Mussel Purification- Commercial Scale Trial of Multi-Layered Purification

MAFF Commission
Technical Report No. 312
May 1987

MAFF R&D Commission 1986/87

SEA FISH INDUSTRY AUTHORITY
Industrial Development Unit

MUSSEL PURIFICATION - COMMERCIAL SCALE TRIAL OF MULTI-LAYERED PURIFICATION

Technical Report No. 312
MAFF R&D Commission 1986/7/8
Project Codes QFA/NBA16

May 1987
M. Jacklin
P. W. Wilson
J. W. Denton

SEA FISH INDUSTRY AUTHORITY
Industrial Development Unit

Technical Report No. 312 May 1987

MUSSEL PURIFICATION - COMMERCIAL SCALE TRIAL OF MULTI-LAYERED PURIFICATION

SUMMARY

To improve the control and cost effectiveness of large scale mussel purification, Seafish has considered the development of a modular, multi-layered purification tank. Laboratory scale trials at Plymouth Polytechnic were successful and gave Seafish the confidence to proceed with a commercial scale trial. This work is part of the 1987-88 MAFF Research Commission, Project NBA 16.

This report describes a series of trials conducted at Monteum Ltd., Shoreham using a modified crustacea holding tank. Mussels were obtained from the Wash and purified using the existing criteria advised by MAFF with the exception that as the trials progressed the number of mussel layers was increased to the maximum of six that the depth of tank allowed. In addition further trials were conducted with increased mussel density in the tank, the use of interleaf boards between layers and purifying mussels in bags. Water temperature, pH and dissolved oxygen were monitored and samples of tank water and mussels, both pre and post purification, were analysed for the bacterium E.coli and Faecal Streptococci. The trials ran from February to April 1987, when trials were curtailed due to the onset of the mussel spawning season, and were concluded during October to November 1987.

Throughout the trials mussels successfuly purified, including those at increased density, demonstrating that multi-layered purification will work. It became clear though, that if adequate oxygen levels were to be maintained throughout the tank, water flow and water temperature have to be controlled.

The use of interleaf boards between layers of mussels did not effect purification although the boards did prevent mud deposition from one layer to another.

Mussels purified successfully when held loosely in bags, but showed significantly less purification when tightly packed. Closely woven ribbon bags tended to retain mud and detritus more than open weave monofilament type.

SEA FISH INDUSTRY AUTHORITY
Industrial Development Unit

Technical Report No. 312 May 1987
MAFF R&D Commission 1986/7/8
Project Codes QFA/NBA16

MUSSEL PURIFICATION - COMMERCIAL SCALE TRIAL OF MULTI-LAYERED PURIFICATION

Contents

Contd....

Contents (contd.)

Contd....

Contents (contd)

APPENDICES:-

SEA FISH INDUSTRY AUTHORITY
Industrial Development Unit

Technical Report No. 312 May 1987

MUSSEL PURIFICATION - COMMERCIAL SCALE TRIAL OF MULTI-LAYERED PURIFICATION

1 INTRODUCTION

With increasing concern over the extent of sewage pollution of mussel beds and escalating application for Closure Orders, the requirement for purification is increasing. This, coupled with planned increases in mussel cultivation, is likely to necessitate the construction of new purification plants.

Purification plant design in the U.K. is specified by MAFF, whose current guidelines are based upon research dating back as far as the 1920's. The guidelines necessitate that purification takes place in large areas of shallow tanks with mussels laid out in a 3in (8cm) deep layer covered by a minimum of 6in (15cm) water depth (further details are given in Appendix III).

Mussels purify themselves by taking in water and particulate material via the inhalent syphon. They pass it into the mouth through the stomach and subsequently expell it as faeces. By this action the stomach and gut contents are flushed out so removing bacteria and other faecal waste. The removal of contaminating organisms is partly due to their physical expulsion and partly due to their natural mortality. This is the basis of the purification process.

The basic concept is that mussels will self-purify when placed in large shallow tanks of clean almost still seawater. Bacteria within the gut and water are bound up in faeces or pseudo-faeces which are subsequently expelled from the animal. This standard design of plant is costly to construct and operate.

It is the purpose of this project to develop more cost effective means of mussel purification. The particular means investigated is the deep stacking of trays of mussels in a high density purification plant, although the water condition and flow rates remain as currently specified by MAFF.

A high density plant requires less ground area than the standard plant, can be more conveniently located, temperature controlled and protected, and is more suited to mechanical handling. The possibility of the mussels at the bottom of the stack being re-contaminated by those above will limit the depth to which stacking is permissible.

An early pilot trial was carried out at Plymouth Polytechnic (Ref 1), where three trays of mussels were stacked on top of each other in a small tank incorporating a closed loop seawater system with U.V. and simulating water conditions in the MAFF standard U.K. purification tanks. This proved successful.

The trials described in this report investigated the deep stacking of trays in a tank of commercial scale in which the potential for re-contamination is greater.

It was also the purpose of these trials to investigate the purification of mussels in bags.

The practice favoured by MAFF for mussel purification is to fill trays with loose mussels. The general procedure is to tip the mussels from a large sack until an even 3in (8cm) deep layer is achieved within each tray. However, if the mussels are retained within the sacks it simplifies the overall handling operations from harvesting to processor. The potential disadvantage is that the sacks would restrain the mussels and retain their detritus thus inhibiting purification. Various types of sack were investigated as an adjunct to the stacking trials.

The project was funded by the MAFF R&D Commission, Project Codes QFA (1986/87) and NBA16 (1987/88). The work was carried out using the tank facilities of Monteum Ltd at Shoreham-By-Sea and technical advice on the conduct of the trials was given by Dr Paul West of MAFF. The assistance given by Monteum Ltd and Dr West is gratefully acknowledged. Brighton Public Health Laboratory carried out the bacterial analysis.

2 OBJECTIVES

The objectives of the trials were:-

(i) To ascertain if mussels will purify adequately in deep stacks in a commercial scale tank.

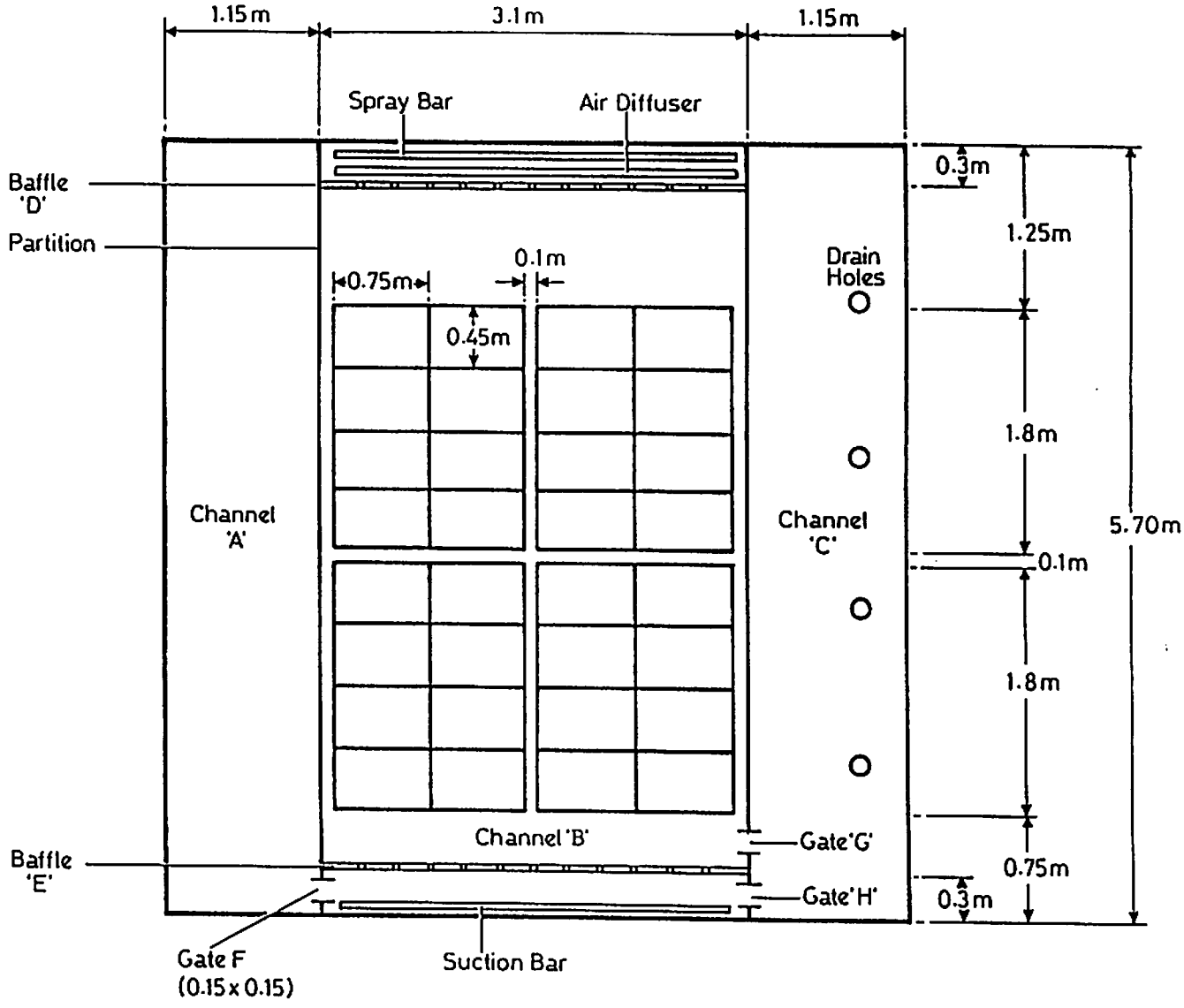
(ii) A secondary objective was to investigate the effects on purification if the mussels are constrained within sacks of various types.

3 TEST RIG AND MATERIALS

3.1 Trial Tank

A concrete tank of dimensions 5.7 x 5.4 x 0.90m deep was available with facilities of plumbed seawater supply, drainage and ultra-violet sterilisation for (18000 l/hr). The tank is located inside a concrete sectional building.

The tank was modified to accommodate the trays that would be used throughout the experiment. Wooden partitions of marine ply were constructed within the main tank to form a central channel, of dimensions 5.7 x 3.1 x 0.90m which would form the 'test tank'. The plan, dimensions and layout of the tank are shown in Figure 1 overleaf.



Plan

Fig. 1 Dimensions and Locations of Tank and Trays

3.2 Pipework and Pump

A new pump of capacity 45,000 l/hr was installed and the pipework replaced with 50mm bore plastic delivery pipe and 76mm bore plastic suction line. A valve controlled the pump output. The plumbing layouts are shown in Figures 2(a) to 2(d) overleaf.

A flowmeter was installed between the pump outlet and the existing U.V. sterilising unit. The meter operated in the range 3000-15000 l/hr.

Valves were installed to permit priming of the pump and suction line.

3.2.1 Spray Bar

The spray bar consisted of 3m long by 50mm diameter bore tube having 6mm diameter spray holes equally spaced along its length (Figure 3).

The bar was fed from a central tee piece coupled via a flexible hose to the U.V. outlet. The spray bar was mounted as high as possible (0.7m) above the maximum tank water level to achieve optimum oxygenation of the water [See Figure 2(b)].

Since the required number of spray holes was best established in practice, and varied with differing flows, spray holes were blocked with PVC tape as required to maintain the powerful jets necessary to provide aeration.

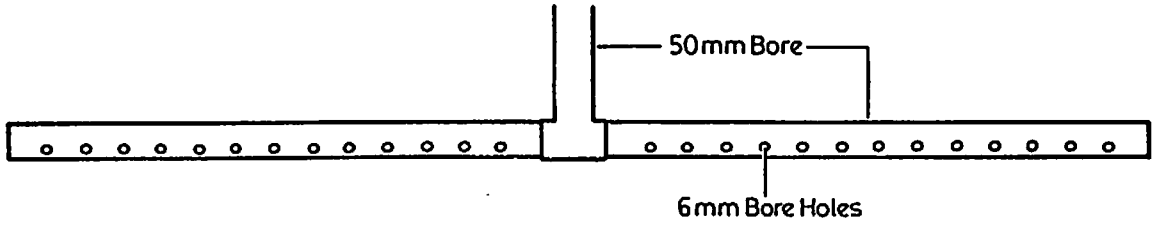
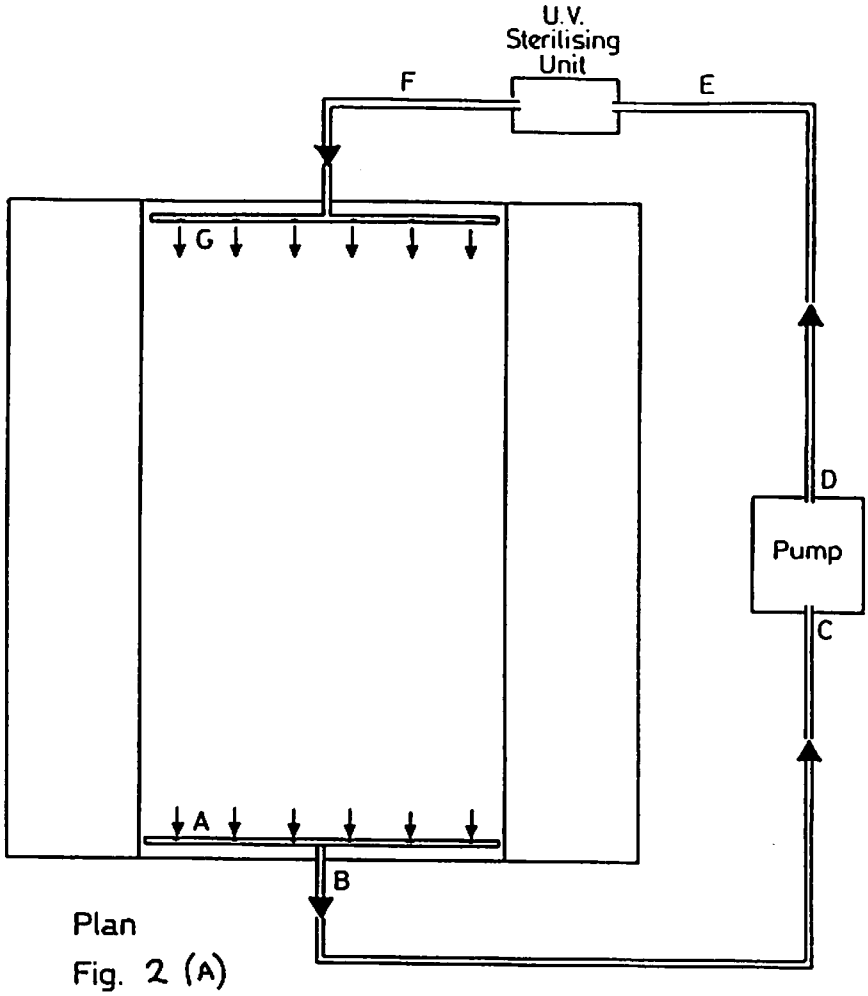


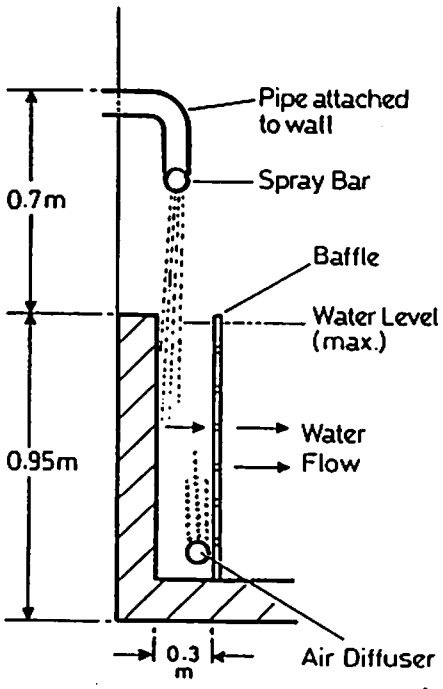
Fig. 3. Spray Bar



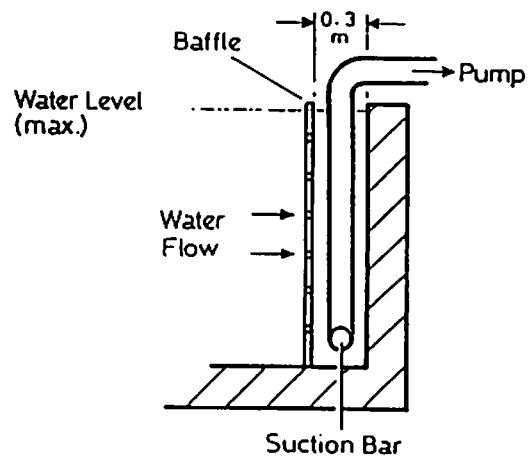
Plan

Fig. 2 (A)

U.V. – 1800 litre Capacity
Pump–4500 litre Capacity
A→B – 50mm Bore
B→C – 75mm Bore
D→E – 50mm Bore
F→G – 50mm Bore
A – 50mm Bore Drilled with 13mm Holes
G – 50mm Bore Drilled with 6 mm Holes



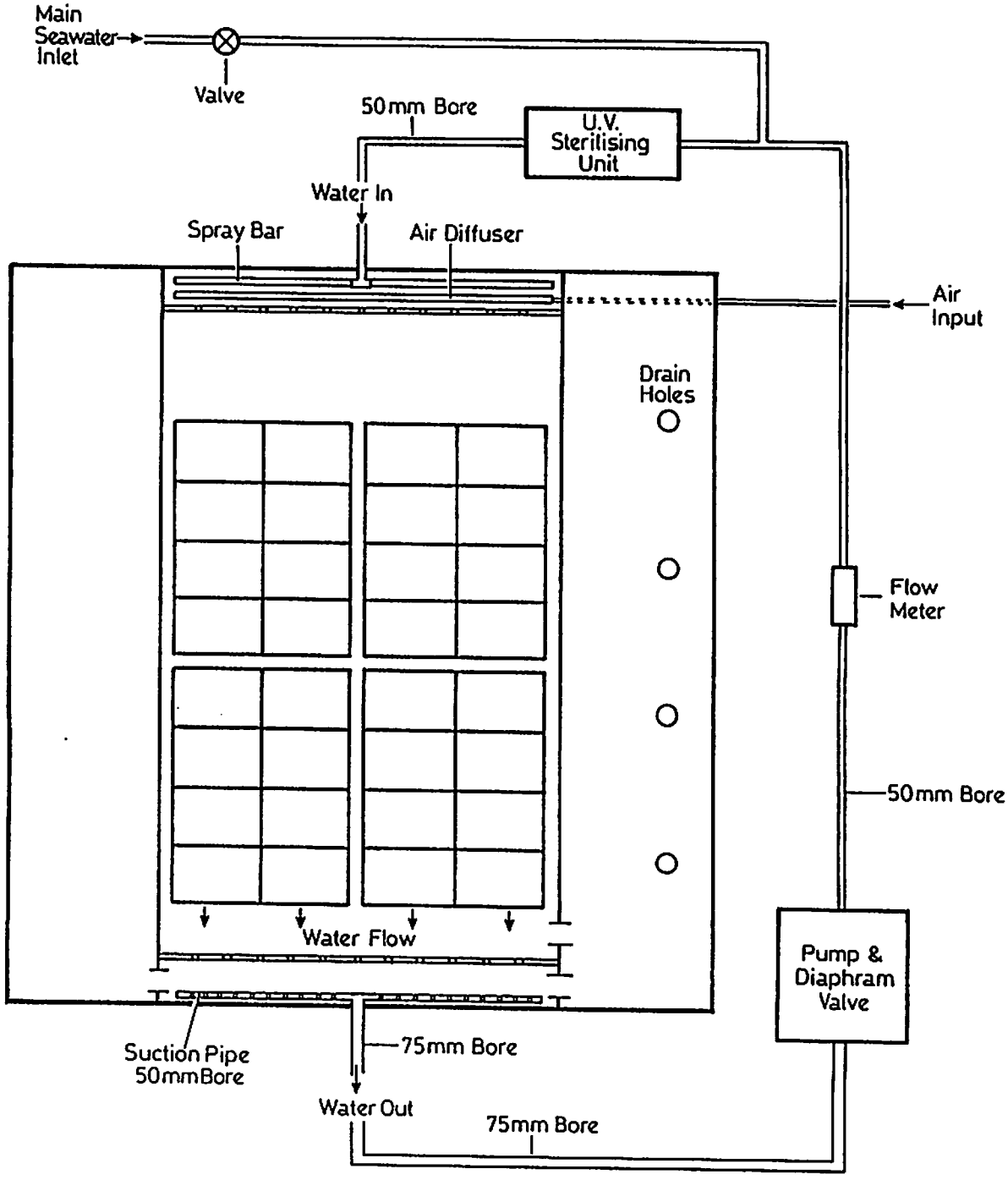
Delivery Side Fig. 2 (B)



Suction Side Fig. 2 (C)

Water Circulation

Fig 2(d)



Water Circulation

3.2.2 Suction Bar

The suction bar comprised of a 3m long, 50mm diameter bore tube which had 13mm diameter holes along its length. In order to achieve even distribution of suction, two tee pieces were installed at 1/3 length and 2/3 length. The tee pieces then combined via 50mm bore tube to a 75mm tee coupled via a 75mm hose to the pump suction.

The suction bar was located 0.05m off the bottom at the outlet end of the tank [See Figure 2(c)]

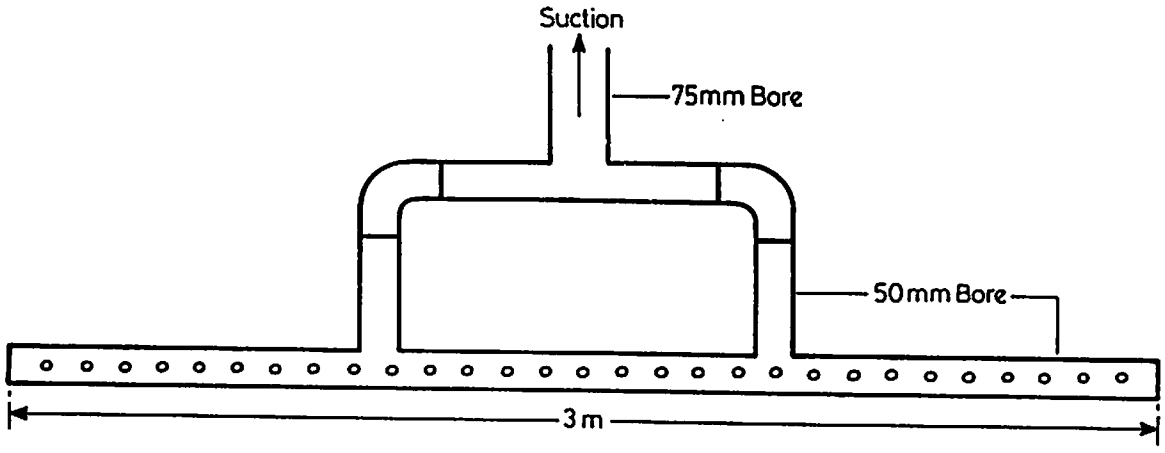


Fig. 4. Suction Bar

3.3 Baffles

Baffles were required at either end of the tank to control the flow of water throughout the working section of the tank [See Figure 2(d)]. The baffle located at the spray bar end of the tank prevented excessive turbulence affecting the mussels and served to even the flow across the width and depth of the tank. The baffle located at the outlet end was similarly to even the flow and ensure suction was effective across the width and depth of the tank. The baffles were positioned 0.3m from either end of the tank and located into vertical runners which allowed removal. The baffles measured 3.1 x 0.9 x 0.08m and were constructed from marine plywood. They were perforated over their full area with 0.03m diameter holes at 0.08m centres.

3.4 Aeration

Aeration of the water was achieved by surface area absorbtion, caused by the entrainment of air from the spray bar, and supplemented by the use of millipore air diffusers connected to an air supply which delivered 8 cubic metres of air per hour. The diffusers were located underneath the spray bar [See Figure 2(b)].

3.5 Trays

The trays used during the trials were Allibert Model No. 41042. The sides and base of the trays are perforated to allow adequate water movement in and around the mussels. The trays are such that in the stacked position an 8cm layer of water would flow over each 8cm layer of mussels (See Figure 5 below).

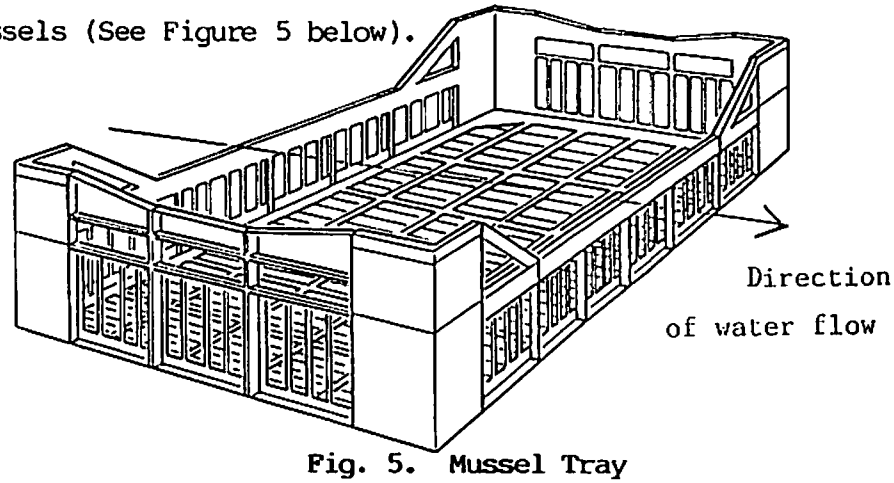


Fig. 5. Mussel Tray

3.6 Bags

Two types of bag material were tested. The first type was the standard close weave plastic ribbon mesh bag commonly used for holding mussels. The second type was made by Seafish from wide mesh (36mm) plastic monofilament. Both bags were approximately 0.45m x 0.8m in size.

3.7 Mussels

These were provided by fishermen from areas around the Welland Cut in The Wash where the River Witham meets the River Welland, and from the proximity of the barrier wall near to Kings Lynn. Both these areas are reputed to be extremely polluted by sewage outfall.

3.8 Microbiological Sampling

Microbiological assessment of mussel and water samples was carried out by Brighton Public Health Laboratory. Analysis was for total Coliforms, E. Coli and Faecal Streptococci using standard methodology (See Appendix II). E. Coli are the standard bacterial indicator organism for sewage pollution, and Faecal Streptococci counts give a 'purification index' of purification activity.

3.9 Control Tanks

The facilities of Monteum Ltd at Shoreham-by-Sea include several purification tanks of standard design. From each batch of mussels to be purified in the trials tank a control sample was taken and purified in one of these standard tanks. These tanks are of concrete construction, are designed for a single layer of mussels in trays and are in the open air.

4 METHODS

4.1 Construction and testing of tank

The rig was assembled during early November 1986 and took approximately one week to complete.

Upon completion the tank was partially filled and the pump run to check performance of the spray bar and the suction bar at various flow rates. The flow meter was calibrated. Trays were subsequently located in the tank and checks of the flow pattern throughout the stack were made using a piece of thread (attached to a wooden rod) which was placed at various positions in the water pathways. The operation of the aeration diffusers was checked up to the full depth of the tank.

The tank was then drained and observations made to ensure that there was no re-suspension of detritus by the flow of water to the drain.

4.1.2 Location of trays within tank

The dimensions of channel B (See Figure 1) allowed a layer of trays 4 wide and 8 long (total 32) to locate on the base of the channel. Trays were positioned upon 8 (45mm x 45mm) battens (2 per tray) which ran underneath the bottom layer of trays and parallel to the sides of the tank. This allowed water to pass underneath the bottom trays and provide room for detritus to accumulate. Each layer of trays started 1.25m from the water inlet end of the tank and finished 0.75m from the water outlet end of the tank. A small central gap (0.1m) was provided along the length and width of the trays to allow access for temperature and dissolved 0_2 measuring probes (See Figure 1).

4.1.3 Tank filling and drainage

The plumbing was so constructed that when filling the tank the incoming seawater was passed from the main feed through the U.V. steriliser and into the tank via the spray bar. During purification, gates F, G and H (See Figure 1) were closed to isolate the central working section of the tank. After purification the gates were lifted slightly and the drains opened.

5 TRIALS

5.1 Sequence of Stacking Trials (February - April 1987)

The rig was tested with an initial run of a single layer of trays to assess purification and compatability with the standard tanks, thence progressing up to 5 layers. Simultaneously with each run of the Trials Tank control mussels were placed in a Control Tank.

Table 1 below relates to the number of layers, weight of mussels and water depth within the tank. Volume relates to actual volume of water in the working section of the tank at stated depth, i.e. total volume less trays and mussels. The flow rates relate to the total water flow rate and to rate per kg of mussels.

Layers	Kg	Depth (m)	Volume (litres)	Flow m^3/hr	Flow L/Kg/hr
1	368	0.21	3301	3.3	8.9
2	736	0.36	5561	5.5	7.5
3	1104	0.51	7811	7.8	7.1
4	1472	0.66	10060	10.0	6.8
5	1848	0.81	12302	12.3	6.6

Table 1

1-5 Layer Stack Conditions in Trial Tank (February - April 1987)

Each tray was filled with 11.5kg of mussels and covered with 7.5cm of water. This equates with the MAFF specification for mussel density and water coverage for standard purification plants. The water within the central working section of the tank was circulated once per hour, again to MAFF specification, the flow rate being increased according to the height of stacking. Because the total volume of water within the central working section (including that beyond the baffles at the inlet and outlet ends of the tank) was circulated once per hour this was approximately equivalent to circulating that immediately surrounding the mussels 1½ times per hour.

In order to operate the trials in a 'worst' situation, mussels were not washed prior to purification. The mussels generally had been hand raked at low water and were therefore heavily contaminated with mud.

On observing the increasing accumulation of detritus in the lower layers there was a slight deviation from this stacking pattern for the 5 layer trial. In this trial the tank was 'split' into two trials. The right-hand side was stacked normally at 5 layers in the manner adopted in previous trials. The left-hand side was modified, however, in that interleave boards and battens were placed between each layer of trays to physically separate each layer (See Figure 6 below). Due to the added thickness of the boards it was only possible to stack this side of the tank 4 trays deep without exceeding the depth of the adjacent 5 stack. The mussel depth within each interleaved tray was increased by a factor of 25% (giving a total 14.5kg in each tray) to ensure that the same mussel to water ratio was maintained in both sides of the tank.

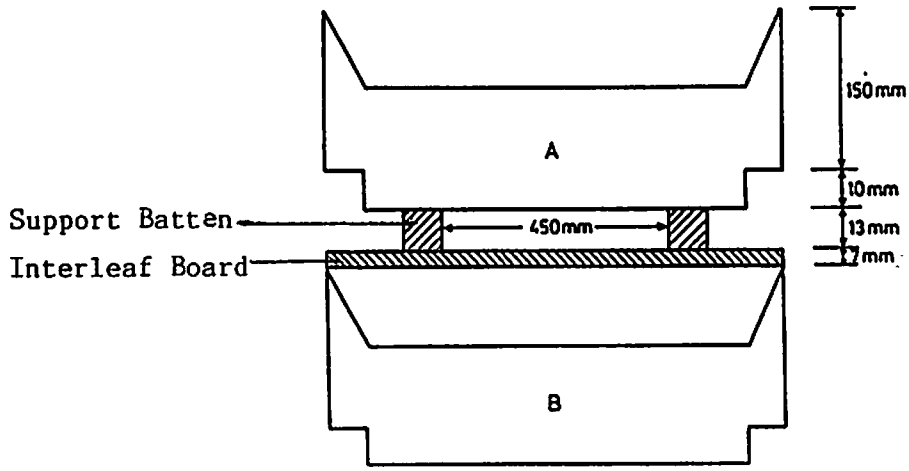


Figure 6. Interleaves between trays.

During April the onset of the mussel spawning season was approaching and following the completion of stacking trials 1-5 high it was decided to carry out one further trial with 6 layers and an increased density of mussels in the trays.

The right-hand side of the tank was stacked 6 high and the left-hand side 5 high with interleaves. The overall mussel loading was increased by approximately 40% so trays on the interleaved side contained 18kg and trays on the straight stacked side contained 16kg. The water flow rate was increased to maintain the flow rate per unit mass of mussels in the tank in line with the 5 layer trial.

At 6 layers the maximum depth of water available in the tank was only just sufficient to cover the mussels.

Layers	Kg	Depth (m)	Volume (litres) (less trays and mussels)	Flow m^3/hr	Flow L/Kg/Hr
6	2976	0.90	12751	19.3	6.5

Table 2

6 Layer Stack Conditions in Trial Tank (April 1987)

Unfortunately spawning of the mussels occured in the tank and so work was postponed until the new mussel season was underway.

5.2 Sequence of Stacking Trials (October - November 1987)

With the restart of the mussel season in October it was decided that two further trials should be carried out.

In the first trial trays were stacked 6 high on the right-hand side of the tank filled with the MAFF specified 11.5kg of mussels. On the left-hand side the trays were stacked 5 high with interleaves (Figure 6) and filled with 14.0kg (20% extra) to maintain the mussel to water ratio.

The second trial was a repeat of the first with the overall mussel density increased by 50% with 17.5kg and 21.0kg in the trays.

Layers	Kg	Depth (m)	Volume (litres)	Flow m^3/hr	Flow L/Kg/hr
6	2224	0.90	13503	22.0 - 18	9.8 - 8.1
6	3360	0.90	12367	22.0	6.5

Table 3

6 Layer Stack Conditions in Trial Tank (October - November 1987)

To ensure adequate salinity the water supply to the holding tank facilities at Monteum is limited to pumping from the river for a few hours on either side of high tide. When the trials were carried out in November there was considerable water demand for other tanks and consequently the Trial Tank took 2-2½ hours to fill. Mussels in the lower trays were therefore immersed in a non-recirculating medium that was not being re-oxygenated until the tank was full and it was noticed that the oxygen concentration at the bottom of the tank dropped significantly while the tank was filling. In the first trial the water flow was therefore initially increased to the pumps maximum capacity of $22m^3$/hr to achieve maximum oxygenation and subsequently reduced to $18m^3$/hr to give a flow rate per unit mass of mussels between those of the one and two layer trials.

In the second trial at maximum pump capacity the flow rate per kg of mussels was much lower and equivalent to that in the earlier five layer trials.

5.3 Individual Trial Sequence

Prior to the start of each of the trials, 3 samples of the mussel consignment to be tested were despatched for analysis of initial contamination as described in section 6.2 and 6.2.1.

The mussels were then loaded into the Trial Tank which was filled with water until the mussels were covered and the trial commenced. Three trays of mussels were placed in the Control tank (3.9) to provide controls as described in section 6.2.2. Water samples were immediately taken from the Trial Tank and analysed as described in section 6.1.4.

The trial was run for 48 hours during which oxygen, temperature and pH were monitored as described in section 6.1.1, 6.1.2 and 6.1.3.

On completion of the trial the water was drained and the mussels removed, and water and mussel samples were taken for analysis as described in section 6.1.4, 6.2.2 and 6.2.3.

In the Control Tank the water was changed every 24 hours (which was standard practice at this site).

5.4 Purification in Bag Trials

In selected trays during the trials mussels were packed into bags rather than being laid loose on the trays. Each type of bag (3.6) was packed in two ways. Firstly, the mussels were packed tightly into the bags as this is standard practice in the industry to aid handling and prevent gaping during distribution. Secondly, the mussels were packed loosely to give the mussels freedom to open during purification.

6 MICROBIOLOGICAL AND CHEMICAL ANALYSIS

Dissolved oxygen, temperature and pH were monitored for both the Trials Tank and the Control Tank. Microbiological assessment of water samples was conducted for the Trial Tank only. Microbiological assessment was conducted on each sample of unpurified mussels and on mussels purified in both Trials and Control Tanks.

6.1 Water Analysis

6.1.1 Oxygen

This was monitored in the Trial Tank at the locations shown by the grid reference below (Figure 7) using a battery operated portable oxygen meter.

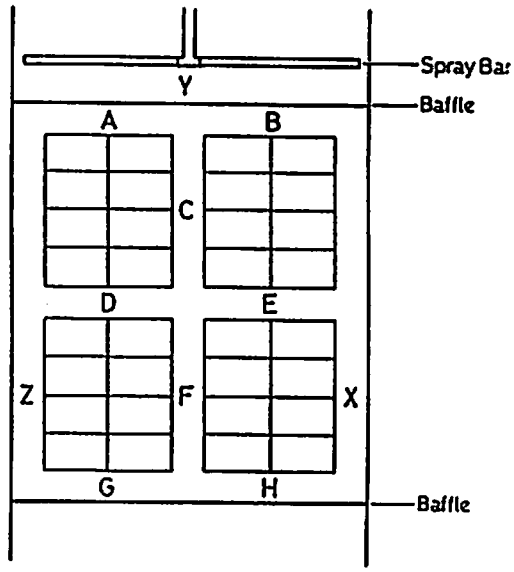


Figure 7. Oxygen measurement points - Trial Tank.

Oxygen content was monitored every 2 hours throughout the day and at 6 hourly intervals throughout the night. Initially readings were taken at the centre of the water column, but as the stack depth increased to 4 layers (0.66m), readings were taken at 1/3 and 2/3 the water depth to give indication of vertical stratification. Oxygen was monitored at the outlet end of the Control Tank.

6.1.2 Temperature

This was measured at location (H) (Figure 7) in the Trial Tank at the same time as oxygen readings were taken. Temperature was monitored at the outlet end of the Control Tank.

6.1.3 pH

This was measured on a daily basis from water entering the Trial and Control Tanks, using a battery operated portable pH meter.

6.1.4 Microbiological Assessment of Water Samples

Microbiological assessment of water samples was for total Coliforms, E.Coli and Faecal Streptococci. Sampling was conducted at the begining (t = 0 hours) and end (t = 48 hours) of purification in the Trial Tank at the locations outlined in the grid pattern below (Figure 8). Each sample was collected from the middle of the water column using a 500ml water bottle.

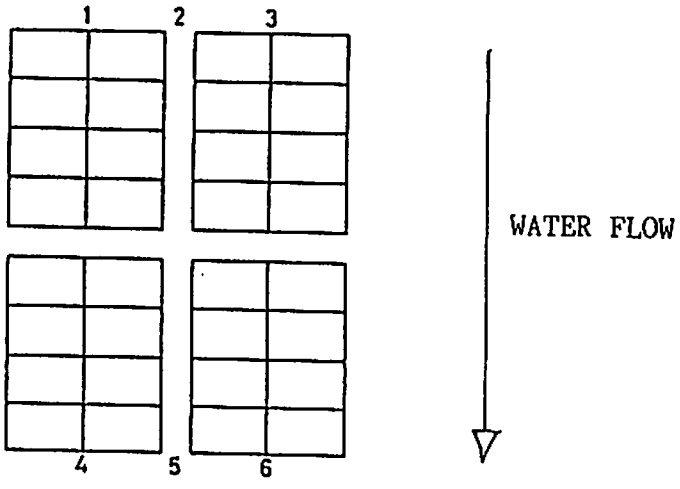


Figure 8. Water sampling locations - Trials Tank

6.2 Mussel Analysis

Microbiological assessment was for total Coliforms, E. Coli and Faecal Streptococci. Samples of unpurified mussels were taken as mussels arrived at Shoreham and samples were taken after purification in the Trial and Control Tank. Further details of the microbiological assessment are given in Appendix II.

6.2.1 Unpurified Mussels

Ten mussels were removed from the incoming mussel consignment. For the 1 layer trial one sample of ten mussels was taken, for subsequent trials three samples of ten were taken.

6.2.2 Control Mussels

Ten mussels were removed from each tray - two from each corner of the tray and two from the centre of the tray. Initially one control tray was used, but as trials progressed it was decided to use three controls: one placed at the beginning of the mussel layer (C1) and two at the end (C2 and C3) (Figure 9).

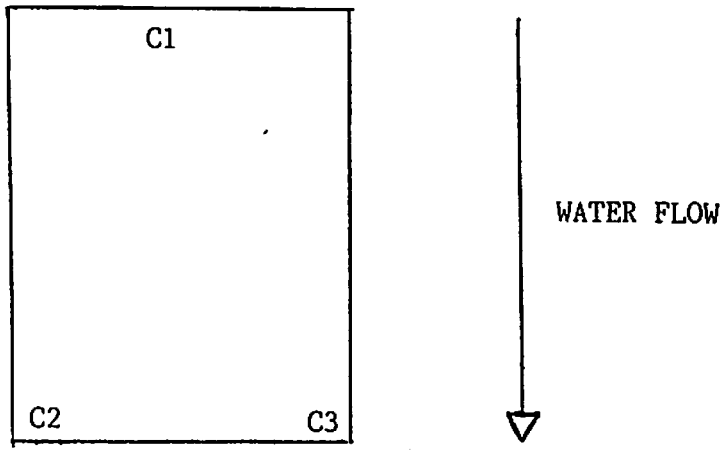


Figure 9. Position of controls in outside Control Tank

6.2.3 Mussels in Trials Tank

Sampling was conducted according to the grid reference in Figure 10 overleaf, again ten mussels per sample - two from each corner and two from the centre of the tray. As the number of layers increased the sampling pattern was taken diagonally downwards along the length of the stack so that samples B1 and B4 were taken from the uppermost layer, samples D1 and D2 and F1 and F2 from middle layers and H1, H2, H3 and H4 from the bottom layer (See Figure 10 overleaf).

Locations of Mussel Samples

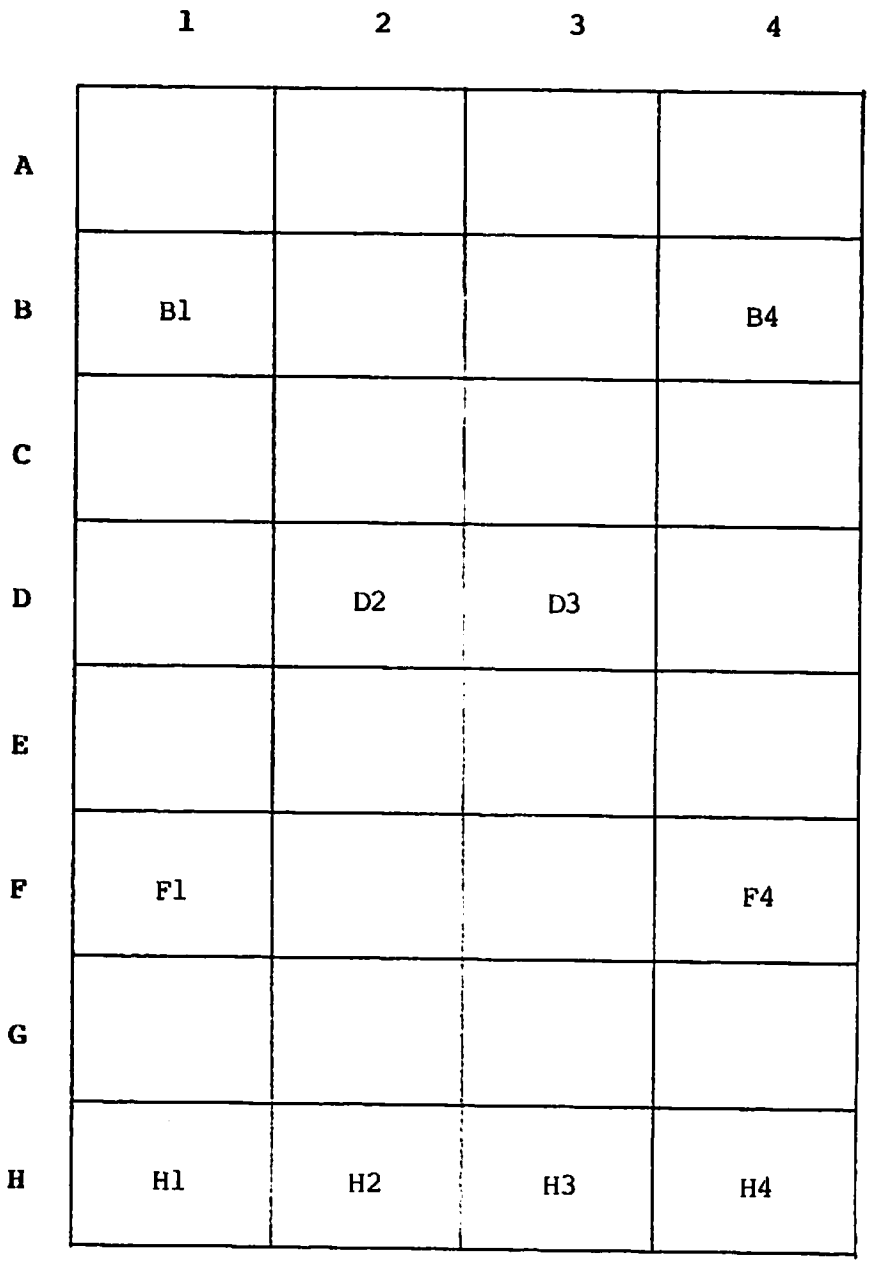


Figure 10. Plan view of trays located in tank. Rows are specified by letters A-H, and columns by numbers 1-4.

This sampling method was chosen as the pattern would follow the anticipated drift of detritus through the stacks with samples H1-4 receiving the greatest potential contamination. For reference the bottom layer is referred to as Layer 1 with layer numbers 2-6 progressing upwards.

Specific sampling location for each successive trial are shown in Appendix I, Tables 17-24.

7 RESULTS

These are set out in Appendix I. The first set of tables (1-8) describe the oxygen temperature and pH readings for the Trial and Control Tank for the duration of each trial.

The second set of tables (9-16) describe the microbiological counts of the water samples taken from the Trial Tank for each trial.

The third set of tables (16-24) describe the microbiological assessment for mussel samples taken for each trial. On each table there is a sketch plan view of the layout of trays within the tank to show where mussels were sampled from. Bagged mussels were placed in row H and the codes indicate the type of bag.

Table 25 presents some analysis relating to changes in pH levels within each of the trials

7.1 Statistical Analysis

The microbiological assessment of the mussel samples was carried out using two different statistical analytical techniques. Total Coliforms and E. Coli were assessed using the most probable number (MPN) technique while the Faecal Streptococci were assessed by direct counting of visible colonies cultured up on the test plates.

As such the results from the two techniques have to be assessed differently. Due to the probability element in the MPN counting technique, the final count is only a probable count. The actual count lies within the range of a third of the value of the probable count number up to three times the value. As such it is only possible to pick out trends from results when using this technique. Individual readings cannot be compared directly. Conversely Faecal Streptococci are asessed by direct counting of visible colonies on a culture plate and so the value of a count using this technique is a true and accurate assessment of the number of colonies present, and readings can be compared directly.

8 DISCUSSION

8.1 Oxygen

Oxygen is probably the single most important parameter in a closed loop purification system in that it is the commodity most quickly depleted from water by respiring animals, and as such has to be monitored and replaced. For these reasons it was decided to measure the oxygen content of the water on a regular basis, (every 2 hours during the day, evry 6 hours at night) to give any indication of over-depletion. It was also for this reason that two aeration systems were built into the tank design to facilitate re-oxygenation of oxygen-depleted water. Firstly the spray bar design ensured that incoming water entrained the maximum amount of oxygen through vigorous turbulence, and secondly, the millipore bubble diffuser provided additional aeration should the water not enter the tank fully saturated. The additional aeration facility proved necessary.

In the one and two layer trials the oxygen entered the tank at about 10.5 mgl^{-1} which is approximately fully saturated and only dropped to 8.5-9.0 mgl^{-1} when sampled at the end of the stack indicating that the mussels respired 1.5-2.0 mgl^{-1} as the water moved over them. From three to six layers the oxygen utilisation increased to 2.5-3.5 mgl^{-1} along the tank.

The temperature of the water increased during this period and it is expected that oxygen consumption should increase as mussel activity increases. A further contributory factor was that the flow rate per unit mass of mussels reduced as the number of layers increased.

There was a marked increase in oxygen consumption in the first six layer trial (6A) which experienced an oxygen drop across the tank of up to 4.5 mgl^{-1}. This increased oxygen utilisation would have been caused by mussels spawning, increased mussel activity due to elevated water temperature and perhaps a reduction in the water to shellfish ratio.

Some fluctuation of readings also occured whilst setting up the flow screens to counter uneven flow across the tank experienced in the latter trials (8.4).

During the first set of trials up to 5 layers (5.1) the oxygen content of the water within the test tank was always above the recommended minimum of 5.0 mgl^{-1}. The six layer trial (6A) is explained above. With the second set of trials at six layers some readings below 5.0 mgl^{-1} were experienced.

Initial low readings were caused by the 2-2½ hours taken to fill the tank (5.2). When the water was circulated and aerated the levels of 4.0 mgl^{-1} measured at the end of the tank increased over a period of approximately 4 hours to the 5.0 mgl^{-1} level.

High water temperatures of 12°C to 13°C no doubt accounted for the overall reduction in oxygen level compared to previous trials, but water flow rate and aeration had an important effect.

In trial 6B the water flow rate was initially set high to improve oxygenation, and then reduced. The effect can clearly be seen in Table 7 of Appendix I with a drop of approximately 1.5 mgl^{-1} across the tank after the pump flow was turned down. Some of this drop may have been caused by a reduction in air supply at the same time but this cannot be very significant as no increase in oxygen level was recorded when the air supply returned to normal.

In trial 6C the water flow rate per unit mass of mussels could not be increased and lower oxygen readings were taken at the end of the tank.

The effect of the air diffusers (3.4) can also be seen in trials 3,4 and 5 (Tables 3, 4, 5 Appendix I) where reduction and increase in oxygen level corresponds to the air being turned off and on.

Oxygen content in the Control Tank was often low, especially with elevated water temperatures when oxygen values fell to 4.0-5.0mgl^{-1}.

8.2 pH

Acidity or pH of the water was monitored because live mussels excrete waste products which cause the acidity of the surrounding water to rise giving a reduction in pH. Too much acidity can inhibit filtration activity and hence purification efficiency of mussels.

The pH of normal seawater is about 7.5 to 8.0 pH units, the pH of incoming seawater used for the trials ranged between 7.6 to 8.2 pH units.

During purification there was a slight drop in pH of between 0.03 to 0.24 pH units and this is not considered significant. There appeared to be little relation with stacking depth or type of tank. An exception was the six layer trial 6A in which the mussels spawned and a drop in pH of 0.73 units occured.

The greatest increase in acidity occurs in the early part of the trial and this is mainly due to the immediate history of the mussels. Before purification mussels have often been standing for 2-4 days on a quayside without access to seawater. In this condition mussels accumulate a reservoir of metabolites which are retained in the cavity between the two shells.

Upon re-immersion into seawater the mussels expell this intra-shell liquor and replace it with clean seawater. It is probable that this sudden release of metabolites caused the immediate pH reduction in the surrounding seawater. Subsequent reduction of pH over the remainder of the trials was small.

8.3 Temperature

Temperature was measured for 2 main reasons; firstly, a considerable difference between the indoor Trial Tank and the outdoor Control Tank was expected, secondly, it was necessary to measure temperature as both oxygen solubility and mussel respiration are temperature dependant.

The advantage of housing the Trial Tank within a building was clearly demonstrated. Although the temperature of the water within the Trial Tank varied from 4.5^{o}C to 13.5^{o}C throughout the trials period, daily fluctuations of only about $\pm$ 1^{o}C were measured. Thus for each trial the mussels experienced little temperature fluctuation over the 48 hour purification period, with only gradual seasonal temperature changes. This contrasted markedly with conditions experienced in the outside Control Tank. Diurnal fluctuations of 6^{o}C were measured and over the trial period from January to April temperature variations from 0-15^{o}C were measured.

During the second set of trials in October and November the Control Tank was not in use but would no doubt have given much higher temperatures. Thus not only did mussels in the Control Tank experience large temperature fluctuations over the 48 hour purification cycle but were near to freezing during the cold weather, and subject to potential heat stress during the warm weather. As the temperature reduces to 0^{o}C mussels begin to cease filtering activity. Conversely at elevated temperatures mussels function much faster and therefore use more oxygen.

However, at these higher temperatures water has reduced capacity for holding oxygen, hence the constant low oxygen readings that were measured in the Control Tank during April. In fact in sunny weather outdoor tanks act like solar panels: the dark coloured mussels absorb radiation and heat-up resulting in increased mussel activity. At the same time rising temperature cause a corresponding reduction in the oxygen carrying capacity of the water.

8.4 Water Flow

The oxygen readings as well as giving direct values for oxygen content, also gave an indirect measurement of water flow, in that if water did not move within an area in the tank, oxygen would have soon become depleted in that area. Thus the oxygen readings were also used as an indication of water movement.

The design of the spray bar, baffles and suction bar was such as to establish uniform water flow throughout the tank. Water flow appeared to be even across the width and depth of the tank for stacks 1, 2 and 3 high. Unequal flow along the lower sides of the tank became apparent during trials of stacks 4 high. This may have been caused by the drainage slope on the floor of the tank. To counteract this the baffle at the outlet end of the tank was raised slightly at the side along which the low oxygen reading was recorded. This remedy was successful.

For stacks 5 and 6 high slightly higher oxygen readings were obtained in the upper half of the tank compared to the lower half. This indicated vertical stratification and that more water was passing through the upper half of the tank - that is, if the assumption that all mussels were respiring at an equal rate is made. To counteract this, in the five layer trial the perforated baffles were raised slightly to facilitate flow along the lower half of the tank. Again this remedy was effective.

As can be seen, if required, the tank had to be 'tuned' at each trial to ensure that balanced flow occured throughout the tank.

This turning procedure was carried out for the first run (6B) of the second set of trials. However, baffle adjustment was not carried out during the 6C trial so as to determine the effect of the stratification over a complete purification cycle. Once established it appeared to remain fairly constant.

8.5 Mussel Mortality

It was noticed after each purification trial, at all layers, that mussels had bound themselves together into clumps by putting out new byssus threads - a process only initiated when mussels are in satisfactory environmental conditions.

Mortality was not a problem in the Trial Tank until the first of the 6 layer increased density trials (6A) when considerable mortality occured and, surprisingly, with higher mortality on the side of the tank with interleaf boards.

Further Seafish work on the general handling of mussels (Ref 2) has showed them to be weak and prone to mortality when handled at high temperatures and particularly during the spawning season. Ambient and tank temperatures were high when the 6A layer trial was carried out and many mussels spawned in the tank.

It was later discovered that mussels in the interleaved side of the tank had been stored at the catching port for 4 days, while those used in the other side of the tank had been stored only for 3 days. Perhaps this length of storage time out of water and the extra day, combined with the seasonal aspects proved too stressful and resulted in high mortality.

Though conditions in the Control Tank were similar to those in the trials tank with regard to high temperatures and low oxygen levels, the controls (3 day dry storage mussels) did not appear to suffer mortality to the same degree. However, there was one major difference in the mode of operation between the 2 tanks. The water in the Control Tank was changed once during the purification cycle.

When the 6 layered trials were repeated later in the year during the October/November period there was no mortality, no indication of spawning and the mussels had purified successfully.

8.6 Microbiological Analysis of Water

Water samples were taken at the beginning and end of each trial run to ascertain whether the water had been cleared of viable bacteria by the end of the trial. This would have occured through two processes; firstly material would have been removed from the water and expelled in faeces or pseudofaeces by the mussels as they purified, and secondly any material which passed through the U.V. sterilisation unit would have been rendered non-viable. The effectiveness of the U.V. system is shown in Tables 10 and 16 where the bacteria counts in the tank water at the start of the trial after passing through the U.V. are shown to be dramatically lower than those from the estuary from which it had been drawn. As can be seen from the tables the counts for total Coliform, E. Coli and Faecal Streptococci were very low by the end of each trial. Higher counts at beginning of the experiments were probably due to initial bacterial release from the mud and the mussels as each trial commenced.

From the figures it appears that the counts were slightly higher in samples taken from the end of the tank indicating that bacteria was being picked up as the water progressed along the length of the tank, particularly as each trial commenced.

8.7 Microbiological Analysis of Mussels

It was intended to use highly polluted mussels for this experiment to determine whether the experiment would be successful whilst conducted under the most unfavourable conditions. Mussels were harvested from two of the most polluted areas in the Wash. Because the mussels were harvested from only one location for each individual trial, they were usually of similar condition and appearance. However, when there seemed to be significant differences within each batch of mussels in the amount of mud and 'debris' in amongst them, the 'dirty' mussels were, as much as possible, placed in the sample locations.

MAFF specify that the purified mussels should have counts of less than 230 E. Coli/100ml sample. However, as can be seen from the initial counts in Tables 17-24 the counts for E. Coli were not exceptionally high so a fair degree of emphasis has to be placed on the reduction in number of total Coliform counts and the Faecal Streptococci counts. There is also a considerable variation in the results between individual samples and thus they are best considered in terms of overall trends.

8.7.1 Mussels Loose in Trays

As can be seen from Tables 17-24 mussels in the Trial Tank that were contained loosely within the trays purified successfully in that the purified E. Coli readings obtained were equal to or less than the MAFF standard of 230 E. Coli/100ml sample. There were 3 readings (all in the single layer trial) where loose mussels did not purify to below the 230 E. Coli/100ml threshold. As high readings were not experienced in these locations in later trials the high readings may have been anomalous results.

Although the unpurified mussels were not highly polluted with regard to the E. Coli counts it seems that they purified from a condition of just polluted to a condition of very clean.

With the exception of the final increased density trial (discussed later), the Faecal Streptococci readings reduced very considerably by approximately a factor of ten at all layers indicating that mussels were actively purifying at all levels.

Initially it was thought that mud deposition from the upper layers could lead to smothering of the lower layers thereby preventing effective purification. For the first three trials deposition did not present a problem in that when the tank was drained down there was no significant visual difference between mussels purified in any of the three layers. As in the first two trials the mud from the three layer trial had passed through the three layers onto the bottom of the tank. It was also noticeable that on each of the successive trials there were increases in the amount of mud and debris deposited on the base of the tank. The mud and debris usually formed the pattern of a rectangular 'shadow' beneath each of the bottom trays.

At the four layer trial however, it was noticeable that the mussels in the bottom trays were covered with more mud than those in the upper layers. The microbiological counts however, were not significantly higher and the mussels had still purified satisfactorily.

The main contrast between deposition was illustrated by the findings in the five and six layer trials in which interleaf boards had been placed between the trays in one half of the tank. Mussels that had purified between the interleaf boards appeared to be very clean at all layers and the mud from each tray had collected on the interleaf board immediately below. There was a small amount of mud on the base of the tank. In contrast mussels on the straight stacked side, especially the bottom two layers, appeared to have more mud in amongst them. This condition was most evident in the bottom layer. However, there did not seem to be a significant difference in microbiological counts between the two sides of the tank (Tables 21-24).

Throughout the trials there appears to be a very slight tendency toward higher bacterial counts at the bottom of the outlet end of the tank compared to the top of the inlet end, although this tendency is difficult to detect within the individual variations in counts and is not considered significant. With the exception of trial 6C, purification in the Trial Tank was always as good as that in the Control Tank and on several occasions was considerably better.

The results for the increased density trial (6C) are shown in Table 24. Although the E. Coli results show a similar reduction, the Faecal Streptococci do not show such an overall reduction as in the previous trials. Low oxygen readings resulting from high temperature and a low water flow rate per kg of mussles probably contributed towards this.

Thus overall the results for trial 6C indicated that the trials facility was at its limit of operation without using deeper trays, a larger pump and temperature control.

8.7.2 Mussels in Bags

The number of samples taken from the bag trials was limited and thus the results should be treated with some caution. However the results were much as expected.

Mussels which had been contained loosely within the wide mesh monofilament 'Seafish' bag retained the lowest counts for 'bagged' mussels, results being similar to those obtained for loose mussels in trays in the same row and layer. Also the 'mud condition' of these mussels was similar to the loose mussels in equivalent positions in that most mud had dropped through the mussels onto the base of the tank.

The mussels purified under these conditions because the loose bag enabled the mussels to open and purify, and the bag afforded little 'resistance' to the passage of mud onto the base of the tank and to the flow of water.

Mussels tightly packed in the wide mesh monofilament bag returned high microbiological counts - often not dissimilar from the counts recorded for the initial polluted mussels. Upon inspection there was a layer of mud on top of the mussels; obviously the result of deposition from above. Also when the bag was untied there appeared to be a similar amount of mud as was present with the unpurified mussels. There was very little mud on the base of the tank underneath the mussels indicating that very little had deposited out. Mussels did not purify under these conditions because they were unable to open their shells and effect purification due to the constraint of the bag. This 'bag constraint' also prevented mussels 'processing' mud through the mussel layer onto the base of the tank.

Mussels loosely packed within the close-weave ribbon mesh effected purification and returned counts that were similar to those of the loose wide mesh experiment but lower than those of the tight wide mesh experiment. Under the conditions experienced within the loose close-weave mesh the mussels could open their shells and purify. However, due to the weave of the fabric much of the mud was retained inside the bag and did not drop through onto the base of the tank. For the same reason a layer of mud was found on top of the bag.

8.8 Overall Considerations

The aforementioned Seafish work on the general handling of mussels has shown them to be very sensitive to physical shocks in addition to high temperatures. Commercial handling practices are likely to drastically reduce the life expectancy of individual mussels in any batch. This may be a significant factor in the variations in the bacterial counts recorded. A proportion of the mussels will be dying, and thus be relatively inactive in the purification tanks.

Considered overall, the results indicate that the critical factors in mussel purification tank design are that there should be a sufficient flow of clean, well oxygenated water at a suitable temperature around the mussels. When these conditions are satisfied then within the limits of the trials the effects of deep stacking and limited increase in the depth of mussels in each layer are not significant. Thus the way is now open for the design of more cost-effective high density purification plant.

However, it must be noted that the trials tank was carefully designed and controlled during operation to establish and maintain the required water conditions around the mussels. AS SUCH IT MUST BE UNDERSTOOD THAT THE RESULTS OBTAINED RELATE ONLY TO THOSE CONTROLLED CONDITIONS AND DO NOT RELATE TO THE STACKING OF MUSSELS IN STANDARD TANKS WHICH WOULD POTENTIALLY BE A DANGER TO HEALTH. ANY PERSON CONSIDERING THE STACKING OF MUSSELS IN PURIFICATION TANKS MUST FIRST CONSULT WITH MAFF.

9 CONCLUSIONS AND RECOMMENDATIONS

1. Successful purification was achieved up to 6 layers which was the maximum capacity of the Trial Tank but it is important to note that this was in the controlled conditions prevailing during the trial and it cannot yet be assumed stacking will be acceptable in other conditions.

2. Significant bacteriological re-contamination did not occur either vertically or longitudically in the Trial Tank.

3. The amount of mud on the bottom layers of mussels did increase with depth but did not adversely effect purification in the Trial Tank.

4. The use of interleaves between layers did not significantly affect purification but did prevent the accumulation of mud on the bottom layers of mussels in the Trial Tank.

5. Purification tended to be slightly better in the indoor, deep stacked Trial Tank than in the outdoor, single layer Control Tank.

6. Maintenance of high levels of dissolved oxygen and the control of temperature appear to be most important considerations in purification.

7. The results indicate that it may be possible to purify mussels in a deeper layer than is currently specified by MAFF provided that the water conditions are correct.

8. Careful design monitoring and operation of the Trial Tank to maintain satisfactory water conditions have been critical to its successful operation.

9. Mussels do not purify if constrained through being packed tightly in bags, but will purify in loose bags.

10. Mud is retained in tight and close mesh bags.

11. Deep stacking offers considerable scope for improvement over traditional shallow purification tanks. Deep stacked tanks take up a small ground area and can thus be housed in a building at a convenient site. The building can provide protection against contamination and allow hygienic operation and also can provide protection against climatic extremes and permit the control of temperature.

12. However, successful purification will not be achieved simply by stacking trays in a traditional purification plant. A deep stacked purification plant must be purpose designed and carefully operated.

13. It is recommended that a design is developed for a commercial deep stacked purification plant and that a prototype plant is built and tested.

10 REFERENCES

REF 1 Christos Ap. Filippopoulos. Seafish Internal Report No. 1320. September 1987. Experimental Investigation of a Stacked Mussel Purification System.

REF 2 McNamara, J. Pollack, A. (1988) Mussel Storage and Handling Trials - Seafish Internal Report No. 1353.

APPENDIX I

Results of Trials of 1-6 layers including:-

Readings of oxygen, temperature, pH taken throughout each trial

and microbiological counts of water and mussels

DATE 12am 27.1.87 - 12am 29.1.87

LAYERS 1

TRIALS TANK															CONTROL TANK		
OXYGEN mgl^{-1} AT EACH POSITION																	
TIME		A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
Tues	1500	10.8	11.0		11.2	11.4		10.4	10.7				4.5	7.80			
	2000	10.7	10.8	11.1	10.7	10.4		10.3	10.2	10.9	11.1		4.5				
Wed	1000	10.4	10.5	10.7	10.2	10.3	10.4	10.2	10.1	10.3	10.8		4.4	7.78			
	1200	9.7	9.6	9.7	8.6	9.1		8.6	8.4	8.6	10.0						
	1400	9.5	9.5	9.4	9.1	9.1	9.8	9.4	8.8	8.5	10.0		5.0		12.0	7.90	2.4
	1600	9.7	9.8	10.1	9.6	9.4	10.2	8.9	8.6	8.7	10.5				11.9	7.89	2.8
	1800	9.8	9.9	10.2	9.1	9.3	10.1	9.0	8.8	8.7	10.4		5.1		12.1		2.2
	2400	9.8	10.0	10.1	9.7	9.4	10.1	9.2	8.7	8.7	10.5						
Thurs	0600	9.9	9.9	10.2	9.4	9.1	10.0	9.0	8.8	8.8	10.4		5.8	7.77	12.2	7.90	1.6
	0900	10.1	10.0	10.2	10.0	9.4	9.8	9.0	8.8	9.1	10.7				12.3		1.7
	1200	10.3	10.4	10.6	10.1		10.4	9.1	9.1	10.0	10.7						

TABLE 1 shows oxygen, temperature and pH readings for the Trial and Control Tanks for the 1 layer trial (see Fig. 7).

LAYERS 2

DATE 12am 17.2.87 - 12am 19.2.87

	TRIALS TANK													CONTROL TANK		
	OXYGEN mgl^{-1} AT EACH POSITION															
TIME	A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
Tues 1300	10.8	10.9	10.9	9.9	11.0	9.7	9.6	9.4	10.1	11.4	10.9	4.3	7.87			
*1530	10.3	10.1	11.0	10.4	10.6	9.1	9.0	9.4	10.5	11.0	10.8	4.5				3.4
1700	10.0	10.1	10.1	9.8	8.5	8.8	8.7	8.4	9.9	10.7	8.8				7.67	
1710	9.5	9.5	9.9	9.8	10.1	10.1	9.2	9.0	9.9	10.1	10.2					
2400	9.9	9.8	10.1	9.8	10.2	9.9	9.0	8.9	9.7	10.2	10.4					
Wed 0600	9.8	9.7	10.0	9.8	10.1	9.5	9.9	8.9	9.7	10.3	9.8					
1000	9.8	9.8	10.1	9.9	9.9	9.4	9.0	8.9	9.9	10.1	9.7		7.85	9.9	7.68	1.6
1200	10.0	9.9	10.0	10.1	9.9	9.5	9.1	9.1	10.0	10.2	9.9	4.6		9.9		
**1400	9.8	9.8	10.1	10.0	10.0	9.6	9.0	9.1	9.6	10.2	9.7					
1600	9.9	9.8	10.4	9.9	9.8	9.6	9.0	8.9	9.7	11.0	9.9	4.9		8.9		2.9
1800	10.4	10.5	10.5	10.0	9.7	9.4	9.0	9.1	9.8	11.3	9.8					
2100	10.0	9.9	10.2	10.0	9.8	9.6	9.0	8.9	9.8	11.0						
Thurs 0600	10.1	9.9	10.3	9.9	9.7	9.9	9.0	9.0	9.7	11.2	9.9					
0930	10.2	10.5	10.6	10.1	9.7	9.3	8.6	8.8	9.6	11.1	9.9	4.6	7.63	10.9	7.65	0.0
1130	10.4	10.2	10.8	10.4	10.0	9.2	9.1	9.0	10.2	7.8	9.9					

TABLE 2 shows oxygen, temperature and pH readings taken for the test tank and standard purification tank for the 2 layer trial. * Aeration Off. ** Aeration On.

DATE 12am 3.3.87 - 12am 5.3.87

LAYERS 3

TRIALS TANK														CONTROL TANK		
OXYGEN mgl^{-1} AT EACH POSITION																
TIME	A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
Tues 1300	9.4	9.7	8.7	8.2	7.8	7.5	7.5	7.6	7.9	9.8	8.2	4.8				
1500	9.6	9.7	8.8	8.5	8.1	9.2	7.6	7.8	8.9	10.7	9.6					
1700	9.6	9.8	8.7	8.6	8.7	9.1	7.7	7.8	8.8	10.7	9.5	4.8	7.6	10.1	7.75	2.8
2400	9.1	9.0	9.2	8.7	8.2	8.7	7.4	7.2	8.8	9.4	8.8					
Wed 0600	9.2	9.1	8.9	8.4	8.1	8.8	7.6	7.9	8.6	9.8	9.1					
0900	9.1	9.2	9.0	8.6	8.1	8.7	7.5	7.7	8.7	9.8	8.6			10.3		2.5
*1200	9.4	9.4	9.4	8.3	8.4	8.7	7.5	7.4	8.6	9.7	8.3					
1400	9.3	9.2	9.0	8.2	8.4	7.7	7.1	7.2	8.3	9.4	7.9					
**1600	9.6	9.1	9.2	8.1	8.3	8.4	6.9	7.0	8.4	9.8	8.7					
1800	9.7	9.2	9.4	8.1	8.4	8.3	7.1	7.4	8.3	9.9	8.6					
2400	9.6	9.3	9.3	8.4	8.4	8.5	7.3	7.2	8.4	9.6						
Thurs 0600	9.5	9.1	8.5	8.2	8.3	8.4	7.4	7.4	8.2	9.6						
0930	9.4	9.0	7.9	7.4	7.8	7.8	7.2	7.5	7.4	9.5	8.8	5.9	7.52		7.61	2.6

TABLE 3 shows oxygen, temperature and pH readings taken for the test tank and standard purification tank for the 3 layer trial. * Aeration off at 1300. ** Aeration on at 1700.

DATE 12am 11.3.87 - 12am 13.3.87

LAYERS 4

TRIALS TANK														CONTROL TANK		
OXYGEN mgl^{-1} AT EACH POSITION																
TIME	A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
Wed 1130	10.6	10.4	10.3	9.0	9.3	8.4	8.6	8.8	9.6	10.7	9.3		7.75		8.02	
1230	10.4	10.3	10.0	8.9	9.1	8.4	8.5	8.0	9.5	10.4				9.2		
1430	10.2	10.1	9.9	8.7	8.4	7.7	7.9	7.8	9.2	10.4	8.5	4.5				6.8
				7.3	7.8	6.8	6.0	6.0								
1630	10.3	10.4	9.7	8.3	8.1	8.0	7.8	6.8	9.5	10.4	9.0					
				7.5	8.1	7.1	6.2	8.2								
1800	10.2	10.4	9.6	8.4	8.3	8.2	7.9	8.3	9.4	10.5	8.9					
				7.8	7.9	7.4	6.4	6.8								
2400	10.3	10.3	9.7	8.5	8.4	8.3	7.8	8.4	9.3	10.3	9.0					
				7.6	7.9	7.0	6.3	7.0								
Thurs *0600	10.2	10.4	9.8	8.5	8.6	8.4	8.1	8.3	9.4	10.5	9.2					
				7.3	7.9	7.2	6.4	7.0	9.4							
9000	9.4	9.5	9.4	8.2	7.9	8.3	7.7	8.1	8.8	9.4	9.1	4.9		10.3		
				6.3	8.8	7.8	6.5	7.6	8.8							
**1100	9.4	9.4	9.1	8.4	8.5	8.0	7.7	8.0	8.6	9.5	8.8			9.9		6.8
	9.4	9.4	9.3	6.6	7.6	6.5	6.5	6.5	8.7	9.5						
1300	9.6	9.6	9.5	8.7	8.4	8.5	8.0	8.0	9.0	10.1					7.99	
				6.7	7.4	6.9	6.7	6.9								
1500	9.7	9.9	9.7	8.8	-	7.6	8.5	8.6	9.1	10.2	8.4					
				6.3	8.0	8.6	6.9	7.6								
1700	9.5	9.7	9.7	8.6	8.0	7.7	7.8	8.7	8.9	10.0	8.6	4.9	7.71			
				6.2		8.4	6.0	7.0								
2400	9.4	9.4	9.6	8.4	8.1	8.6	7.7	8.7	9.1	10.2						
				6.6		7.9	6.2	6.5								
Fri 0600	9.3	9.3	9.4	8.2	7.9	8.4	7.8	8.6	8.9	10.1						
				6.3		7.8	6.5	7.0								

TABLE 4 shows oxygen, temperature and pH readings for the test tank and standard purification tank for the 4 layer trial. * Aeration off at 0800. ** Aeration on at 1200.

NOTE When two readings are given the upper figure denotes a reading taken at one third the water depth and the lower figure denotes a reading taken at two thirds the water depth.

DATE 12am 18.3.87 - 12am 20.3.87

LAYERS 5

	TRIALS TANK													CONTROL TANK		
	OXYGEN mgl^{-1} AT EACH POSITION															
TIME	A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	$OXY_{Mgl^{-1}}$	PH	TEMP°C
Wed 1230												5.5	7.80			
1445	9.3	9.1	8.8	7.6	7.8	8.1	7.4	7.3	8.2	9.5	7.9		7.64			
			8.7	7.5	7.6	7.4	6.8	6.8	7.4		7.8		7.67			
1715	8.9	8.9	8.8	8.0	8.6	8.6	8.4	8.5	8.3	10.0	8.2					
			8.8	7.2	7.5	8.5	8.3	8.3								
2250	8.6	8.9	9.1	8.2	8.9	8.8	8.8	8.6	7.7	10.2	8.9					
			9.0	6.4	7.2	8.2	8.5	8.7	8.0		9.2					
Thurs 0600	9.1	9.3	9.8	8.3	9.2	9.0	8.5	8.9	8.4	11.2	9.3	6.2		5.8		2.6
			9.5	6.3	7.9	9.2	8.2	8.7	8.7		9.2					
o**0930	11.0	11.1	10.1	9.3	9.6	6.6	8.7	9.3	8.5	11.5	9.7				7.49	
			10.0	6.7	8.4	9.7	8.6	8.2	9.5		9.8					
***1210	9.3	9.5	9.3	8.1	8.2	8.2	7.3	7.6	8.1	10.0	8.3	6.5	7.7			
			9.3	8.0	8.1	8.0	6.9	7.1	8.0		8.3		7.69			
1400	9.7	9.5	9.8	8.4	8.8	8.8	8.6	8.9	8.6	11.0	9.1	6.3				7.4
			10.0	7.4	7.4	8.7	8.6	8.7	8.8		9.0					
1600	8.8	8.9	9.1	8.6	8.8	8.7	8.6	8.9	8.3	10.2	9.0	6.5		4.1		8.4
			8.9	7.6	7.7	8.7	8.7	8.8	8.8		9.2					
1800	8.3	8.5	9.0	8.4	8.8	8.7	8.7	8.8	8.7	10.3	8.9	6.6		4.1		7.9
2400																
Fri 0600	o_2 probe inoperative															
0900												6.3	7.66		7.57	4.3
													7.68			

TABLE 5 shows oxygen, temperature and pH readings for the test tank and standard purification tank for the 5 layer trial. ** Aeration off at 1130. *** Aeration on at 1340. o Baffle at G raised 1cm.

NOTE When two readings are given the upper figure denotes a reading taken at one third the water depth and the lower figure denotes a reading taken at two thirds the water depth.

DATE 12am 30.3.87 - 12am 1.4.87

LAYERS 6 (A)

	TRIALS TANK													CONTROL TANK		
	OXYGEN mgl^{-1} AT EACH POSITION															
TIME	A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
0715													8.26			
1000	9.0	8.9	9.0	6.8	6.9	3.8	5.2	3.4	7.1	9.7	5.7	7.7	7.66	6.0	7.52	9.9
	9.0	8.9	9.3	7.2	6.9	5.8	7.1	5.8	7.8		6.9					
1200	9.0	9.1	9.0	7.1	7.0	4.5	6.0	4.1	7.0	9.0	7.1					
	9.1	9.1	9.1	7.3	6.7	5.7	6.9	5.0	7.9							
1400	9.3	9.2	8.9	7.5	7.1	5.9	7.0	5.0	7.3	9.7	7.0	8.1		5.7		11.7
	9.4	9.2	8.9	7.0	8.4	5.9	7.0	4.7	7.7		7.4					
1600	9.0	9.0	8.8	6.9	6.7	5.6	6.5	4.4	7.1	9.2	6.8	8.4		4.9		12.2
	8.9	9.1	8.8	7.1	6.3	5.7	7.0	4.5	7.8		6.7					
1800	9.0	9.1	9.0	7.2	6.8	5.7	6.3	4.5	7.1	9.4	6.9	8.5	7.50	4.9	7.44	12.4
	9.0	9.2	9.1	7.5	6.3	5.9	7.1	4.3	8.0		6.6					
°2400	9.2	9.3	9.2	7.8	7.6	6.0	7.0	5.2	7.3	9.1	7.6	8.9		4.2		10.9
	9.2	9.3	9.1	7.7	6.5	5.9	6.7	4.6	8.0		7.7					
0600	9.2	9.2	9.2	7.6	7.6	6.2	7.0	5.2	7.5	9.7				4.4		11.0
	9.0	9.3	9.1	7.7	6.8	5.5	6.7	4.3	8.0							
°° 0900	9.3	9.2	9.1	7.6	7.4	6.3	6.9	5.4	7.6	9.6	7.4	11.4*	7.49	4.8	7.63	12.5
	9.3	9.2	9.1	7.3	6.4	5.0	6.3	4.2	8.1	9.6	7.5					
1100	8.4	8.4	8.5	7.6	7.0	5.8	6.3	5.9	7.4	9.0	7.2	9.9		5.8		12.2
	8.2	8.3	8.6	7.1	6.1	5.4	6.0	5.2	7.9		7.0					
1300	8.4	8.5	8.6	7.5	7.0	6.2	6.4	6.5	7.1	9.1	7.5	10.3		4.7		13.2
	8.4	8.5	8.6	7.5	6.0	5.0	5.8	5.2	7.6		7.2					
1500	8.7	8.7	8.6	7.9	7.6	6.5	6.7	6.2	7.5	9.2	7.9	9.6		4.1		15.0
	8.7	8.7	8.6	7.5	6.7	5.2	6.1	5.3	7.7		7.4					
1700	8.8	8.9	8.8	7.8	7.6	6.7	6.8	6.6	7.6	9.3	7.9	9.7	7.53	4.3	7.47	15.2
	8.8	8.8	8.6	7.5	6.1	5.6	6.4	5.6	7.7		7.7					
2400	8.9	9.0	8.7	7.9	8.0	6.7	6.8	6.4	7.7							
	8.8	8.9	8.8	7.6	7.0	5.6	6.2	5.5	7.3							
0600	9.2	9.2	9.0	8.1	8.3	6.9	7.1	7.0	7.8	9.4		10.2		4.9		8.9
	9.0	9.1	9.0	7.8	8.1	6.2	6.5	6.0	8.1							

TABLE 6 shows oxygen, temperature and pH readings for the test tank and standard purification tank for the first 6 layer trial. °End baffle raised 1cm at side X. °°End baffle raised 1.2cm at side X. * Suspect reading.

NOTE When two readings are given the upper figure denotes a reading taken at one third the water depth and the lower figure denotes a reading taken at two thirds the water depth.

DATE 11am 27.10.87 - 12am 29.10.87

LAYERS 6 (B)

TRIALS TANK														CONTROL TANK		
	OXYGEN mgl^{-1} AT EACH POSITION															
TIME	A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
0200	8.0	8.1	7.9	5.5	6.1	5.5	4.2	4.0	4.6	8.0	6.0	12.7	7.86			
0630	7.5	7.8	7.7	5.4	6.5	6.1	5.1	5.1	5.1	8.0	7.2	12.6				
! 0930	9.1	8.9	8.6	6.1	7.0	7.7	5.6	5.8	6.4	9.5	7.8	12.6				
*1230	7.6	7.4	7.3	4.8	5.5	5.4	3.9	4.2	5.2	8.2	6.4	12.5				
o 1530	8.1	8.2	7.1	5.6	6.8	5.6	4.2	5.2	5.5	8.2	7.5	12.6				
1800	6.8	7.1	6.6	4.8	5.7	5.1	3.5	4.9	4.9	7.5	6.3	13.6				
**2130	7.7	8.0	7.5	6.7	7.1	5.9	4.7	5.9	6.6	8.3	7.8	13.6				
2400	7.6	7.8	7.2	6.1	7.0	5.7	4.6	5.6	6.5	8.3	7.6	13.1				
0600	7.4	7.6	6.9	5.6	6.7	5.5	4.8	5.5	6.2	8.2	7.4	13.0				
0930	7.8	8.3	7.6	5.9	7.2	6.0	4.9	6.0	6.6	8.9	7.9	12.7				
1200	8.0	8.1	8.3	6.5	6.6	7.0	5.2	5.9	6.2	8.7	8.0	12.5	7.72			

TABLE 7 shows oxygen, temperature and pH readings for the test tank and standard purification tank for the second 6 layer trial.

NOTE Standard tank not in use. * Aeration reduced at 11.20. ** Aeration increased. ! Flow reduced from 22-18m^3/hr. o Baffle raised 1cm at side Z.

DATE 10.11.87 - 12.11.87

LAYERS 6 (C)

		TRIALS TANK													CONTROL TANK		
		OXYGEN mgl^{-1} AT EACH POSITION															
TIME		A	B	C	D	E	F	G	H	Z	Y	X	TEMP°C	PH	OXY Mgl^{-1}	PH	TEMP°C
Tues	1400	7.6	7.5	7.2	4.9	5.1	5.5	4.3	4.5	5.1	7.7	5.3	11.8	7.53			
	1645	7.7	7.8	7.6	4.4	5.2	6.2	4.2	4.3	5.3	7.9	5.2	11.9				
	2030	7.8	7.7	7.5	6.1	6.2	6.6	5.2	5.2	6.4	8.0	6.3	12.0				
	2345	8.0	7.9	7.8	6.0	6.4	6.7	5.3	5.3	6.4	8.0	6.4	11.8				
Wed	0620	7.9	7.9	7.6	5.9	6.1	6.6	5.2	5.1	6.3	7.9	6.4	12.4				
	0945	8.0	7.9	7.8	5.5	6.4	6.4	4.6	5.2	6.4	8.0	6.1	12.6				
					6.3	6.1											
	1200	8.0	8.1	7.8	5.8	6.3	6.7	4.7	5.4	5.7	8.0	6.0	12.5				
					6.9		6.8	5.7	4.8	6.4		6.3					
	1500	8.0	8.1	8.0	5.2	6.4	6.7	3.8	5.2	6.0	8.0	6.2	12.8				
				7.9	6.2	5.9	6.7	4.8	4.5	6.9		6.2					
	1700	8.1	8.1	7.7	5.8	6.6	6.4	4.5	5.2	6.1	8.2	6.3	12.9				
					6.7	6.2	6.4	5.4	4.9	6.3		6.0					
	2045	8.2	8.1	8.0	5.4	6.9	6.4	4.6	5.6	5.8	8.0	6.3	13.0				
					6.1	6.0	7.0	5.6	5.1	6.4		6.1					
	2330	8.0	8.0	7.8	5.4	6.6	6.3	4.3	5.4	5.5	8.0	6.1	13.3				
					6.2	5.8	6.4	5.6	4.5	6.6		6.0					
Thurs	0620	7.9	7.9	7.6	5.0	6.2	6.3	4.3	4.9	5.3	8.0	5.9	13.0				
					5.5	5.5	6.6	5.4	4.8	6.4		5.8					
	0945	8.0	8.1	7.7	5.4	6.6	6.3	4.5	5.3	5.6	8.0	6.0	13.1	7.48			
					6.6	5.3	6.6	5.6	4.9	6.6		5.8					

TABLE 8 shows oxygen, temperature and pH readings for the test tank and standard purification tank for the third 6 layer trial.

NOTE Standard tank not in use. When two readings are given the upper figure denotes a reading at one third the water depth and the lower figure denotes a reading taken at two thirds the water depth.

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 1

START

POSITION	TC	EC
1	19	2
2	21	4
3	18	2
4	38	9
5	31	3
6	25	3

TABLE 9 above shows the microbiological counts for total Coliforms, E. Coli, for 100ml water samples taken at the beginning of trial (1 Layer)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 2

START

POSITION	TC	EC	FS
1	94	55	46
2	130	80	70
3	103	80	70
4	100	100	70
5	120	100	65
6	115	88	80
Estuary Water	4500	1500	1500

TABLE 10 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning of trial (2 Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 3

	START			END		
POSITION	**TC**	**EC**	**FS**	**TC**	**EC**	**FS**
1	7	1	1	1	1	2
2	7	1	3	1	1	24
3	5	1	1	1	1	11
4	8	2	6	2	1	27
5	14	1	8	3	1	34
6	9	1	5	1	1	95

TABLE 11 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning and completion of trial (3 Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 4

	START			END		
POSITION	TC	EC	FS	TC	EC	FS
1	1	1	1	1	1	1
2	1	1	3	1	1	1
3	1	1	2	1	1	2
4	1	1	8	1	1	15
5	3	1	4	1	1	11
6	1	1	23	1	1	1

TABLE 12 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning and completion of trial (4 Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 5

	START			END		
POSITION	**TC**	**EC**	**FS**	**TC**	**EC**	**FS**
1	20	13	23	9	2	7
2	17	13	24	6	4	10
3	9	9	26	12	4	8
4	26	23	31	3	1	5
5	29	26	26	10	10	6
6	27	27	19	16	4	9

TABLE 13 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning and completion of trial (5 Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 6 (A)

	START			END		
POSITION	**TC**	**EC**	**FS**	**TC**	**EC**	**FS**
1	2	1	5	1	1	4
2	3	1	14	3	1	3
3	2	1	7	1	1	8
4	35	17	100	8	6	21
5	45	25	10	4	1	16
6	47	33	20	5	1	18
Spray Bar	60	48	35	1	1	1

TABLE 14 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning and completion of trial (6 Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 6 (B)

	START			END		
POSITION	**TC**	**EC**	**FS**	**TC**	**EC**	**FS**
1	500	15	17	8	1	1
2	490	31	30	1	1	1
3	64	24	40	9	1	131
4	700	50	33	13	1	116
5	800	73	53	16	1	125
6	800	40	67	10	1	131

TABLE 15 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning and completion of trial (6B Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR WATER SAMPLES

NO. LAYERS 6 (C)

	START			END		
POSITION	**TC**	**EC**	**FS**	**TC**	**EC**	**FS**
1	1400	400	22	7	1	1
2	1800	300	16	1	1	34
3	600	300	26	6	4	52
4	2600	600	35	9	1	47
5	2000	300	26	11	1	23
6	2400	300	5	8	2	34
Estuary Water	5700	2000	26			
Spray Bar	1100	600	4	11	1	1

TABLE 16 above shows the microbiological counts for total Coliforms, E. Coli, and Faecal Streptococci for 100ml water samples taken at the beginning and completion of trial (6C Layers)

Note: positions shown in Fig. 8 in report

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 1

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	340 270 400			400 330 100	Layer 1
C					
D		68 40 200	5400 1100 400		Layer 1
E					
F	170 10 700			220 93 100	Layer 1
G	110 68 1700				Layer 1
H	800 45 ML 100	800 490 MT 600	170 110 RL 200	5400 3500 RT 1100	Layer 1

Initial	9200
	470
	17000
Control	1300
	1300
	1200

TABLE 17

Shows a plan view for the trays containing the mussels in the 1 layer trial. The top number in each tray represents the total Coliform count for the sample taken from that location. The middle number represents the E. Coli count and the lower number represents the Faecal Streptococci count.

ML - monofilament bag loosely packed.
MT - monofilament bag tightly packed.
RL - ribbon bag loosely packed.
RT - ribbon bag tightly packed.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the standard Control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 2

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	10 10 200			20 10 400	Layer 2
C					
D		20 10 300	20 10 400		Layer 1
E					
F	68 20 400			45 20 500	Layer 1
G					
H	140 20 ML 600	45 20 RL 200	120 45 MT 1000	20 20 RT 400	Layer 1

Initial	9200	5400	3500
	170	170	40
	7200	6000	2800
Control	68	45	110
	45	20	20
	900	300	400

TABLE 18

Shows a plan view for the trays containing the mussels in the 2 layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E. Coli count and the lower number represents the Faecal Streptococci count.

ML - monofilament bag loosely packed.
MT - monofilament bag tightly packed.
RL - ribbon bag loosely packed.
RT - ribbon bag tightly packed.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 3

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	78 10 100			45 10 400	Layer 3
C					
D		130 20 300	130 45 400		Layer 2
E					
F	130 45 300			220 10 400	Layer 1
G					
H	93 68 ML 400	190 93 MT 1000	78 20 RL 300	230 78 RT 500	Layer 1

Initial	1100	400	170
	120	92	20
	5200	2300	1500
Control	1300	700	140
	330	140	110
	23900	64900	800

TABLE 19

Shows a plan view for the trays containing the mussels in the 3 layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E. Coli count and the lower number represents the Faecal Streptococci count.

ML - monofilament bag loosely packed.
MT - monofilament bag tightly packed.
RL - ribbon bag loosely packed.
RT - ribbon bag tightly packed.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the Control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 4

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	20 10 100			78 20 300	Layer 4 (top)
C					
D		20 10 100	20 10 600		Layer 3
E					
F	10 10 800			45 10 300	Layer 2
G					
H	20 10 600	93 40 MT 1300	110 20 ML 800	68 10 600	Layer 1

Initial	400	470	
	110	93	
	1300	3500	
Control	130	110	330
	78	170	330
	900	1400	400

TABLE 20

Shows a plan view for the trays containing the mussels in the 4 layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E. Coli count and the lower number represents the Faecal Streptococci count.

ML - monofilament bag loosely packed.
MT - monofilament bag tightly packed.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the Control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 5

SPRAY BAR

Layer Mussels Sampled from Column 1+2		1	2	3	4	Layer Mussels Sampled From Column 3+4
	A					
Layer 4	B	68 20 100			78 45 300	Layer 5 (Top)
	C					
Layer 2	D		170 61 2600	170 45 1000		Layer 3
	E					
Layer 1	F	78 10 20			130 20 700	Layer 2
	G					
Layer 1	H	220 20 ML 800	220 68 300	9200 5400 MT 11700	330 78 RL 1700	Layer 1

Initial	9200 340 26500	3500 240 16300	3500 280 21700
Control	170 78 500	1100 140 800	170 45 1800

TABLE 21

Shows a plan view for the trays containing the mussels in the 5 layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E. Coli count and the lower number represents the Faecal Streptococci count.

ML - monofilament bag loosely packed.
MT - monofilament bag tightly packed.
RL - ribbon bag loosely packed.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the Control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 6 (A)

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	40 10 100			93 40 800	B1 Layer 5 B4 Layer 6
C					
D		110 20 100	93 20 100		Layer 3
E					
F	68 20 100			40 10 300	Layer 1
G				470 78 100	Layer 1
H		790 130 300	5400 260 MT 5600	170 45 ML 100	Layer 1

Initial	9200 200 10100	1300 270 400	210 140 3400
Control	110 20 100	270 40 200	78 45 100

TABLE 22

Shows a plan view for the trays containing the mussels in the 6(A) layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E.Coli count and the lower number represents the Faecal Streptococci count.

ML - monofilament bag loosely packed.
MT - monofilament bag tightly packed.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the Control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 6 (B)

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	710 40 100			210 110 0	B1 Layer 5 B4 Layer 6
C					
D		110 40 0	790 70 100		Layer 3
E					
F	130 130 200			270 130 200	Layer 1
G					
H	130 0 0	130 20 100	1100 230 0	330 170 100	Layer 1

Initial	7900	14000	14000
	45	1700	490
	3600	5400	5000
Control	700	330	390
	50	70	260
	0	0	100

TABLE 23

Shows a plan view for the trays containing the mussels in the 6(B) layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E.Coli count and the lower number represents the Faecal Streptococci count.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the Control Tank.

MICROBIOLOGICAL COUNTS FOR MUSSEL SAMPLES - TRIAL 6 (C)

SPRAY BAR

	1	2	3	4	Layer Mussels Sampled From
A					
B	1300 20 300			490 60 700	Bl Layer 5 B4 Layer 6
C					
D		1300 20 400	490 20 400		Layer 3
E					
F	410 20 5700			490 0 1200	Layer 1
G					
H	1700 0 8200	210 30 3100	2200 40 3500	160000 40 4000	Layer 1

Initial	11000	320	340
	170	110	170
	1100	1300	3900
Control	1100	2200	790
	40	40	20
	0	100	200

TABLE 24

Shows a plan view for the trays containing the mussels in the 6(C) layer trial. The top number for each sample represents the total Coliform count for the sample taken from that location. The middle number represents the E.Coli count and the lower number represents the Faecal Streptococci count.

Initial counts are the counts for the unpurified test mussels. Control counts are the counts for the mussels purified in the Control Tank.

TRIALS		CONTROLS	
T1	7.80 - 7.77 (.03)	CONT.	7.90 - 7.90 (0)
T2	7.87 - 7.63 (.24)		7.67 - 7.65 (.02)
T3 NOT START	7.6 - 6.53 (.08)		7.75 - 7.61 (.14)
T4	7.75 - 7.71 (.04)		8.02 - 7.99 (.03)
T5	7.80 - 7.68 (.12)		7.49 - 7.57 (-.06)
T6(A) * SPAWNED	8.26 - 7.53 (.73)		7.52 - 7.47 (.05)
T6(B)	7.86 - 7.72 (.14)		-
T6(C)	7.53 - 7.48 (.05)		-

TABLE 25

Describes range and reduction of pH measurement in the Trial and Control Tanks during each of the trials.

APPENDIX II

Microbiological Analysis Technique for Mussels and Water

1 MUSSEL ANALYSIS TECHNIQUE

1.1 Removal of Shell Contents

The hands of the examiner were washed before removal of meat and liquor from mussels. Sterile scalpels were used to open the mussels and approximately 35-55 gr of meat and intervalvular liquor was collected in sterile petri dishes. Before each mussel was opened the blade of the scalpel was wiped clean, dipped into industrial methylated spirit, passed through a flame and allowed to cool. The mussel flesh was cut into small pieces and transferred into a sterile pre-weighed small plastic bag.

1.2 Homogenisation and Dilution of Sample

Samples of mussel meat and liquor were weighed to the nearest gram. It was necessary to homogenise and dilute the sample in order to achieve a consistency which permitted ease and accuracy of inoculation into culture media. One part, by weight, of sterile 0.1% (w/v) peptone water (Bacteriological peptone 0.1%) was added to the sample and the mixture homogenised for at least 90 seconds in a Stomacher (Seward Laboratory, Model No. BA6021). Twenty (20ml) of the homogenate was poured into a sterile wide-bottomed glass jar. To that was added 80ml of sterile 0.1% (w/v) peptone water. Therefore the final dilution of the homogenate was 1:10.

2 BACTERIOLOGICAL METHODS

2.1 Determination of Total Coliforms (TC), E.coli (EC)

Using 10ml sterile pipettes, 10ml of homogenate was transferred to each of five tubes containing 10ml "double strength" Mineral Modified Medium Base (MMMB) (OXOID) and an inverted small glass tube (Durham vial). Aliquots 1ml of the homogenate was transferred to each of 5 tubes containing 10ml "single strength" MMMB (OXOID) and an inverted small glass tube. Aliquots 0.1ml of the homogenate was transferred to each of 5 tubes containing 10ml "single strength" MMMB (OXOID) and an inverted small glass tube.

After the contents of each tube were thoroughly mixed by gentle swirling, the 3 sets of 5 tubes were incubated at 37°C in a warm air incubator. Tubes were examined visually after 48 hours for bacterial growth, acid positive and gas formation in the Durham vial. Tubes gas positive and acid positive were subcultured by using a 3mm diameter wire loop, to one tube containing 10ml Brilliant Green Bile (20%) Broth (BGBB, OXOID) and an inverted small glass tube, and incubated at 37°C for 24 hours. Subcultures were also taken to another tube of BGBB and these were inserted at 44°C in a thermostatically controlled water bath for 24 hours. Tubes of BGBB incubated at 44°C showing gas formation were inoculated in tubes containing 5ml of Tryptone water (OXOID). The new set of tubes were incubated at 37°C for 24 hours.

Total Coliforms: The number of tubes of BGBB incubated at 37°C showing gas formation at each were dilution recorded and the corresponding Most Probably Number (MPN) was determined by reference to standard tables (American Public Health Association, 1984).

E. Coli (EC): The number of tryptone water tubes incubated at 37°C showing Indole production after adding few drops of Kovac's reagent were recorded and the corresponding MPN was determined by reference to standard table (APHA, 1984).

2.2 Faecal Streptococci (FS)

Aliquots 2ml of the homogenate was pipetted into each of 5 petri dishes. To that was added 15.20ml of freshly made Slanetz and Bartley agar (OXOID). The set of petri dishes was incubated at 37°C for 48 hours. Counts of FS were determined by adding up the number of red colonies on all 5 plates. The sum of red colonies represents FS per gram. For the first week and the first half of the second week only 1ml of the homogenate was added to each of 5 tryvli dishes.

Water samples were taken and examined by membrane filtration. Samples were taken at 0-48 hours. By the membrane filtration technique 100ml of seawater was filtered through Whatman filters port size 0.45u placed in Millipore filter holder. After filtration the membrane was removed with sterile forceps and transferred directly to absorbent pads saturated with 4ml growth medium (Membrane Lauryl Broth PHLS/SCA, 1980 b) (pH 7.4-7.5). The sample was incubated at 44°C for 24 hours.

Standards	Esch. coli per 100gm flesh	
United Kingdom	0 - 500	Satisfactory
	600 - 1500	Suspicious
	1500	Unsatisfactory
E.E.C.	300	Satisfactory
U.S.A.	230	Satisfactory

APPENDIX III

GENERAL CRITERIA FOR MUSSEL PURIFICATION AS ADVISED BY MAFF

1. Mussels to be laid out in a single 8cm layer, which approximates to 34kg/m^2.

2. Mussels should be covered by a minimum of 15cm depth of water.

3. Volume of water within tank should be circulated at the rate of once per hour through a U.V. steriliser.

4. Oxygen content of water should not drop below 5mg/l.

5. Purification cycle should operate for not less than 36 hours.

6. Water temperature must not be less than $5^{o}C$.