited volume at which up-scaling was performed (TRL5 at best), there is limited scope for patenting, licensing, or selling the extraction technique. Further funding is required to bring the process to pilot scale and to increase the TRL to 6 or, ideally, 7. A non-exhaustive range of potential Irish and EU enterprise and innovation funding includes:

- Enterprise Ireland Innovation Voucher, Innovation Partnerships and R&D grants (if SME involved in exploitation)
- BIM Innovation Platform
- EU Framework Programme 9
- EU Blue Bio Initiative
- EU Research Initiative Actions
- European Research Council Grant
- Irish Research Council Grant
- Royal Society of Chemistry Innovation and Technology Development Grant

Ideally development funding in conjunction with an industrial partner would be sought. To fully exploit the commercial potential of the results of this project a partner is required with experience in up-scaling, process development, sales, and marketing.

4.3 IP Considerations

Advice and guidance were sought from the TU Dublin Hothouse office, which specialises in Intellectual Property considerations, patent application and invention disclosures. Examination of the exploitable results from this project did not warrant patent application or invention disclosure however it did encourage further development of the production process. Although the material used and the product produced were not sufficiently different from what is already in the public forum, the way in which the steps were applied was considered innovative and thusly encouraged to be kept within the project consortium and project reports. The principle of informing as to "what was done but not how it was done" is therefore applied and is covered under the IP agreement established by all consortium partners at the outset of the project proposal.

4.4 Policy Considerations

The further development of an up-scaled commercial crab chitin process is supported by several pieces of European and Irish legislation and policy. This is especially so in the context of applying the E^3 principles in seeking to achieve sustainable commercial production. Such policies include:

- UN 2030 Sustainable Development Goals
- EU Innovation 2020
- Water Framework Directive
- Maritime Spatial Planning Directive
- National Marine Planning Framework
- Euro Green 2020
- BIM Seafood Innovation and Business Planning Scheme
- National Waste Management Strategy
- Ireland 2040
- Fianna Fáil, Fine Gael, Green Party Programme for Government 2020

Not only do these policies support the exploitation and development of this work but this project enhances the scope of many of the policies.

4.5 Considerations for future work

There exists a series of considerations which ought to be addressed for future exploitation:

- Sourcing raw crustacean material from other end users, e.g. restaurants, home waste and recycling centres. This would involve setting up a relatively sophisticated supply chain, involving segregation and pre-sorting.
- Sourcing raw crustacean material from other producers both in Ireland and further afield to expand the application of the technologies to other species and waste streams.

- Investigate the feasibility of financing and developing an onsite or central processing facility in Ireland to allow crab processors to directly control chitin production and generate a new source of income.

5 Conclusion

Based on this report some key conclusions can be drawn regarding the commercialisation of chitin production from crab waste stream material:

- There is a good supply of low cost raw starting material, however this is not easily accessible outside of crab processing factories.
- There is strong demand for high-quality chitin product for use in specialised materials.
- Price competition is based on quality and purity. The BlueShell chitin product needs to focus on only the highest quality and purity.
- Key aspects for reducing environmental impact have been identified.
- The current process is at TRL 4/5. This needs to be extended to 6/7 to ensure exploitation.
- There are ample policy and funding supports to progress this work to commercial production, supply, and sale.