# Commercial Trial of a Multi-Layer Mussel Purification Tank with Re-use of Artificial Seawater

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SEA FISH INDUSTRY AUTHORITY
Seafish Technology

# COMMERCIAL TRIAL OF A MULTI-LAYER MUSSEL PURIFICATION TANK WITH RE-USE OF ARTIFICIAL SEAWATER



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## SUMMARY

Seafish have successfully carried out trials with multi-layer stacking of mussels and re-use of artificial seawater in order to develop a more cost effective and controlled method of mussel purification. As a result the concept of an approved modular tank design has developed, with a purification facility being made up of any number of these tanks housed in a building.

Shellfish processor, Heiploeg and Lynn Shrimpers Ltd., wished to proceed with such a system using eight purification tanks, but first it was necessary to conduct a trial with a single tank to demonstrate its effectiveness and establish operational criteria. Seafish approached local engineering company, Bead Engineering, and agreement was reached on a joint trial involving all three parties. Discussions were held with Department of Health, MAFF and the local Environmental Health Department at Kings Lynn and a trials programme drawn up and agreed upon.

This report describes the trials which subsequently took place following the design of the stainless steel multi-layer tank by Bead Engineering, to Seafish specification, and its installation at the premises of Heiploeg Lynn. The trials were conducted by Seafish as part of its 1988/89 MAFF Research Commission, Project NBA 16. A total of eighteen purifications were made with the tank using a single initial mix of artificial seawater to which ten percent make-up was added at each use. Bacteriological analysis showed that mussels purified satisfactorily throughout the trials and there was no detrimental effect on mussel storage life. It became clear during the trials that water temperature needed to be kept between 5°C and 10°C to ensure satisfactory purification. This makes it essential to house the tank in a building in order to maintain temperature control. Following the first twelve trials the effect of overloading trays/boxes with mussels was investigated and it was possible to establish a safety margin on the nominal load of 15kg per box.

Before the trials were completed a provisional licence to operate the purification tank was granted by the Department of Health to Heiploeg Lynn, pending an official operating approval document being drawn up. A list of operating conditions were drawn up by Seafish and sent to both MAFF and Dept. of Health.

Toward the end of the trials an alternative, deeper, purification box was used with some success, and also a foam collector developed to remove organic waste from the artificial seawater during purification.

Seafish would like to acknowledge the help given during the trials by the following:

Bead Engineering, Kings Lynn.
J. Williamson and his staff of Heiploeg and Lynn Shrimpers Ltd.,
Mr. S. Williamson of J.J. Shellfish.
Graham English, Skipper of "TARIKI" and his crew.
Dr. Shepherd (former head of dept.) Dr. P. White (head of dept.) and Mr. R. Hardy of The Public Health Laboratory, Norwich.

Mr. R. Murphy and Mr. V. George of Environmental Health, Kings Lynn. Mr. C. Rodgers, Fish Diseases Laboratory, MAFF, Weymouth. Mr. J. Wickens, Fisheries Laboratory, MAFF, Conwy.

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## 1. INTRODUCTION

The existing criteria upon which existing large scale mussel purification is based requires mussels to be spread in large shallow tanks with access to a supply of clean seawater. This results in an open air purification plant that requires a lot of land, is labour intensive and generally remote from the source of mussel supply. To make purification more cost effective, Seafish have considered the development of a multi-layer system with partial re-use of artificial seawater. Such a purification plant would have a reduced land and labour requirement and enable it to be housed in a building, thereby offering much improved control of environmental and hygiene conditions. It would also enable the plant to be sited near to the source of mussel supply, thus reducing transport costs. Seafish have carried out successful mussel purification trials both with multi-layer stacking (Ref. 1) and the re-use of artificial seawater (Refs. 2 & 3), and have developed the concept of an approved modular tank design. At present purification tanks in the U.K. must comply with a generalised set of MAFF criteria and consequently

the detail design and construction of each proposed purification tank design must be considered individually. Seafish consider that an approved tank design with associated operating criteria would be more appropriate and reduce the time taken to obtain a licence to operate the tank from the Dept. of Health.
Heiploeg and Lynn Shrimpers Ltd., a shellfish processor at Kings Lynn, Norfolk, wished to build a mussel purification plant based on this concept with eight identical stainless steel tanks. However MAFF were concerned that multi-layer purification, re-use of artificial seawater and stainless steel tanks had not been tried together and indicated that a further commercial scale trial was necessary. Seafish discussed this with Heiploeg Lynn and local company Bead Engineering, already involved in crab processing, and decided that a joint trial be carried out using a single modular purification tank. A tank with a capacity of 1.5 tonnes of mussels would be made to Seafish specification by Bead and installed at the premises of Heiploeg Lynn in time for the mussel season (September 1988 to April 1989). Several tanks of this capacity were considered more effective than a single large tank as they could be used in sequence and give a regular process flow, maximising labour and equipment utilisation.

After discussion with the Dept. of Health London, Seafish produced a trials programme for a commercial scale trial using a single multi-layer purification tank to purify mussels. The trials would demonstrate the effectiveness of the system and help define operational criteria, and if successful lead to approval for the tank to be operated commercially. A small, single layer Control Tank, used in previous trials (Refs. 2 & 3), and operating to existing MAFF criteria would be run throughout the trials to obtain comparative data.

This work was funded by the 1988-89 MAFF Research Commission, Project NBA16 (Mussel Purification).

## 2. OBJECTIVES

**2.1**

To demonstrate the effectiveness of combined multi-layer stacking and re-use of artificial seawater (ASW) in stainless steel tanks for purifying mussels.

**2.2**

To conduct trials to ascertain the onset and rate of mortality of purified mussels at each re-use of ASW.

**2.3**

To establish operating criteria for the re-use of ASW in a multi-layered purification tank.

**2.4**

To obtain approval from the Department of Health for the Seafish multi-layer purification tank with re-use of ASW.

## 3. TRIALS SITE

The purification tank and ancillary equipment were installed at the premises of Heiploeg Lynn, Alexandra Dock, Kings Lynn. Heiploeg Lynn provided a temporary 30m x 10m x 10m building, exclusively for the trials, with power and fresh water supply on a well drained concrete hard standing.

# 4. TRIALS EQUIPMENT

## 4.1 Purification Boxes

The purification tray requires open mesh sides to enable water to flow through it and an open mesh base to allow waste to fall through. Plastic stack/nest type boxes were considered for use with the purification tank due to their space saving when nested empty and ease of stacking when loaded. Two boxes were considered suitable:

### 4.1.1 Paxton CV480P

This has external dimensions of 600mm x 500mm x 185mm and a capacity of 35 litres. See Figure 1. Internal depth is 165mm.

### 4.1.2 Allibert 11037

This has external dimensions of 600mm x 400mm x 236mm and a capacity of 37 litres. See Figure 2. Internal depth is 205mm.

The Paxton box was chosen as its internal depth enabled the MAFF criteria of 75mm depth of mussels and 75mm depth of free water over their surface to be adhered to. The Allibert box was considered more robust but being deeper would require mussels to be in layers well in excess of 75mm if an equivalent load of mussels were to be stowed in a given volume. Nevertheless allowance was made in the purification tank design to use both types of box.

## 4.2 Pallet

The boxes are to be stacked on pallets to enable mechanical handling, the pallets remaining in the purification tank during purification. This necessitates therefore a pallet with an open type platform to enable waste from the boxes to fall through it onto the purification tank base. Several suitable pallets were available, the one chosen being a Paxton PH1 with external dimensions of 1208mm x 1007mm x 165mm.

### 4.3 Multi-layer Purification Tank

The purification tank was designed and constructed by Bead Engineering to Seafish specification and holds a total of 96 boxes, stacked six high on four pallets (Figure 3). The tank is constructed in type 316 stainless steel with pipework and fittings in ABS and UPVC plastics. Excluding external fittings and water cascade, the tank has external dimensions of 5600mm x 1250mm x 1850mm, and has an internal volume of $11m^3$. A basic outline is shown in Figure 4.

The tank has its own circulation pump, ultra violet light sterilizer, flowmeter and control valve, with water drawn from one end and delivered to the other via a water cascade to aerate the water.

Flow screens allow a uniform flow of water through the tank. An oil-free air blower is also fitted to allow additional aeration via air diffusers mounted on the tank base beneath the cascade. All equipment is mounted on the back of the cascade end of the purification tank, as shown in Figure 5, to be clear of the box handling operations and spray from water wash down.

To enable the waste to be flushed from the tank after purification the tank base slopes to one end. This has raised the overall height of the tank and so to maintain workable access a walkway is fitted along one side, as shown in Figure 6.

The tank was installed by Bead Engineering inside the building provided by Heiploeg Lynn.

### 4.4 Reservoir

A cylindrical reservoir for holding the artificial seawater whilst the purification tank was emptied and refilled with mussels was made by Bead Engineering and installed alongside, as shown in Figure 7. The reservoir is 2260mm in diameter and 3000mm deep with a capacity of $10m^3$.

### 4.5 Artificial Seawater Make-up Tanks

Two plastic tanks of 750 litre capacity were supplied to premix salts for use in the trials. A portable submersible pump was used to transfer salt water to the reservoir.

### 4.6 Control Mussel Purification Tank

Seafish installed on site a small Control Tank capable of holding a single tray of mussels, as shown in Figure 8. The tank has its own U.V. sterilizer, pump, flow control and aeration and is designed to operate within the existing MAFF criteria for mussel purification.

### 4.7 Environmental Chamber used for Storage Trials

Manufactured by Cee-Tel Thermal Equipment Ltd., the test chamber installed at IDU premises, Hull, has a 1m x 1m x 1m chamber in which any required temperature between -30°C and 100°C can be maintained. Computer control enables any pre-set temperature/time regimes to be run within the chamber and monitored.

### 4.8 Instrumentation

Water temperature, pH, dissolved oxygen and salinity in the purification tank were measured using portable electronic meters. Ammonia, Nitrite and Nitrate levels were measured on site using chemical test kits. Further details are given in Appendix I.

## 5. ARTIFICIAL SEAWATER (ASW)

MAFF Laboratory Leaflet No. 39 (Ref. 4) gives information on artificial seawater and salinity requirements for shellfish. The salt content or salinity of seawater is usually expressed as number of parts by weight of salt in one thousand parts by weight of water. The unit 'parts per thousand' is indicated by the symbol °/oo. Salinities of 22°/oo, 27°/oo and 30°/oo are specified, all using five basic salts. A salinity of 27°/oo was chosen for re-use even though a lower salinity of 22°/oo is considered satisfactory for mussels. This was to allow a margin for error in the commercial environment, to avoid the danger that if salinity fell too low the mussels would not purify. Details are given in Appendix II.

## 6. MUSSEL SUPPLY

Heiploeg and Lynn provided fresh mussels as required for the trials. These were supplied by one vessel to try and minimise the effect on post purification mortality trials of different handling methods. Mussels were landed in nominal 25kg sacks on the quay opposite the trials site (Figure 9) and brought round by lorry.

## 7. BACTERIOLOGICAL ANALYSIS

Several quantitative methods exist in the U.K. for the examination of bivalve molluscs for bacteriological sewage contamination. These include roll tubes, pour plates and most probable number (MPN) techniques, but apparently there is no national standard method. For the purpose of this trial MAFF requested the use of an MPN method specified by P.A. West and M.R. Coleman (Ref. 5). Norwich Public Health Laboratory was approached by Seafish and an agreement reached for samples to be taken on a seven day week basis throughout the trials. At the start of each trial three mussel samples were taken and at the end a further five with two water samples as well. Mussel sampling technique and location are detailed in Appendix III. At Norwich the mussel and water samples were examined for *Escherichia coli* (*E. coli*) and Group D faecal streptococci. Faecal streptococci were to be used as an indication that purification had occured in the absence of sufficient numbers of *E. coli*.

At the end of purification, counts of *E. coli* should all be less than 230 *E. coli*/100g and in water less than 2 *E. coli*/100ml. Counts of streptococci in purified mussels are not well defined but more than 1000/100gm would be suspicious.

## 8. TRIALS PROCEDURE

The trials were progressed in two distinct stages. First a continuous run of twelve consecutive purifications were made on a repeat procedural basis to prove the system and obtain Department of Health approval. Following the successful completion of Stage I a second series of six individual trials were conducted on a one or two trials per week basis, mainly to determine the effects of tray overfilling and inadequate washing of the unpurified mussels. All trials involved the operation by Seafish staff of both multi-layer and control tanks, with each purification being monitored. For each purification the control tank was filled with freshly made artificial seawater. The multi-layer tank is filled with 90% of the water from the previous trial and 10% freshly made. At the end of the purification cycle water at the bottom of the tank contains mud, shell and faeces and is flushed away losing 10% of the water to prevent any contamination of the next purification cycle.

### 8.1 Trials 1-12 Standard Purification

#### 8.1.1 Filling Purification Tank with Mussels

96 boxes were filled with 15-16kg of mussels (Figure 10) to give a nominal tank load of 1500kg. After washing the boxes of mussels with a freshwater hose they were loaded manually into the multi-layer tank and stacked on four plastic pallets located inside. The samples for bacteriological analysis were taken whilst filling the purification tank. The control tank contained a single tray of mussels.

#### 8.1.2 Filling Purification Tank with Artificial Seawater

For the first use 100% freshly made artificial seawater was transferred from the reservoir into the purification tank through the U.V. until the top layer of mussel boxes were covered. Thereafter the tank was filled with 90% from the previous trial and 10% freshly made. The control tank was always filled with freshly made.

Once filled both tanks had U.V., water pump and aeration switched on. The water flow was set to change the water in the tanks once per hour.

### 8.1.3 Monitoring Trials

pH, ammonia, nitrite and nitrate levels were measured at the end of each trial, with salinity checked at the start. Water temperature and dissolved oxygen levels were monitored throughout each trial.

### 8.1.4 Emptying Purification Tanks

The trials operated on a continuous 48 hour cycle with tanks being emptied and refilled every second day. The duration of purification during each cycle was a minimum of 42 hours.

In the multi-layer tank, firstly water samples were taken from both ends of the tank and then the water was pumped back to the reservoir via the water circulation suction. This is positioned so that 90% of the water has been removed when suction is lost. Boxes of mussels were removed manually and washed, and samples for bacteriological analysis and mortality holding trials were taken. With the mussels removed the tank drain was opened and the tank flushed out. The control tank was totally drained and samples taken.

After purification the remaining mussels were usually re-bagged and sent away for commercial sale, but only on the understanding that they were to be treated as unpurified mussels requiring purification in a licensed plant prior to consumption.

### 8.1.5 Holding Trials

After each purification cycle samples of 40 mussels was taken from both multi-layer and control tanks. These were put into an insulated container and taken to IDU, Hull. Here they were put into shallow trays and held in a moist condition at 15°C in the Environmental Chamber. Mortality in each sample was assessed daily.

## 8.2 Trials 13-18 Non Standard Purification

Apart from the changes described below the trials procedure followed that above in 8.1.

### 8.2.1 Tray Overfilling

For trials 13, 15, 16 and 17 the boxes were filled with 20-21kg of mussels to give a nominal tank load of 2000kg. In trials 13 and 15 the unpurified mussels were washed as normal but in trials 16 and 17 the mussels were not washed.

### 8.2.2 Alternative Trays

In trials 15 and 17, forty of the deeper boxes (4.1.2) were stacked on the pallets at both ends of the purification tank. The shallower standard boxes being used on the other two pallets. This was done to obtain data on deeper layers of mussels. The deep boxes were also part of a handling trial, the boxes having been filled at sea (Figure 11) and transferred directly to the tank.

### 8.2.3 Foam Hood

The cascade and aeration system produce a considerable amount of foam on the surface of the tank when in operation. Foam has a large surface area for bacteria and waste materials to attach to and its continuous removal was considered worthy of investigation. A special foam collection hood was constructed and used during Trial 14. Further details are given in Section 10.8.

### 8.2.4 Handover Trial

Throughout the trials the boxes had been weighed to ensure consistency of biomass within the tank. Commercially this would not be practical and the boxes would be filled 'by eye' on a volumetric basis. For Trial 18 the staff at Heiploeg Lynn filled the boxes without weighing and were then randomly checked by Seafish.

## 9. TRIALS RECORD

The summary record is given below. No delays were encountered as a result of bad weather effecting mussel supply.

| TRIAL NO | DATES | MUSSEL CONDITION | COMMENT |
|---|---|---|---|
| 1 | 22/24 Nov | Fresh * | Building still open at both ends |
| 2 | 24/26 Nov | Fresh * | Building still open at both ends |
| 3 | 26/28 Nov | Fresh * | |
| 4 | 28/30 Nov | Fresh * | Cockle shell put into cascade trough |
| 5 | 30 Nov/ 2 Dec | Fresh * | |
| 6 | 2/4 Dec | Fresh * | |
| 7 | 4/6 Dec | Fresh * | |
| 8 | 6/8 Dec | Fresh * | Preliminary trial with foam removal |
| 9 | 8/10 Dec | Fresh * | |
| 10 | 10/12 Dec | Fresh * | |
| 11 | 12/14 Dec | Fresh * | |
| 12 | 14/16 Dec | Fresh * | |
| 13 | 11/13 Jan | Mixed | 20kg per tray |
| 14 | 16/18 Jan | 2 day old | Foam hood trial |
| 15 | 18/20 Jan | Fresh * | 20kg per tray |
| 16 | 23/25 Jan | Fresh | 20kg per tray mussels not washed |
| 17 | 25/27 Jan | Fresh * | 20kg per tray mussels not washed |
| 18 | 30 Jan/ 1 Feb | Fresh | Mussels not weighed |

* Indicates mussels taken from same vessel.

# 10. RESULTS & DISCUSSION

## 10.1 Bacteriological Results

In order to show that satisfactory purification has occurred it is necessary to have high initial counts in the mussels before purification and final counts of less than 230 E. coli per 100gm. The results of sample bacteriological analysis for E. coli and faecal streptococci are shown in Tables 10.1, 10.2 and 10.3. The counts of E. coli before purification vary considerably but individual trials, throughout the trials period, do have high counts. With the exception of Trial 1 the post purification values of E. coli are all well below 230 with nearly all at 20 or less, indicating that mussels have purified within both multi-layer and control tanks. Values for faecal streptococci follow in a similar manner and although there are a few high counts these are not considered significant when compared to those for E. coli.

The first twelve trials were carried out with a standard procedure (8.1) in order to obtain Dept. of Health approval for the multi-layer purification tank with boxes filled with 15kg of washed mussels. A further six trials were done to establish the effects of change to those standard procedures and are discussed in 10.5, 10.8 and 10.9. The bacteriological results show that in spite of this abuse the mussels continued to purify satisfactorily even though the same initial batch of artificial seawater (with 10% make up at each trial) was used for all eighteen trials.

The only set of results that were not entirely satisfactory were those from Trial 1. Although high initial counts did fall, in both the multi-layer and control tanks they did not reach the same level as the following trials. The single count of 3,500 E. coli may on its own be considered a rogue result but two values, one in both multi-layer and control tanks are greater than 230. This is considered to be an effect of water temperature and is discussed in Section 10.3.

The results for the water analysis are, with exception of Trial 6, satisfactory with no E. coli or faecal streptococci found at the water cascade and only isolated incidence at the suction. In Trial 6 values of 3 E. coli were shown at both suction and cascade. This is an isolated case and coincided with water samples being taken after the tank had started to drain down instead of before.

## 10.2 Post Purification Mortality Trials

Previous trials by Seafish (Ref. 6) have shown a relationship between mussel storage temperature and mortality. Storage for one day at 15°C approximates to three days storage at 5°C and three and a half days storage at 0°C. Samples of mussels from both control and multi-layer tanks were taken after each trial and held at 15°C. The incidence of mortality on a daily basis is shown in Figures 12 and 13.

The object of the mortality trials was to compare differences between re-used and freshly made artificial seawater. Figure 14 shows a comparison between re-used water in the multi-layer tank and freshly made in the control at both 10% and 50% mortality. Although differences do occur within each trial there is no obvious overall difference in mortality at either level between re-used artificial seawater and freshly made.

In previous laboratory trials (Ref. 2 and 3) quite significant differences in mussel mortality were found between individual trials and were attributed to different handling methods at sea and storage times before purification. An attempt to overcome this was made by obtaining fresh mussels from the same vessel for each trial. This has had some effect as the differences between trials follow a more even pattern with a general reduction in storage life being shown, possibly the effect of seasonal change on the intrinsic condition of the mussels.

One handling change that did occur was at the end of Trial 3 when the catching vessel, which had previously size graded mussels by hand sieve, fitted a mechanical trommel. These machines speed up the sorting of mussels but give much physical abuse to the mussels with a subsequent effect on their mortality (Ref. 6). A reduction in the period to the onset of mortality can be seen after Trial 3.

When mussels were taken from the multi-layer tank for mortality trials they were taken from both the top and bottom of the tank. This was done to see if there were any differences resulting from re-ingestion of detritus by the mussels at the bottom of the tank. The results are shown in Figure 15 and although differences occur within each trial there is no overall trend in favour of either top or bottom of the purification tank.

## 10.3 Water Temperature

The water temperatures in the multi-layer tank and ambient for each trial are shown in Figure 16. The straight lines simply join plotted points and do not represent a constant rate of temperature change between readings. During the first three trials the water temperature remained between 3.5°C and 5.5°C. Thereafter water temperatures remained between 6.0°C and 10°C. Although ambient temperatures varied considerably the water temperature within each trial varied by no more than 2.0°C and in most cases was only 1.0°C. This demonstrates the effect of enclosing the purification tank within a building.

Purification relies upon the shellfish functioning and it is important therefore that the tank water is warm enough to permit this. Mussel filtration activity is effected by water temperature and can become increasingly less active once temperatures fall below 5°C (Ref. 7). MAFF recommend a minimum water temperature of 5°C (Ref. 8). Trial 1 was the only trial in which the water temperature remained below 5.0°C (mean of 4.0°C) and also the only trial in which purification was not considered satisfactory (8.1). Although initial counts in this trial were high this also applied to several other trials in which purification was satisfactory and in which water temperatures were greater than 5.0°C.

It was observed during the trials that mussel activity appeared to increase at water temperatures of 8.0°C or more (10.4), and can be seen in increased oxygen demand. Unfortunately as water temperature rises its ability to hold oxygen decreases and consequently a maximum water temperature needs to be specified. The mild winter enabled the purification tank to be operated at water temperatures of 10°C with which it coped, giving no cause for concern. Nevertheless, as discussed in 10.4 on oxygen, a maximum water temperature of 10°C is recommended.

### 10.4 <u>Oxygen</u>

Persistent low levels of dissolved oxygen in the water can inhibit filtration activity and a level of at least 50% of air saturation value at the appropriate temperature and salinity is recommended by MAFF (Ref. 8). In general this represents a requirement for a minimum dissolved oxygen level of 5mg/l within the purification tank. Dissolved oxygen levels (mg/l) throughout the trials in the multi-layer tank are shown in Figure 17. The vertical bars represent mean oxygen levels during purification along both sides of the trials tank at both top and bottom layers, and remain above the level of 5mg/l.

The means of maintaining adequate dissolved oxygen levels in the purification tank is to pass sufficient re-oxygenated water through it. In any purification system it is therefore vital that the design allows a uniform water flow throughout the tank. In a multi-layer system this is made more difficult in that it is necessary to not only maintain flow across each layer but also ensure that stratification between layers does not occur. In Figure 17 it can be seen that there is little difference in oxygen level between top and bottom of the purification tank or from left to right hand side. This is essential if the tank is to purify mussels successfully and has been achieved by careful consideration of the flow screens installed at either end of the tank. Trials 14 and 15, which show apparent anomalies are described in Section 10.8 in which a foam hood was used.

It was observed during the trials that mussel activity increased with water temperature with mussels becoming particularly active at 8.0oC or more. This can be seen in Figure 17, with increased oxygen consumption at the higher water temperatures. Clearly, if dissolved oxygen levels above 5mg/l are to be maintained to ensure that purification occurs, then a maximum water temperature must be specified. Figure 18 shows mean oxygen levels during the trials at both ends of the tank. By fitting straight lines through these points both the fall in overall level of dissolved oxygen and also the increase in oxygen consumption with temperature can be seen.

If we extrapolate the lower, suction end of the tank line down to the 5mg/l level of dissolved oxygen it can be seen that this fits a water temperature of 12°C. This is based on the first twelve trials when 15kg of mussels were put in each box. If the mass of mussels in each box is more than 15kg then a greater level of oxygen consumption would be expected. This is shown by the points denoting trials where 20kg per box was used. If box loading is specified at 15kg then it is inevitable that a certain amount of overweight will occur in practice. This will increase the slope of the mean oxygen level line at the suction end of the tank such that a level of 5.0mg/l will be reached before 12.0°C. A maximum purification tank water temperature of 10.0°C is thus recommended for this design of tank.

If tank design is modified to provide a greater input of oxygen, for example by increasing water flow rate or further direct aeration, then a greater box loading or operating temperature may be permissible.

## 10.5 Purification Tank Operation

### 10.5.1 Box Filling

In previous trials by Seafish (Ref. 1) it was shown that if mussels were constrained and not given space to open and filter, then purification was not satisfactory. In a multi-layer system it is essential that boxes are not overfilled as when immersed in water the mussels require free space. This means that clearance must be left between the top surface of mussels in a box and the bottom of the box stacked above (Figure 10). The standard trial was with a load of 15.0kg, but satisfactory purification also occurred with 20.0kg in the boxes. At 20kg there is only about 30mm of clearance between the mussels and the box above, and is considered to be adequate although of course the depth of mussels in each box is now 45mm greater than the 75mm specified by MAFF (Reference 7). At loadings greater than 20kg in these boxes it is possible that purification may be impaired. In a commercial situation the stipulated box load will inevitably at times be exceeded. By stipulating a load of 15kg a safety factor of at least 33% is known to exist. If 20kg were stipulated then the safety factor may be small for these boxes.

### 10.5.2 Aeration

Water was re-oxygenated by two methods. The first was a water cascade mounted some 600mm above the water surface behind a flow screen (Figure 19). The cascade consisted of a trough mounted across the width of the tank, filled with cockle shells and fed by the circulation pump. The cockle shell evened the flow to form a uniform water curtain. The second means of aeration was to put an air diffuser at the bottom of the tank in the well formed by the flow screen and the end wall. Care must be taken with the air supply as a considerable amount of foaming occurs if this is too great. Up until Trial 10 an air supply of 10 litres/minute (l/m) was used but was increased to 20 l/m as higher oxygen demand was encountered with higher water temperatures and tray overloading. At 30 l/m excessive foaming occurred.

It was observed during the trials that two types of foaming occurred. During the day a fine bubbled, creamy foam developed in small quantities at the cascade end of the tank. This was similar to the foam that developed with excessive air supply. At night though, the foam became very course bubbled and in far greater quantities than during the day (Figure 20). The reason for this is not known but may relate to mussel activity at night, although they appeared active throughout the 42 hour purification cycle.

### 10.5.3 Tank Operation

Filling and emptying procedures were straightforward, it being necessary only to operate valves to alter direct flow either to the reservoir or from it (Figure 5). By using the tanks own circulation pump to drain down the water, flow conditions were maintained, thereby causing no turbulence which could lead to re-ingestion of bacterially loaded material by the mussels. In addition the water was drained from the purification tank and back into it again via the U.V. sterilizer to minimise the possibility of any cross contamination.

The tank suction (Figure 21) was fixed such that on emptying the tank, suction was lost when 90% of the water had been removed to the reservoir. This ensured that 10% would be replaced at each re-use. The water remaining in the tank is flushed out with the waste in the bottom of the tank when the tank drain is opened.

### 10.5.4 Box Emptying

A good sign of mussel activity and hence purification is the mussels embyssing themselves during the 42 hours. This occurred throughout the trials with no obvious weakening as the trials progressed. Unfortunately the mussels not only attach themselves to one another but also to the boxes, particularly the underside of the box above. Consequently when unloading the boxes manually as much as half the mussels from the box were attached to the one above (Figure 22). It was noticed that the mussels became more heavily embyssed in the lower layers of the tank.

## 10.6 Water Analysis

### 10.6.1 Ammonia

Figure 23 shows the ammonia levels at the end of each purification cycle expressed in terms of ammonium concentration in parts per million (ppm). During the first twelve trials, which were run continuously, the level in the re-used water increased up to 20 ppm. The water to mussel ratio in the tank was 6.4 l/kg. The control tank levels remain within 2 to 5 ppm. These results are similar to a previous laboratory trial (Ref. 3) where continuous re-use on a much reduced scale also resulted in 20ppm at a water to mussel ratio of 7.5 l/kg.

After a break of 26 days, during which the water was left circulating around the purification tank, several trials were carried out with increased mussel loading and a water to mussel ratio of 4.6 l/kg. The foam hood described in Section 10.8 was used in trials 14 to 18. The ammonia level rose to 30 ppm but fell back to 20 ppm after Trial 18 which operated on a reduced box load. No adverse effect was found in either mussel mortality or tainting of mussel meat.

### 10.6.2 Nitrite

Figure 24 shows the nitrite levels at the end of each purification expressed in terms of total nitrite in ppm. The level remained at or below 0.5ppm for the first twelve days in the re-used water, after which the level started to rise reaching 2.0 ppm at the end of Trial 12. The level in the control tank remained below 0.5ppm for the first eighteen days after which it too rose up to 1.0 ppm. After the break in trials of 26 days the nitrite level in the re-used water was at approximately 4 ppm after which there was a sudden decline to 1.0 ppm coinciding with Trial 13. The level then started to rise again. The build up of nitrite may indicate the effect of nitrifying bacteria, attached to the surfaces of the tanks, breaking down ammonia. The activity continuing between Trials 12 and 13. Immediately before Trial 13 the multi-layer tank surfaces were cleaned with high pressure cold water. This may have accounted in some way for the nitrite drop, but it may also be the effect of further nitrifying bacteria converting the nitrite to nitrate. Nitrite is toxic to mussels but at what level is not clear. No effect on mortality was observed during the trials.

### 10.6.3 Nitrate

Nitrate levels were consistently low during the trials. The test equipment available allowed only measurement in 10 ppm bands and all readings taken proved to be in the less than 10 ppm range.

### 10.6.4 pH

Figure 25 shows the pH in both multi-layer and control tanks taken at the end of each trial. After the first trial the pH of the re-used water remained almost constant at 7.5 to 7.8. The level in the control tank was consistently higher at 7.7 to 8.1. Both hydrogen ions and carbon dioxide ($CO_2$) can cause a reduction in pH, the hydrogen ions giving cause for concern. If low pH is caused by dissolved $CO_2$ this can easily be blown off by direct and vigorous aeration. A plastic container of the tank water was taken during Trial 8 and aerated for two hours during which the pH rose from 7.6 to 8.2 thus indicating the removal of $CO_2$.

The tap water at Kings Lynn had a particulary low pH of 7.1. Vigorous aeration of the make up water increased this to 8.0 after three hours.

### 10.7 Artificial Seawater Re-use

By taking measurements of water levels within the reservoir during the tank filling and emptying operations the volume of water in the purification tank with 1,500kg of mussels was controlled at 9.15m$^3$. At the end of each purification 8.15m$^3$ of water was pumped back to the reservoir which represents a loss of 1.0m$^3$ or 10.8%. This water was replaced at the next purification with 1.0m$^3$ of freshly made artificial seawater.

In commercial practice the weight of mussels may well exceed 1,500kg (10.5.1) which in turn will displace water and alter the ratio. At 20kg per box instead of 15kg there will be an additional 480kg of mussels which at 0.7 l/kg will displace 0.336$m^3$ of water. With a total water volume of 8.81$m^3$ (9.15$m^3$ - 0.336$m^3$) a make up of 1$m^3$ now corresponds to 11.3%. In practice therefore the make up should always be greater than 10%.

An essential aspect of the trial was to demonstrate that artificial seawater could be re-used for purifying mussels. After 18 trials there was no adverse effect on mortality and satisfactory purification continued, even though levels of ammonia and nitrite rose and fell, presumably with bacterial action within the tank. No effect on mussel filtering activity or formation of byssal threads was observed and the impression was that the water could have continued to be re-used. Unfortunately this could not be done as the project approached the end of the financial year and the end of mussel season in the Wash area.

After the first twelve trials the results were discussed with MAFF as a preliminary stage in obtaining approval and agreement reached that the artificial seawater could be used ten times (with 10% replenishment) before all the water should be replaced. On the basis of further successful purifications with the same water the number of re-uses should now be subject to further consideration. During the trials the salinity of the artificial seawater remained between 25°/oo and 28°/oo.

### 10.8 Foam Hood

Foam has a large surface area for bacteria and organic waste to attach itself to, and its removal is often a part of water recirculation systems. Foaming is generally created by direct aeration and the foam removed together with the bacteria and waste. With the re-use trials proceeding beyond the initial twelve proving trials it was considered worth trying to set up such a system within the multi-layer purification tank.

This had to be at the suction end of the tank which was clear of the electrics and controls and easy for access to wash down. A hood was made in plywood (Figure 26) which sat on the end of the tank spanning the flow screen and overhanging the end of the tank. A baffle was fitted inside to enable foam to run off onto the concrete floor (Figure 27). Aeration was provided by installing an air diffuser over the suction pipes at the bottom of the tank.

Use of the hood commenced during Trial 14 with several options of aeration. Used on its own with direct aeration at the suction end only it was found that oxygen levels at the cascade end became uneven. It was found necessary to have diffusers on at both ends of the tank. The action of aeration at the suction tended to unbalance the water flow by creating a localised flow at the top of the flow screen and giving an increase in oxygen level at the top of the tank in this area. However, this did not appear to create any 'dead' spots and was not considered a problem. It was found necessary to get the air flow right as too little meant no foam and too much an excessive water loss. A total air flow of 20 l/min was found most suitable feeding diffusers at both suction and cascade.

The foam hood worked well and was used for the remaining four trials. However, the need for it may depend upon the final outcome of number of re-uses that are permitted before the water has to be replaced.

## 10.9 <u>Deep Box Trial</u>

In Section 4.1 reference is made to an alternative box to the one used, which was 40mm deeper internally (Figure 2). This box is more robust and was being used in handling trials at sea to try and overcome some of the physical damage and shock incurred when using sacks. The boxes would fit into the purification tank and so in Trials 15 and 17 these boxes were filled at sea and loaded at the suction and cascade ends of the purification tank at 25 boxes per pallet, stacking five high. The loading of the tank is shown in Table 10.2. Rows 1 and 8, column 1 were filled with 15kg of mussels. All other boxes were filled with 20kg. Bacteriological counts of pre-purified mussels were not very high and so final results for E. coli of 20 are less significant. However, values for faecal streptococci were higher and give similar low results to those obtained in other trials, indicating purification was occurring.

The concept of filling boxes at sea and landing them directly to the purification tank is good but loading them directly into the purification tank must be treated with caution. It is important to ensure that boxes are not overloaded (10.5.1), and as it is not practical to expect fishermen to only half fill boxes at sea it will be necessary to empty some of the mussels out or to insert spacers between the boxes in the tank. This could lead to boxes being put directly into the purification tanks in a grossly overloaded condition. For the purification tank to function it is also important to have the correct number of boxes to fill the tank. This is best achieved by keeping the purification boxes on the premises were they can be kept clean and under some control.

## 10.10 Purification Tank Approval and Operating Criteria

After the first 12 trials the results obtained were discussed with MAFF at the Fish Diseases Laboratory, Weymouth. They were satisfied with the results and gave technical approval to the Dept. of Health. On February 13th 1989 Dept. of Health issued a provisional licence number to Heiploeg Lynn to operate the purification tank commercially pending an official operating approval document being drawn up. MAFF must provide the Dept. of Health with an operating specification for the plant and this was also discussed. The following is the list of provisional operating conditions that were sent by Seafish to both MAFF and Dept. of Health.

**OPERATING CONDITIONS FOR MULTI-LAYER TANK FOR PURIFICATION OF MUSSELS**

1. **Box Loading**

*1.1 15kg of mussels per box (nominal 75mm deep with 75mm free space above).

1.2 Boxes stacked six high within tank.

2. **Operation**

* 2.1 Mussels washed before purification.
* 2.2 Water flow 9.2m3/hr (one change per hour).
* 2.3 U.V. steriliser 6 x 30 watt tubes.
2.4 Air supply to water 20 l/minute.
* 2.5 Dissolved oxygen level in water no less than 5.0mg/l.
* 2.6 Minimum water temperature 5oC.
2.7 Maximum water temperature 10oC.
2.8 Minimum purification cycle of 42 hours.

3. **Artificial Seawater**

3.1 Artificial seawater to be made up from five basic salts as defined in MAFF Laboratory Leaflet No. 39. Artificial Seawater for Shellfish Tanks. Mixture to give a salinity of 27 parts per thousand.

3.2 After each purification cycle 10% of the seawater must be replaced with fresh artificial seawater.

3.3 Artificial seawater as defined in 3.1 and 3.2 above may be used for 10 successive purification cycles only.

* Denotes existing criteria for mussel purification.

The successful purification of mussels in the multi-layer purification tank at Kings Lynn depends upon an even flow of sufficiently oxygenated water through the mussel boxes. This has been achieved by the unique design of the tank and clearly it cannot be interpreted as justification for any multi-layer system that may be presented by others.

It has been equally important to ensure that water temperature within the purification tank remains between 5°C and 10°C. This has been achieved by housing the tank in a building and is considered to be a condition of operation of the purification tank.

## 10.11 Costs

### 10.11.1 Artificial Seawater

If we take the basic make-up salts as described in Appendix II and assume a mains water cost of £1.00 per cubic metre the cost of a cubic metre of artificial seawater (ASW) is £6.86. The multi-layer purification tank will require $9m^3$ for its initial fill and with ten uses allowed with $1m^3$ make-up a water cost per tonne of mussels will be:-

| | | £ |
|---|---|---|
| $9m^3$ of ASW | = | 61.74 |
| $1m^3$ x 9 | = | 61.74 |
| | | 123.48 |

| | | |
|---|---|---|
| Mussels purified in 10 cycles | = | 15 tonnes |
| ASW cost per tonne of mussels | = | £8.23 |
| The cost per 25kg sack | = | 20.5p |

### 10.11.2 Purification Tank

The costs given below are those for the single trials tanks and reflect the design and production cost of a one-off unit. These costs, subject to rises in material cost, would come down with further production. In addition it must be remembered that the reservoir will service several purification tanks.

| | £ |
|---|---|
| Multi-layer purification tank - steelwork only | 8,000 |
| Reservoir | 6,700 |
| Walkway | 1,000 |
| Pump and pipework | 2,500 |
| Air blower and pipework | 800 |
| U.V. steriliser | 1,000 |
| Installation/transport | 2,000 |
| | 22,000 |

| | |
|---|---|
| Plastic trays | 700 |
| Pallets | 120 |
| | £820 |

## 11. CONCLUSIONS AND RECOMMENDATIONS

**11.1**

The multi-layer stacking of mussels combined with the re-use of artificial seawater in a Type 316 stainless steel tank successfully purified mussels.

**11.2**

Artificial seawater (with 10% replenishment after each purification cycle) was re-used successfully for eighteen successive purifications.

**11.3**

Storage trials after purification, with mussels from both the multi-layer tank with re-used ASW and a standard single layer tank with freshly made ASW, showed no differences in mortality throughout the eighteen trials.

**11.4**

Throughout the trials a gradual increase in the onset of mortality was shown in mussels taken from both the multi-layer and the standard tanks and was considered to be a seasonal effect.

**11.5**

There was no difference in purification or mortality between mussels purified at the top and bottom or either end of the multi-layer tank.

**11.6**

The multi-layer purification tank was given provisional approval to operate commercially by the Department of Health pending official operating approval documentation.

**11.7**

The number of uses of the artificial seawater was provisionally set at ten. It is recommended that consideration is given to this being increased.

**11.8**
Levels of ammonia up to 30 ppm and nitrite up to 4ppm in the re-used water had no apparent effect on mussel purification or mortality. A foam hood was successfully fitted to the multi-layer tank during the last few purifications to remove organic waste from the water, but the need for foam removal is not proven.

**11.9**
It is considered essential that multi-layer purification tanks operate within defined operating conditions.

**11.10**
The design of the tank, including the flow screens and stacking system must create an even flow of oxygenated water through the mussels.

**11.11**
Water temperature in the tank must be maintained between 5°C and 10°C to maintain oxygen levels above 5mg/l and ensure sufficient mussel activity for satisfactory purification. This requires the tank to be housed within a building. In the one instance during the trials where water temperature remained below 5°C, mussels did not purify satisfactorily.

**11.12**
To ensure oxygen levels remained above 5mg/l in the trials tank within the specified temperature range, direct aeration via air diffusers was required at a rate of 20 litres per minute.

**11.13**
Box loading must be controlled. With 20kg instead of 15kg of mussels in the boxes used mussels still purified satisfactorily. Nevertheless 15kg is stipulated to give sufficient safety margin in the commercial environment.

An alternative, deeper, purification box was given limited trial. It appeared satisfactory but its use cannot be proven as bacteriological counts were not high prior to purification.

**11.14**

The way is now clear for the construction of a commercial scale, mechanised, multi-tank high density purification facility re-using artificial seawater.

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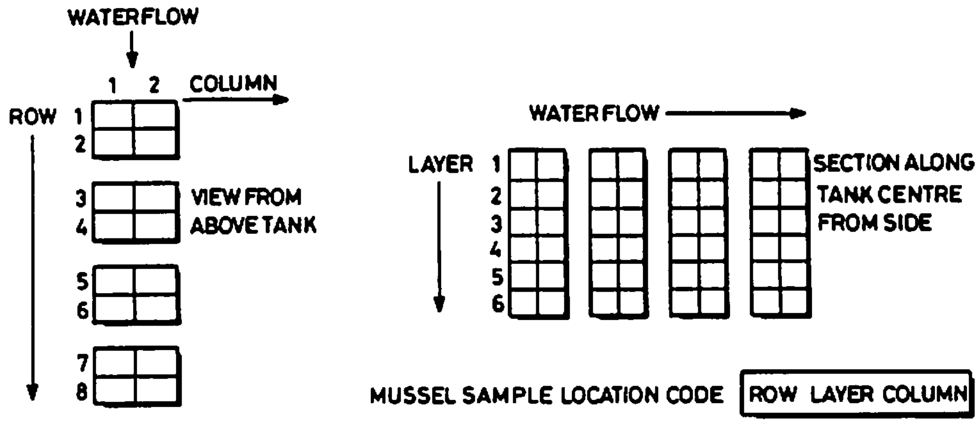
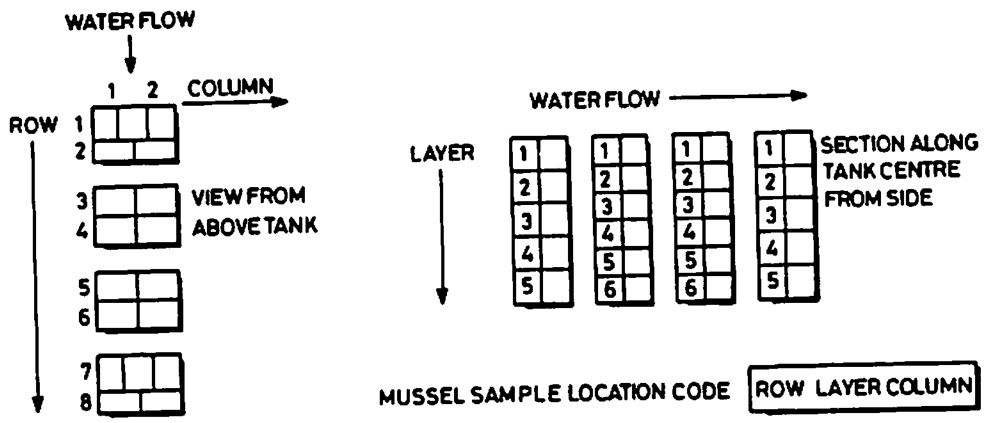



| TRIAL No. | PRE-PURIFICATION MUSSEL SAMPLE E.Coli per 100 gm | | | POST PURIFICATION MUSSEL SAMPLE E.Coli per 100 gm. | | | | | | POST PURIFICATION WATER E.Coli per 100ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 111 | 542 | 861 | 111 | 542 | 861 | 862 | ( - ) random | control | cascade | suction |
| 1 | 5,400 | 16,000 | >18,000 | 130 | 490 | 130 | 130 | (811) 3,500 | 490 | NIL | NIL |
| 2 | 790 | 140 | 230 | <20 | <20 | <20 | 20 | (811) <20 | <20 | NIL | NIL |
| 3 | 260 | 210 | 1,300 | <20 | <20 | <20 | <20 | (811) <20 | <20 | NIL | NIL |
| 4 | 330 | 330 | 230 | <20 | 20 | <20 | <20 | (162) <20 | 20 | NIL | NIL |
| 5 | 490 | 170 | 80 | <20 | 20 | 20 | <20 | (262) <20 | 20 | NIL | NIL |
| 6 | 1,700 | 1,300 | 5,400 | <20 | <20 | <20 | 110 | (451) <20 | <20 | 3 | 3 |
| 7 | 2,400 | 3,500 | 16,000 | <20 | 20 | <20 | <20 | (661) 50 | <20 | NIL | NIL |
| 8 | 170 | 140 | 700 | <20 | 20 | <20 | <20 | (161) 20 | <20 | NIL | NIL |
| 9 | 460 | 1,400 | 790 | <20 | <20 | <20 | 20 | (722) <20 | <20 | NIL | NIL |
| 10 | 40 | 40 | 40 | <20 | <20 | 20 | <20 | (241) <20 | <20 | NIL | NIL |
| 11 | 790 | 3 500 | 3 500 | <20 | <20 | <20 | 20 | (451) <20 | <20 | NIL | NIL |
| 12 | 490 | 330 | 220 | <20 | <20 | <20 | <20 | <20 | <20 | NIL | NIL |
| 13 | 16,000 | 3,500 | 1,400 | <20 | <20 | <20 | <20 | (561) 20 | <20 | NIL | 1 |
| 14 | 220 | 50 | 9,200 | <20 | <20 | 20 | 20 | (711) 20 | <20 | NIL | NIL |
| 15 | 80 | 330 | 230 | SEE TABLE 10·2 | | | | | | NIL | NIL |
| 16 | >18,000 | >18,000 | >18,000 | <20 | 20 | <20 | <20 | (223) <20 | <20 | NIL | 2 |
| 17 | 2,400 | 330 | 100 | SEE TABLE 10·2 | | | | | | NIL | NIL |
| 18 | 1,300 | 1,300 | 490 | <20 | <20 | 20 | <20 | (812) <20 | <20 | NIL | NIL |

Bacteriological Analysis of Mussels for E.coli. Table 10·1




For Trial 15 a combination of two box types were used in the multi-layer tank

ALLIBERT 11037 used in rows 1, 2, 7 & 8. Being deeper these boxes stack only 5 high

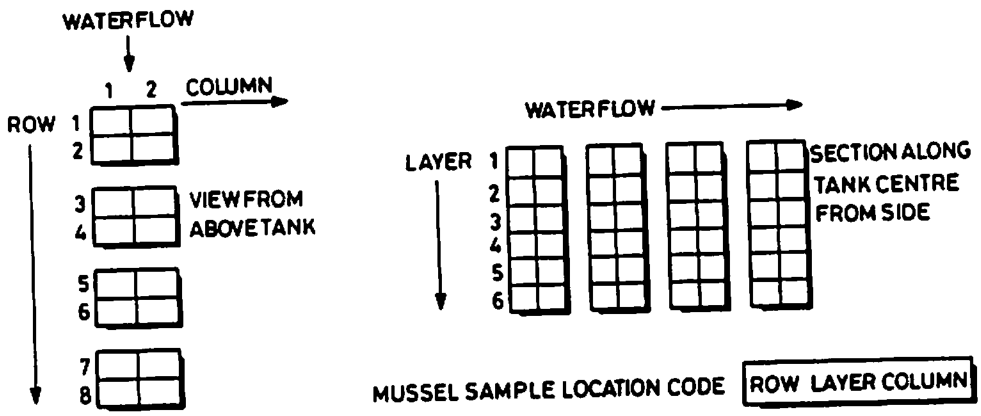
PAXTON 4820P used in rows 3, 4, 5 & 6.

| PRE-PURIFICATION | | | | |
|---|---|---|---|---|
| SAMPLE LOCATION | TRIAL 15 | | TRIAL 17 | |
| | E. coli 100 gm-1 | F.S. 100 gm-1 | E. coli 100 gm-1 | F.S. 100 gm-1 |
| 1 1 1 | 80 | 1,300 | 2,400 | 790 |
| 5 4 2 | 330 | 230 | 330 | 2,400 |
| 8 5 1 | 230 | 1,400 | 100 | 2,400 |

F.S. Faecal Streptococci

| POST PURIFICATION | | | | |
|---|---|---|---|---|
| SAMPLE LOCATION | TRIAL 15 | | TRIAL 17 | |
| | E. coli 100 gm-1 | F.S. 100 gm-1 | E. coli 100 gm-1 | F.S. 100 gm-1 |
| 1 1 1 | <20 | 40 | <20 | 20 |
| 1 5 6 | <20 | 20 | - | - |
| 3 1 1 | <20 | 40 | - | - |
| 5 4 2 | <20 | <20 | <20 | 130 |
| TOP of BOX 8 1 2 | 20 | <20 | <20 | 50 |
| BOTTOM of BOX 8 1 2 | <20 | <20 | | |
| TOP of BOX 8 5 2 | 20 | 50 | <20 | 130 |
| BOTTOM of BOX 8 5 2 | <20 | 50 | | |
| 8 5 1 | - | - | <20 | 20 |
| CONTROL | 20 | <20 | <20 | 130 |

Bacteriological Analysis of Mussels Trial 15 Table 10.2




| TRIAL No. | PRE-PURIFICATION Faecal Streptococci per 100 gm. | | | POST PURIFICATION Faecal Streptococci per 100 gm. | | | | | | WATER F. Strep. per 100 ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 111 | 542 | 861 | 111 | 542 | 861 | 862 | ( – ) random | control | cascade | suction |
| 1 | 3,500 | 3,500 | 2,400 | 140 | 790 | 80 | 790 | (811) 170 | 340 | NIL | NIL |
| 2 | 490 | 490 | 460 | 20 | <20 | <20 | <20 | (811) <20 | <20 | NIL | NIL |
| 3 | 170 | 70 | 130 | <20 | <20 | <20 | 20 | (811) <20 | <20 | NIL | NIL |
| 4 | 340 | 460 | 80 | <20 | <20 | 20 | <20 | (162) <20 | <20 | NIL | NIL |
| 5 | 220 | 490 | 130 | <20 | 1,300 | 2,400 | <20 | (262) 250 | 230 | NIL | NIL |
| 6 | 2,400 | 2,400 | 5,400 | 20 | <20 | 20 | 110 | (451) 20 | <20 | NIL | NIL |
| 7 | 5,400 | 2,400 | 2,400 | 70 | 20 | 20 | 80 | (661) 50 | 20 | NIL | NIL |
| 8 | 1,100 | 460 | 1,100 | <20 | <20 | <20 | <20 | (161) <20 | <20 | NIL | NIL |
| 9 | 270 | 790 | 330 | <20 | 20 | <20 | <20 | (722) <20 | 50 | NIL | NIL |
| 10 | 1100 | 790 | 490 | <20 | <20 | 20 | <20 | (241) 20 | <20 | NIL | NIL |
| 11 | 490 | 220 | 110 | <20 | 20 | 3,500 | 20 | (451) 20 | 80 | NIL | NIL |
| 12 | 310 | 230 | 230 | <20 | 150 | 1,100 | 110 | <20 | <20 | NIL | NIL |
| 13 | 200 | 220 | 1,300 | <20 | 20 | 20 | 20 | (561) 80 | 20 | NIL | NIL |
| 14 | 230 | 220 | 2,400 | <20 | <20 | 130 | 80 | (711) 50 | <20 | NIL | NIL |
| 15 | 1,300 | 230 | 1,400 | SEE TABLE 10·2 | | | | | | NIL | 2 |
| 16 | 16,000 | 240 | 5,400 | <20 | 70 | <20 | 170 | (222) <20 | 90 | NIL | NIL |
| 17 | 790 | 2,400 | 2,400 | SEE TABLE 10·2 | | | | | | NIL | 1 |
| 18 | 2,400 | 3,500 | 2,400 | 140 | 560 | 150 | 230 | 260 | 130 | NIL | NIL |

Bacteriological Analysis of Mussels for Faecal Streptococci

Table 10·3

# APPENDIX 1

## INSTRUMENTATION USED DURING THE PURIFICATION TRIALS

1. Temperature : Comark 2001 digital thermometer with probe (°C).

2. pH : Wissteck-Werkstatten pH 91 portable meter.

3. Oxygen : Dryden 9070 portable dissolved oxygen meter (mg/l).

4. Salinity : Dryden portable meter with seawater probe.

5. Ammonia : Merck Aquaquant colourmetric test kit suitable for the analysis of seawater. As supplied this kit uses an indephenol blue method of determination to give a measure of ammonium in parts per million (ppm). The range of the kit is 0-8ppm. To extend its range to 0.8ppm for the trial, water samples were diluted with distilled water.

6. Nitrite : Merck Aquamerck colourmaster test kit suitable for the analysis of seawater. Gives concentrations of nitrite in the range 0.025 to 20ppm.

7. Nitrate : Seatest Marine Aquarists Multi Kit. Although suitable for measurement of ammonia ph, Nitrite and Nitrate using colourmetric determinations the nitrate test only was used. The test gave concentration of nitrate nitrogen in the range 0 to 100ppm.

## APPENDIX II

## ARTIFICIAL SEAWATER MIXTURE

Five basic salts as defined in MAFF Laboratory Leaflet No. 39 (Ref. 4) were mixed in the following proportions to give a salinity of 27 parts per thousand in 1000 litres of water.

| | | |
|---|---|---|
| Sodium Chloride | $NaCl$ | 21.08kg |
| Magnesium Sulphate | $MgSO_4$ | 5.18kg |
| Magnesium Chloride | $MgCL_2$ | 4.12kg |
| Flake Calcium Chloride | $CaCl_2$ | 1.06kg |
| Potassium Chloride | $KCl$ | 0.50kg |

Commercial grade salts were obtained from a bulk supplier in 50kg sacks other than sodium chloride which came in 25kg sacks.

Cost are given below for sacks bought on individual and per tonne basis.

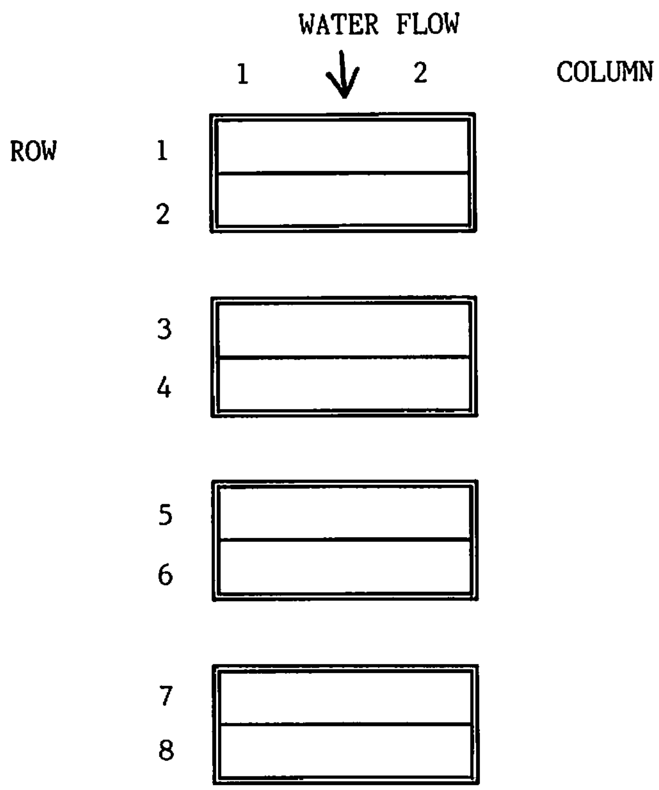
| | £ per sack (individual) | £ per sack (per tonne basis) |
|---|---|---|
| $NaCl$ | 4.63 | 3.59 |
| $MgSO_4$ | 17.90 | 12.45 |
| $MgCl_2$ | 11.45 | 9.71 |
| $CaCl_2$ | 17.35 | 12.40 |
| $KCl$ | 38.25 | 11.75 |

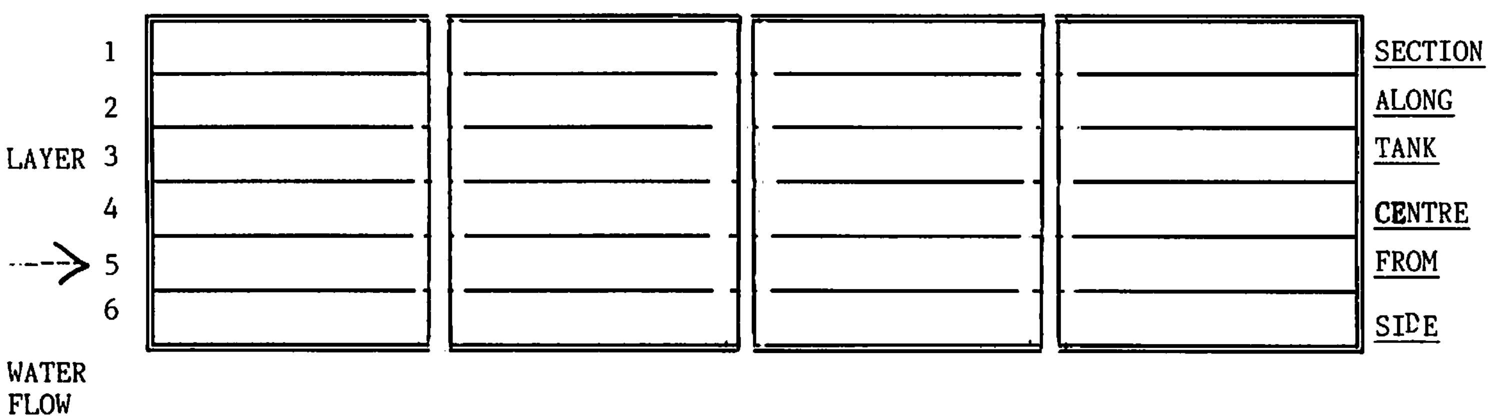
If the sodium and magnesium salts are bought in bulk the salt cost per 1000 litres of water will be:

| | £ |
|---|---|
| $NaCl$ | 3.02 |
| $MgSO_4$ | 1.29 |
| $MgCL_2$ | 0.80 |
| $CaCl_2$ | 0.37 |
| $KCl$ | 0.38 |
| | 5.86 |

## APPENDIX III

## BACTERIOLOGICAL ANALYSIS - SAMPLING TECHNIQUE




For the purpose of sample location the above definition was used

## 1. Mussel Sampling

Each mussel sample for bacteriological analysis contained a sufficient number of mussels to provide 50gm of shelled mussel meat (usually 12-15 mussels) and was taken from an individual box. Mussels were taken from the four corners and centre of the box and placed in a clean coded bag and sealed.

## 2. Mussel Samples - Purification

3 individual mussel samples were taken from the following locations :-

**Sample**

1. Row 1 Layer 1 Column 1
2. Row 5 Layer 4 Column 2
3. Row 8 Layer 6 Column 1

## 3. Mussel Samples - Post Purification

The following individual mussel samples were taken from the following locations:-

**Sample**

1. Row 1 Layer 1 Column 1
2. Row 5 Layer 4 Column 2
3. Row 8 Layer 6 Column 1
4. Row 8 Layer 6 Column 2
5. See text below
6. Control Tank

As the water flows through the tank the level of dissolved oxygen reduces. Waste also falls through the mussel layers to the base of the tank, consequently the space at the end and bottom of the tank is the most likely location for any problems to occur. Two samples (3 and 4) were to be taken from here. Sample 5 was an additional sample that was taken at random.

## 4. Water Samples

Single water samples were taken just before the end of each purification cycle at both cascade and suction ends of the purification tank.