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SR653 - Evaluation of ‘free of flesh’ shell criteria; implementation and uptake evaluation

Aquatic Water Services Ltd

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FINAL REPORT

EVALUATION OF 'FREE OF FLESH' CRITERIA IMPLEMENTATION AND UPTAKE EVALUATION

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For

Sea Fish Industry Authority

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WATER SERVICES LIMITED

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WATER SERVICES LIMITED

EXECUTIVE SUMMARY

In 2010 Sea Fish Industry Authority commissioned Aquatic Water Services Ltd (AWS) to undertake a study to help define Free Of Flesh (FOF) status for shell waste for a variety of shell types and a number of prescribed cleaning processes (Ref: FitzGerald , 2010). This work was required to maintain an exemption for 'clean' shell use for Technical Purposes within the Animal By-Product Regulations.

The findings of the initial study showed that whilst many shellfish types could be effectively cleaned some species were both hard to clean and difficult to assess their FOF status due to high residual test levels of flesh test parameters within shells. This project represents a continuation of this project to help find a way forward with these problem areas by developing an alternative testing protocol based upon the use of a leach methodology and to explore options for a combination cleaning process which can produce FOF shell for difficult to clean shell types such as crab.

A hybrid cleaning process consists of two stages:

-Stage 1. A Physical cleaning stage involving a washing process whereby flesh components are transferred from shell to water. This could include a floatation method such a brine or Dissolved Air Floatation.

-Stage 2. A Degradation stage involving the breakdown of residual flesh from the shell. This could involve the use of microbes, enzymes or specific chemicals.

The objective of this study was to test the potential components of a hybrid cleaning process in order to assess whether a suitable combination process could be produced to enable a FOF cleaning process for difficult to clean species such as crab.

The previous FOF study had demonstrated that protease enzymes were effective in polishing shell to reach FOF standards. It was therefore reasoned that it could be possible to use this medium to target flesh removal from shell to allow indirect testing of flesh content. However, despite the enzyme effectively dissolving the flesh the current study has not been able to develop an effective leach methodology. In consequence, Crustacean species with high residual organic level remain both difficult to clean and difficult to demonstrate FOF status.

Direct shell testing indicated that a hydrid cleaning processes can remove most flesh to <3% flesh in crab although testing lacks the precision (due to high residuals) to confirm FOF status. Crab was found to be a challenging shell type to clean as each sub-component (e.g legs, claws, carapace, tails and pouch) presented distinct properties. However, a number of findings were obtained:

-*Stage 1, Pre-crushing.* The success of a hybrid process is strongly dependant on optimising flesh removal in the Stage 1 Physical process which in turn is driven by a pre-cleaning crushing stage to liberate flesh trapped in enclosed shell components.

-*Stage 1 Washing*. Resuspension of fine flesh particles and soluble components removed a significant proportion (~1.5%) of flesh in a liquor. However, dense threads of white meat remained closely associated with the shell.

-*Stage 1 Floatation.* Although brine floatation works with mollscan flesh it was not effective at density separation on crab meat. Dissolved Air Floatation (DAF) was effective at floating off both white meat and with the addition of polymer fine particles from the liquor. However, it could not liberate these components when they were 'tapped' within a mass of shell fragments.

-*Stage 1 Vortex Separation.* Floatation in an agitated upwelling system using a hydrodynamic floatation method was effective in separating dense white meat from crab shell fragments.

-*Stage 2 Enzyme Degradation.* Enzymes rapidly broken down flesh components when presented in small fragments and was an effective second stage cleaning method even without warming of the solution.

-*Stage 2 Microbial Degradation.* A bacterially mediated breakdown method in an aerated system did remove both odour and flesh fragments although a couple of days were required for effective removal.

Whilst a hybrid cleaning process possibly could provide FOF shell in a difficult to clean shell type such as crab its use must be considered in a commercial context. Some components of crab (e.g tails) are particularly difficult to effectively clean and could magnify the system requirements for a combined waste thereby undermining financial viability. Alternative economic options may exist which could use the hybrid technological approach:

-*Separation of stages*. The Stage 1 Physical process with crushing could be used for compaction of waste volume with flesh separation for possible utilisation of flesh. Stage 2 polishing of shell to attain FOF status would then need to be based upon economic returns for the FOF shell by-product.

-*Separation of shell components.* Some components are easier to clean than others (e.g claw) with other components remaining difficult to clean even after crushing (e.g tail). It may therefore be more cost effective to segregate waste and not treat these difficult components.

-*Optimisation for specific shell components*. Targeted cleaning of crab shell sub-components with potential value (e.g carapace) could focus on a washing followed by an enzyme treatment to present advantages over traditional chemical methods.

-*Optimisation for specific by-products*. High quality flesh by-products may have greater value than FOF shell and as such the separation process may be optimise removal of this fraction.

In summary, the economics of producing a FOF hybrid process are challenging for difficult to clean shell types such as crab. There could be scope for an individual processor to tailor a cleaning process to achieve their own specific needs, or for perhaps a regional facility to handle bulk quantities of material from a number of contributing processors. In both cases success of the scheme is likely to be influenced by segregation of the waste streams by the processor at point of production.

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‘FREE OF FLESH’ SHELLFISH WASTE CHARACTERISATION

1.0 INTRODUCTION

1.1 Overview

Aquatic Water Services Ltd (AWS) was commissioned by Sea Fish Industry Authority in January 2010 to undertake a project to assess shell cleaning processes and to undertake testing on a range of different shellfish to determine suitable criteria and tolerance limits to help define Free Of Flesh (FOF) shell. This project further explores some of the limitations raised in the initial study.

The current Animal By-products Regulation (ABPR) permits the use of ‘Free Of Flesh’ shell for certain defined technical uses. However ‘free of flesh’ is not defined and it is currently unclear which shellfish can be produced to free of flesh standards. Historically, ‘free of flesh’ was taken to mean free of visible signs of flesh (i.e. a visual inspection) but there were concerns from the environmental regulators about whether the legal framework was effectively interpreted and implemented.

The ABPR was revised in 2011 and allows Member States to define ‘free of flesh’ and develop their own standards within the implementing regulation. Shell which achieves a ‘free of flesh’ status can be taken out of the scope of the ABPR. The findings of the previous study (Ref: FitzGerald, 2010) have been adopted by Seafish within their guidance (Ref: Sea Fish) and in turn by DEFRA (Ref: DEFRA).

Ref: Sea Fish (http://www.Sea Fish.org/media/Publications/FS58_10_10_defining_free_of_flesh_shell.pdf)
Ref: Defra (http://animalhealth.defra.gov.uk/managing-disease/animalbyproducts/disposal-of-shell-fish-shells.htm)

The previous FOF project reviewed the FOF status and criteria of a full range of shellfish types and cleaning processes with the objective of allowing regulators to define FOF to maintain the current exemption under ABPR. The intrinsic nature of the shell presented a complex residual background which lead to the conclusion that only a range of chemical and physical observations could be used to determine FOF status and no one parameter was wholly effective in determining FOF status was possible when directly assessing shell. In addition, the study highlighted which shellfish types presented difficulties along with which cleaning processes/combinations which could potentially achieve FOF status.

This follow-on project seeks to build on the previous study by assessing two key components:

-Developing and evaluating an enhanced FOF protocol
-Evaluating the degree to which FOF status can be achieved in difficult to treat shellfish waste

1.2 Standardisation of ‘Flesh’ Leach Test

1.2.1 Leach Test Requirement

Residual organics and protein are present not only in flesh but also in the shell itself – particularly in the case of crabs. In consequence, the previous study identified that direct testing of these parameters in the shell was not reliable for some species (e.g such as Crustacea). It was suggested that a more representative way of assessing FOF status in these difficult types of species may be to develop a leach methodology which can selectively target the flesh stripping it from the shell so allowing testing of a liquor sample free from the interference of the shell.

1.2.2 Enzyme Leach Testing

A number of leach options were tested in the previous study (Ref: FitzGerald 2010) with varying levels of success. Some leach options were effective at liquidising the flesh (e.g sodium hydroxide) but were discounted as they can also leach test products from shell as well as flesh in addition to making the resulting liquor difficult to test.

Enzymes selected to break specific proteins were found to be effective in the breakdown of flesh in the previous study. Commercially available protease enzymes can be obtained in a consistent strength standardised at 100TU (Trypsin Units). The product 439 is available from a UK company (Biocatalyist Ltd) and was utilised in the current project.

1.2.3 Leach Optimisation

The use of a standard strength protease solution allows testing of a range of dilutions to assess the optimum concentration / temperature profile. Once an effective concentration/temperature profile has been identified the process can be further optimised by testing various leachate:shell ratios. The objective of the leach optimisation was to attempt to provide a simple but effective methodology which could be easily replicated on a variety of shell types.

1.2.4 Leach Analysis

Previous shell testing options included organic content (i.e volatile solids from ashing), total nitrogen, ATP and direct protein test kits. Whilst organic volatile content was found to be the best laboratory based parameter in the direct shell testing total N may still have some merit once removed from the shell matrix and was therefore included in the testing regime.

Through the course of the study Total Carbon and COD (Chemical Oxygen Demand) were also explored as alternative measure of organic content. COD is commonly recognised wastewater parameter to assess total organic content but is likely to overestimate flesh content as even refractory organics will be oxidised. BOD (Biological Oxygen Demand) was also considered as it is a widely accepted measure of readily degradable organic content. However, the BOD test requires respiration of active bacteria which is likely to be compromised in the enzyme degradation processes tested which would tend to be supported by the limited test results (Appendix A4).

1.3 Hybrid Cleaning Processes

1.3.1 Cleaning Process Components

The previous study identified three classes of cleaning process:

- Cook Separation
- Physical Washing
- Chemical/Biological Degradation

Whilst some shellfish types could be effectively cleaned by a single process some complex shell types such as gastropods and crustacea are difficult to clean to ensure meeting FOF grade. This study explores options for a hybrid cleaning process bringing together a combination cleaning process.

1.3.2 Shellfish Types

FitzGerald 2010 showed that crab was one of the most difficult species to effectively clean. This is result of a number of factors:

-Shell form is complex with difficult to reach components (e.g joints in claws)
-Dirty shell with a high level of organic debris
-Difficult to test shell with high levels of residual organics and protein

As crab is also a major species landed with limited and expensive disposal options, there is a pressing commercial reason to resolve the issues for this processing sector.

1.3.3 Staged Process Development

Crab waste is typically cooked as part of the normal crab boiling process and as such a 'cook separation' process (such as is effective for cockles and queen scallop) is unlikely to provide any further benefits for this species. Therefore a physical separation and degradation process combination is considered further through a series of benchtop testing. Process optimisation requires balancing both processes as reduced performance in stage 1 would increase the demands on stage 2. An outline of both stages is provided below:

-*Stage 1 – physical separation (brine floatation / Dissolved Air Floatation).*
This stage will involve the use of a water based washing phase followed by a physical process to move the flesh away from the shell to allow separation. Some flesh types float away from shell in a concentrated brine allowing removal with the supernatant. An alternative floatation method is Dissolved Aeration Floatation (DAF) which is a common waste water cleaning process which is particularly effective for organic debris. The DAF method relies on the injection of supersaturated 'white water' which allows microbubble formation on particles allowing debris to float to the surface to form a scum which can be removed. Performance of DAF can be enhanced for fine particles by the addition of specialist polymers which help for larger flocs more capable of bubble retention.

-*Stage 2 – biological / degradation (enzyme / microbial)*.
The previous study showed that specialist protease enzymes can be effective in degrading flesh into a liquor allowing washing out. Degradation can also be brought about by bacteria which can also secrete proteases to breakdown food products.

2.0 LEACH TEST DEVELOPMENT

2.1 Testing Methodology

2.1.1 Shell:Leachate Ratios

Based on the previous study a 1:4 w/w shell:leachate ratio was adopted as a starting point. This provided sufficient volume of leachate to complete immerse all shell types tested whilst maximising concentration of the test substance in the leach liquor. Testing was performed in 1L Duran pyrex bottles with 200g of shell :800ml of liquor (see Plate 2.1).

2.1.2 Digestion Temperatures

Enzyme performance is temperature dependant to an optimum of 50°C. Ambient testing (at room temperature of 20°C) was compared to the optimum 50°C using a hot plate. As availability of temperature control is unlikely for most prospective testing, a simple method was sought to try and increase the temperature profile and 'kick-start' an enzyme leach. To this end a 'boil start' method was developed which involved immersing the test shell in a quantity of boiling water (minus the volume of enzyme) at the onset of the test. This was also seen as potentially helping to liberate and re-hydrate flesh prior to digestion. Care was needed to avoid adding the enzyme at the onset of adding the boiling water as enzymes are specialist proteins themselves which are denatured by heat. In consequence, cooling profiles were studied in order to ensure that enzymes when added would provide a 50°C starting temperature. It was found that 30 minutes cooling time was required to allow the shell boiling water temperature to drop to a 'safe' level before the addition of the enzyme which was added chilled (as stored in the fridge to maintain integrity).

2.1.3 Leach Concentrations

Enzyme performance will be dependant on the relationship between enzyme and substrate quantity and form (see Section 2.1.4 below). With low flesh levels an accordingly low concentration of enzyme may be sufficient to break down flesh. In contrast, excessive high flesh contents may exceed enzyme breakdown capacity in a weak solution. Leach concentrations of 1%, 2.5%, 5%, 10% and 20% were tested.

2.1.4 Shell Form

It should be noted that the physical form of the flesh substrate is critical to enzyme performance as big chunks of flesh will present a much lower surface area for active degradation than for fine particles. In consequence, there is likely to be a need to crush test shell to some degree as a physical pre-treatment to the leach test. During the current tests test samples were crushed to pass a 12mm (~0.5 inch) grade grid.

2.1.5 Leach Analysis Method Screening

Initial tests performed on the organic content of the shell leachate were made by testing Total Carbon and Total Nitrogen (TN). Results provided conflicting results (see Section 2.2.2, Appendix A1) and there was concern that the testing methodology was compromised by the shell matrix. In order to explore this hypothesis some simple flesh surrogate (crab-stick) and direct crab flesh tests were performed with a wider range of test parameters (including COD). The crabstick surrogate, although bearing no relationship to actual crab flesh, was of a consistent quality and form which was easy to measure and visually observe digestion.

2.1.6 Controls

Tap water blank controls were tested against enzyme solutions to assess leaching potential within some tests. In addition, enzyme control samples were also needed to be submitted for testing to assess organic and total N content of the enzyme solutions themselves. Controls of 1% and 20% were tested and analysed for Total Carbon, Total N and COD.

A crab flesh sample of known mass was also used as a 'control' for a complete digest. The flesh sample was liquidised in a blender and submitted for analysis.

2.1.7 Flesh Leach Methodology

In addition, to the shell leach tests some crab flesh leach tests were also undertaken to assess leach performance without the potential interference from the shell matrix. As with the shell testing 1L test volumes were utilised within Duran bottles (see Plate 2.1) although for 'flesh' tests a much smaller quantity of flesh (12-20g) replaced the 200g of shell to emulate a similar flesh content (i.e <10% flesh).

100ml sub-samples obtained after set periods and diluted with water to 500ml before being submitted for analysis. Results were factored by x5 to correct concentrations to the original pre-dilution level.

As with shell leach results enzyme sample results were also corrected for the contribution of the test parameter derived from directly from the enzyme by using the enzyme control results (see Section 2.2.1).

2.2 Leach Results

2.2.1 Controls

Enzyme Controls

Enzymes are proteins which themselves contain Total Carbon and Total N and as such controls of enzyme solutions were analysed to allow corrections to be made to the shell leachate results. Controls of 20% and 1% were tested and analysed for Total Carbon, Total N and COD with the results shown in Table A1 and A3 respectively. Total Carbon and Total N for intermediate enzyme concentrations (2.5%, 5% and 10%) were calculated by factoring the enzyme control. Cross comparison between the x2 enzyme controls gave good agreement for Total Carbon but more uncertainty for Total N as shown below:

Enzyme Conc.	*Source*	*Total Carbon*	*Total N*
5% (calc.)	20% Sample (Table A1)	7189 mg/l	268 mg/l
5% (calc.)	1% Sample (Table A3)	7250 mg/l	170 mg/l

Water Controls

The previous FOF study included water leaches which were shown to be ineffective in removing all observed flesh. In consequence, only limited number of tap water blank controls were tested in order to provide some comparison against enzyme performance (see Appendix A). The water controls showed a gradual leaching of the test parameter over time (see Figures 1 and 2: data series shown in yellow) which in most cases were lower than the corresponding enzyme leaches.

Flesh Controls and Comparison with Empirical Factors

Crab flesh samples were submitted both as 'solid' samples (see Table 1) and as a liquidised sample (see Table A4). Flesh results have been used with empirical data to provide a 'theoretical' complete digest level whilst the liquidised flesh results have been used to provide a 'sampled' complete digest level within the flesh leachate tests (see Figure 2).

Table 1 results for the crab flesh show good agreement between the vortex separated and the shop purchased crab with ~38.5-38.8% Total Carbon. It should be noted that of the 87.6% volatile solids 38.8% was therefore Total Carbon. The remainder of the 87.6% is therefore attributable to nitrogen, hydrogen and oxygen. The 12.4% of ash will be from phosphorus, salt and other trace minerals.

Table 1: Analysis of Crab Flesh

Sample ID No	Description	Total C (mg/kg)	Volatile Solids (500 C) %	Total N (mg/kg)
2438157	Crab meat - vortex separation	385120 (38.5%)	-	>103600
2446562	white crab meat -Purchased	388360 (38.8%)	87.6	>103600

It is interesting to compare these test results with the theoretical empirical levels of Total Carbon and Total N. It is not possible to provide an exact C:N ratio of protein as each amino acid has a slightly different formula. The common amino-acid glycine C_2H_5NO) has a Total Carbon content of 40.7% - which is very close to the 38.8% obtained from testing. It is therefore possible to calculate potential volatile solids (% flesh) by factoring up the Total Carbon % by x2.5. It is also therefore proposed to utilise the empirical relationship (i.e Total N content of 23.7%) for Total N to calculate potential volatile solids (% flesh) by factoring up the Total N % by x4.2.

2.2.2 Crab Shell Leaching

Table 2 presents the programme of enzyme testing for x7 conditions under ambient, 'boil start' (see Section 2.1.2) and 50°C held conditions. Samples were periodically removed at various time intervals over a 48hr period. Physical observations were made on all tests and strategic samples were sent for analysis.

Table 2: Summary of Shell Leach Testing and Analysis Programme

Test	Enzyme Conc.	T0	30 mins	1hr	24hr	48hr
Ambient	5%	x	x	x	x	x
Ambient	10%	x	x	x	x	x
Ambient	20%	x	x	x	x	x
Boil Start	5%		x	x	x	x
Boil Start	10%		x	x	x	x
Boil Start	20%		x	x	x	x
50C held	20%		x	x	x	x

Key:		
	x	*Test run*
	x	*Test Sample sent for analysis*

Total Carbon results when corrected for the original enzyme contribution were often negative raising the possibility of some unforeseen reaction (e.g liberation of CO_2). Corrected Total N results were generally positive numbers although with no discernable trend and are therefore not plotted. In view of the first batch of results (Table 2 shaded cells) no further retained samples were submitted for analysis. Results are discussed in Section 3.1.

Some further direct testing of shell has been undertaken (pre and post enzyme leaching) to help determine result viability on a mass balance basis (See Section 3.2.1).

2.2.3 Crab Surrogates Leaching

A sequence of typical digestion is illustrated in Plates 2.2, 2.3 and 2.4 for T0, T6 and T24hrs. As can be seen enzyme action was rapid leaving no discernible solid after 6hrs.

Plate 2.1
Shell Enzyme Leach

Plate 2.2
Crab Stick Enzyme Leach – T0

Plate 2.3
Crab Stick Enzyme Leach – T6

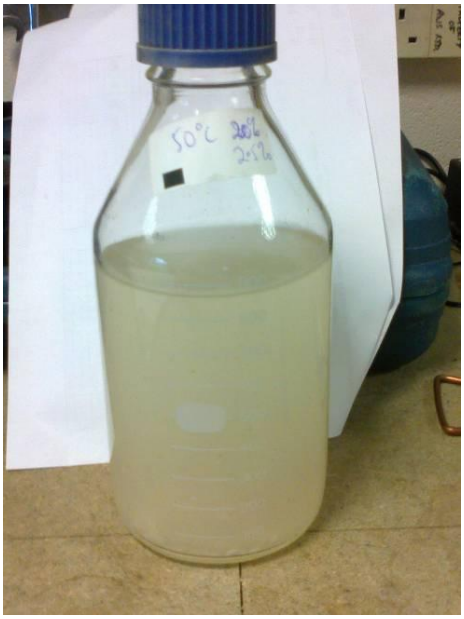


Plate 2.4
Crab Stick Enzyme Leach – T24
(Note: clearer than T6 – more dissolved?)

It should be noted that the control test with a tap water leach remained completely intact after 24hrs soaking. Analytical results are listed in Table A2 and presented for Total Carbon, COD and total N in Figure 1. Generally positive results were obtained with credible trends. Control samples exhibited a low but gradual increase as soluble components leached into the water. Enzyme leaches generally increased in parameter concentrations in line with visual observations of degradation. However, as the same starting mass of crabstick was used for all tests a constant concentration end point would have been expected for all tests which undermined the result viability.

2.2.4 Crab Flesh Leaching

Test Set 1

Crab flesh obtained from previous physical separation exercises (see Section 2.2.1) was utilised in a further series of enzyme leach tests to try and provide some quantitative relationship for real 'flesh' in the absence of shell material. Results are listed in Table A3 which showed conflicting performance for the different test parameters. In general Total Carbon and COD results were variable with some negative levels once corrected for enzyme contribution. In contrast, Total N results showed a consistent trend with increasing levels as shown in Figure 1. Unfortunately, all tests should ideally leach a similar level of total N which is not indicated from the results. Indeed the 'control' sample in tap water showed a higher increase in total N than some of the ambient temperature enzyme leaches – despite the fact that visually all the enzyme crab samples showed the removal of the flesh whilst the control still retained the flesh. It should be noted that the 'flesh' samples did however still contain observable shell fragments and ligaments which were clearly visible following enzyme digestion. It is possible this factor may have compromised some of the consistency between samples although subsequent flesh sample analysis comparison between Test Set 1 and Test Set 2 (see Table 1) indicated minimal variation in flesh quality.

Test Set 2

Some further testing with exactly measured quantities of purchase crab flesh (i.e with no crab shell) were repeated to try and provide more reproducible results and remove potential uncertainty related to shell interference. 20g of purchased white crab meat were accurately measured using a milligram balance and retested using the same enzyme test criteria as explored in Test Set 1.

Results are listed in Table A4 with timeseries plots for Total Carbon, COD and Total Nitrogen presented in Figure 2. In addition, to the tap water leach blanks 'theoretical' and 'sample' maxima levels for complete digestion are also plotted to provide an indication of leach efficiency. These results are discussed in Section 4.1.

2.2.5 Scallop Test Sample Leaching

A FOF king scallop sample was obtained to provide some comparison between the direct shell testing and indirect leachate testing. The limited results (see Table 3) from the scallop leach indirect testing do generally concur with the direct shell testing indicating that ~0.5% flesh was present. As can be seen the Total Carbon and Total N results for the direct testing were below the laboratory reporting limit with limited resolution on the volatile solids. The leach results show a much higher level of analytical resolution at this low flesh level highlighting the potential advantage of leach testing.

Table 3: Scallop Direct Shell and Indirect Leachate Comparison

Sample ID No	Description	Total C (mg/kg)	Volatile Solids (500 C) %	Total N (mg/kg)
2446566	Direct testing - *Note 1, 2*	<7500 (<1.88% flesh)	0.5	<2600 (<1.09% flesh)
2288858	Indirect testing - (200g in 1L) *Note 1, 2, 3*	494 (0.62% flesh)	-	13 (0.02% flesh)

Note 1: Total C converted to flesh using x2.5 empirical factor (See Section 2.2.1)
Note 2 Total N converted to flesh using x4.2 empirical factor (See Section 2.2.1)
Note 3: As 200g sample results are x5 to provide output in mg/kg

Unfortunately, the uncertainty over the wider reproducibility of the leach methodology currently limits the extent to which this technique can be applied.

3.0 HYBRID CLEANING PROCESS

3.1 Physical Separation

3.1.1 Crushing

Effective flesh removal with any physical separation process is dependant on an effective crushing process prior to separation to allow liberation of flesh from enclosed shell fragments (e.g joints of claws). In order to provide a source of crushed crab a shell crusher was designed in consultation with Hydrok Ltd (Plate 3.1). The crusher consisted of a hopper descending to dual rotating rollers equipped with interlocking breaking bars. Roller gap and bar separation was selected to allow breaking/snapping of shells without excessive crushing (Plate 3.2). Broken shell fragments then dropped into a central hopper for collection.

Plate 3.1
Shell Crusher
(Note: horizontal breaker blades)

Plate 3.2
'Single' Crushed Crab Shell
(Note: intact leg in centre of frame)

Plate 3.3
Ineffective Crab Claw Crushing
(Note: flesh still encased in shell/ligaments)

A number of factors influenced crusher performance. A higher feed rate, or slower rotation speed, provided a much higher crushing potential as more material is squeezed in between the roller gap. Particle shape also had a big influence on crusher performance. Although crab carapace was easily crushed a low feed rate of for crab claw and legs reduced performance as the horizontal bar configuration allowed these long fragments to drop parallel to the blades cracking the shell but not fully separating joints (Plate 3.3). Crusher performance for this difficult material was enhanced with a couple of passes to effectively crush leg joints. Crab shell grain size for 'single' and 'double' crushes are provided in Table 4 overleaf.

Table 4: Summary of Crusher Performance

Crab Shell type	Grain Size		
(mixed waste)	% >12mm	% 2-12mm	% <2mm
Single crushed *(Note 1)*	26	74	0
Double crushed *(Note 2)*	6	59	36

Note 1: Average of x3 0.15kg samples

Note 2: 1kg sample

Table 4 above shows that a double crush was effective in reducing the quantity of large fragments (>12mm) which were more capable of encasing flesh thereby preventing subsequent separation. Crab legs in particular were difficult to crush owing to their form and required multiple crush passes as highlighted in Table 5 below.

Table 5: Influence of Shell Material on Crusher Performance

Parameter	Unit	Crab leg	Crab claw
Mass shell	(kg)	3.5	4.5
Volume Uncrushed	(L)	10	10
Volume Crush 1	(L)	8	6
Volume Crush 2	(L)	6	-
Volume Crush 3	(L)	4	-

Crusher performance was also influenced by the use of water to help purge mashed material from the rollers. Although this may be useful from a process perspective it was not used where 'washing' assessments were needed to quantify flesh liquors (see Section 3.1.2 below).

This crusher unit was subsequently tested on crab, whelk, scallop, oysters and slipper limpets.

3.1.2 Physical Separation - Washing

The essence of the degradation stage of a hybrid process will be to polish residual flesh left by the physical separation process. If the first stage physical separation is not effective then excessive flesh residues could extend the duration of the second stage process and undermine economic viability.

At the time of undertaking the degradation testing an effective physical separation process had not been developed (Section 3.1.5). In consequence, it was necessary to manually undertake a physical pre-treatment to wash shell and remove most flesh. This was achieved by undertaking x2 passes of crab waste through the crusher (Section 3.1.1) before agitating with water in a cement mixer for 5 minutes. A couple of washing trials were undertaken using different washing regimes as outlined in Tables 6 and 7 below.

Table 6: Washing Performance for Different Shell Waste Material (Single Wash)

Shell	Shell	Water	Flesh (Solid)		Flesh in Liquor Wash				
Type	Mass		Mass	% Flesh	Total Carbon	Total N	Total Carbon	Total N	Ratio
			(Wet)	(Wet)	(Dry)	(Dry)	(Dry)	(Dry)	
	(kg)	(L)	(kg)		mg/l	mg/l	%	%	
Crab leg	3.5	17.5	0.175	5.0%	990	414	0.49%	0.21%	2.4
Crab claw	4.5	17.5	0.2	4.4%	1643	686	0.64%	0.27%	2.4

Table 7 used a set volume (10L) of two types of crab wastes which yielded shell:water ratios of 1:5w/w and 1:4w/w for leg and claw respectively. As the liquors were decanted (and sampled) it became apparent that a significant level of white meat was retained at the base of the supernatant. Solid flesh fragments were collected and weighed to calculate flesh recovery (see Plate 3.5).

Plate 3.4
Physical Washing in Cement Mixer

Plate 3.5
Crab Flesh Recovered

A further washing test was conducted using a higher shell:water ratio but with a series of repeat washes as shown in Table 7 below. Decant liquors for the x3 washes were analysed to obtain an indication of the washable load as shown below:

Table 7: Washing Performance Using Multiple Washes (Mixed Crab Shell Waste)

Wash No.	Flesh (Solid)			Flesh in Liquor Wash					
	Mass	% Flesh	% Flesh	Total C	Total N	Ratio	Total C	Total N	
	(Wet)	(Wet)	(Dry)	(Dry)	(Dry)	(Dry)	(Dry)	(Dry)	Average
	(kg)			(mg/l)	(mg/l)		% Flesh	% Flesh	% removal
Wash 1	0.041	0.82%	0.08%	2181	1068	2.0	1.09	0.90	69.2%
Wash 2	0.026	0.52%	0.05%	725	302	2.4	0.36	0.25	21.3%
Wash 3	0.017	0.34%	0.03%	290	151	1.9	0.14	0.13	9.5%
Overall	0.084	1.68%	0.17%			2.1	1.60	1.28	

Table 7 above shows that the majority of the organic liquors were removed in the initial wash (~70%) with diminishing returns in subsequent washes. It is uncertain what impact a shorter duration wash cycle would have on performance.

Consideration to the solid flesh and washable 'liquor' flesh components will be given in Section 4.2.

This triple washed crab with removed flesh was considered to be representative of a well physically separated shell mass. Close inspection revealed that only low levels of flesh appeared to be retained in association with leg joints and ligaments. This shell material was utilised for subsequent enzyme (Section 3.2.2) and bacterial degradation (Section 3.2.3) testing.

3.1.3 Brine Floatation

Some commercial separation equipment is based on the use of brine to float off cooked cockles from the shells. The previous study used brine floatation with 20% w/w salt to float flesh during crushing to help remove flesh. This was seen to work acceptably for some species such as whelks and slipper limpets. However, the dense threads of crab leg white meat were not effectively floated away from the shell. Further testing on this option was not pursued.

Plate 3.6
Brine (20%) Separation on Slipper Limpet
(Note: shell – left / flesh recovery – right)

3.1.4 Dissolved Air Floatation

Dissolved Air Floatation (DAF) is a common waste water treatment technique used to 'float' solids to form a surface scum by the addition of air saturated water. Air is dissolved in water by subjecting air and water to high pressure with sufficient contact area and time. The resultant water is supersaturated when released at lower pressure forming a 'white water' which releases microbubbles of air which through surface tension adhere to particles which then float to the surface where they are periodically scraped away using a paddle and trough.

In commercial DAF systems white water is generated either by spraying water in pressure vessel or by the use of turbine pumps where air and water are both introduced and pressurised within the pump itself. For the purposes of this trial a microbubble diffuser and a pressure vessel were employed.

Microbubble Diffuser. An initial attempt was made to use a microbubble diffuser (see Plates 3.7-3.8) produced by Hydrok Ltd submerged in a tank with 5kg crab to ~100L water. Little surface separation could be observed and the attempt was aborted as insufficient saturation could be obtained.

Plate 3.7
Microbubble Diffuser with baffle plates

Plate 3.8
Microbubble Diffuser in Operation

Pressure Vessel 'White Water' Production. A Sartorius pressure vessel (see Plate 3.9) was used in all subsequent Stage 1 DAF trials to support both jar tests and bench top DAF trials. White water was manually obtained by placing 1L of tap water within the 5L pressure vessel and pressurising using a standard air compressor to 5bar. The vessel was then vigorously shaken to ensure saturation.

Plate 3.9
Pressure Vessel for 'White water' production

Plate 3.10
Jar Test Equipment

Impact of Polymers on DAF Efficacy. The efficacy of DAF processes can be further enhanced by addition of long chain polymers which can bind smaller particles together allowing retention of microbubbles. Zetag ™ a common polymer used in waste water treatment processes to bind organic particles in sludge dewatering systems was tested in shell waste water tests.

The use of polymers to improve flesh removal in Stage 1 processes is problematic. Jar tests on crab shell liquor demonstrated a marked increase in sludge formation which corresponded to a marked improvement in liquor clarity indicating that the polymer was effective in the removal of small solid particles. However, the same poly volume additions did not have the same impact when added to a liquor / shell mixture. In mini-DAF tests it was apparent that instead of small particles forming a floc in suspension they in fact adhered onto the shell itself.

Jar Test Performance. Jar Test equipment (see Plate 3.10) are waste water treatment industry standard equipment to assess the treatability of waste waters through the use of polymers and coagulants. The equipment consists of a motorised set of stirring paddles which allow controlled mixing and settlement tests with a number of test 'jars' running in parallel. The objective of jar tests is to allow optimisation of chemical additive doses under fixed conditions.

Initially a 1:4 crab shell:water mix were placed within the standard 500ml jars. Unfortunately, the paddles jammed with the shell fragments and did not circulate the contents. The jar were replaced by a 2000ml jug which provided better performance and allowed limited testing and assessment of relative performance. However, flesh removal rates were probably reduced as flesh particles buried in the shell mass were unable to float as they were trapped under the shell fragments. It was recognised that a shallow tank system was needed to allow better liberation of flesh particles to float to the surface. A bench test mini-DAF tank was constructed as described in the following section.

Bench Test Mini-DAF Tank. To overcome the limitations of the jar test equipment a rectangular tray (Plate 3.11) was used as the basis for a bench scale mini-DAF tank by construction of a shaped scraper and removing a slot in the tray side-wall for scum decanting. This configuration allows the shell to be spread to a shallow depth facilitating flesh floatation once DAF white water is added.

Plate 3.11
Dissolved Air Floatation of Crab
(Note: flesh scum being scraped from tray)

Plate 3.12
DAF Cleaned Shell and Separated Flesh
(Note: Numerous crab eyes in flesh fraction)

Shell:water ratios of 1:4 were used before the addition of 1L of white water (20%) decreased the shell fraction to 1:5. Although this system provided improved performance relative to the jar tests delivery of the white water to such a wide tank did not provide optimum delivery to flesh detritus. It is probable that floatation performance may also have been reduced with wasted bubble formation on shell fragments. It was concluded that white water DAF process would be a useful means to clean flesh fragments from a waste water flow ideally after another upstream flesh separation process.

3.1.5 Flow Separation

Observation of the crab shell/flesh wash mixture revealed that whilst much of the flesh (particularly from the carapace) was readily broken down to small particles easily washed out of the shell in a liquor. However, the white meat, particularly from the legs, formed dense 'threads' of flesh which were hard to separate from the shell by washing alone. Although this flesh settled out it was marginally easier to resuspend than crab shell itself suggesting a slightly lower density. A series of tests were performed to see if a flow separation process could be developed.

Flume Tests. In order to ascertain the critical resuspension velocity of the different shell and flesh particles a square section drainpipe was modified into a flume to allow test particles to be placed within a controlled environment and subjected to gradually increased measured flow rates until resuspension occurred. Flow into the flume was provided by a submersible pump placed into a sump to recirculate water around the flume. A laminar flow was ensured by positioning of a drilled baffle plate whilst outlet flow depth was controlled by a retained weir plate on the discharge end of the flume.

Flow rates was measured by two methods:

-Direct current meter measurement using a dopper current meter. Current meter readings were presented on a top box as m/s with a 1 second integration period. A number of successive readings were averaged and presented in Table 8

-Calculated flows using a rise test to measure the volumetric flow which was then applied to the cross sectional area of the flume.

Table 8: Flume Crab Flesh Resuspension Data

Flume test Test No	63 mm channel - weir wall = 40 mm sec/10 litres	sec/litre	ml/sec	l/sec	Velocity
1	41.80	4.18	239.23	0.239	0.095
2	42.20	4.22	236.97	0.237	0.094
3	41.60	4.16	240.38	0.240	0.095
4	41.20	4.12	242.72	0.243	0.096
5	42.30	4.23	236.41	0.236	0.094
6	41.90	4.19	238.66	0.239	0.095
Average	**41.83**	**4.18**	**239.06**	**0.239**	**0.095**

Results of the flume testing (Table 8) showed that small crab shell particles were resuspended at ~0.15m/s whilst the white meat flesh was resuspended at ~0.095m/s. This suggests that there is a potential narrow flow window where flesh can be moved whilst retaining the shell.

Vortex Separation Tank Tests. Vortex separation is a flow technique used in settlement technology for the separation of dirty water from grit. In essence, the water/solid flow enters a circular tank allowing settlement of solids and the removal of water from a central outlet position. It was hoped to modify this process so that central velocities could be within the target 0.10-0.15m/s flow window identified from the flume tests with a potential to remove flesh without shell.

Plate 3.14
Vortex Flow Separator
(Note: inverted central cone outlet)

Plate 3.15
Upwelling Flow Separator
(Note: shell in base circulating)

In order to test the flume data with a mass of shell a recirculating rotary flow tank test was set up to see if physical observation with a mass of mixed crab/flesh matched the flume data. Initially, a 560mm diameter circular tank was provided with a flow rate of ~500ml/Sec with flow introduced at a tangential angle to induce a circular motion where velocity is reduced across the radius of the tank before reaching an outlet at the centre of the tank (see Plate 3.14). Whilst the flow at the margin of the tank exceeded 0.15m/s as measured by the in-situ flow monitor flesh separation was not successful as flow rates decreased too rapidly towards the centre of the tank and did not retain the flesh in suspension. In addition, continued addition of mixed dirty crab was seen to provide a debris field with reduced velocity in the boundary layer allowing flesh to settle out in between crab stationary shell fragments.

A second 260mm circular tank was testing with a similar inlet/outlet configuration with the intention that the same flow in a smaller diameter tank should help ensure velocities were higher allowing continual movement of the shell tumbling along the tank bottom so that flesh could be liberated. This tank differed slightly in that it had a conical bottom and a downward central discharge. Flow measurements in this smaller dimension tank were only possible at the margin of the tank where they exceeded 0.15m/s. Test 1 with 0.5kg of dirty crushed crab legs provided moderately good performance and flesh removal with ~0.15kg recovery (30% by weight). This was the highest proportion of flesh recovered in Vortex tests and probably reflects the 'dirty' initial quality of the crab rather than the efficacy of the process relative to later tests.

Observation through the clear plastic of the tank revealed that some flesh was trapped beneath the shell which rapidly dropped to the centre of the cone. Consideration was then given to provision of an upwelling flow to resuspend flesh particles.

Upwelling Tank Tests. The 260mm diameter circular tank using the previous vortex separation was modified by using the central pipe as the inlet by corking the central pipe and drilling multiple inlet jet holes to improve shell resuspension. A new discharge pipe was provided at mid-height which allowed collection of flesh in an external sieve (Plate 3.16).

Plate 3.16
Upwelling Separator Outlet

Plate 3.17
Upwelling Clean Shell and Flesh Outputs

When 0.5kg of dirty crab was introduced good flow separation was achieved whilst shell was seen to freely rise and settle within the tank. Some dead-spots of low flow persisted (possibly due to slight variations in pipe perforation) where shell settled potentially trapping flesh. Manual agitation provided some additional flesh although increasing efforts only yielded an increasingly greater proportion of ligaments which were the next most readily resuspended shell fragments.

Table 9 summarises the proportion of flesh removal with each test which became successively less (see Upwelling 2a-2c) although an overall flesh removal of 11% (wet) was obtained. Despite this very thorough cleaning, the combined 11% of flesh removed is still significantly lower than the 30% obtained on a previous batch. Physical observation of the sample revealed no obvious flesh content which suggests that the variation in flesh removal was a function of crab shell quality rather than changes in the Vortex performance following the modifications.

Table 9: Summary of Vortex Flesh Separation

Upwelling	Pumps	Flow rate	Quality	Shell weight (g)	Flesh Removed		
Test	(No.)	(l/sec)		(g)	(g)	(% wet)	(% Dry)
2a	1	0.5	Good	500	30	6.00%	0.60%
2b	1	0.5	Average *(Note 1)*	500	15	3.00%	0.30%
2c	1	0.5	Average *(Note 1)*	500	10	2.00%	0.20%
3	2	0.66	Average *(Note 1)*	1000	40	4.00%	0.40%
4	2	0.58	Good	1000	25	2.50%	0.25%

Note1: Flesh with ligament

Addition of a larger 1kg of dirty crab changed the resuspension performance in the cone with a reduced amount to shell movement and less flesh liberation. Although all previous pump flow rates were 500ml/second in common with previous experiments it was decided to increase the flow rate with a second pump in order to increase resuspension. Flows at 666ml/second provided a much higher proportion of shell indicative to excessive flow rates yet despite this flesh removal rates were not as good. An intermediate flow rate 580ml/second did provide a good quality crab flesh with little shell although the reduced recovery rate (relative to the 0.5kg batch) suggests that retention was still occurring in the cone. It was concluded that a continuous process would need to ensure a controlled removal of clean shell to allow ongoing effective separation.

Samples of Vortex clean shell and collected flesh were obtained for analysis. Results are tabulated below:

Table 10: Analysis of Crab Shell after Vortex Separation

Sample ID No	Description	Total C (mg/kg)	Volatile Solids (500 C) %	Total N (mg/kg)
2438156	Vortex clean crab shell (legs)	215120	-	31760
2446564	Vortex clean crab shell (claw)	121770	18.8	23250
2446565	Vortex clean crab shell (carapace/tails)	304050	26.6	24680

Table 10 data above indicates that the quality of the crab shell is highly variable between different shell components (legs, claws and carapace/tails). Although the test parameters still show considerable levels of organics and Total N discussion in Section 4 indicates that this is still actually quite clean and that Vortex separation has been successful.

3.2 Degradation

3.2.1 Enzyme Treatment

Enzyme leach methodology development (see Section 2) was used to provide data on potential enzyme treatment as part of a degradation process. Post-enzyme digestion results in Table 10 below can be compared with Vortex cleaned shell in Table 9.

Table 11: Analysis of Crab Shell After Enzyme Treatment

Sample ID No	Description	Total C (mg/kg)	Volatile Solids (500 C) %	Total N (mg/kg)
2446558	Amb-2.5%-24hrs- clean vortex shell	71070	17.9	12540
2446559	Boil start- 50C - 2.5% -24hrs- vortex shell	76400	18.2	13660

Results are discussed in Section 4.2.

3.2.2 Bacterial Degradation

5kg of physically separated washed shell (see Section 3.1.2) was placed in a aeration tank with 20L of activated sludge and 60L of water. A blower passed continuous aeration via a micro-bubble diffuser into the tank to maintain a suitable dissolved oxygen level for microbial respiration. Plate 3.19 shows the activated sludge degradation process in operation.

Plate 3.18
Microbial Cleaned Crab Shell

Plate 3.19
Activated Sludge Digest

Test samples were obtained at the onset and after 24hrs and 48hrs (Plate 3.18) as presented in Table 12 which shows that Total Carbon and Total N content roughly halfed every 24hrs producing low % flesh levels. Some caution is required in the use of the enzyme leach methodology to access FOF status (see Section 4.1). However, visual inspection of the shell sample could also detect no observable flesh or odour of crab.

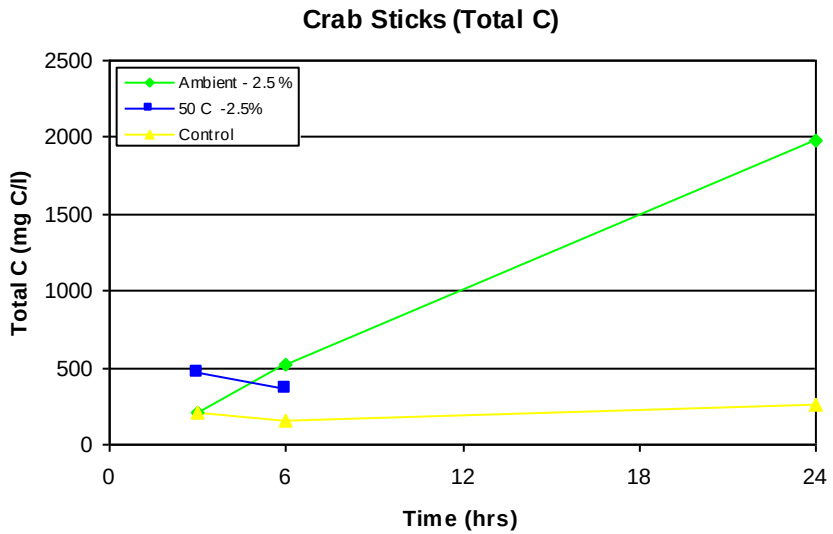
Table 12: Enzyme Leach Data Showing Successive Drop in Residual Flesh Levels for Microbial Samples

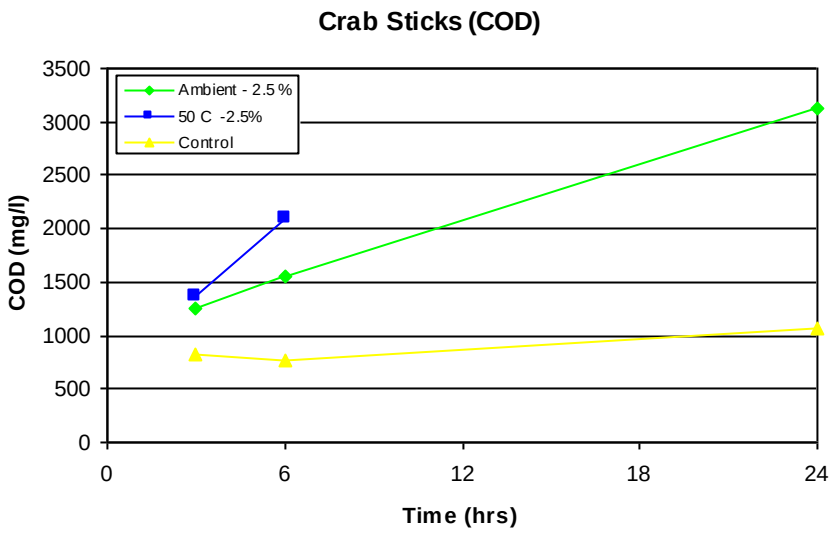
Sample	Leach Analysis		Flesh retained		Flesh Removed	
	Total C	**Total N**	**Total C**	**Total N**	**Total C**	**Total N**
	(mg/Kg)	(mg/Kg)	(%)	(%)	(%)	(%)
T 0 (start)	2364	628	0.61%	0.27%	-	-
T 1 (24 hrs)	1347	229	0.35%	0.10%	0.26%	0.17%
T 2 (48 hrs)	676	191	0.17%	0.08%	0.44%	0.19%

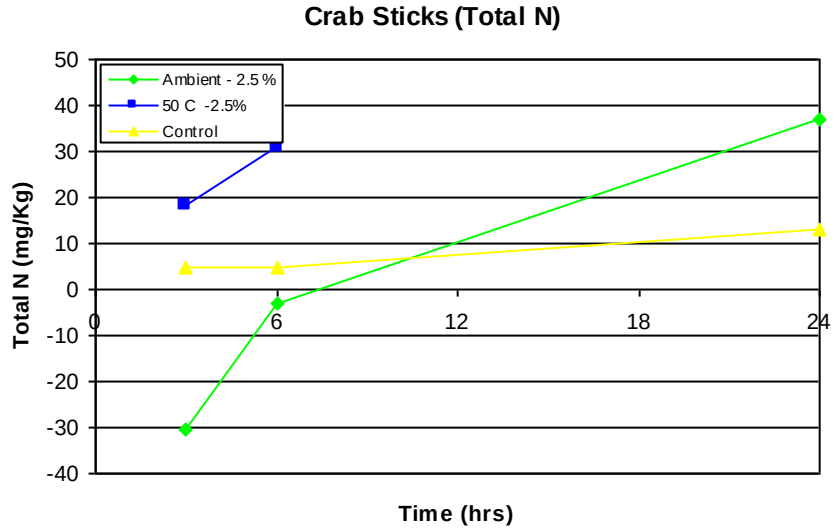
Note 1: 100% crab flesh contains 38.8% Total C (by testing), 23% Total N (assumed from empirical ratio)

It should be noted that shell would need to be rinsed with water following a microbial degradation process as any residual bacterial activated sludge on the shells would contribute to the Total Carbon and Total N if analysed. As activated sludge readily settles out to provide a clear supernatant (this process is the central component in most waste water treatment processes) the clear supernatant could be used to rinse processed shell. An illustration of a potential process using microbial degradation is provided in Figure 4.

Figure 1: Set 1 - Summary of Enzyme Leach Results







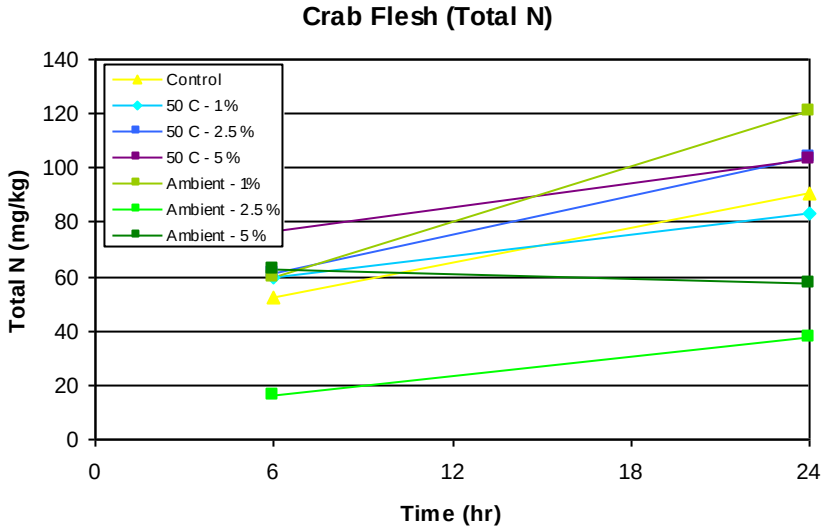
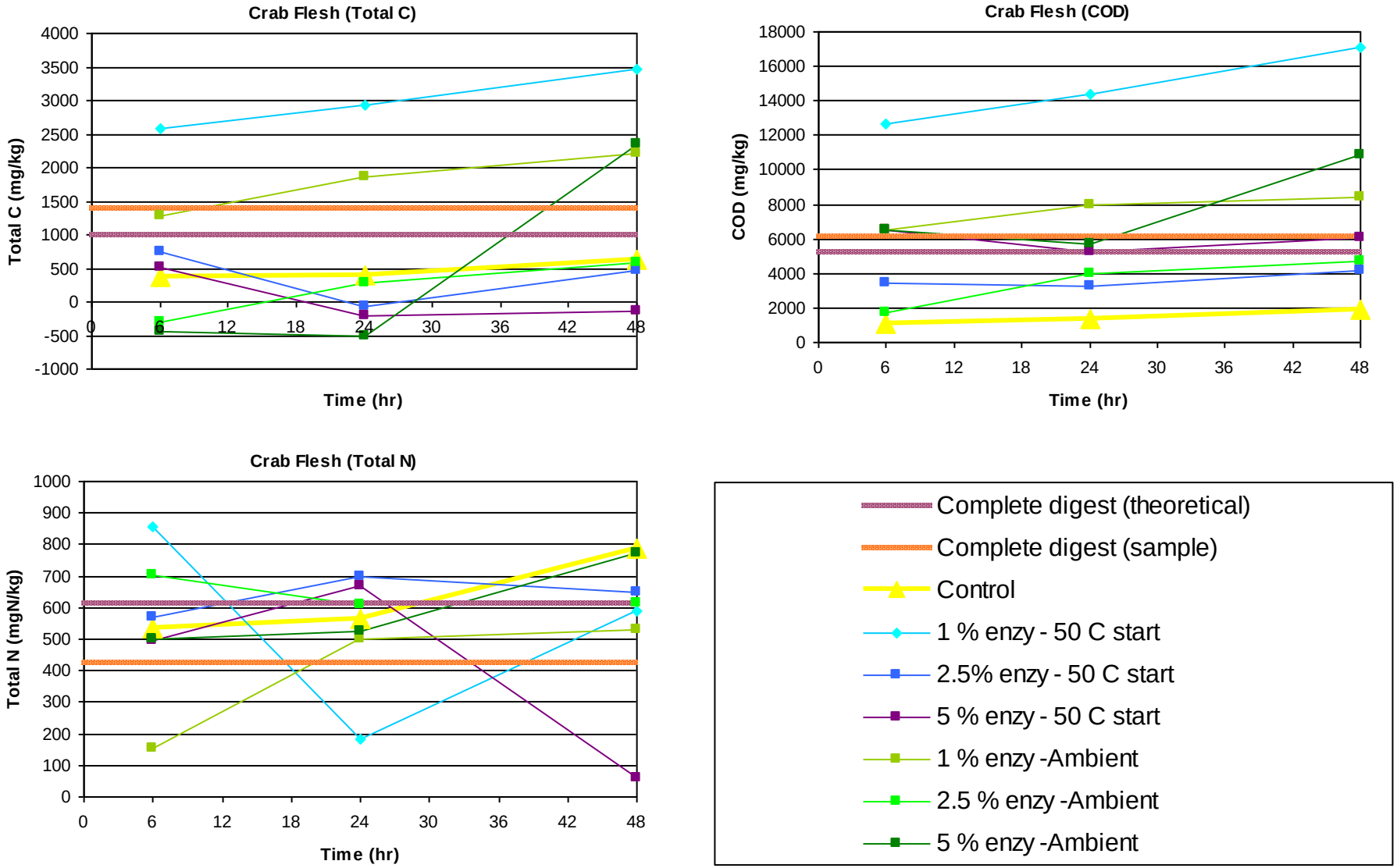


Figure 2: Set 2 - Summary of Enzyme Leach Results



4.0 DISCUSSION

4.1 Development of a Leach Methodology

Section 2 outlines the development of a leach methodology using the commercially available standard protease solution 439 produced by Biocatalysts Ltd and the initial results obtained. The previous Sea Fish FOF study had shown that shells soaked in this product were significantly cleaner than other leaching/cleaning methods. It was reasoned that the enzymes which crack proteins would specifically target available flesh which would be liquidised by the enzymes into the water medium which could then be easily and cheaply tested free from the matrix effects encountered with direct testing of the shell.

The focus of the current project was to develop a standardised leach methodology so that a reliable testing protocol could be easily adopted by anyone wishing to assess FOF status. The proposed method development consisted of the following steps:

-Temperature / concentration profiles to select the best combination for further testing

-Shell leach optimisation to test a range of shell:leach ratios with varying organic content

-Method robustness to assess reproducibility with triplicate repetitions and sensitivity using standard addition of known flesh quantities

-Test a range of FOF shell types previously tested by direct shell analysis

In the event once the enzyme control results had been subtracted there was no consistent temperature /concentration timeseries profiles and even some negative values. In theory all leach concentrations should exceed the starting level provided by the enzyme (i.e all results should be positive). It was predicted that with increasing leach time or temperature the quantity of flesh digested should also rise until the solution was saturated. Once all available flesh has been broken down a maximum concentration should be achieved. It was anticipated that the temperature/concentration profiles would indicate that the 'boil start' with the highest enzyme concentration (20%) would give the fastest flesh degradation. In practice leach testing on shell (see Table A1) provided no such relationships.

This approach was used on a number of test samples obtained from other components of the current study and for a FOF scallop sample. In some cases such as the microbial degradation work (Section 3.2.2) the results for both Total Carbon and Total N parameters provided a 'sensible' output. The scallop FOF test sample (see Section 2.2.5) also demonstrated low levels of leached Total Carbon and Total N equating to <0.6% flesh consistent with the expectation of the visually clean FOF sample and the low level direct shell testing results. However, the poor reproducibility of the leach methodology does not provide a sufficient level of confidence in the results.

It was surmised that perhaps the sampling regime was flawed (digested samples have a high 'sludge' level associated with digested flesh) and as such more simple flesh leach models were attempted using crab flesh (obtained from the physical separation stages) and crabsticks as a homogenous test matrix. Although results were improved conflicting results (see Table A2 and A3) were still obtained. A final tranche of leach testing was performed using pure white crab meat and carefully controlled conditions. Test Set 2 included comparison against 100% digest flesh samples and theoretical empirical data which still indicated poor performance and confirmed the low level of reliability for the technique.

In summary, the currently tested leach method has not provided a robust testing protocol. The fact remains that the previous FOF study (Ref: FitzGerald, 2010) did not find a wholly effective direct shell testing regime for shell types with a high residual organic levels. There is still a need for a more targeted leach method which can better remove flesh into a shell free liquor to allow indirect testing. Until this can be devised it will be difficult to demonstrate that shell types such as crab can be produced to FOF standards.

As an alternative caustic washes (sodium hydroxide) of various strengths were tested in the original FOF study with some success. One advantage of caustic is that it should contain no organic or total N content and therefore not require control corrections. Disadvantages are the obvious handling issues as well as the potential need to pH neutralise the leachate prior to submission for analysis.

4.2 Development of a Hybrid Cleaning Process

The previous Sea Fish FOF Criteria Report (Ref: FitzGerald 2010) identified that some species (e.g particularly crustaceans such as crab and nephrops) were hard to clean to FOF status with a single cleaning process. A hybrid cleaning process was proposed to optimise a number of cleaning stages. Section 3 outlines the work undertaken to devise a composite treatment train encompassing a physical separation stage to remove gross flesh contents followed by a degradation stage to polish away residual flesh. Aspects of this treatment train are considered below:

Solid Flesh Removal

Effective crushing is an essential pre-requisite for solid flesh removal. In the case of crab flesh fragments are intimately trapped within shell structures such as leg joints where they are attached to ligaments. These structures must be physically opened up to allow the subsequent washing stage to be effective (Section 3.1.1).

Solid flesh recovery rates during the washing stage were highly variable between batches (Section 3.1.2) with typical wet weight recovery of <6%. Flesh content can be higher due to batch variation (e.g Test 1, maximum of 30% (wet) obtained) or with increase washing (e.g Test 2, 11% with triple washing). In the case of the Test 1 batch physical observation (see Plate 3.3) showed a high level of flesh (30%) which had been relatively poorly picked. Discussion with the processor revealed that some crab components were hand picked to provide quality meat product free from shell – at the expense of increased meat loss in the waste relative to mechanical recovery. The quality of hand picking will obviously be operator dependant.

Enhanced Vortex separation during the cleaning stage increases the level of flesh removal potentially at the expense of by-product quality. Section 3.1.5 describes a triple washing in Test 2 (Table 7) where flesh recovery is boosted from 6% (wet) to 11% (wet) but with the later washes containing increased proportion of ligament.

The proportion of solid flesh removal will also be influenced by the type of waste component used. For example, brown crab meat seems to physically breakdown more easily than white meat which was retained as distinct ‘threads’. In consequence, a carapace waste is likely to contribute an increase proportion of load in the liquor phase relative to claw or leg waste.

Comparisons between dry and wet flesh content indicate a moisture content of ~90% (i.e dry weights are 10%) of the equivalent wet weights – although this is likely to be highly variable. In consequence, solid flesh recoveries of even 30% (wet weight) equate to just 3% (dry weight). This is likely to equal or exceed the load provided by the liquor phase.

It is apparent from Figure 3 that effective solid flesh removal is critical to the success of the physical washing stage. Any solid flesh retained in the shell mass will pass forward to the Stage 2 degradation process and will therefore have a significant impact on both the loading and residence time to this subsequent step.

Soluble/Fine Particles Flesh Removal
Liquor flesh removal during the washing stage was effective at stripping a significant amount of soluble liquor with fine particles with a high organic content (see Section 3.1.2). Two washing tests yielded an equivalent flesh content of ~0.6-1.6% (on a dry weight basis).

Residual Flesh Removal
Enzyme removal of residual flesh is effective at producing clean shell (see Table 11). Unlike the microbial degradation method enzymes work rapidly with even short exposures producing protein breakdown when flesh with a high surface area is exposed. As indicated previously the crushing process will have a significant role in the whole treatment process as lumps of solid flesh adhered to shell will not digest so readily. The economics of enzyme treatment will require scrutiny as there is no current information on the degree to which an enzyme solution can be reused on shell waste. Theoretically, the enzyme solution can be reused many times allowing for the loss of volume with shell output and with flesh sludge liquor. In practice some trials would be needed to assess the rate of degradation and operational efficacy as experience has shown that after a while the enzyme solution starts to smell..

Section 3.2.2 considers an activated sludge system where shell is fully immersed in a bacterial medium with air bubbled through the suspension. Although this provided a high rate of degradation it is an energy intensive process. Alternative processes using a recirculation of a bacterial liquor sprayed onto the shell treatment process might be a lower cost option to build and operate – however, care would be needed to ensure that any flesh on the shell is sufficiently immersed in the bacterial liquor to reach beneficial treatment and consistent treatment. Further trials to optimise potential commercial systems would be required to establish the most appropriate process. A critical factor will be the site footprint as lower cost systems operating at a low intensity are accordingly slower processes and therefore require a larger area.

4.3 Relative Stage Performance of a Hybrid Process

Table 13 compares direct shell analysis results for crab at various cleaning stages from the current study against the results from the previous FOF study (Ref: FitzGerald 2010. Although there is some slight variation in the scope of determinands used with a greater emphasis on Total Carbon in the current study, there tends to be quite good agreement with the common enzyme leached samples.

The Mass Balance of flesh removed from clean shell should in theory match loads removed in solid and liquor phases. In general the direct shell results ~20%flesh is removed of which only ~5% is accounted for by Stage 1 and 2 processes. This may be an under-estimation of Stage 2 degradation performance for which it has not been possible to adequately test using the leach methodology. However, the 20% flesh dry weight would exceed 100% flesh wet weight (at 90% moisture) and as such it is probable that much of this volatile load is bound with the shell. The change in shell volatile levels does however provide an indication of how clean the shell is.

An overview of the crab shell quality is provided below:

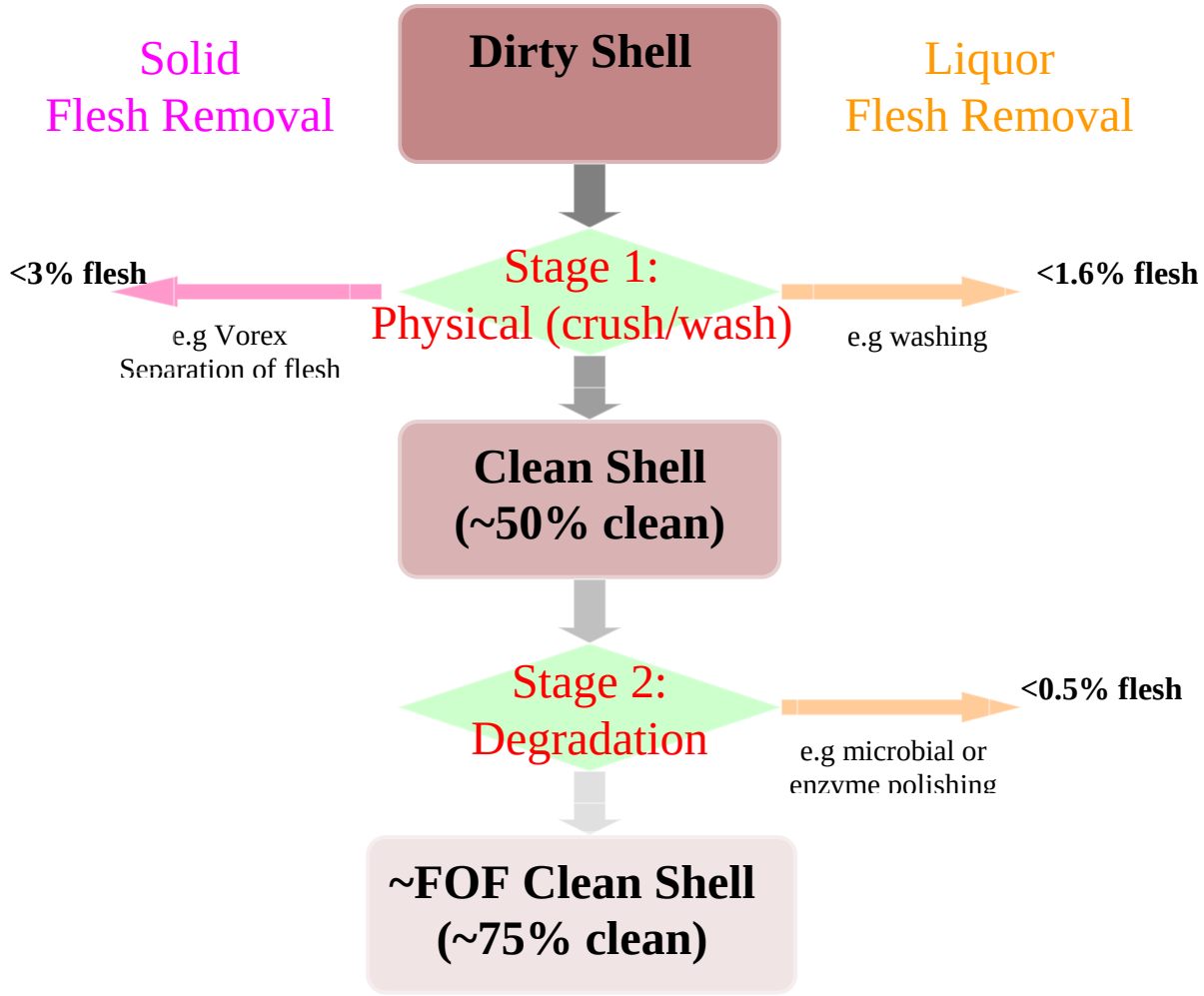
> -*Dirty Shell:* Although there was variation in the level of flesh retained in ‘dirty’ crab High Total N contents of 30,000-55,000mg/l were obtained with volatile solids of ~30-40%. If ~17% volatile solids if attributable to the clean shell this equates to ~10-20% volatile solids as flesh (i.e dirty shell).

-*Stage 1 Cleaned Shell*: The Vortex separated shell shows high variation dependant on shell type (e.g legs, claw, carapace/tail) with volatile solids levels of ~20-30%. If ~17% volatile solids is attributable to the clean shell this equates to ~3-10% volatile solids as flesh (i.e ~50% clean)

-*Stage 2 Cleaned Shell:* Both the current and the previous study indicates 'clean' crab shell has a volatile solids of ~17-19% and a total N content of ~11,000-14,000mg/l. This concurs with the theoretical total N content attributed to chitin and protein in the shell calculated in FOF, 2010 of between 9,000-21,000mg/l. If ~17% volatile solids if attributable to the clean shell then there is the potential for ~1-3% flesh (i.e ~75% clean). Against the 1% flesh definition it is marginal whether the shell has attained FOF status.

Figure 3 below provides a flow diagram of a hybrid process with indications of the relative proportions of flesh removed by the various stages.

Figure 3: Flow Diagram of Hybrid Cleaning Process



Note 1: Flesh quantities on a dry weight basis

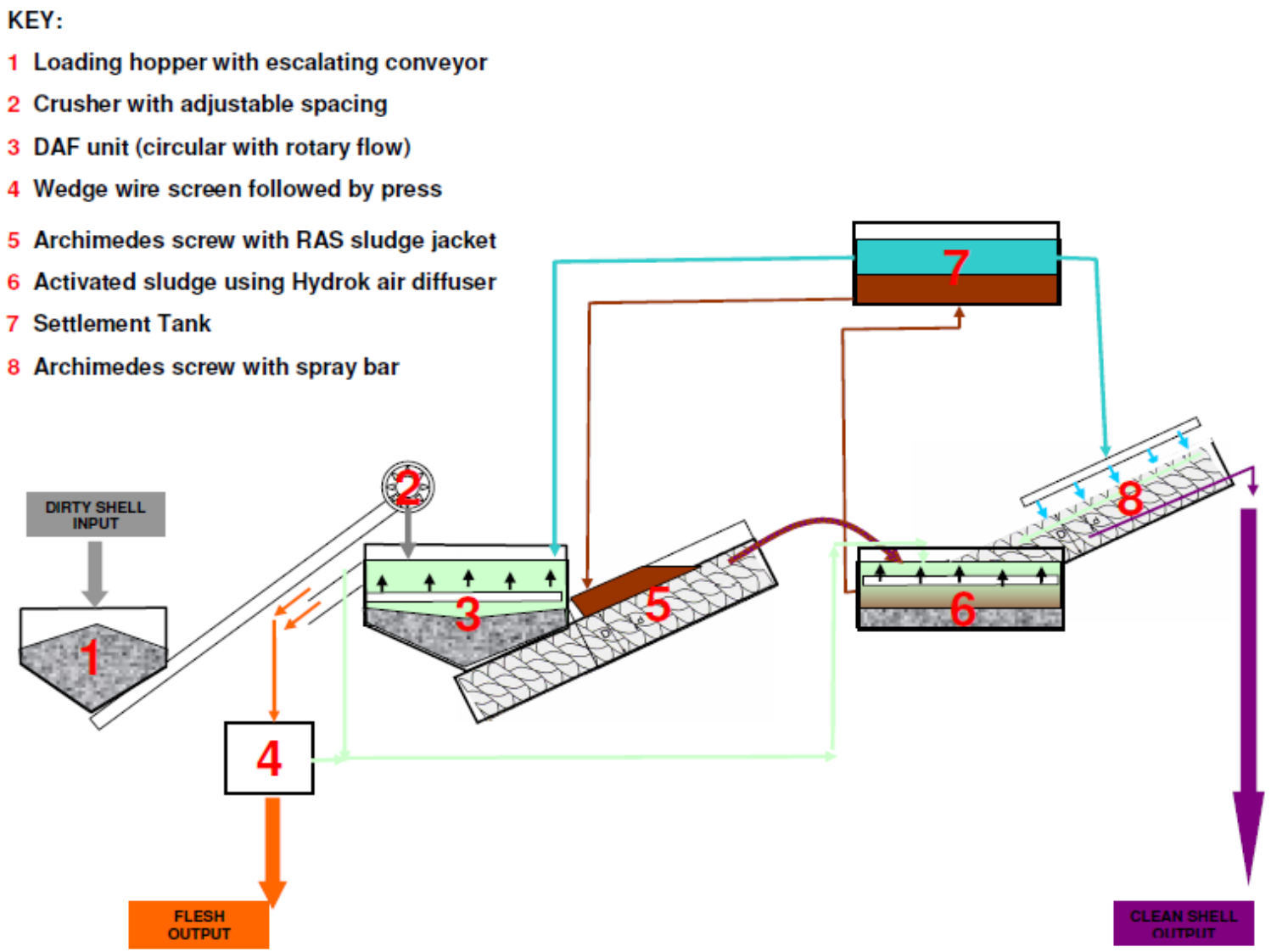
As can be seen the majority of the flesh load is removed in Stage 1 but the potential to attain FOF status is a function of Stage 2 performance. Removal of the last few % volatile solids may dictate the residence time in the Stage 2 process. This in turn will set the footprint size of the treatment process. An illustration of a potential hybrid process is provided overleaf.

Table 13: Reduction in Flesh Content Through a Combination Cleaning Process for Crab Shell

Crab treatment	**Sample No.**	**Odour**	**Flesh %**	**Total C**		**Vol Matter**	**Total N**	
			(Vis.)	**(mg/Kg)**	**(Calc. Flesh %) (Note 4)**	**(%)**	**mg/Kg**	**(Calc. Flesh %) (Note 5)**
Dirty Shell								
Dirty - from Fresh (cooked)	*Note 1*	Slight	90	-	-	29.6	30940	99.2
Dirty - from Frozen (cooked)	*Note 1*	Slight	90	-	-	37.6	53350	171.0
Post-Stage 1 Physical Cleaning								
Vortex clean crab shell (legs)	2438156	None	0 (Note 2)	215120	56	-	31760	101.8
Vortex clean crab shell (claw)	2446564	None	0	121770	32	18.8	23250	74.5
Vortex clean crab shell (carapace)	2446565	None	0 (Note 2)	304050	79	26.6	24680	79.1
Post-Stage 2 Degradation *(Note : Ref: FOF, 2010 calculated FOF crab to have Total N levels of ~9,000-21,000 mg/l)*								
24 hrs 20% caustic	*Note 1*	Slight	5	-	-	16.8	13860	44.4
Enzyme control - 439L	*Note 1*	None	5	-	-	23.1	11570	37.1
T 0 - clean vortex shell	2446557	None (Note 3)	0	89220	23	19	12620	40.4
Amb-2.5%-24hrs- clean vortex shell	2446558	None (Note 3)	0	71070	18	17.9	12540	40.2
Boil start - 2.5% -24hrs- vortex shell	2446559	None (Note 3)	0	76400	20	18.2	13660	43.8

Note 1: Results from previous FOF report, 2010
Note 2: High occurrence of ligaments in legs, High occurrence of feathery appendages in tail (disposed with carapace)
Note 3: A slight enzyme induced small (not 'crab' smell)
Note 4: Based on assumption that Total C provides 38.8% of volatile solids (=flesh)
Note 5: Based on assumption that Total N x 6.25 = Protein content and Protein in crab is 19.5% of flesh (Ref: FOF, 2010)

Figure 4: Illustration of Potential Hybrid Process for Shell Cleaning



4.4 Demonstration of FOF Status

This project has not been successful in the determination of a robust leach protocol to allow demonstration of FOF in shell types with high residual organics (e.g crustaceans). The project has shown that for some crab shell components it is likely that FOF shell has been produced – however, other crab shell components are hard to clean which could undermine the process. Due to inherent variability in flesh levels between components, and indeed between batches, it will be challenging to ensure all batches of shell reach FOF quality unless the system has sufficient spare capacity built in to work under 'worst case' scenario. In view of this any crab FOF facility will require a high intensity of monitoring to demonstrate consistent batch-by-batch performance using a HACCP model to identify the hazards which could compromise the process. It may then be possible to design a process matched to the waste stream load with set operating criteria (e.g treating crab shell with critical limits set on proportion of crab tails).

In Summary, in the case of crab, there are therefore two key problems:

-It is not yet possible to prove that any clean crab shell attains FOF status.

-It is hard to consistently produce FOF crab shell

4.5 Economic Uptake for a Potential Hybrid FOF System

Whilst it may be technically possible to produce a FOF shell for even difficult to clean species such as crab, the underlying driver will always be economics. This will be a balance between external disposal cost for shell waste against internal costs for a dedicated cleaning system. Whilst some internal costs (e.g capital purchase) may be fixed, revenue costs such as spare manpower capacity are variable depending on individual processors circumstances and operations. The balancing of the costs for cleaning shell against the potential by-product income streams (e.g flesh for potting bait and FOF shell for specific Technical applications) will also be processor specific.

The economics of using a complete hybrid FOF system will be strongly influenced by whether economies of scale can be obtained and therefore may be unattractive for a single processor. Furthermore, it is probable that most processors will have insufficient site footprint to encompass a complete hybrid system. This is especially the case in difficult to clean species where some components are particularly problematic. However, some economic options may exist which could use this technological approach:

-*Separation of stages*. The Stage 1 Physical process with crushing could be used for compaction of waste volume to allow increased storage with whilst flesh separation possible utilisation of flesh e.g potting bait applications (Ref: FitzGerald 2007). Stage 2 polishing of shell to attain FOF status would be an economic decision based upon whether an application with a sufficient economic return is available for the FOF shell by-products (Ref: FitzGerald 2008.)

-*Separation of shell components.* Crab shell waste has a number of discrete components (e.g legs, claws, carapace, tail and pouch) all of which have distinct properties. Some components are easier to clean than others (e.g claw) with some components remaining difficult to clean even after crushing (e.g tail). It is possible that devising a FOF cleaning process for a mixed crab waste will not be economic – yet separation of waste streams in the processing facility (e.g segregation of tails) could then make the FOF treatment of the remaining streams viable if unfavourable components are removed.

-*Optimisation for specific shell components*. In the case of crab there is a potential market to produce FOF carapace prior to heat treatment and filling with dressed crab. Stage 2 degradation processes could be enhanced if Stage 1 physical processes (i.e crushing) are restricted. Pressure washing followed by protease enzyme digestion could be a cost effective cleaning technique as it leaves no residuals and requires no pH correction.

-*Optimisation for specific by-products*. Flesh by-product separated in the cleaning process may present opportunities for increased revenue to subsidise the cleaning operation. The cleaning process will need to be tailored to the quality requirements of the product. For example, the Vortex separation system can obtain flesh of quite high quality with a low content of shell or ligament or be adjusted to increase removal rates with a compromise of reduced quality.

In summary, the economics of producing a FOF hybrid process are challenging for difficult to clean shell types such as crab. There could be scope for an individual processor to tailor a cleaning process to achieve their own specific needs, or for perhaps a regional facility to handle bulk quantities of material from a number of contributing processors. In both cases success of the scheme is likely to be influenced by segregation of the waste streams by the processor at point of production.

Market drivers will be essential to progress this concept. Recent advances in FOF shell utilisation for scallop in the UK have been strongly driven by the opening of significant volume markets with at a moderate rate of return. A comparable market need of sufficient value could have a similar effect for crab FOF production. The 'Catch-22' is that it is hard to develop a market for a specific product using FOF crab when no FOF crab is available for the market to try. There maybe scope for pump priming a suitable trial scale facility with a focus on developing market applications in order to bridge this gap.

5.0 CONCLUSIONS & RECOMMENDATIONS

In 2010 Sea Fish Industry Authority commissioned Aquatic Water Services Ltd (AWS) to undertake a study to help define Free Of Flesh (FOF) status for shell waste for a variety of shell types and a number of prescribed cleaning processes. This work was required to maintain an exemption for 'clean' shell use for Technical Purposes within the Animal By-Product Regulations.

The findings of the initial study showed that whilst many shellfish types could be effectively cleaned some species were both hard to clean and difficult to assess their FOF status due to high residual test levels of flesh test parameters within shells. This project represents a continuation of this project to help find a way forward with these problem areas by developing an alternative testing protocol based upon the use of a leach methodology and to explore options for a combination cleaning process which can produce FOF shell for difficult to clean shell types such as crab.

Although not all objectives have been positively attained a number of findings can be drawn:

5.1 Potential for a Leach Methodology to Assess FOF Status

In theory a leach method should provide a more representative indirect sample for analysis than the direct analysis of shell. In practice, there are difficulties in obtaining consistent and repeatable results with difficult shell types such as crab. This project has taken forward the use of specific proteases which appear to be effective in producing FOF shell. Indeed visually the flesh breaks down and is present in the test leachate indicating that the method works. Despite this chemically the results of Total Carbon, Total N and COD testing are inconsistent. In consequence, it has not been possible to produce a robust reproducible protocol.

The implications are that it will be hard to prove FOF status for shell types with high residual organics regardless of how clean they may actually be. The need for an effective leach method still remains although divining an effective methodology is elusive.

One prospect may be to return to caustic leaches which although effective at breaking down flesh may require pH correction prior to analysis. A limited series of targeted tests could be performed to establish viability before engaging a larger scale project if results are promising.

5.2 Potential for a Hybrid Cleaning Process to Achieve FOF Status

The current project has shown on a small scale that it is possible to achieve FOF shell in difficult to treat species such as crab with a combination cleaning process. Unfortunately, whilst visual observation indicates the shell is FOF the poor performance of the enzyme leach methodology has not been able to analytically verify this. Direct analysis of crab shell for volatile solids indicates levels of 18-19% which is line with expectations for clean shell (Ref: FitzGerald, 2010), however, the high residual organics in the shell cannot be differentiated.

The success of the hybrid cleaning process is strongly dependant on an effective crushing stage to liberate flesh from complex shell structures such as crab leg joints. The absence of a good crushing stage will prevent the action of the subsequent physical separation stage.

The selection of an effective physical separation stage in crab should allow removal of dense white meat muscle fibres. Brine floatation was not effective with this material whilst Dissolved Air Floatation by itself did not separate flesh from the shell matrix. Only flow separation in a suitably designed vessel allowed free removal of this flesh which was recovered with good selectivity.

A number of potential degradation options are available to breakdown residual flesh in a tertiary polishing stage. These include caustic soaking, protease enzymes and microbial action. A good physical separation stage will strongly influence the success of a degradation stage as excessive load could overload performance particularly if large chunks of flesh persist.

A commercial hybrid cleaning system based upon the small scale results of this study is an engineering possibility. The financial viability of such a system will be strongly dependant on the market output for the potential by-products to help offset set-up capital costs (see Section 4.4). It should be noted that aspects of a hybrid process could be considered for specific objectives in the context of specific financial drivers such as crushing for space saving waste compaction and flesh recovery and utilisation.

6.0 REFERENCES

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APPENDIX A

Shell and Enzyme Leach Results

Table A1: 'Shell' Enzyme Sample Results

Sample ID No	Type of Sample	Description	Notes	Leach Period	Enzyme (%)	Total C (mg/l)	COD (mg/l)	Total N (mg/l)
2288840	Enzyme control			-	20%	28755		1071
-	Enzyme control			-	10% (calc)	14377		536
-	Enzyme control			-	5% (calc)	7189		268
-	Enzyme control			-	1% (calc)	1438		54
2288841	Enzyme study	Washed crab shell	*1,2*	T0	5%	92		927
2288842	Enzyme study	Washed crab shell	*1,2*	T0	10%	-2424		0
2288843	Enzyme study	Washed crab shell	*1,2*	T0	20%	-2588		-547
2288847	Enzyme study	Ambient (Washed crab shell)	*1,2*	T+48hr	5%	384		280
2288848	Enzyme study	Ambient (Washed crab shell)	*1,2*	T+48hr	10%	-2232		178
2288849	Enzyme study	Ambient (Washed crab shell)	*1,2*	T+48hr	20%	-2300		282
2288850	Enzyme study	Boil Start (Washed crab shell)	*1,2*	T+48hr	5%	-1511		726
2288851	Enzyme study	Boil Start (Washed crab shell)	*1,2*	T+48hr	10%	280		371
2288844	Enzyme study	Boil Start (Washed crab shell)	*1,2*	T+30min	20%	1860		272
2288845	Enzyme study	Boil Start (Washed crab shell)	*1,2*	T+1hr	20%	-1040		368
2288846	Enzyme study	Boil Start (Washed crab shell)	*1,2*	T+24hr	20%	-2157		255
2288852	Enzyme study	Boil Start (Washed crab shell)	*1,2*	T+48hr	20%	-473		49
2288853	Enzyme study	50C (Washed crab shell)	*1,2*	T+48hr	20%	-3679		376
2288859	Enzyme study	Flesh (10g)	*1*	T+24hr	20%	3609		-718
2446560	Enzyme study	Ambient (Vortex crab shell)	*1,2*	T+24hr	2.5%	79	4105	413
2446561	Enzyme study	Boil start (Vortex crab shell)	*1,2*	T+24hr	2.5%	288	3765	459
2288837	Microbial' Sample	T0 Stage 1	*1,2*	T+24hr	20%	2364		628
2288838	Microbial' Sample	T1 (1day degradation)	*1,2*	T+24hr	20%	1347		229
2288839	Microbial' Sample	T2 (2day degradation)	*1,2*	T+24hr	20%	676		191
2288856	Physical Sample	DAF - No poly	*1,2*	T+24hr	20%	309		316
2288857	Physical Sample	DAF - 60ml poly	*1,2*	T+24hr	20%	2171		609
2288858	Test Sample	FOF Scallop	*1,2*	T+24hr	20%	494		13

Note 1: Analytical results corrected by subtraction of appropriate enzyme control results
Note2: 200g of shell leached in 800ml of enzyme solution

Table A2: Set 1 - 'Crab Stick' Enzyme Sample Results

Sample ID No	Procedure	Notes	Leach Period	Total C mg/l	COD as O2 (redox) mg/l	Total N (mg/l)
2372208	Control		3	205	825	<5
2372215	Control		6	151.5	765	<5
2372223	Control		24	258.5	1065	12.85
2372206	50 C start -2.5%	*1*	3	473.5	1375	18.05
2372213	50 C start -2.5%	*1*	6	363.5	2100	30.9
2372203	Ambient - 2.5 %	*1*	3	205	1245	-30.45
2372210	Ambient - 2.5 %	*1*	6	517.5	1550	-3
2372221	Ambient - 2.5 %	*1*	24	1977	3125	36.8

Table A3: Set 1 - 'Crab Flesh' Enzyme Sample Results

Sample ID No	Procedure	Notes	Leach Period	Total C mg/l	COD as O2 (redox) mg/l	Total N (mg/l)
2372236	Control		6	176	670	52.05
2372244	Control		24	343.5	1190	90.8
2372240	50 C start - 1 %	*1*	6	430	-3315	59.37
2372248	50 C start - 1 %	*1*	24	403.5	1990	82.97
2372241	50 C start - 2.5 %	*1*	6	536	1300	61.1
2438153	50 C start - 2.5 %	*1*	24	509.5	2385	103.7
2372242	50 C start - 5 %	*1*	6	488.5	-150	76.45
2438154	50 C start - 5 %	*1*	24	-650	1400	103.4
2372237	Ambient - 1%	*1*	6	452	1910	59.32
2372245	Ambient - 1 %	*1*	24	728.5	2810	120.67
2372238	Ambient - 2.5 %	*1*	6	-592	-1615	15.85
2372246	Ambient - 2.5 %	*1*	24	-1625	-1935	37.5
2372239	Ambient - 5 %	*1*	6	-222	1250	62.35
2372247	Ambient - 5 %	*1*	24	-610	250	57.45
2372243	Mixed crab shell /flesh - 5%	*1*	6	2616	6800	436
2438155	Mixed crab shell /flesh - 5%	*1*	24	400	7400	530.45
2438158	1 % control enzyme (439 L)		-	1450	5070	33.98
-	2.5% enzyme (calc.)		-	3625	12675	84.95
-	5% enzyme (calc.)		-	7250	25350	169.9

Note 1: Analytical results corrected by subtraction of appropriate enzyme control results

Table A4: Set 2 - 'Crab Flesh' Enzyme Sample Results (20g of white crab meat)

Sample ID No	Procedure	Notes	Leach Period	TOC as C mg/l	BOD as 02mg/l	COD as O2mg/l	Total N (mg/l)
2446563	20g crab total (homogenised)		-	1394.2	<3000	6156	424
2446536	Control (in water)		6	373.5	875	1065	534.85
2446543	Control (in water)		24	397		1320	563.1
2446550	Control (in water)		48	619	1550	1930	788
2446537	1 % enzy -Ambient	*1*	6	1270	2632	6550	154
2446544	1 % enzy -Ambient	*1*	24	1872	3132	7950	501
2446551	1 % enzy -Ambient	*1*	48	2214.5	2882	8450	529
2446538	2.5 % enzy -Ambient	*1*	6	-313.5	-1545	1685	703
2446545	2.5 % enzy -Ambient	*1*	24	286	-795	4005	609
2446552	2.5 % enzy -Ambient	*1*	48	571	205	4705	615
2446539	5 % enzy -Ambient	*1*	6	-442	-3340	6550	499
2446546	5 % enzy -Ambient	*1*	24	-500	410	5675	523
2446553	5 % enzy -Ambient	*1*	48	2358.5	-1090	10875	770
2446540	1 % enzy - 50 C start	*1*	6	2589	-143	12640	858
2446547	1 % enzy - 50 C start	*1*	24	2939	5882	14410	182
2446554	1 % enzy - 50 C start	*1*	48	3465.5	6882	17130	591
2446541	2.5% enzy - 50 C start	*1*	6	750	-1445	3435	571
2446548	2.5% enzy - 50 C start	*1*	24	-60.5	-1295	3245	697
2446555	2.5% enzy - 50 C start	*1*	48	468.5	1705	4205	649
2446542	5 % enzy - 50 C start	*1*	6	505.5	-4840	6525	493
2446549	5 % enzy - 50 C start	*1*	24	-203.5	-1090	5275	669
2446556	5 % enzy - 50 C start	*1*	48	-128	-2590	6075	61

Note 1: Analytical results corrected by subtraction of appropriate enzyme control results

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